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Survival of *Escherichia coli* O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water

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Abstract

Many foodborne outbreaks of enterohemorrhagic *Escherichia coli* O157:H7 infection have been associated with the consumption of contaminated vegetables. On-farm contaminations through contaminated manure or irrigation water application were considered likely sources of the pathogen for several outbreaks. Field studies were done to determine the survival of *E. coli* O157:H7 on two subterranean crops (carrots and onions), and in soil fertilized with contaminated manure compost or irrigated with contaminated water. Three different types of composts, PM-5 (poultry manure compost), 338 (dairy manure compost) and NVIRO-4 (alkaline stabilized dairy manure compost), and irrigation water were inoculated with an avirulent strain of *E. coli* O157:H7 at 10^7 cfu g^{-1} and 10^5 cfu ml^{-1}, respectively. A split-plot block design plan was used for each crop, with five treatments (one without compost, three with each of the three composts, and one without compost but with contaminated irrigation water applied) and five replicates for a total of 25 plots, each measuring $1.8 \times 4.6\,m^2$, for each crop. Composts were applied to soil as a strip at a rate of 4.5 metric tons ha^{-1} before carrots and onions were sown. Contaminated irrigation water was sprayed once on the vegetables at the rate of 2 l per plot for this treatment 3 weeks after carrots and onions were sown. *E. coli* O157:H7 survived in soil samples for 154–196 days, and was detected for 74 and 168 days on onions and carrots, respectively. *E. coli* O157:H7 survival was greatest in soil amended with poultry compost and least in soil containing alkaline-stabilized dairy manure compost. Survival profiles of *E. coli* O157:H7 on vegetables and soil samples, contaminated either by application of contaminated compost or irrigation water, were similar. Hence, preharvest contamination of carrots and onions with *E. coli* O157:H7 for several months can occur through both contaminated manure compost and irrigation water.

Keywords: Compost; Manure; Manure-amended soil; *E. coli* O157:H7; Carrots; Onions

1. Introduction

Reported outbreaks of foodborne illness associated with fresh fruits and vegetables in the USA have nearly tripled since 1973 (Bean et al., 1997; Tauxe et al., 1997). The increased consumption of raw and sparsely cooked vegetables, coupled with the importation of fresh produce from countries where produce handling is compromised by lower sanitation standards, has generated a heightened concern for food safety (NACMCF, 1999). An outbreak of enterotoxogenic *Escherichia coli* infection associated with eating carrots occurred in 1993 in the USDA (CDC, 1994). Although the original source of contamination of implicated produce has seldom been identified, manure from farm animals has been highly suspected as a leading vehicle of pathogen transmission (Doyle, 2000a,b). Sources of microbial pathogens on fresh produce at the preharvest stage include feces, irrigation water, inadequately composted manure, soil, air, animals, and human handling (Beuchat, 1996; Buck et al., 2003).

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Large amounts of animal manure are applied to agricultural land in the USA. An estimated 1.36 billion tons of manure are produced annually in the United States, of which approximately 90% is generated by cattle (US Senate Agriculture Committee, 1998). Animal manure is frequently used as a fertilizer and soil conditioner. Adding manure to the soil has agronomic benefits through the addition of plant nutrients (nitrogen, phosphorus and potassium) and organic matter (Gagliardi and Karns, 2002b). Manure nutrients help build and maintain soil fertility. Manure can also improve soil tilth, increase water-holding capacity, lessen wind and water erosion, improve aeration, and promote beneficial organisms (Gagliardi and Karns, 2002a). However, animal manures frequently contain enteric pathogenic micro-organisms (Pell, 1997) and land spreading of manure can lead to pathogen entry to the food chain.

