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Effect of Proximity to a Cattle Feedlot on *Escherichia coli* O157:H7 Contamination of Leafy Greens and Evaluation of the Potential for Airborne Transmission

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The impact of proximity to a beef cattle feedlot on *Escherichia coli* O157:H7 contamination of leafy greens was examined. In each of 2 years, leafy greens were planted in nine plots located 60, 120, and 180 m from a cattle feedlot (3 plots at each distance). Leafy greens (270) and feedlot manure samples (100) were collected six different times from June to September in each year. Both *E. coli* O157:H7 and total *E. coli* bacteria were recovered from leafy greens at all plot distances. *E. coli* O157:H7 was recovered from 3.5% of leafy green samples per plot at 60 m, which was higher ($P < 0.05$) than the 1.8% of positive samples per plot at 180 m, indicating a decrease in contamination as distance from the feedlot was increased. Although *E. coli* O157:H7 was not recovered from air samples at any distance, total *E. coli* was recovered from air samples at the feedlot edge and all plot distances, indicating that airborne transport of the pathogen can occur. Results suggest that risk for airborne transport of *E. coli* O157:H7 from cattle production is increased when cattle pen surfaces are very dry and when this situation is combined with cattle management or cattle behaviors that generate airborne dust. Current leafy green field distance guidelines of 120 m (400 feet) may not be adequate to limit the transmission of *E. coli* O157:H7 to produce crops planted near concentrated animal feeding operations. Additional research is needed to determine safe set-back distances between cattle feedlots and crop production that will reduce fresh produce contamination.

The consumption of fresh produce increasingly has been linked to human food-borne disease (1–4). In particular, leafy vegetables, such as spinach and lettuce, have become significant vehicles for the transmission of food-borne pathogens. Produce-associated outbreak surveillance data from the Centers for Disease Control and Prevention (CDC) for the period from 2000 to 2009 showed that among produce commodities, leafy greens were the most frequently linked to outbreaks (5). An analysis of published reports of U.S. fresh produce-associated outbreaks with an attribution risk ranking tool categorized the combination of leafy greens and *Escherichia coli* O157:H7 with the highest risk ranking score, followed by *Salmonella enterica* in tomatoes ranking second and *S. enterica* in leafy greens ranking third (6). For all U.S. food-borne disease outbreaks caused by Shiga toxin-producing *E. coli* (STEC) in the period of 1998 to 2008, leafy vegetables were the second most common implicated commodity, following beef (2). In addition, spinach, lettuce, and cilantro were commodities most associated with the recovery of STEC in produce market basket surveys conducted within the USDA Agricultural Marketing Service Microbiological Data Program from 2002 to 2012 (7). Leafy greens typically are consumed raw, so protecting these products from microbial contamination in the preharvest environment is critical to reducing the risk for food-borne illness. Potential sources of pathogens for preharvest produce contamination include contaminated soil and soil amendments (manures, biosolids, and composts), contaminated irrigation water or runoff water from livestock operations or manure-amended fields, livestock, wild animals, birds, and insects (8–13).

Cattle are a significant reservoir of the zoonotic pathogen *E. coli* O157:H7 (14). For this reason, outbreaks of *E. coli* O157:H7 disease linked to the consumption of spinach and lettuce have focused attention on cattle as a potential source of this pathogen for produce contamination. Food safety guidelines published by the California Leafy Green Products Handler Marketing Agreement (15) propose an interim guidance distance of 400 ft (120 m) between concentrated animal feeding operations and crops but also admit that there is a lack of scientific data supporting this guidance. In particular, the significance or magnitude of the risk of transport of *E. coli* O157:H7 from animal production facilities to produce crops by wind, dust emissions, or insects is unknown. Although the airborne transport of *E. coli* has been observed, data regarding emission rates from livestock production or transport distances is limited (9, 16–18). Millner and Suslow (18) collected 1,000-liter bioaerosol samples at distances of 30 to 400 ft downwind of a cattle feedlot; they recovered high *E. coli* concentrations from air at 30 ft and lower *E. coli* concentrations at 100 ft, and they did not detect *E. coli* at distances of 200 ft or greater. In a separate

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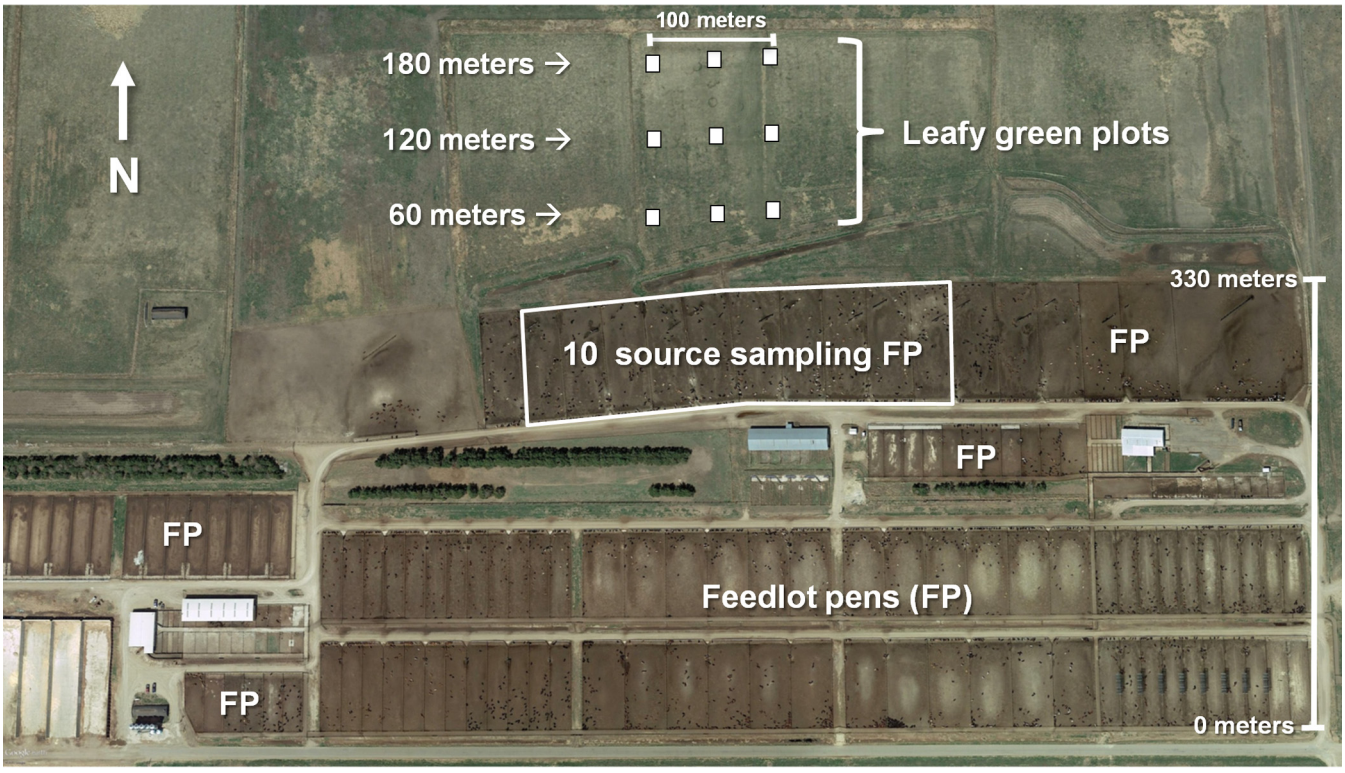

