E. coli 0157:H7 Frequently Asked Questions

Critical Issue Report 2006.3
The Organic Center

I INTRODUCTION

The tragic and serious outbreak of *E. coli* O157:H7 infections linked to fresh spinach from California has focused national attention on food safety practices and polices. The scope and seriousness of the outbreak has raised hard questions about:

- Farming systems – Are there practices or production inputs used by conventional farmers, or organic producers that increase or decrease the risk of *E. coli* food contamination?
- Beef cattle and dairy cow manure management and methods used to apply manure to crop fields.
- Animal husbandry, animal health, and feeding practices.
- Irrigation and surface water quality, and the movement of *E. coli* bacteria down into ground water.
- Sanitation in processing plants, and worker sanitation in plants and in the field.
- Use of fertilizers that might contain *E. coli*.
- The adequacy of current public and private policies, quality control programs, and regulations applicable to conventional and organic farming.

There is an enormous body of science on pathogenic *E. coli* O157 that provides important clues to where this dangerous organism comes from, how it can make its way to food, other ways people are exposed, and further steps that can be taken to reduce the terrible burden of disease linked to pathogenic *E. coli* strains. This "Critical Issue Report" provides a roadmap to published research, and teams of scientists conducting cutting edge work to better understand *E. coli* epidemiology. Each reference cited in this report appears in the bibliography, most with full abstracts. As new information emerges, this report and the bibliography will be updated and reposted to The Organic Center's website.

Standing manure water in a vegetable 'test plot'. Photo: Washington State University (WSU).

ABOUT *E. coli*

There are over 225 serotypes (unique strains) of *E. coli*, the majority of which are not dangerous (CIDRAP, 2006). Indeed, *E. coli* bacteria are essential to the healthy functioning of human and animal digestive systems. But some serotypes have picked up "pathogenicity islands" – extra genetic material that can turn a harmless bacterium into a dangerous threat to either people or certain animal species.

The *E. coli* O157:H7 serotype is among the most dangerous when people are exposed, and causes tens of thousands of illnesses annually. Children and the elderly are the most vulnerable to serious complications. This serotype, however, causes no harm to cattle because it does not bind to the walls of their GI (gastrointestinal) track. In people, *E. coli* O157:H7 binds to the cells that line our GI tract, leading to bloody diarrhea, and in some cases, a series of dangerous, even life-threatening complications.

An "outbreak" of illnesses from *E. coli* O157 is defined as two or more cases from the same direct cause or source of exposure. Outbreak investigations provide the government with insights into prevention and research priorities. The 8,598 cases associated with 350 outbreaks reported to CDC in 1982-2002 accounted for less than one-tenth of 1% of the total number of cases during that 20-year period (Rangel et al., 2005).

II THE BURDEN OF HUMAN DISEASE TRIGGERED BY *E. coli*

How bad is the California fresh spinach E. coli *outbreak compared to past outbreaks?*

Very bad, and no one knows how many additional cases may still be reported. Based on the timing of the announcements and recalls, and the latency period for infections, the total number of reported cases from fresh spinach will hopefully remain below 200.

The California fresh spinach outbreak is one of the worst ever reported in produce. The CDC has calculated that between 1982 and 2002, the median number of cases per *E. coli* outbreak caused by produce is 20 (Rangel et al., 2005). In its analysis of outbreaks from 1990 to 2003, the Center for Science in the Public Interest estimated that there were, on average, 51 cases of illness per outbreak linked to produce (Smith DeWaal et al., 2006). So, the California spinach outbreak is about four, to as much as 10-times the size of the average produce-triggered outbreak.

Still, outbreaks linked to beef products cause far more total cases each year, even though CDC estimates fewer cases per average outbreak linked to hamburger, compared to cases linked to produce (8 cases per ground beef outbreak, versus 20 cases in produce outbreaks) (Rangel et al., 2005).

Some of the most serious known outbreaks include the 1992-1993 cases in the Pacific Northwest triggered by undercooked Jack-in-the-Box hamburgers, an outbreak that encompassed 501 illnesses. Contaminated sprouts in Japan in 1996 sickened more than 6,300 schoolchildren, and contaminated ground water in Canada triggered 1,436 illnesses and 6 deaths (CIDRAP, 2006).

How many illnesses are caused by E. coli *O157 each year?*

In 1999 the Centers for Disease Control published a comprehensive review of foodborne illnesses in the United States (Mead et al., 1999). A team of CDC scientists estimated that about 73,000 people are sickened annually by *E. coli* O157:H7, leading to some 2,000 hospitalizations and 60 deaths. In addition, CDC reported that three other pathogenic serotypes caused a combined 195,580 cases of human illness annually, for a total for all pathogenic *E. coli* of 269,060 cases (Mead et al., 1999).

Is there any more recent information on the number of cases?

Yes, and there is good news in it.