Many outbreaks of infection have been associated with water or food directly or indirectly contaminated with animal manure (CDC, 1996, 1999, 2001; Cieslak et al., 1993). Contaminated manure can contact the produce directly through its use as a soil fertilizer or indirectly through infiltration of irrigation water or water used to wash the produce (Doyle, 2000b). Although *E. coli* O157:H7 has been isolated from sheep (Kudva et al., 1998), pigs (Chapman et al., 1997), deer (Fischer et al., 2001) and goats (Pritchard et al., 2000), it is generally accepted that cattle, with a prevalence of up to 36.8% (Chapman et al., 1997), are the primary reservoir of this pathogen. The presence of *E. coli* O157:H7 in cattle illustrates the complex, interrelated nature of the environment, livestock production practices, food safety, and microbial ecology. Once ingested, *E. coli* O157:H7 persists in the rumen and colon of the animal, contaminating the feces, which subsequently acts as a vehicle in the horizontal transmission to uninfected animals (Faith et al., 1996). In a study by Chapman et al. (1997), samples of rectal feces were collected immediately after slaughter from 400 cattle each month for a 1-year period. *E. coli* O157 was isolated from 752 (15.7%) of 4800 cattle, including 246 (13.4%) of the 1840 beef cattle and 268 (16.1%) of the 1661 dairy cattle being culled. The monthly prevalence of *E. coli* O157 in cattle was 4.8–36.8% and was at its highest in spring and late summer. Contamination from bovine feces has been implicated in a number of foodborne outbreaks of *E. coli* O157:H7 infection (Morgan et al., 1988; Besser et al., 1993). Once in foods or food ingredients, *E. coli* O157:H7 displays a remarkable ability to survive harsh environmental conditions. Of particular concern in this regard is its ability to survive well under refrigeration conditions (NACMCF, 1999) and its atypically high acid tolerance. For example, investigation of an outbreak of *E. coli* O157:H7 infection associated with apple cider in southeastern Massachusetts revealed that *E. coli* O157:H7 presumphvely from manure used as a fertilizer in the orchard in apple juice survived, for 20 days at a pH value below 4, conditions previously considered sufficient to inhibit the growth and survival of bacterial pathogens and non-toxigenic strains of *E. coli* (Besser et al., 1993).

Cross-contamination of produce with manure or improperly composted manure used on the farm can be a source of pathogen during preharvest. Although competition with soil micro-organisms and adverse environmental conditions can reduce pathogens, there is little information regarding the degree to which these pathogens can survive in manure-amended soils or in soils irrigated with contaminated water and also on vegetables that are grown on those soils.

The primary objective of this study was to investigate the survival characteristics of *E. coli* O157:H7 on carrots and onions and in surrounding soil, when manure composts of different types or irrigation water contaminated with the pathogen were applied to soil in fields typical of those used for vegetable production.

2. Materials and methods

2.1. Bacterial strain, culture conditions, and preparation of inocula

E. coli O157:H7, strain B6914, without the *stx1* and *stx2* genes, was used for all experiments. The strain was obtained from J.S. Karns and J.V. Gagliardi (USDA Agricultural Research Service, Beltsville, Maryland). In addition, *E. coli* O157:H7 strain B6914 contains genes for green fluorescent protein (GFP) and ampicillin resistance on a stable plasmid (Gagliardi and Karns, 2002b). The GFP marker enabled enumeration of *E. coli* O157:H7 in the presence of the natural flora of manure.

A pure culture of the strain was held at −80°C in 25% glycerol until needed. When required, a sterile Tryptic soy broth (Difco Laboratories, Detroit, Michigan) containing 100 μg of ampicillin per ml (TSB/A) was inoculated with a loop from the frozen culture and incubated overnight at 37°C in a shaking water-bath. A tryptic soy agar (Difco) containing 100 μg of ampicillin per ml (TSA/A) plate was streaked for pure culture from this broth and incubated overnight at 37°C. A single colony from the TSA/A plate was inoculated into 10 ml of TSB/A and incubated at 37°C for 16–18 h with agitation (150 rpm) to grow to the mid-log phase. A 0.5-ml suspension of the isolate was transferred to 100 ml of TSB/A and incubated for 16–18 h with agitation (150 rpm). The culture was harvested at the mid-log phase, three times pelleted by centrifugation at $5000 \times g$ for 20 min, and washed by 0.1% peptone-water. Final cell pellets were resuspended in 0.1% peptone-water to

achieve an optical density (OD) of 0.5 at 630 nm. *E. coli* O157:H7 strain B6914 cells were serially diluted in sterile peptone-water and inoculated into manure composts to achieve a density of 10^7 cfu g^{-1}. The cell density of the inoculum was confirmed by plating on TSA/A plates.

2.2. Inoculation of compost and irrigation water

Composts used included PM-5 (poultry manure compost), 338 (dairy manure compost) and NVIRO-4 (alkaline stabilized dairy manure compost), and were provided by Patricia Millner at USDA-ARS, Beltsville, Maryland. The composts were prepared as described by Islam et al. (2004a).