FIG 1 Google Earth image of the feedlot, showing the locations of the 9 leafy green plots sited 60, 120, and 180 m from the north edge of the feedlot. The 10 feedlot pens immediately south of the plots in the northernmost row of feedlot pens were designated for source sampling and are outlined.

study, bundles of fresh spinach were staged at distances of 0, 60, and 150 ft downwind from the loadout area of a cattle feedyard, where substantial dust can be generated when cattle are being moved to or from the feedyard (19). Air sampling did not detect airborne pathogens; however, *E. coli* O157:H7, *Salmonella*, and generic *E. coli* organisms were found on the spinach following dust generation by cattle, with little effect of distance from the loadout area on the numbers of pathogen-positive samples or the levels of generic *E. coli* on the spinach. Further research is needed to determine set-back distances or buffer zones that will effectively reduce the risk of airborne *E. coli* O157:H7 contamination of produce crops.

The objectives of this study were to determine the impact of the proximity to a beef cattle feedlot on *E. coli* O157:H7 contamination of a leafy green produce crop and to evaluate the potential for airborne dissemination of *E. coli* O157:H7.

MATERIALS AND METHODS

Study site and experimental design. The study was conducted from May to September in both 2011 and 2012. Beginning in May of each year, leafy greens were planted in each of nine plots (6.1 by 9.1 m) that were located 60, 120, and 180 m (200, 400, and 600 ft, respectively) from the nearest row of feedlot pens at the 6,000-head-capacity beef cattle feedlot at the U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska (three plots at each distance) (Fig. 1). The plots were planted in a field north of the feedlot in order to take advantage of the prevailing south winds that are typical during the spring and summer in this region. Subplots within each plot were planted every 2 to 3 weeks to provide leaves for sampling from June through September. Spinach (Renegade F1) was planted in 2011; in 2012, mustard greens (Tendergreen) and turnip greens (Purple Top White Globe) were planted in addition to spinach. The plots were enclosed with 2.4-m-high fence panels to exclude deer; poultry netting (91.4 cm high) was fastened to the bottom of the fence panels to exclude rabbits. The fence panels were constructed of galvanized 6-gauge wire with 10.2-cm by 10.2-cm spacing (Oklahoma Steel & Wire, Madill, OK).

Irrigation water was hauled in by truck as needed and applied by gentle overhead spraying to simulate the common industry practice of overhead sprinkle irrigation. On a given day, the entire planted areas of all of the plots were irrigated to give the same amount of water. From 7.0 to 20.5 mm of water was applied depending upon moisture need. Water samples were periodically collected throughout each project season both at the source well and coming out of the tank. For each sampling, two 10-ml samples from each site (well and tank) were tested for *E. coli* O157:H7 using the enrichment and immunomagnetic separation (IMS) procedures described below. Total *E. coli* was determined by spread plating 200 μl of each water sample onto each of 5 plates of CHROMagar ECC (DRG International, Inc., Mountainside, NJ), which were incubated at 37°C overnight and examined for blue *E. coli* colonies. Additionally, soil samples (two per plot) were collected and analyzed before the first planting in May. Each soil sample was a composite of several soil cores taken from half of the plot; the soil corer was cleaned and sanitized with 70% isopropyl alcohol between samples. The composited soil was mixed, and 10-g samples were tested for *E. coli* O157:H7 and total *E. coli* as described below.

The 10 feedlot pens (approximately 30 by 90 m each) most adjacent to the plots were designated for source sampling (Fig. 1). Feedlot personnel ensured that cattle were housed in these pens nearly continuously from May through mid-September in each year. The pens typically were stocked with 60 to 80 head per pen. The feedlot cattle working schedule was obtained in order to evaluate the impact of these activities (moving cattle in and/or out of the pens for weighing, vaccination, treatment, shipping, etc.) on the experimental data. To assess environmental conditions,

weather data were recorded by an on-site weather station (Vantage Pro2; Davis Instruments, Hayward, CA).

Sampling and microbial analyses of leafy greens and FSM. Leafy greens and feedlot surface manure (FSM) were collected and analyzed six times in each of 2011 and 2012: once in June, twice in July, twice in August, and once in September. For leafy greens, the target on each sampling day was to collect 30 samples from each plot, for a total of 270 samples each day, with 90 samples from each plot distance of 60, 120, and 180 m from the feedlot. If the number of samples in one plot was limited, additional samples were collected from other plots at the same distance. In August 2011, spinach available for sampling was limited because of poor germination and growth, so on 4 August 2011 and 29 August 2011, 210 and 248 samples were collected, respectively. Plots at 180 m were sampled first, and plots at 60 m were sampled last. Leafy greens were collected using clean latex gloves and scissors to cut randomly sampled leaves from throughout the plot, and individual samples were placed in separate ziplock bags. Between individual samples, a new pair of gloves was donned and scissors were sanitized with 70% isopropyl alcohol.

At each sampling time, 10 FSM samples were collected from each of the 10 feedlot pens, from behind the concrete feedbunk apron (source samples; 100 FSM samples each sampling time). Separate teams of personnel collected leafy greens and FSM in order to avoid any inadvertent contamination of the crop via footwear or clothing worn in the feedlot pens. The FSM samples (ca. 250 to 300 g each) were collected directly from the pen surface by hand, using a clean latex glove for each sample, and were placed in separate ziplock bags. Samples were transported to the laboratory for processing within 1 h of collection.