Government agencies teamed together to form FoodNet a few years ago. This program collects and analyses the latest data on foodborne illnesses and outbreaks (see http://www.cdc.gov/foodnet/). Based on the most recent data from FoodNet, there has been about a 29% reduction in the number of *E. coli* O157 cases since 1996-1998 (FoodNet, 2006). An aggressive program targeting ground beef safety through the development and adoption of Hazard Analysis Critical Control Point (HACCP) food safety programs in meat slaughter and processing plants is credited with triggering this progress. In its 2005 FoodNet update, CDC states –

> *"The decline in the incidence of STEC O157 infections observed in recent years suggest that coordinated efforts by regulators and industry have been effective in reducing contamination and illness related to ground beef" (FoodNet, 2006).*

Based on the CDC's estimate of a 29% decline in the number of *E. coli* O157 cases from the 73,000 reported in 1999, ***there are likely to be about 51,830 cases of* E. coli *O157 illnesses in the U.S. in 2006.***

Accordingly, the current fresh spinach outbreak is likely to account for less than 0.4% of the total number of *E. coli* O157:H7 cases this year.

Has the government projected the economic costs of the illnesses triggered by E. coli*?*

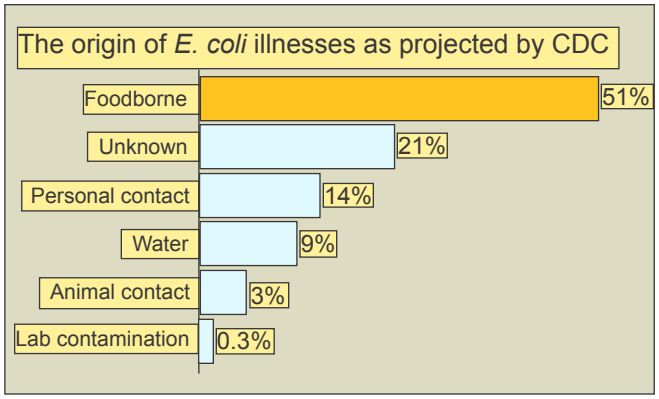
Yes. Illnesses caused by *E. coli* lead to an estimated $405 million in costs annually, according to a report published by government scientists in 2005 (Frenzen et al., 2005). The authors state "The high cost of illnesses due to O157 STEC [*E. coli*] infections suggests that additional efforts to control this pathogen might be warranted."

The comparative costs per case of foodborne illness triggered by *E. coli* O157, *Campylobacter*, and *Salmonella* bacteria drive home the unique risks posed by pathogenic *E. coli* serotypes. According to estimates published by USDA's Economic Research Service in 2000, the average case of *E. coli* O157 illness costs about $11,210, while the average case of *Campylobacter* and *Salmonella* imposed costs of $611 and $1,790 respectively.

How are people exposed to E. coli *O157?*

Many different ways.

CDC projects that about 52% of *E. coli* illnesses come from foodborne transmission, 14% from people-to-people contact, 9% from water, 3% from contact with animals, 0.3% from exposures in laboratories, and 21% from unknown cases (Rangel et. al., 2005).

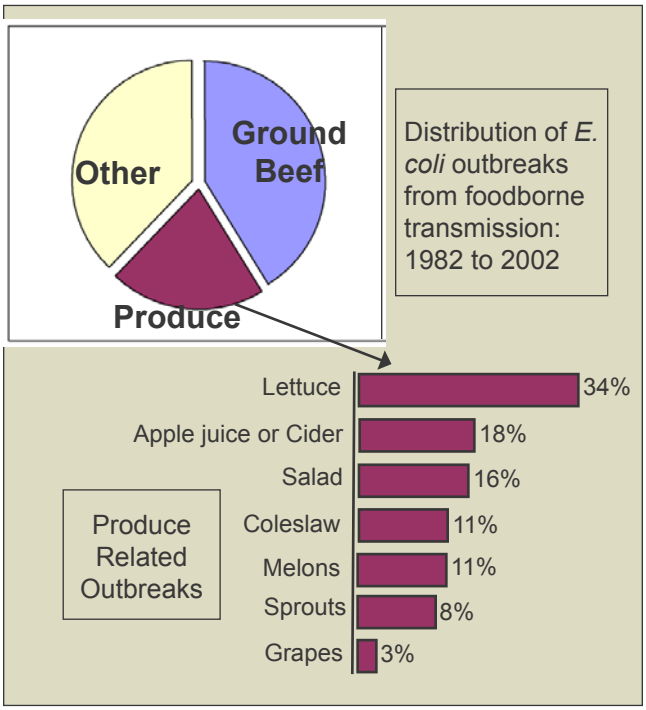


For outbreaks involving exposure to *E. coli* O157 from food, ground beef caused 41% of reported outbreaks between 1982 and 2002, and produce caused another 21% (Rangel et al., 2005). Fruit juices, sprouts, and dairy products from raw milk were other important sources of foodborne transmissions.