Each of the three poultry/dairy manure composts was inoculated with *E. coli* O157:H7 at 10^7 cfu g^{-1}. Irrigation water was inoculated with 10^5 *E. coli* O157:H7 ml^{-1}. The compost was weighed for each plot and mixed in with the same amount of soil from the same plots at a rate of 4.5 metric tons (wet wt) ha^{-1} as strips. This compost/soil mixture was then applied over the rows. Carrots and onions were direct seeded in the rows to which the compost/soil mixture had been applied on the previous day. No chemical treatments were applied for weed control. A split-plot block design plan was followed for each crop, with five treatments (one without compost, three with each of three composts, and one without compost but with contaminated water applied), and five replicates for a total of 25 plots for each crop. Each plot measured $1.8 \times 4.6\,m^2$.

2.3. Planting vegetable seeds

Vegetable crops, carrots (*Daucus carota*, L.) and onions (*Allium cepa*), for this study were produced on the Horticulture Farm of the Coastal Plain Experiment Station of the University of Georgia, Tifton, Georgia. Both carrots and onions were produced according to production guidelines by the University of Georgia Cooperative Extension Service (Lorenz and Maynard, 1988) and by Kelley et al. (1998). Carrot (Choctaw variety, Solar Seed Co., Eustis, Florida) and onion (Georgia Pride variety, Taylor Farms, Tifton, Georgia) seeds were procured from a local supplier. Seeds were directly sowed in late October 2002 by a plate seeder with an attachment of a spreader shoe to scatter the seeds in a narrow band. The pattern was twin rows that were 75 mm apart, with two twin rows on each bed. The seeds were spaced 50 mm apart within the row, which gave a final stand of about 60 plants m^{-1} of twin row. A roller was used to firm the soil over the seeds which were planted at a depth of 6 mm. Light irrigation was required frequently during warm, dry periods for adequate germination. A hand sprayer was used to spray contaminated irrigation water on the vegetables only once at the rate of 2 l per plot to each of the five plots for this treatment, 3 weeks after carrots and onions were seeded.

2.4. Sampling of soil and vegetables

At selected time intervals (0, 7, 14, 21, 35, 42, 49, 70, 77, 84, 91, 105, 112, 119, 126, 140, 147, 154, 161, 168, 175, 182, 189, 196, and 203 days) from each plot for each crop, ca. 100 g of soil was aseptically collected in a sterile plastic bag from around a randomly selected plant 2.5 cm deep from the surface. Analysis of carrots and onions began at day 21, when the vegetables were large enough for sampling. From each plot, a randomly selected plant was pulled from the soil and only the edible carrot root or onion bulb was collected aseptically in sterile plastic bags as plant samples. The samples were transported to the laboratory in a cooler with ice, placed into a walk-in cooler at 4°C within 4 h of collection, and analysed within 48 h.

2.5. Isolation of E. coli O157:H7 from soil and vegetable samples

In a sterile Whirl-Pak bag, 10 g of each soil sample and 90 ml of 0.1% peptone-water were pummeled in a Stomacher for 30 s at low speed. Forty-five ml of 0.1% peptone-water was added to approximately 5 g of each carrot roots or onion bulbs sample in a sterile Whirl-Pak bag and rinsed by rubbing and vigorously agitating by hand for 30 s. *E. coli* O157:H7 counts in the peptone of soil samples and in peptone-wash water of vegetable samples were determined as described by Islam et al. (2004b). Serial dilutions (1:10) of each sample were prepared with 0.1% peptone-water, and 0.1 ml portions of each dilution were spread onto TSA/A plates. Plates were incubated at 37°C for 24 h, and colonies of *E. coli* O157:H7, which were fluorescent under UV light, counted. Randomly selected colonies that were green and fluoresced under UV light were confirmed to be *E. coli* O157:H7 by an *E. coli* O157:H7 latex agglutination test (Oxoid Ltd., Hampshire, England). When the pathogen was not detected by direct plating, 1 g of soil sample or 1 ml of vegetable rinse suspension was added to 99 ml of universal preenrichment broth (Zhao and Doyle, 2001) and incubated at 37°C for 24 h with agitation (150 rpm). Dilutions of cultures were surface plated on TSA/A plates.