E. coli O157:H7 presence and total *E. coli* levels were determined for each leafy green sample. Ten grams of leaves was weighed into a sterile filtered bag (Nasco, Ft. Atkinson, WI), 90 ml of tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD) was added, and the bag contents were mixed well by hand massage. For determination of total *E. coli*, 750 μl was removed into a sterile tube and 50 μl was spiral plated onto CHROMagar ECC using an Autoplate 4000 spiral plater (Spiral Biotech, Inc., Norwood, MA). CHROMagar ECC plates were incubated at 37°C overnight, and blue *E. coli* colonies were counted. The limit of detection of this direct plating was 200 CFU g^{-1}. The remainder of the 1:10 dilutions of samples in TSB were incubated for 7 h at 37°C, held at 4°C overnight, and then subjected to IMS to determine the presence of *E. coli* O157:H7 as described below.

E. coli O157:H7 presence and levels were determined for each FSM sample. Ten grams of FSM was measured into a sterile filtered bag, 90 ml of TSB was added, and bag contents were mixed as described above. A 750-μl aliquot was removed, and 50 μl was spiral plated onto CHROMagar O157 (DRG International) containing 5 mg $liter^{-1}$ novobiocin and 2.5 mg $liter^{-1}$ potassium tellurite (ntCHROMO157). The ntCHROMO157 plates were incubated at 42°C overnight, and presumptive mauve-colored colonies were tested with *E. coli* O157 latex agglutination reagents (Oxoid Ltd., Basingstoke, United Kingdom). Agglutination-positive colonies were counted and confirmed by multiplex PCR as described below. The remaining FSM samples in the bags were incubated for 7 h at 37°C and then held at 4°C overnight before IMS to recover *E. coli* O157:H7.

For IMS, 1,000 μl of leafy green sample enrichments was added to 20 μl of anti-O157 Dynabeads (Invitrogen Corp., Carlsbad, CA) in individual wells of 96-well deep-well blocks. For IMS of FSM sample enrichments, 500 μl of enrichment was added to 500 μl of phosphate-buffered saline with Tween (PBS-Tween; Sigma, St. Louis, MO) and 20 μl of anti-O157 Dynabeads in individual wells of 96-well deep-well blocks. Beads and samples were mixed by shaking at room temperature for 20 min. The IMS beads were removed from the sample, washed twice in 1-ml volumes of PBS-Tween, and concentrated into 100 μl of PBS-Tween using a Kingfisher 96 magnetic particle processor. Fifty μl of the concentrated IMS beads was spread plated onto ntCHROMO157 and incubated at 37°C for 22 to 24 h. Presumptive colonies were tested with *E. coli* O157 latex agglutination reagents. Agglutination-positive colonies were isolated and confirmed by multiplex PCR for genes for O157, H7 flagellin, intimin, and Shiga toxins 1 and 2 (20). The *fliC* primer sequences were those of Gannon et al. (21), and PCR conditions were those of Paton and Paton (22). Confirmed *E. coli* O157:H7 isolates were subjected to pulsed-field gel electrophoresis (PFGE) subtyping using the CDC PulseNet protocol and the restriction endonuclease XbaI (23). Subtypes were defined as isolates that had indistinguishable PFGE patterns.

The specificity of CHROMagar ECC for total *E. coli* enumeration was confirmed using one set of leafy green samples. One to two blue colonies from each positive CHROMagar ECC plate were streaked for isolation onto a fresh plate, and isolated colonies were tested by PCR using primers based on specific 16S rRNA gene sequences for *E. coli* (24). The PCR conditions were as described by Gonzales et al. (25).

Air sampling. MAS-100 Eco microbial air samplers (Merck KGaA, Darmstadt, Germany) were used to collect air samples, when the wind was from the south, southeast, or southwest. On each sampling day, air samples were collected at each of the nine leafy greens plots and at three locations at the north edge of the feedlot pens. Four air samplers were used in order to collect air samples simultaneously at sites located 0, 60, 120, and 180 m from the feedlot. CHROMagar O157 containing 1.0 g $liter^{-1}$ sodium pyruvate was used for determination of *E. coli* O157:H7 (CHROMO157-SP), and CHROMagar ECC containing 1.0 g $liter^{-1}$ sodium pyruvate was used for determination of total *E. coli* (CHROMECC-SP). At each site, 1-m^3 air samples were collected onto two plates each of CHROMO157-SP and CHROMECC-SP ($n = 6$ for each bacterial group, at each of 0, 60, 120, and 180 m from the feedlot). On a given sampling day, the entire air sampling process took approximately 2.5 h and was conducted in either the morning or afternoon. Pen surface conditions (i.e., dusty, muddy) were recorded, as were wind speed and direction, which were confirmed later with weather station data. CHROMO157-SP and CHROMECC-SP plates were incubated at 37°C for up to 48 h and examined for characteristic target colonies.

Statistical analyses. The number of leafy green samples in each plot that were positive for *E. coli* O157:H7 or total *E. coli* for each sample date were reported as a percentage. Total *E. coli* concentrations in air samples were reported as CFU/m^3 of air. The experimental unit was the plot; for air sample data analyses, three air sampling sites along the feedlot pen fences were designated as plots located 0 m from the feedlot. Least-squares means were analyzed using the General Linear Models procedure (GLM; SAS Institute, Inc., Cary, NC). For *E. coli* O157:H7 or total *E. coli* in leafy greens, the model included the effects of year, sample date, distance, and all interactions; for air sample data, total *E. coli* concentrations on each sample date were analyzed separately across distance. Distance was tested, with plot(distance) as the error term. Least-squares means are presented in the text and tables. For all statistical analyses, differences were considered significant at $P < 0.05$ and were considered tendencies at P values of less than 0.10 but greater than 0.05.

RESULTS AND DISCUSSION

Because the prevalence of *E. coli* O157:H7 shedding by cattle may vary from year to year, the study was conducted in each of 2 years to improve our chances that the magnitude of this pathogen shed by the cattle was adequate to accomplish the study objectives. The seasonal variation of shedding of *E. coli* O157:H7 by cattle is well recognized, with the prevalence of shedding typically being highest during the warmer months (26, 27). However, numerous studies also have demonstrated the transient nature of *E. coli* O157:H7 shedding (28, 29); thus, we sampled FSM and leafy greens multiple times within each year and collected numerous samples at each interval. Climatic conditions in a given location also vary annually, so conducting the 2-year study provided the opportunity to examine the potential impacts of various environmental conditions on the dissemination of *E. coli* O157:H7 from cattle produc-