What is known about cases of E. coli *illness linked to produce?*

In the study of outbreaks caused by produce from 1982 to 2002 (Rangel et al., 2005), CDC concluded that lettuce accounted for 34%, 18% were from apple cider or apple juice, 16% from salad, 11% from coleslaw, 11% from melons, 8% from sprouts, and 3% from grapes.

Produce-triggered outbreaks occurred most commonly from food eaten in restaurants; there were 15 restaurant-triggered produce outbreaks out of a total of 38 produce outbreaks. Investigations reveled that ***almost half of these were caused by cross-contamination during food preparation.***



What is known about cases of E. coli *illness linked to animal products?*

Meat and dairy products have been identified as the most common source of human illnesses caused by *E. coli*. In recent years the number of disease outbreaks and severity of cases linked to consumption

of livestock products has declined. Progress in reducing the frequency of *E. coli* O157 contamination in animal products, coupled with increasing frequency of illnesses linked to fresh produce, helps explain the growing concern of the food industry and regulators in the wake of the current spinach outbreak.

In a study triggered by a sharp increase in reported *E. coli* O157 cases in New Jersey in 1994, Mead et al. (1997) analyzed risk factors for *E. coli* illness. They concluded that patients in the outbreak were much more likely to have eaten a hamburger than controls, and that 80% of the hamburgers consumed by ill people had been cooked at home. Furthermore, they highlighted the direct role consumers must play in assuring that safe food bought at the supermarket remains safe right up to the time it is consumed –

> ***"Adequate hand washing by food preparers could have prevented 34% of* E. coli *O157:H7 infections in the study population" (Mead et al., 1997).***

What about other routes of exposure?

In addition to exposures to *E. coli* O157 through food, children have been infected with the bacterium at petting zoos (Chapman et al., 2000). About 11% of the cattle fecal samples tested for *E. coli* O157 at Minnesota county fairs in 2000 and 2001 tested positive (Cho et al., 2006).

Contaminated drinking water, and water in lakes and pools where people swim have also triggered cases of illness linked to *E. coli* O157. A study of an outbreak triggered by beverages sold by vendors at an agricultural fair in Ohio implicated a local source of contaminated water used to mix drinks, and reported a seasonal increase in illness frequency linked to the fair schedule across 15 Northeast Ohio counties (Crump et al., 2003). Varma et al. (2003) linked another outbreak to airborne *E. coli* O157 bacteria in dust in a multi-purpose community building at an agricultural fairground in Lorain County, Ohio. Dust samples from the building remained infectious with *E. coli* O157 bacteria for 42 weeks.

Is there a connection between antibiotic use on livestock farms and E. coli *O157 frequency or virulence?*

This is a complex question under active study by scientists.

Studies have found widely varying levels of antibiotic resistance among *E. coli* O157 serotypes. In a 2000 study by the CDC's National Antibiotic Resistance Monitoring System (NARMS), ten percent of *E. coli* O157 isolates were resistant to one or more antibiotic, and 7% were multidrug resistant (NARMS, 2000). A study focused on isolates from cattle, humans, swine, and farms collected during the years 1985 to 2000. The team found that 39% of the *E. coli* isolates were resistant to one or more antimicrobials (Schroeder et al., 2002).

Some evidence suggests that the selection pressure imposed on livestock enteric bacteria by subtherapeutic antibiotic use sets the stage for the kind of genetic mutations that are required to turn harmless *E. coli* into toxin-producing, dangerous serotypes (Galland et al., 2001). It is known that multiple antibiotic resistance genes sometimes move from one bacterium to others on plasmids. The genes triggering the production of *Shiga*-like toxins by certain *E. coli* serotypes may move in the same way, and in some cases, with plasmids carrying antibiotic resistance genes (Schroeder et al., 2002). The role and impacts of antibiotic use in triggering this process are not understood and are subject to active debate.

III Sources of *E. coli* O157

Where does E. coli *O157 come from?*

The vast majority of *E. coli* O157 bacteria start out in the digestive systems of beef and dairy cattle (Valcour et al., 2002; CIDRAP, 2006). *E. coli* O157 bacteria enter the environment when they are shed in the manure passed by infected beef and dairy animals. Once in the environment, several other species of animals can serve as a reservoir of *E. coli* O157, without suffering any adverse consequences.

A study in Louisiana concluded that deer were not a significant reservoir for *E. coli* O157, since only 0.3% of hunter-harvested deer tested positive (Dunn et al., 2004). A similar study in Nebraska found that only 0.25% of 1,608 deer samples tested positive (Renter et al., 2001).

Is it normal for cattle to have E. coli *O157 in their digestive tracts?*

E. coli O157 is commonly found at low-levels in most U.S. beef cattle and dairy herds. Less than one percent, to rarely over 10% of animals tested, are found to shed *E. coli* O157. Still, its presence in cattle is not normal. Infection rates tend to be higher in the spring and summer.