2.6. Moisture and pH analyses

Moisture content and pH of manure-amended soil from the field were determined by procedures as described by Islam et al. (2004b). Moisture was determined by drying 5 g of soil at 105°C for 24 h in a Precision drying oven (Precision Scientific, Winchester, Virginia) and then weighing the residual. The pH of

manure-amended soil was determined by adding 10 g of soil to 250 ml of distilled water. The suspension was stirred for 5 min and then allowed to settle for 5 min. The pH of the liquid was determined with an Accumet Basic pH meter (Fisher Scientific, Pittsburg, Pennsylvania).

2.7. Statistical analyses

The experimental design was a split plot (Gomez and Gomez, 1994), where crop was the main plot and treatment was the sub-plot. Each treatment was replicated five times and each sample from a treatment was plated in duplicate at each sampling time. Hence, *E. coli* O157:H7 reported for each data point represents the mean of ten values. Data were analysed by the General Linear Model procedure of the Statistical Analysis System (SAS, 2001).

3. Results

E. coli O157:H7 survived for at least 154 days in all the amended soil samples on which carrots or onions were grown (Figs. 1 and 2). However, in soil samples from carrot fields where poultry manure compost PM-5 and dairy cattle manure compost 338 were applied, survival was up to 196 days. *E. coli* O157:H7 was detected for 168 days on carrots and for 74 days on onions post-application of contaminated compost or

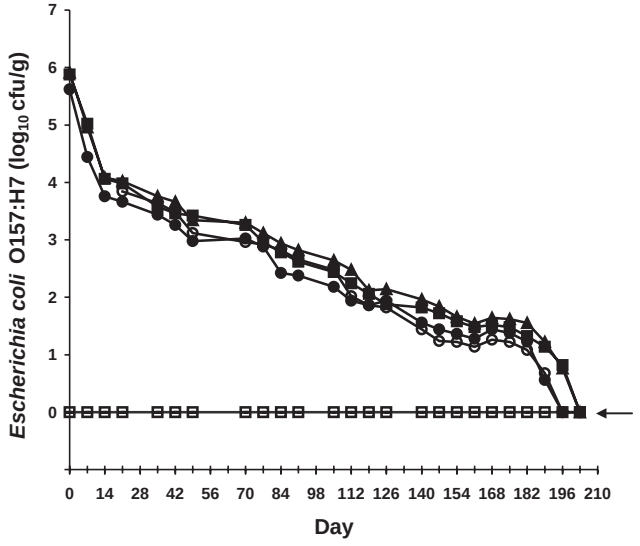

Fig. 1. Survival of *E. coli* O157:H7 in inoculated compost-amended or inoculated water-irrigated soil samples from fields used for growing carrots. Treatments included: no compost (□), poultry manure compost (■), dairy cattle manure compost (▲), alkaline-stabilized dairy cattle manure compost (●), and contaminated irrigation water (○). Contaminated irrigation water was applied at 3 weeks after seeds were planted. Arrow (←) indicates not detectable by enrichment culture.

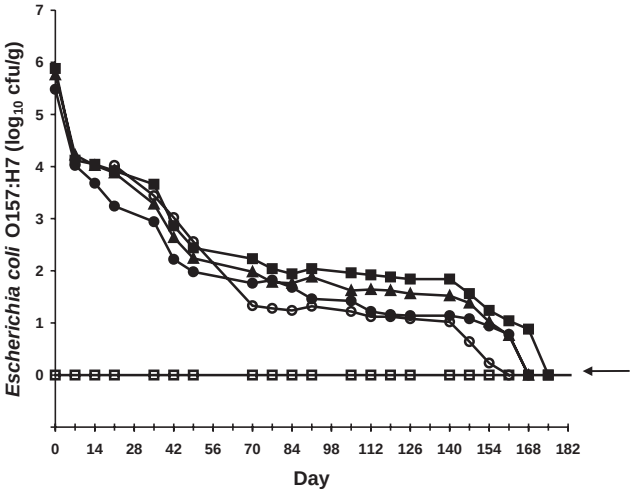

Fig. 2. Survival of *E. coli* O157:H7 in inoculated compost-amended or inoculated water-irrigated soil samples from fields used for growing onions. Treatments included: no compost (□), poultry manure compost (■), dairy cattle manure compost (▲), alkaline-stabilized dairy cattle manure compost (●), and contaminated irrigation water (○). Contaminated irrigation water was applied at 3 weeks after seeds were planted. Arrow (←) indicates not detectable by enrichment culture.