TABLE 1 Weather data during the 5-month project periods in 2011 and 2012

Yr and mo	Precipitation (mm)	Avg temp (°C)	No. of days with high temp (≥32.2°C)	Dominant wind direction[b]	Avg wind speed (mph)	Avg daily high wind speed (mph)	No. of days with high wind (≥25 mph)
2011							
May[a]	128	15.4	3	SE	10.5	24.0	21
June	60	21.6	6	ESE	7.4	25.5	14
July	105	26.1	15	ESE	5.6	23.1	13
August	140	23.1	7	SE	4.8	20.7	5
September	18	15.7	2	ESE	4.4	19.2	3
Entire 5-month project period	452	20.3	33	ESE	6.6	22.6	56
2012							
May	87	18.8	5	SSE	7.8	26.8	19
June	96	23.5	10	S	7.4	26.9	17
July	60	27.1	25	S	4.1	18.2	3
August	51	22.7	12	S	4.3	20.9	9
September	16	18.1	6	SSE	4.0	19.8	11
Entire 5-month project period	310	22.0	58	S	5.5	22.5	59

[a] The on-site weather station was installed on 27 May 2011. Weather data for May 2011 were obtained from a weather station at the University of Nebraska South Central Agricultural Laboratory located approximately 2 miles from the experimental site at the feedlot.
[b] ESE, east-southeast; SSE, south-southeast.

tion. Weather data are summarized in Table 1 for the 5-month project periods in each year. Average annual precipitation for Clay Center, Nebraska, is 731 mm, with average rainfall of 484 mm during the 5-month period from May to September (National Climatic Data Center; http://www.ncdc.noaa.gov/). Rainfall during May-September 2011 was 452 mm (93% of average rainfall) but only 310 mm during May-September 2012, which was 64% of the average rainfall (Table 1). The average temperatures during May-September in 2011 and 2012 were 20.3 and 22.0°C, respectively. Although these average temperatures were similar, there were an additional 25 days during the 5-month project period in 2012 for which the high temperature was 32.2°C or greater (33 days in 2011 and 58 days in 2012). Winds were predominantly from a southerly direction during each project period, being more from the east in 2011 and more from the south in 2012. The average wind speed, average daily high wind speed, and the number of days with high winds of ≥25 mph were comparable in 2011 and 2012.

We initially proposed to conduct all experiments using spinach. However, in the first year we found spinach production to be a challenge when temperatures were very hot during July and August. The hot temperatures substantially inhibited the germination and growth of the spinach that was planted during these months. Thus, in 2012 we also planted mustard greens and turnip greens as alternative trap crops. Both mustard and turnips germinated and grew well compared to spinach when temperatures were hot. However, both mustard and turnips contain glucosinolates and glucosinolate breakdown products, which can have potent antimicrobial properties. To confirm that these antimicrobial compounds did not inhibit *E. coli* O157:H7 growth during the short nonselective enrichment of mustard and turnip greens, we conducted an inoculation and recovery experiment wherein 20 samples each of freshly cut spinach, mustard greens, and turnip greens were inoculated with the same low level (4.8 cells per gram of leafy greens) of a 5-strain cocktail of bovine *E. coli* O157:H7. When subjected to our enrichment and IMS procedure as described above, *E. coli* O157:H7 was recovered from 100% of each sample type, demonstrating that mustard greens and turnip greens could be used as substitute trap crops for spinach.

Irrigation water and soil from the plots were sampled and analyzed to assess any potential contributions to crop contamination in this experiment. Irrigation water, collected from the well and coming out of the truck tank, was tested three times during both 5-month study periods, and it was always negative for both *E. coli* O157:H7 and *E. coli*. Among soil samples collected from the plots before planting in May, one soil sample from a plot located 60 m from the feedlot was positive for *E. coli* O157:H7 in 2012. The PFGE subtype of this soil isolate was unique from the PFGE subtypes of *E. coli* O157:H7 isolates from leafy greens (discussed further below), suggesting that the preplanting soil was not a significant source of the pathogen. All other soil samples were negative for *E. coli* O157:H7 and *E. coli*.

The FSM in the nearest 10 feedlot pens was examined as a means to confirm the presence of the pathogen in the most probable source material (Fig. 1) and also to provide *E. coli* O157:H7 strains for PFGE subtyping to infer linkages to strains that were found in the leafy greens. The percentage of positive FSM samples on each sample date ranged from 49 to 94% in 2011 and from 47 to 97% in 2012 (Table 2). Because of the constant mixing and dispersion of excreted feces by hoof action on the feedlot pen surface, the prevalence of this pathogen in FSM is not a direct measurement of the prevalence of fecal shedding by the cattle; however, these data do indicate that the cattle were shedding *E. coli* O157:H7 throughout both project periods. The average percentages of *E. coli* O157:H7-positive FSM samples in each year, 73.3% in 2011 and 71.7% in 2012, were similar ($P > 0.05$). In addition, the average percentages of FSM samples in which *E. coli* O157:H7 was detectable by direct plating for each year were not different ($P > 0.05$) (Table 2). Over both years, 9.2% of FSM samples had enumerable levels of *E. coli* O157:H7, which is similar to our previous observations of 12.8% of FSM samples with enumerable levels of *E. coli* O157:H7 (30) and suggests the presence of cattle that were shedding high concentrations of the pathogen.

On 13 August 2012, 43% of FSM samples contained levels of *E.*

TABLE 2 Percentage of *E. coli* O157:H7-positive and -enumerable samples of FSM and average percentage of *E. coli* O157:H7-positive leafy green samples per plot[a]

Leafy green sample date	% of *E. coli* O157:H7-positive (-enumerable[b]) samples in feedlot surface manure[c]	Avg % of *E. coli* O157:H7-positive leafy green samples per plot at[d]:		
		60 m	120 m	180 m
28 June 2011	49.0 (9.0)	0 A	0 A	1.1 A
5 July 2011	81.0 (10.0)	4.4 A	2.2 A	0 A
20 July 2011	67.0 (2.0)	2.9 A	0 A	1.1 A
4 August 2011	94.0 (9.0)	1.4 A	0 A	0 A
29 August 2011	62.0 (9.0)	0 A	0 A	0 A
12 September 2011	87.0 (7.0)	0 A	0 A	2.4 A
All dates, 2011	73.3 (7.7)	1.5 A	0.4 A	0.8 A
18 June 2012	83.0 (7.0)	4.4 A	3.3 A	1.1 A
2 July 2012	66.0 (2.0)	6.6 A	3.3 AB	0 B
23 July 2012	47.0 (3.0)	1.7 A	1.8 A	0 A
13 August 2012	97.0 (43.0)	2.2 A	1.1 A	0 A
27 August 2012	77.0 (6.0)	12.2 A	2.2 B	0 B
10 September 2012	60.0 (3.0)	6.4 A	12.2 B	15.5 B
All dates, 2012	71.7 (10.7)	5.6 A	4.0 AB	2.8 B
All dates, 2011 and 2012	72.5 (9.2)	3.5 A	2.2 AB	1.8 B