Duncan et al. (2000) point out in a review that "Normally *E. coli* is greatly outnumbered in the ruminant gut by anaerobic bacteria, producers of weak acids inhibitory to the growth of this [*E. coli* O157] species."

There are millions of harmless *E. coli* bacteria in the digestive systems of all cattle. When cows are fed high-energy, grain-based rations, the pH in their digestive systems changes to favor *E. coli* O157. Stress and illness can also increase the susceptibility of cattle to *E. coli* O157, as does holding cattle off feed (Duncan et al., 2000). Sanitary practices on the farm play a direct role in whether and how quickly *E. coli* O157 infections spread through a cattle herd (LeJeune et al., 2001; Scott et al., 2006; Rice et al., 2000; McGee et al., 2002).

Research published in 2001 found only 45 fecal samples (0.2%) of manure from beef feedlots was positive for *E. coli* O157, out of a total of 24,184 samples (Galland et al., 2001). Another study found *E. coli* O157:H7 in 40 of 3,152 (1.3%) fecal samples, and 40 of 2,058 (1.9%) cattle (Sargeant et al., 200). None of the cattle tested had *E. coli* in more than one sample over the 11-month sampling period. Significant differences were detected in prevalence among sample collection dates. *E. coli* O157 was also detected in 3 of 199 (1.5%) water samples.

A study focused on beef calves before they were sent to feedlots and found that 2.5% of the animals shed *E. coli* O157 prior to entering the lots. (Dunn et al., 2004).

Hancock et al. (1994) found that only 10 out of 3,570 fecal samples of dairy cattle from the Pacific Northwest tested positive for *E. coli* O157 in a 1994 study. They also found *E. coli* O157 in 10 of 1,412 samples of manure from beef cattle, or just 0.71%. Several of the same scientists conducted a similar survey in 1997 and found *E. coli* O157 in 1% of fecal samples from beef cattle. Research published by another team of researchers based at Washington State University found that 7.4% of 1,440 samples of manure and farm samples contained Shiga-toxigenic *E. coli* strains (STEC), of which *E. coli* O157 is "the predominant STEC serotype associated with human disease in the Untied States." (Cobbold et al., 2004).

During the summer in Louisiana dairy herds, 38.5% of herds and 6.5% of animals tested positive for *E. coli* O157 in manure (Dunn et al., 2004). LeJeune et al. (2004) reported that 13% of 4,790 bovine fecal samples from cattle feedlots in the U.S. tested positive for *E. coli* O157.

Studies in Europe have found very little *E. coli* O157 in both beef cattle and dairy herds.

A study by Albihn et al. (2003) in Sweden found that just 1.2% of 3,071 fecal samples tested positive. An outbreak of human illness impacting about 100 people in Sweden in 1995 ***triggered the establishment of a national surveillance program testing for E. coli O157 in all cattle at slaughterhouses.*** A similar study in Finland found only 1.3% of dairy fecal samples testing positive for *E. coli* 057 (Lahti et al., 2001).

Buncic et al. (1997) tested 371 cows from 55 dairy farms in New Zealand and found only two animals shedding *E. coli* O157 positive manure (about one-half of one percent).

E. coli O157 was found in just two of 2,446 samples of pig manure in a Swedish study (Eriksson et al., 2003).

IV *E. coli* O157 IN BEEF AND DAIRY CATTLE

In a unique study focusing on *E. coli* O157 cases in Canada, scientists used 80 indicators of livestock density to study linkages between farm animals and *E. coli* illnesses. The strongest positive association between *E. coli* case frequency and a livestock density indictor was the ratio of beef cattle population to human population (Valcour et al., 2002).

Liquid manure being pumped with a "honey wagon." Photo: Tim McCabe NRCS

A variable reflecting the application to cropland of raw manure via a manure spreader, or as liquid slurry, was the second most significant variable explaining the geographic distribution of *E. coli* illnesses. In discussing a major multiple-pathogen outbreak in the Walkerton, Ontario area, including many cases triggered by *E. coli* O157, the authors state –

> *"Strong evidence suggests that the contamination of Walkerton's water supply was due to manure runoff from a nearby farm that entered a shallow well supplying the municipal water system." (Valcour et al., 2002)*

Significant progress in the prevention of *E. coli* O157 illnesses from ***ALL*** sources will require three things:

- Better understanding of the complex microbial ecology within the bovine digestive system;
- Changes in animal feeding and husbandry practices; and
- Practical steps to reduce the number of infected animals, slow the transmission from animal to animal, and minimize the severity of individual cases.

Does E. coli *O157 reach cattle through their feed?*

Yes. Several studies have found that a few percent to over 10% of feed samples test positive for *E. coli* O157.