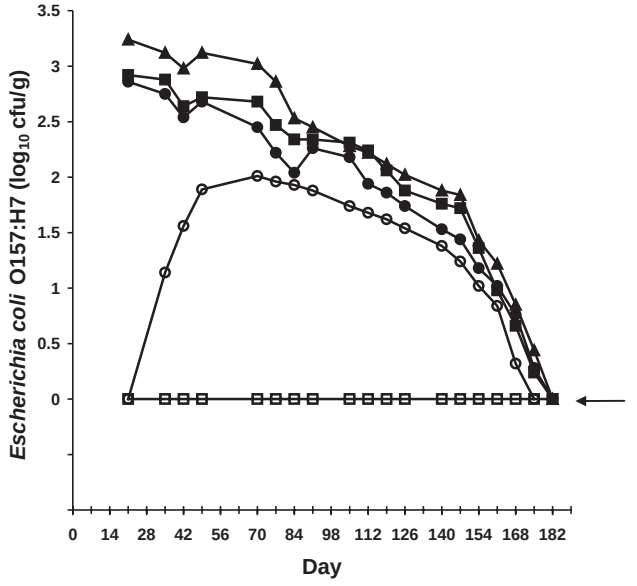

Fig. 3. *E. coli* O157:H7 counts on carrots grown in fields containing inoculated compost-amended or inoculated water-irrigated soil. Treatments included: no compost (□), poultry manure compost (■), dairy cattle manure compost (▲), alkaline-stabilized dairy cattle manure compost (●) and contaminated irrigation water (○). Carrots were harvestable at day 126. Contaminated irrigation water was applied at 3 weeks after seeds were planted. Arrow (←) indicates not detectable by enrichment culture.

water to the soil (Figs. 3 and 4). For the plots treated with contaminated composts, initial *E. coli* O157:H7 cell numbers ranged from 2.9 to 3.2 $\log_{10}$ cfu g^{-1} of carrots (Fig. 3) and from 2.0 to 2.4 $\log_{10}$ cfu g^{-1} of onions (Fig. 4) at the initial day of sampling (day 21). *E. coli*

O157:H7 cell numbers progressively declined on both carrots and onions with time, but did so more rapidly on onions. At approximate dates when onions (day 140) and carrots (day 126) were harvestable, *E. coli* O157:H7 counts were 0 and 1.5–2.0 $\log_{10}$ cfu g^{-1} on the vegetables, respectively. Overall, *E. coli* O157:H7 survived better in either soil or on crops if dairy or poultry manure were added than the alkaline compost. It appears in Figs. 3 and 4 that *E. coli* O157:H7 grew between days 21 and 28 on carrots and onions irrigated with *E. coli* O157:H7-contaminated irrigation water. Growth did not occur but rather the results represent the presence of *E. coli* O157:H7 on the vegetables before and after the contaminated irrigation water was applied. The 21-day samples were obtained before the contaminated water was applied, so no *E. coli* O157:H7 was detected, whereas samples assayed at day 28 and thereafter had been irrigated with the contaminated water.

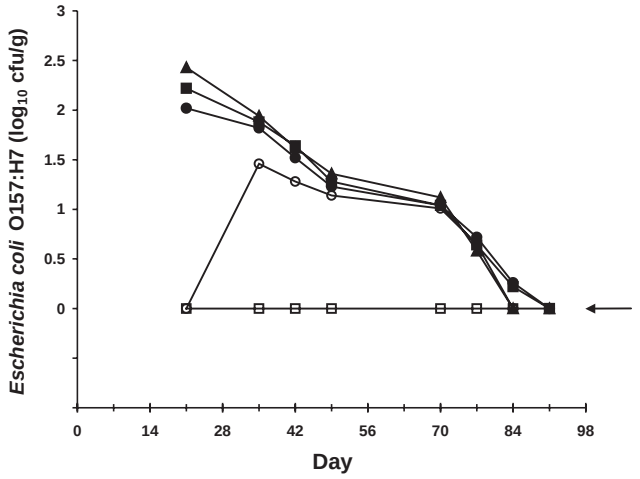

Fig. 4. *E. coli* O157:H7 counts on onions grown in fields containing inoculated compost-amended or inoculated water-irrigated soil. Treatments included: no compost (□), poultry manure compost (■), dairy cattle manure compost (▲), alkaline-stabilized dairy cattle manure compost (●) and contaminated irrigation water (○). Onions were harvestable at day 140. Contaminated irrigation water was applied at 3 weeks after seeds were planted. Arrow (←) indicates not detectable by enrichment culture.