[a] On each sample date, 100 feedlot pen surface samples were collected, and 270 leafy green samples were collected (30 leafy green samples from each of 9 plots, for 90 leafy green samples per plot distance).
[b] Enumerable means detectable by direct plating. The limit of detection for direct plating was 200 CFU g^{-1}.
[c] FSM samples typically were collected on the same days that leafy greens were sampled, but on 20 July 2011, 4 August 2011, and 29 August 2011, the FSM samples were collected 1, 3, and 5 days, respectively, before leafy green samples were collected.
[d] The leafy greens were spinach for all sample dates, with the exception of 13 and 27 August 2012, when a mixture of turnip and mustard greens was sampled, and 10 September 2012, when only turnip greens were sampled. Within rows, least-squares means followed by different letters (A and B) are significantly different ($P < 0.05$).

coli O157:H7 detectable by direct plating, which was significantly higher ($P < 0.05$) than was seen on any other sample date (Table 2). While the average concentrations of *E. coli* O157:H7 among enumerable FSM did not differ by sample date ($P > 0.05$) (data not shown), a high proportion of the FSM samples with high concentrations of the pathogen, including 2 of 2 samples with >5.00 log CFU g^{-1} of FSM, 3 of 4 samples with ca. 4.00 log CFU g^{-1} of FSM, and 13 of 24 samples with ca. 3.00 log CFU g^{-1} of FSM, were sampled on this day. The majority of enumerable FSM samples (80 of 110) had <3.00 log CFU g^{-1} of FSM of *E. coli* O157:H7. Among the enumerable FSM samples, the average concentrations of *E. coli* O157:H7 in 2011 and 2012 were 2.71 and 2.91 log CFU g^{-1} of FSM, respectively, and were not different ($P > 0.05$) (data not shown).

The average percentages of *E. coli* O157:H7-positive leafy green samples per plot at each distance are shown in Table 2. In general, *E. coli* O157:H7 was recovered from leafy greens at low rates, but it was found at all three plot distances tested. There was no significant effect of distance on the percentage of *E. coli* O157:H7-positive samples per plot on any sample date in 2011 ($P > 0.05$). Over the whole year, 1.5% of spinach samples per plot at 60 m were positive for the pathogen, and this was not significantly different from the 0.4% at 120 m or the 0.8% at 180 m.

The recovery of *E. coli* O157:H7 from leafy greens was higher in 2012 than in 2011 ($P < 0.05$). On three sample dates (2 July 2012, 27 August 2012, and 10 September 2012), there was an effect of distance on the percentage of *E. coli* O157:H7-positive samples per plot ($P < 0.05$). Interestingly, on 10 September 2012 the percentage of *E. coli* O157:H7-positive samples per plot at 180 m was significantly higher at 15.5% ($P < 0.05$) than the 6.4% of positive samples at 60 m. However, when averaged over all sample dates in 2012, the 5.6% of positive samples per plot recovered at 60 m was significantly higher than the 2.8% of positive samples per plot recovered at 180 m ($P < 0.05$). When data from both years are considered together, the 3.5% of positive leafy green samples per plot at 60 m was higher than the 1.8% of positive samples at 180 m ($P < 0.05$), indicating a decrease in *E. coli* O157:H7 contamination as distance from the feedlot is increased.

The higher recovery of *E. coli* O157:H7 from leafy greens in 2012 was due in large part to the recoveries of the pathogen on 27 August 2012 and 10 September 2012 (Table 2). These two sample dates were preceded by several weeks of very little rainfall and several days of high temperatures. Average August precipitation for Clay Center, Nebraska, is 87.9 mm; from 1 August 2012 through 10 September 2012, there was only 51.6 mm of rainfall. In the 2 weeks before sampling on 10 September 2012, only 0.51 mm of precipitation fell (data not shown). During the same time the average daily high temperature was 32.4°C, with 8 days for which the high temperature was 32.2°C or greater. In the 2 weeks before the 27 August sampling, the only recorded precipitation was 27.7 mm on 26 August 2012, and the average daily high temperature was 28.1°C, with 3 days with high temperatures that were 32.2°C or greater. The high percentages of *E. coli* O157:H7-positive leafy green samples per plot for each plot distance on 10 September 2012 are further associated with a large rearrangement of cattle in the northernmost row of feedlot pens on 6 and 7 September 2012. Over these 2 days, a total of 450 head of cattle (including 300 head from the 10 adjacent pens; Fig. 1) were moved out of their pens and replaced with a similar number of cattle. The high temperatures and low rainfall had made the feedlot pen surfaces very dry and dusty. Furthermore, winds often were breezy (average daily high wind speed of 21.9 mph) during this 4-week period from 13 August through 10 September 2012 and were predominantly from the south, likely transporting the pathogen north from the feedlot into the plots.

Another contributing factor to the higher recovery of *E. coli* O157:H7 from leafy greens on 27 August and 10 September 2012 may be the higher prevalence and levels of the pathogen on the feedlot surface on 13 August 2012 (Table 2). As noted above, a significantly higher percentage of FSM samples collected on that date had concentrations of *E. coli* O157:H7 high enough for detection by direct plating. While the occurrence of the pathogen in leafy greens on that same sample date was relatively low, subsequent dissemination of high levels of *E. coli* O157:H7 from the feedlot surface to the plots may have increased the contamination of leafy greens that were sampled on the later dates. Numerous studies have evaluated the risks associated with preharvest contamination of leafy vegetables and have found that *E. coli* O157:H7 can persist on spinach and lettuce for days up to weeks (31–38). Both growth chamber and field studies indicate that after inoculation onto leafy greens to mimic a contamination event, populations of *E. coli* O157:H7 initially decrease rapidly, followed by a more gradual rate of decline of remaining cells. The persistence of

TABLE 3 Average percentage of total *E. coli*-positive leafy green samples per plot at 60, 120, and 180 m from the feedlot[a]

Sample date	Avg % of *E. coli*-positive leafy green samples per plot at[b]:		
	60 m	120 m	180 m
28 June 2011	10.6 A	7.8 A	13.3 A
5 July 2011	45.6 A	32.2 A	22.2 A
20 July 2011	41.5 A	25.5 AB	13.0 B
4 August 2011	42.3 A	80.0 B	92.2 B
29 August 2011	38.3 A	16.1 AB	3.0 B
12 September 2011	9.6 A	6.9 A	15.8 A
All dates, 2011	31.3 A	28.1 A	26.6 A
18 June 2012	33.3 A	25.5 A	7.8 A
2 July 2012	57.8 A	37.8 A	45.6 A
23 July 2012	13.1 A	2.2 A	11.1 A
13 August 2012	1.1 A	1.1 A	0 A
27 August 2012	72.0 A	46.7 B	25.6 B
10 September 2012	60.6 A	71.1 A	63.3 A
All dates, 2012	39.6 A	30.7 AB	25.6 B
All dates, 2011 and 2012	35.5 A	29.4 AB	26.1 B

[a] On each sample date, 270 leafy green samples were collected (30 leafy greens samples from each of 9 plots, for 90 leafy green samples per plot distance).
[b] Within a row, least-squares means followed by different letters (A and B) are significantly different ($P < 0.05$). The leafy greens were spinach for all sample dates, with the exception of 13 and 27 August 2012, when a mixture of turnip and mustard greens was sampled, and 10 September 2012, when only turnip greens were sampled.