In 2003 research, Davis et al. (2003) found *E. coli* O157 in four of 2,365 feed samples tested. Dodd et al. (2003) found *E. coli* O157 in 10.3% of feed samples taken from feeding troughs in Midwestern feedlots. Curiously, the Dodd study found no correlation between *E. coli* O157 numbers and general coliform bacteria counts. In 1998 work, Lynn et al. (1998) found no samples of feed that contained *E. coli* O157, despite the fact that 30% of the 209 samples tested contained other *E. coli* serotypes.

Can farmers do anything to reduce E. coli *O157 levels in their animals?*

Yes, they can do a lot.

Gilbert et al. (2005) found that enterohaemorrhagic *E. coli* [EHEC] levels were 100-times higher in cattle feed a high grain ration, compared to animals on a roughage-based diet. Herriot et al. (1998) reported that dairy heifers feed corn silage were more likely to have *E. coli* O157, compared to heifers not feed silage.

Runoff from this livestock yard may enter a nearby stream and degrade water quality. Photo: Tim McCabe, NRCS

Dozens of published studies show that animal rations and animal husbandry play direct roles in triggering *E. coli* O157 colonization of the bovine digestive system (e.g., McSweeney et al., 2004; Russell et al., 2000; Diez-Gonzalez et al., 1998).

In 1998, the first study in a major scientific journal was published on the impact of dietary changes on *E. coli* shedding (Diez-Gonzalez et al., 1998). This paper reported that switching beef cattle in a feedlot to a high-roughage diet for the last week before slaughter triggers a dramatic decline in *E. coli* O157 numbers. This promising finding has been replicated multiple times and is now widely accepted in the animal health community.

Reducing *E. coli* O157 levels in cattle headed for slaughter significantly reduces the risk of *E. coli* O157 contamination on meat products, but does relatively little to prevent the buildup of *E. coli* O157 in manure and manure storage lagoons. For this reason, many routes of *E. coli* exposure would not be impacted, except perhaps marginally, by changing how beef animals are fed the last week they spend in feedlots.

Few beef feeders have adopted the practice because of fear that a switch in diet during the last week in a feedlot may cause a drop in quality grade from Prime to Choice, or Choice to Select. Either drop in grade would be accompanied by a significant loss in income. Some organic meat producers and processors encourage, or require that their growers switch beef animals in feedlots to a high-forage diet in the last week prior to slaughter as an added food safety measure.

Garber et al. (1999) showed that dairy farms using concrete alleys and flushing systems were 8-times more likely to test positive for *E. coli* O157 than farmers using other manure removal systems. They also showed that *E. coli* levels tended to be higher in the summer than the winter.

Hutchinson et al. (2005) found that young calves still receiving milk had "significantly lower levels and prevalence of *E. coli* O157." *E. coli* pathogen levels were also reduced on farms that included some sort of bedding. Cattle on diets composed mostly of grass had less likelihood of infection with *E. coli* O157.

V ENVIRONMENTAL FATE AND MOVEMENT

How can E. coli *O157 move from cattle farms to crop fields?*

Once in the environment, *E. coli* O157 can move in several ways around agricultural landscapes. The two most common ways are through the land application of raw, uncomposted manure, and through runoff of manure or lagoon water into streams and irrigation ditches.

Fertilizer application applied directly into irrigation lateral for flood application, Yuma, AZ. Photo: Jeff Vanuga, NRCS

Wild animals and birds can become infected, serve as a reservoir, and move *E. coli* O157 bacteria across a landscape, although studies assessing the importance of wildlife in transmitting *E. coli* O157 infections have generally concluded that wildlife plays a modest, or no role in most regions (Rangel et al., 2005; Valcour et al., 2005).

How long does E. coli *O157 last in the environment?*

This is a complicated question. *E. coli* O157 environmental fate is driven by many factors. In general, the bacterium lasts longer in warmer, wetter climates.

E. coli O157 is known to survive in soil for between one and six months, and sometimes longer. Many factors can extend, or reduce, survival in soil. Heat, lack of soil microbial activity, and moisture tends to extend the time period *E. coli* O157 survives in soil. Survival times decline in soils that have high levels of microbial activity, in cool or cold soils, and under dry conditions. Jiang et al. (2002) report that *E. coli* O157 lasted for 77 days in manure-amended soils at 5 degrees C, and longer than 226 days at 15 degrees C.

Incorporating manure into soils and tillage reduces survival times (Boes et al., 2005). ***Soils on organic farms have also been shown to accelerate the decline in* E. coli *levels, compared to similar soils under conventional management (Franz et al., 2005).***

On the farm, *E. coli* O157 is known to persist in water troughs for several weeks to a few months. It can last for comparable periods in feed troughs. Transmission through water and feed troughs is considered a major source on on-farm movement from animal to animal. Berry et al. (2005) found that *E. coli* O157 survives in cattle feedlot soils under a wide range of manure and moisture conditions for up to 133 days.