Table 1
Average monthly temperature and rainfall for Tifton, GA

Month	Average temperature (°C)			Average rainfall (mm)
	Maximum	Minimum	Mean	
October	26.3	12.8	19.7	56
November	21.4	8.3	15.1	74
December	16.9	4.3	10.8	96
January	14.8	2.7	8.8	125
February	16.8	4.1	10.6	118
March	21.2	8.6	15.1	123
April	25.5	12.4	19.1	96
May	29.1	16.6	23.0	103
June	31.9	20.2	26.2	108

Source: Southeast Regional Climate Center, Columbia, South Carolina.

Vegetables were grown from late October 2002 to late June 2003 at Tifton, Georgia. Monthly average temperatures and precipitation for the Tifton area are presented in Table 1. The average monthly temperature varied from a maximum of 31.9°C in June to a minimum of 2.7°C in January (Table 1). Average rainfall varied from a maximum of 125 mm in January to a minimum of 56 mm in October (Table 1). Mineral and nitrate composition and pH values of the three different compost preparations are presented in Table 2. NPK values are highest for dairy manure compost and lowest for alkaline-stabilized dairy manure compost (Table 2). The pH values of composts PM-5, 338 and NVIRO-4 before being applied to the field were 8.1, 8.7 and 7.5, respectively (Table 2). Throughout the study period of nearly 200 days, the pH values of the soil from both carrot and onion fields, applied with manure composts, for all of the treatments ranged from 6.1 to 8.3. Moisture contents of the soil varied widely from 2% to 13%, depending on rainfall (data for pH and moisture content not shown).

Due to an unusually cold winter for Georgia in 2002–03, the general growth of the vegetables was very slow and the yield was below normal. Harvest data for the two crops revealed that greatest yields were from the dairy manure-amended soil. At day 126, when the carrots were ready for harvest, the average weight of a carrot grown on dairy manure compost-amended soil was 157 g, whereas the average weight of a carrot grown on poultry manure compost-amended soil or alkaline

Table 2
Mineral and nitrate composition and pH of compost preparations[a]

Types of composts	Weight of the chemicals (kg ha^{-1} of compost)									NO_3 (ppm)	pH
	Cu	Fe	Mn	Zn	Ca	Mg	K	P	NO_3		
Poultry manure compost # PM-5	1.28	3.48	13.29	1.90	5397	411	16595	333	1693	756	8.1
Dairy manure compost # 338	12.19	58.25	73.00	31.45	9203	813	55688	357	6989	3210	8.7
Alkaline-stabilized dairy manure compost # NVIRO-4	1.19	3.60	57.75	4.79	8066	402	3330	62	185	83	7.5

[a] Average of four samples.

stabilized manure compost-amended soil was 147 or 129 g, respectively. Similarly, at day 140, when the onions were ready for harvest, the average weight of an onion grown on dairy, poultry or alkaline-stabilized manure compost-amended soil was 124, 118, or 115 g, respectively. These results conform to the nutritional composition of the composts, with dairy manure compost having the greatest nitrate and mineral contents and alkaline-stabilized dairy manure compost having the least (Table 2).

4. Discussion

Animal manures frequently contain enteric pathogenic micro-organisms and land spreading can lead to pathogen entry in to the food chain. Root crops and leafy vegetables have the greatest risk of contamination from manure application to soil. Such vegetables can also become contaminated with contaminated water during irrigation, rinsing, processing, and cooling. Several foodborne disease outbreaks have been attributed to the consumption of both carrots and green onions, due to contamination with enterotoxigenic *E. coli*, *Shigella* spp., or hepatitis A (CDC, 1994; CDC, 2003). Cross-contamination of the produce with fecal waste or improperly composted manure used on the farm was suggested as a possible source of these pathogens during preharvest. In this study, results revealed that *E. coli* O157:H7 from inoculated animal manure compost can survive for more than 6 months in the soil of vegetable fields, and could be detected for at least 10 weeks on onions and 5 months on carrots, which were exposed to southern Fall–Winter environmental conditions. These findings indicate that *E. coli* O157:H7 can be transmitted through animal manure to carrots and onions in an agricultural production environment.