E. coli O157:H7 on leafy greens is further suggested by the finding that spinach sampled >66 days after planting was more likely to be contaminated with generic *E. coli* (39). In addition to the potential for greater probability of contamination due to longer exposure, leafy greens at later stages of maturity may be at greater risk for contamination (40, 41). In the current study, there was poor correlation between the number of days after planting and the percentage of leafy green samples that were positive for *E. coli* O157:H7 at any distance from the feedlot (R^2 values from 0.002 to 0.056) or when data were pooled over distance (R^2 of 0.049) (data not shown).

The total *E. coli* on leafy greens was determined in the case that *E. coli* O157:H7 was not detectable and also to provide supplemental information regarding the dissemination of this bacterial species from the feedlot. The average percentages of total *E. coli*-positive samples per plot at each distance are shown in Table 3. The findings of such high percentages of *E. coli*-positive leafy greens was unexpected, especially given that the limit of detection for the direct spiral plating was 200 CFU g^{-1}. To our knowledge, CHROMagar ECC has not been tested for its specificity in the recovery and quantitation of *E. coli* from leafy green samples. To confirm specificity, we tested 148 presumptive *E. coli* isolates, purified from blue colonies on CHROMagar ECC plates from a set of plated leafy green samples, for the presence of 16S rRNA gene sequences specific for *E. coli* (24). These specific primers amplify a 96-bp fragment in *E. coli*, which was obtained for all 148 of the screened isolates. Within the distances that we tested, the high percentages of total *E. coli*-positive samples seem to preclude the use of this measure as an indicator for *E. coli* O157:H7 contamination of leafy greens grown near cattle feedlots. However, the data do suggest factors that influence the dissemination of this species from cattle production facilities, as well as the risk for produce contamination.

Total *E. coli* was recovered from leafy greens at all distances. As seen for *E. coli* O157:H7, on many sample dates there was no effect of distance on the percentage of *E. coli*-positive samples per plot. There was a significant effect of distance ($P < 0.05$) on 20 July 2011, 4 August 2011, 29 August 2011, and 27 August 2012; with the exception of 4 August 2011, the percentage of *E. coli*-positive leafy green samples per plot was higher at 60 m than at 180 m from the edge of the feedlot. The reverse trend was seen on 4 August 2012, where the percentage of *E. coli*-positive leafy green samples per plot was higher at 180 m than at 60 m from the feedlot. However, when averaged over both years, the 35.5% of positive samples per plot at 60 m was significantly higher than the 26.1% of positive samples at 180 m ($P < 0.05$), indicating that, like *E. coli* O157:H7, the percentage of leafy greens contaminated with *E. coli* decreased as distance from the feedlot increased. Similarly, *E. coli* concentrations suggested that contamination levels decreased in leafy greens planted at the farther plot distance. Among those samples that had levels of *E. coli* that were detectable by direct plating, the average counts were 3.15, 3.19, and 2.92 log CFU g^{-1} at 60, 120, and 180 m, respectively (data not shown). *E. coli* concentrations at 180 m were significantly lower ($P < 0.05$) than those at 60 and 120 m, which did not differ ($P > 0.05$).

In addition to the higher contamination rate of leafy greens planted at 180 m compared to that at 60 m, the data collected on 4 August 2011 are notable for the high percentages of *E. coli*-positive samples per plot (Table 3). The 92.2% of *E. coli*-positive leafy green samples per plot at 180 m on that date was higher ($P < 0.05$) or tended to be higher ($P < 0.10$) than percentages for samples collected on any other sample date. Additionally, for those samples that had enumerable *E. coli* on this date, the average *E. coli* counts were significantly higher ($P < 0.05$) than average counts obtained on most other sample dates, at 4.13, 5.95, and 5.70 log CFU g^{-1} at 60, 120, and 180 m, respectively (data not shown). When processing the leafy greens for microbial analysis on this sample date, we noted that nearly all of the leaves were unusually dirty or dusty, regardless of plot and distance. In the 2 weeks before 4 August 2011, there had been considerable cattle management activity in the northernmost row of feedlot pens, including many of the 10 adjacent feedlot pens. This included the removal of approximately 300 head of cattle for shipping (20 and 21 July 2011), placement of approximately 300 head into the empty pens (20, 21, and 26 July 2011), and movement of cattle for weighing and sorting (20 and 21 July 2011). Because of the hot temperatures, most of this activity took place in the early mornings between 5:30 a.m. and 8:00 a.m. For one of these mornings, feedlot personnel reported the generation of substantial dust that hung in the air, drifting slowly north above the feedlot and the field containing the leafy green plots. Calm winds of 1 to 6 mph from the south were confirmed from weather data recorded on 20 July 2011 (data not shown). The feedlot pens were in a dusty condition, as only 5.84 mm of rain fell in the 12 previous days and the average daily high temperature during this 12-day period was 33.2°C. Gravitational settling of the feedlot dust onto the field north of the feedlot likely accounts for the dust and high concentrations of *E. coli* on the leafy greens. In addition, the apparent slow settling of the dust may have played a role in the higher percentages of *E. coli*-positive samples and higher concentrations of *E. coli* found on leafy greens that were planted at 180 m compared to 60 m from the

TABLE 4 Average total *E. coli* concentrations in air samples collected at the north edge of the feedlot pens and at each of the leafy green plots