Fenlon et al. (2000) studied the environmental fate of *E. coli* O157 following application of dairy slurry (liquid manure) on a clay loam soil in grass pasture. *E. coli* O157 was detected on the grass for only one week. There was limited transport of bacteria down

into the soil (2%), and about 7% drained off the field following a rainfall event. The authors concluded that heavy rains could lead to considerable losses of *E. coli* O157 to leaching and surface runoff. Silage made from grass containing *E. coli* O157 led to a significant increase in *E. coli* O157 counts, under certain conditions.

Cote et al. (2005) applied pig manure to cucumber fields to track the persistence of *E. coli* and Salmonella bacteria. In sandy loam soils, the bacteria were undetectable after 56 to 70 days.

Entry et al. (2005) applied dairy manure and compost to potato fields and found no increase in coliform or *E. coli* levels after seven days. Potato skins had higher coliform and *Enterococcus* spp. levels following application of dairy manure, compared to composted dairy manure.

Gagliardi et al. (2002) found that *E. coli* persisted for 92 days on alfalfa roots in a soil microcosm study, but disappeared in as few as 25 days in fallow soils and on some crops.

How long does E. coli *O157 last in raw cattle manure applied to cropland?*

Survival times vary greatly as a result of soil and weather conditions. Avery et al. (2005) found that *E. coli* O157 bacteria were still viable in 77% of a number of organic wastes two months after land application. They concluded that storage of wastes will help reduce bacteria numbers, but cannot be counted on to totally eliminate *E. coli* O157.

Siphon tubes used for furrow irrigation on Romaine lettuce, Yuma, AZ. Photo NRCS

In a study of organic and conventional lettuce production systems including applications of dairy manure spiked with *E. coli* O157, Franz et al. (2005) found that *E. coli* O157 levels declined faster in organic soils than conventional. ***The level of roughage in the cattle diets significantly influenced* E. coli *levels (the more the roughage, the lower the level).***

How effective are the National Organic Program's (NOP) animal manure and compost requirements in preventing E. coli *O157 contamination of farm crops?*

On certified organic farms, the NOP prohibits applications of raw animal manure for a minimum of 90 days before crop harvest in the case of crops for which there is no possible contact between the soil and the harvested portion of the crop. For all other crops, no raw manure may be applied within 120 days of harvest. Organic farmers are encouraged to apply composted animals manure, and explicit guidelines are set forth governing how compost must be made.

The NOP requirements, and other practices followed by organic farmers to prevent *E. coli* O157 contamination, are not infallible, nor are the practices used by conventional farmers, who are bound by no government food-safety driven restrictions on when and how animal manures can be applied.

Most scientists who have studied the impacts of manure management, composting systems, and manure and compost application methods on *E. coli* survival in the soil and on crops agree that further research is needed to assure adequate margins of food safety. The results of most published research supports the conclusion that under normal conditions, properly managed and applied manure and compost, on organic and conventional farms, does not pose a food safety threat. But published research also shows clearly that margins of safety are not great enough to eliminate risk under combinations of unusual conditions that might include odd weather, unusual pest problems, operator error, equipment failures, lack of effort in sanitizing equipment, or lack of knowledge.

Building compost windrows in Massachusetts.

What about the NOP rules applicable to compost?

The NOP rule applies to compost applied on certified organic farms. It must be made in a manner that results in a temperature in the compost pile between 131 degrees F and 170 degrees F (55 to 77 degrees C) for 3 days when an in-vessel or static aerated pile system is used; or, at temperatures between 131 and 170 degrees F for 15 days using a windrow composting system, during which the rows must be turned at least five times.

Jiang et al. (2003) studied the fate of *E. coli* O157 during the composting of cow manure spiked with high levels of *E. coli* O157. They found that the pathogen became undetectable after 7 to 14 days at 50 degrees C. They recommend that compost contaminated with high levels of *E. coli* O157 should be held for 1 week, and preferably 2 weeks at a minimum temperature of 50 degrees C. Based on these findings, the NOP requirements would appear to be adequate. Other studies have found the NOP requirements to be excessively strict. For example, Lung et al. (2001) found that *E. coli* O157 lasted only 72 hours during composting at 45 degrees C.

Ingham et al. (2004) tested the adequacy of the NOP manure application rules in a study in Wisconsin using noncomposted diary cattle manure applied to vegetable production fields.

Within 90 days after application, *E. coli* levels generally had declined 1,000-fold. Levels remained detectable in enriched soils for up to 168 days. In many plots, *E. coli* O157 was not detected after about 100 days, leading the authors to conclude "the 120-day limit provided [in NOP rules] provided an even greater likelihood of not detecting *E. coli* on carrots." Still, the authors concluded that the 120-day restriction did not absolutely guarantee that all produce would be free of *E. coli* at harvest, especially short season produce like radishes.