The survival characteristics of *E. coli* O157:H7 in the environment is of fundamental importance to predict accurately the risks associated with farm practices such as the spreading of manure. Results of a study by Wang et al. (1996) indicate that *E. coli* O157:H7 can survive in feces for a long period of time (up to 70 days) and retain its ability to produce verotoxins. Composting is frequently used at many farms, which is a managed treatment in which the heat generated by microbial action in the process may kill many micro-organisms (including pathogens). Ideally, if the composting is carried out properly, the end product should be free of most pathogenic micro-organisms. However, due to the variability of environmental conditions, temperatures throughout the entire heap of composting materials may not be adequate to kill foodborne pathogens. Furthermore, during storage on the farm, the compost may be contaminated by raw manure which can contain *E. coli* O157:H7 or other pathogens. Therefore, like raw manure, improperly composted manure can be the source of foodborne pathogens. Several studies have revealed that *E. coli* O157:H7 can survive for an extended period of time, ranging from a few months to 21 months, in manure heaps or manure-amended soil under various environmental conditions (Jiang et al., 2002; Jones, 1999; Kudva et al., 1998). Our study examined the influence of vegetable-growing conditions on the survival of *E. coli* O157:H7, and results revealed that survival of this pathogen in the vegetable-growing soil was comparable to that in soil not containing vegetables.

Our studies revealed that the persistence of *E. coli* O157:H7 in soil is dependent on the type of vegetable grown in the soil, with inactivation more rapid in soil in which onions are grown than in soil in which carrots are grown. Furthermore, *E. coli* O157:H7 in soil can contaminate for many weeks the surfaces of soil-grown vegetables; however, the pathogen persists on carrots at considerably higher cell numbers (10^2–10^3 cfu g^{-1}) for longer periods of time (>15 weeks) than on onions (ca. 10^2 cfu g^{-1} for ca. 1 week and ca. 10^2 to <10 cfu g^{-1} for ca. 7 weeks). Survival of *E. coli* O157:H7 was better on carrots than on onions, which confirmed the results from our earlier study in a growth chamber (Islam et al., 2004c). This may be due to the presence of high concentrations of antimicrobial phenolic compounds in onions compared to carrots (Sofos et al., 1998). Phenolic compounds are commonly present in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antimicrobial activity (Elnima et al., 1983; Zohri et al., 1995).

In addition, Kudva et al. (1998) has reported that the Shiga toxin type 1 and 2 genes in *E. coli* O157:H7 had little or no influence on bacterial survival over 21 months in manure and manure slurry. Hence, results from our study with a non-virulent strain of *E. coli* O157:H7 should be extrapolatable to the virulent strain.

The long-term survival of *E. coli* O157:H7 in manure-amended soil emphasizes the need for appropriate farm waste management to curtail the environmental spread of this pathogen. United States Department of Agriculture (USDA) National Organic Program standards specify an interval of at least 120 days between the application of non-composted manure as fertilizer and harvesting of vegetables whose edible portions contact soil fertilized in this way (USDA, 2000). Based on our results, we conclude that the USDA 120-day interval between manure application and harvesting food crops may not be adequate for Georgia's climate and that at least a 210-day interval be considered. This appears to be sufficient time for this pathogen to be reduced to undetectable levels in the soil and not to contaminate vegetables.

5. Conclusions

Application of *E. coli* O157:H7-contaminated manure compost to the production field or irrigation with *E. coli* O157:H7-contaminated water may result in contamination of crops in the field for several months. Our study has demonstrated that carrots and, to a lesser extent, onions grown in soil containing contaminated manure or irrigated with contaminated water results in contamination of the edible portion of the plants. The danger of improper manure handling can be manifest as direct contamination of produce, water supplies, animals, or even humans. Since the source of contamination in most infection outbreaks is not conclusively determined, the risks from improper manure handling are probably underestimated. Through this study in actual field conditions, we contend that elimination of pathogens from manures used as fertilizer is a critical intervention point for managing the pathogen problem on crops used as feed and food and in managing the microbial safety of the water supply. The impacts of on-farm practices, which may result in *E. coli* O157:H7 becoming associated with carrots and onions, or for that matter other vegetables, have not been sufficiently explored. The levels of *E. coli* O157:H7 used in this study are far greater than what would likely be found on an agricultural field; however, under natural conditions, even a low level of contamination of *E. coli* O157:H7 with a low infective dose could present a human health hazard.

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