Sample date	Feedlot pen surface conditions	Wind direction; avg wind speed/avg high wind speed[a] (mph)	Avg. *E. coli* concentrations (CFU/m^3 of air) in air at:			
			Edge of feedlot (0 m)	Leafy green plots		
				60 m	120 m	180 m
15 August 2011	Muddy	SE; 10.3/16.8	42.2 A[b]	7.2 B	3.0 B	2.2 B
18 August 2011	Moderately dusty	SSE; 10.7/20.2	2.3 A	2.7 A	2.7 A	0.8 A
22 August 2011	Dusty	S; 12.7/22.9	10.2 A	4.0 B	4.0 B	2.2 B
26 August 2011	Very dusty	SSE; 7.5/14.8	41.7 A	5.8 B	2.5 B	1.7 B
1 September 2011	Muddy	SSW; 8.5/15.4	3.7 A	1.0 B	0.2 B	0.0 B
6 June 2012	Moderately dusty	SE; 11.1/19.4	26.5 A	5.7 B	2.2 B	1.7 B
7 June 2012	Moderately dusty	SSE; 10.9/19.6	15.3 A	2.2 B	0.8 B	0.8 B
22 June 2012	Moderately muddy	SSE; 11.3/19.4	9.3 A	3.3 B	0.7 B	0.3 B
26 June 2012	Dry	SE; 11.7/19.1	4.7 A	1.8 B	1.5 B	0.3 B
27 June 2012	Dry	SW; 13.7/22.4	1.8 A	0.7 AB	1.3 AB	0.2 B
6 July 2012	Very dusty	SSW; 6.8/12.8	4.0 A	4.5 A	2.0 A	3.5 A
17 July 2012	Moderately dusty	S; 6.1/11.5	3.4 A	1.5 B	0.5 B	0.8 B
25 July 2012	Moderately dusty; freshly scraped[c]	SSW; 8.8/15.8	0.0 A	0.0 A	0.0 A	0.2 A
15 August 2012	Very dusty	S; 9.5/16.5	16.0 A	3.0 B	3.2 B	1.2 B
24 August 2012	Very dusty	SSE; 11.2/20.1	837.2 A	16.7 B	10.0 B	5.3 B

[a] Dominant wind direction, average wind speed, and average high wind gust speed during the approximately 2.5-h air sampling periods are given. Wind data were recorded at 15-min intervals by an on-site weather station. SSW, south-southwest.
[b] The sample date-by-distance interaction was significant ($P < 0.0001$), so total *E. coli* concentrations on each sample date were analyzed separately across distance. Within a row, values followed by different capital letters (A and B) are significantly different ($P \leq 0.05$).
[c] In the week preceding 25 July 2012, the 10 adjacent feedlot pens were scraped clean of accumulated manure before placement of new cattle.

feedlot on 4 August 2011. Typically, the concentration of viable microorganisms decreases downwind during airborne transport, because of both gravitational settling and biological inactivation (42). Airborne bacteria may be inactivated by exposure to UV radiation or the desiccating conditions of high temperatures and/or low humidity (42, 43). However, airborne dissemination during the early morning hours may have minimized these damaging exposures and enhanced the survival of *E. coli*. A similar situation may account for the 10 September 2012 data (Table 2), where the percentage of *E. coli* O157:H7-positive samples per plot at 180 m was higher at 15.5% ($P < 0.05$) than the 6.4% at 60 m. As described above, the feedlot pen surfaces were very dry and dusty when cattle were moved on 6 and 7 September 2012. The approximately 240 head of cattle that were moved from 7 of the 10 adjacent feedlot pens on 6 September 2012 were moved starting at about 7:00 a.m.; although a similar hanging dust cloud was not reported and cannot be confirmed, winds that morning were calm (2 to 3 mph) and from the east-southeast during the time the cattle were being driven.

The percentages of *E. coli*-positive leafy green samples per plot at all distances were notably lower on 13 August 2012 than on all other sample dates (Table 3). This result may be due to the cleaning and removal of FSM source material from many of the nearby pens a few weeks before this sample date. From 16 to 20 July 2012, cattle were removed from 11 pens in the northernmost row of feedlot pens, including 9 of the 10 source sampling pens (Fig. 1), and the manure was scraped and removed from the pens before putting new cattle in the pens on 24 and 25 July 2012.

Air samples were collected on a number of days in each year. *E. coli* O157:H7 was not detected in air samples collected at any distance from the feedlot in either year. However, *E. coli* was consistently detected in these samples. Average total *E. coli* numbers in 1-m^3 air samples collected at the north edge of the feedlot pens (0 m) and at the leafy green plots located at 60, 120, and 180 m north of the feedlot on each sampling date are shown in Table 4. For the majority of sample dates, *E. coli* concentrations in air samples were significantly higher at the edge of the feedlot than at the plots ($P < 0.05$). On the other days, there was no significant difference in the levels of *E. coli* in air at the edge of the feedlot compared to levels in air at the plots at any distance ($P > 0.05$). The *E. coli* concentrations in air samples collected at the leafy green plots were low, and there were no differences in *E. coli* concentrations in air at 60, 120, and 180 m from the feedlot ($P > 0.05$) on any sample day, although a numerical drop in *E. coli* concentrations often was observed as distance from the feedlot increased.

Our approach to examining the airborne transport of *E. coli* O157:H7 and *E. coli* included collecting air samples under a variety of situations and conditions in order to gather information about the factors that may impact the airborne dissemination of these organisms. *E. coli* concentrations in air samples collected on 24 August 2012 were notably higher than in those collected at the same distances on all other sample dates (Table 4). As noted above, there were high temperatures and low precipitation in the weeks preceding this sample date, and the feedlot pen surfaces were dry and dusty. Furthermore, there was a higher-than-normal degree of cattle activity in the feedlot pens during the air sampling period. The cattle in 9 of the 10 adjacent pens had been put in on 24 and 25 July 2012, recently enough that they were still very curious about our air sampling activities and moved in close to the fence to watch. On 24 August 2012, the temperature was much cooler than it had been on the previous days; the high temperature on that day was 23.8°C, compared to the daily highs of 29.5 to 33.6°C of the previous 4 days. The cattle were behaving rambunctiously with the combination of curiosity and cooler temperatures, and their running, butting, and crowding behaviors generated substantial dust from the pen surface, thereby increasing the detection of *E. coli* in the air. This is consistent with observations made in the early evening, when cattle often are most active, at

large cattle facilities adjacent to lettuce and leafy greens fields in major production regions in California (18).

In contrast, few to no *E. coli* bacteria were detected in air samples that were collected on 25 July 2012 (Table 4). These air samples were collected within a few days of the manure being scraped and removed from the pens in the northernmost row of the feedlot on 16 to 20 July 2012 (including 9 of the 10 most adjacent feedlot pens) (Fig. 1). Thus, when the FSM source material was substantially removed, minimal *E. coli* was detected in the air samples. This finding provides further evidence that removal of the FSM from the pens reduced leafy green contamination with *E. coli* on 13 August 2012 (Table 3).