Islam et al. (2004) used dairy manure and several types of compost, and irrigation water that was spiked with *E. coli* O157 to study persistence in the field under vegetable production systems. *E. coli* O157 was found to persist for 154 to 217 days and was detected on parsley and lettuce for up to 77 and 177 days after seedlings were planted.

A Norwegian research team fertilized organic lettuce with cow manure, compost, and slurry and found no difference in the bacteriologic quality of the lettuce at harvest (Johannessen et al., 2004).

Work by Mukherjee et al. (2006) in Minnesota found that the use of manure or compost aged more than 12 months on organic vegetables reduced *E. coli* levels by 19-fold.

Manure overflows this storage area and threatens nearby streams with degraded water quality. Photo Tim CCabe, NRCS

References

Albihn,A., E.Eriksson, C.Wallen, and A.Aspan. 2003. "Verotoxinogenic Escherichia coli (VTEC) O157:H7--a nationwide Swedish survey of bovine faeces." *Acta Vet.Scand.* 44:43-52.
Abstract: In the autumn of 1995 the first outbreaks of enterohemorrhagic Escherichia coli O157:H7 including ca 100 human cases were reported in Sweden. From outbreaks in other countries it is known that cattle may carry these bacteria and in many cases is the source of infection. Therefore, the present study was performed to survey the Swedish bovine population for the presence of verotoxin-producing E. coli (VTEC) of serotype O157:H7. Individual faecal samples were collected at the 16 main Swedish abattoirs from April 1996 to August 1997. Of 3071 faecal samples, VTEC O157 were found in 37 samples indicating a prevalence of 1.2% (CI95% 0.8-1.6). All 37 isolates carried genes encoding for verotoxin (VTI and/or VT2), intimin, EHEC-haemolysin and flagellin H7 as determined by PCR. Another 3 strains were of serotype O157:H7 but did not produce verotoxins. The 37 VTEC O157:H7 strains were further characterised by phage typing and pulsed-field gel electrophoresis. The results clearly show that VTEC O157:H7 is established in the Swedish bovine population and indicate that the prevalence of cattle carrying VTEC O157:H7 is correlated to the overall geographical distribution of cattle in Sweden. Results of this study have formed the basis for specific measures recommended to Swedish cattle farmers, and furthermore, a permanent monitoring programme was launched for VTEC O157:H7 in Swedish cattle at slaughter

Avery,L.M., K.Killham, and D.L.Jones. 2005. "Survival of E. coli O157:H7 in organic wastes destined for land application." *J.Appl.Microbiol.* 98:814-822.
Abstract: AIM: To determine the persistence of Escherichia coli O157 in contrasting organic wastes spread to land and to assess the potential environmental risk associated with the disposal of these wastes to land. METHODS AND RESULTS: Twenty-seven organic wastes originating from slaughterhouses, wastewater treatment plants (raw and treated sewage), creameries and farms (bovine slurry), were inoculated with E. coli O157:H7 and incubated at 10 degrees C. Although pathogen numbers gradually declined in all the wastes, albeit at different rates even in the same waste type, E. coli O157:H7 was still viable in 77% of organic wastes tested after 2 months. CONCLUSIONS: Long-term storage of organic wastes led to a significant and gradual decline in E. coli O157:H7 numbers. Consequently, storage may be a useful means of reducing the pathogen load of wastes destined for land application. However, in most cases, long-term storage cannot be expected to completely eliminate E. coli O157:H7 from waste. SIGNIFICANCE AND IMPACT OF THE STUDY: Our results indicate that current legislation may be insufficient to protect the environment from E. coli O157:H7 contamination from untreated wastes spread to land

Berry,E.D. and D.N.Miller. 2005. "Cattle feedlot soil moisture and manure content: II. Impact on Escherichia coli O157." *J.Environ.Qual.* 34:656-663.
Abstract: The moisture and manure contents of soils at cattle feedlot surfaces vary spatiotemporally and likely are important factors in the persistence of Escherichia coli O157 in these soils. The impacts of water content (0.11-1.50 g H2O g(-1) dry feedlot surface material [FSM]) and manure level (5, 25, and 75% dry manure in dry FSM) on E. coli O157:H7 in feedlot soils were evaluated. Generally, E. coli O157:H7 numbers either persisted or increased at all but the lowest moisture levels examined. Manure content modulated the effect of water on E. coli growth; for example, at water content of 0.43 g H2O g(-1) dry FSM and 25% manure, E. coli O157:H7 increased by 2 log10 colony forming units (CFU) g(-1) dry FSM in 3 d, while at 0.43 g H2O g(-1) dry FSM and 75% manure, populations remained stable over 14 d. Escherichia coli and coliform populations responded similarly. In a second study, the impacts of cycling moisture levels and different drying rates on naturally occurring E. coli O157 in feedlot soils were examined. Low initial levels of E. coli O157 were reduced to below enumerable levels by 21 d, but indigenous E. coli populations persisted at >2.50 log10 CFU g(-1) dry FSM up

to 133 d. We conclude that E. coli O157 can persist and may even grow in feedlot soils, over a wide range of water and manure contents. Further investigations are needed to determine if these variables can be manipulated to reduce this pathogen in cattle and the feedlot environment