Interestingly, *E. coli* also could be detected in air samples when the feedlot pen surfaces were muddy. As an interesting comparison, on 7 June and 22 June 2012, air samples were collected under similar conditions of wind direction and speed (Table 4). The pen surfaces were moderately dusty on 7 June 2012 and moderately muddy on 22 June 2012. However, on both days, *E. coli* concentrations in air samples were at comparable levels, suggesting that the airborne transport of *E. coli* from feedlot pen surfaces is not confined only to dry, dusty conditions.

Although *E. coli* O157:H7 was not detected in air samples collected downwind from the feedlot, the routine detection of *E. coli* suggests that the airborne transport of *E. coli* O157:H7 from feedlots also can occur. Our previous work has found that total *E. coli* typically is homogenously present at levels of 10^5 to 10^6 CFU g^{-1} of FSM on feedlot pens containing cattle (44, 45). These studies (44, 45) and additional work (30) further indicate that *E. coli* O157:H7, when present in FSM, occurs at much lower concentrations than does total *E. coli*. This is further suggested by the findings of the current work (Table 2); while 72.5% of all FSM samples were positive for *E. coli* O157:H7, only 9.2% of FSM samples had levels of 200 CFU g^{-1} or greater. Thus, given the generally low concentrations of total *E. coli* that were detected in air, detection of *E. coli* O157:H7 would be unlikely.

However, it is important to note that caution is recommended in interpreting the results obtained from microbial air samplers that rely upon cell growth for detection of airborne bacteria. As noted above, exposure to UV radiation, dehydrating conditions (e.g., high temperature and low relative humidity), and other unfavorable conditions may injure cells during airborne transport (42, 43). Furthermore, stress imposed during the collection process also may damage cells (46, 47). We collected air samples using MAS-100 Eco microbial air samplers, which work by aspirating air through a perforated lid and impacting this air onto the surface of agar media in a standard petri dish. Previous work has reported that impaction stress can injure bacteria and reduce their recovery on agar media (47, 48). This issue can be exacerbated when selective agar medium is used for bacterial estimation, such as the CHROMagars O157 and ECC that we used to recover *E. coli* O157:H7 and total *E. coli*, respectively (47). We added sodium pyruvate to the CHROMagars to improve the detection of any injured target bacteria in the air samples (49, 50).

Based on our results, more sensitive air sampling techniques are needed for the detection of *E. coli* O157:H7 in air samples collected downwind from cattle production facilities. Culture-independent molecular detection methods (such as PCR, quantitative PCR, or nucleic acid sequencing) and/or instruments that can collect and concentrate microorganisms from larger volumes of air over longer periods of time can improve sensitivity or be used to better describe the diversity of microbes in bioaerosols (51). While determining the presence and levels of viable pathogens in bioaerosols is fundamental for assessing the risks associated with their airborne transmission, these culture-independent techniques can provide the complementary information needed to get a more complete picture of the extent to which *E. coli* O157:H7 is transported as a bioaerosol and how far the pathogen can be transported from cattle production. There are a number of good reviews describing current knowledge and research needs for the issues associated with airborne transport of pathogens from livestock production operations and animal wastes (42, 46, 52).

All *E. coli* O157:H7 isolates from leafy greens (75 isolates) and FSM (870 isolates) were subtyped by PFGE. Two PFGE subtypes were found among the 14 leafy green isolates in 2011, and these were 2 of the 3 predominant PFGE subtypes found among FSM samples in that year. The three predominant FSM subtypes represented 75.2% of the 440 FSM *E. coli* O157:H7 isolates in 2011. In 2012, there also were three predominant PFGE subtypes among the 430 FSM *E coli* O157:H7 isolates (82.1% of FSM isolates), two of which also were predominant subtypes in 2011. These three PFGE subtypes also were the predominant subtypes of 2012 leafy green *E. coli* O157:H7 isolates, representing 88.5% (54/61) of the leafy green isolates. The long-term predominance of genetic subtypes among *E. coli* O157:H7 isolates found on a feedlot or farm has been documented in a number of studies (53–56). Shere et al. (56) found persistent subtypes within individual farms in a 14-month study examining *E. coli* O157:H7 in calves and environmental samples (water, feed, flies, and birds) at four different dairy farms. LeJeune et al. (55) found that most *E. coli* O157:H7 cattle isolates were one of four closely related genetic subtypes that persisted at a commercial beef feedlot for at least 4 months.

This work has provided a long-term and detailed look at the dissemination of *E. coli* O157:H7 in the environment downwind from a cattle production facility and has indicated the risk for contamination of leafy green produce grown near such a facility. In both years of this study, the PFGE subtypes of *E. coli* O157:H7 found in the leafy greens also were found in FSM, thereby providing a direct link to the cattle and the feedlot. While airborne dissemination from the feedlot was indicated, these observations do not exclude other possible mechanisms of pathogen dissemination from livestock. The potential for pest fly species to transmit *E. coli* O157:H7 was examined as part of the current study and will be described in a separate report. Furthermore, we recorded feedlot activity in the nearest row of feedlot pens. The USMARC feedlot is a research feedlot but is managed much like a commercial feedlot. Various cattle management activities took place throughout the project seasons at more distant sections of the feedlot, and cattle also were present in pastures near the feedlot; these other potential sources may have impacted our observations. Although *E. coli* O157:H7 was not detected in air samples, the associations of leafy green contamination and total *E. coli* in air samples with the combination of dusty, windy conditions and cattle management activities that involve animal movement or active behaviors indicate that airborne transmission plays a role in dissemination of these organisms from cattle operations. This is further suggested by the lower extent of leafy green contamination and airborne transmission of *E. coli* from the feedlot surface when the feedlot surface manure was removed from the pens. Overall, *E. coli* O157:H7 and total *E. coli* results suggest that there is a decrease in contamination as distance from the feedlot is increased; however, the high per-

centages of leafy greens contaminated with *E. coli* suggest great risk for planting fresh produce 180 m or less from a feedlot. What does this mean for produce growers? It means that the current buffer zone distance guidelines of 400 feet (120 m) may not be adequate to reduce the risk of produce contamination near cattle feedlots, depending on various site-specific modifying factors. The maximum distance that we examined was 180 m, and at that distance we found *E. coli* O157:H7-positive leafy greens and also found total *E. coli* in air samples. Further work will be needed to determine adequate buffer zone distances that will reduce the risk of pathogen contamination of fresh produce grown near cattle feedlots.

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