Boes,J., L.Alban, J.Bagger, V.Mogelmose, D.L.Baggesen, and J.E.Olsen. 2005. "Survival of Escherichia coli and Salmonella Typhimurium in slurry applied to clay soil on a Danish swine farm." *Prev.Vet.Med.* 69:213-228.
Abstract: A pilot study was carried out on a Danish swine farm infected with multi-resistant Salmonella Typhimurium DT104 (MRDT104). We aimed to (1) investigate to which degree the decline of Escherichia coli and Salmonella in swine slurry applied to farmland depended on the application method; (2) estimate the survival times of E. coli and Salmonella in the soil surface following deposition of naturally contaminated pig slurry; and (3) simulate survival of Salmonella in different infection levels using E. coli data as input estimates. Slurry was deposited by four different methods: (1) hose applicator on black soil followed by ploughing and harrowing; (2) hose applicator on black soil followed only by harrowing; (3) hose applicator on a field with winter-wheat seedlings without further soil treatment; (4) slurry injector on a field with winter-wheat seedlings without further soil treatment. E. coli and Salmonella could not be detected at all in soil following treatment 1. Following the other treatments, E. coli was not detected in soil samples after day 21 and Salmonella was no longer detected after day 7. Simulation results showed that clinical (4 log CFU g(-1)) and sub-clinical Salmonella levels (2500 CFU g(-1)) would fall below the detection limit within 10 or 5 days, respectively. Analysis of samples from 62 Danish MRDT104-infected swineherds showed that nearly 75% of these herds had low levels of MRDT104 (< 10 CFU g(-1)) in their slurry. Our results show that ploughing and harrowing of soil amended with contaminated pig slurry was an effective means to reduce environmental exposure to E. coli and Salmonella on this clay-soil farm

Buncic,S. and S.M.Avery. 1997. "Escherichia coli O157:H7 in healthy dairy cows." *N.Z.Vet.J.* 45:45-48.
Abstract: Faecal samples were taken from 371 cows originating from 55 dairy farms and slaughtered at one slaughterhouse; tonsils were taken from 215 of these animals. Escherichia coli 0157:H7 was found in the faeces of only two animals and was not found in any tonsils. The farm supplying the first positive cow detected at the slaughterhouse was visited 3 months later and 160 animals (80 cows and 80 heifers) were tested by rectal swabs; E. coli 0157:H7 was not isolated

Chapman,P.A., J.Cornell, and C.Green. 2000. "Infection with verocytotoxin-producing Escherichia coli O157 during a visit to an inner city open farm." *Epidemiol.Infect.* 125:531-536.
Abstract: Two cases of Escherichia coli O157 infection occurred in children after visiting an inner city open farm. Subsequently faecal samples collected from animal pens and samples of composted mixed animal manure and vegetable waste were examined for E. coli O157 by enrichment culture, immunomagnetic separation and culture of magnetic beads to cefixime tellurite sorbitol MacConkey agar. Strains of E. coli O157 were characterized by hybridization with DNA probes for VT1, VT2 and eaeA, plasmid profile analysis, phage typing and pulsed field gel electrophoresis (PFGE).
Verocytotoxin-producing E. coli O157 strains were isolated from faecal samples from a cow, a horse, 3 breeds of pigs, 2 breeds of sheep and 2 breeds of goats and from 2 samples of compost which had been processed for 3 months. All strains were phage type 21, hybridized with probes for VT2 and eaeA but not with one for VT1, harboured 92 and 2 kb plasmids and gave indistinguishable banding patterns with PFGE. Although only two culture-confirmed cases of infection had been identified, the farm had over 100,000 visitors per year and so it was closed as a precaution both to allow a thorough investigation and to prevent further cases. The investigation identified many factors

which may have contributed to transmission of E. coli O157 infection. Most of these were readily resolved by appropriate corrective measures and as there were no further cases associated with the farm during the ensuing 4 weeks it then re-opened. These cases highlight the risk, especially to young children, of acquiring zoonotic infections during visits to open farms and emphasize the need for adequate guidance and supervision before and during such visits

Cho,S., F.Diez-Gonzalez, C.P.Fossler, S.J.Wells, C.W.Hedberg, J.B.Kaneene, P.L.Ruegg, L.D.Warnick, and J.B.Bender. 2006. "Prevalence of shiga toxin-encoding bacteria and shiga toxin-producing Escherichia coli isolates from dairy farms and county fairs." *Vet.Microbiol.*
Abstract: Shiga toxin-encoding bacteria (STB) and shiga toxin-producing Escherichia coli (STEC) were detected and isolated from dairy cattle and their farm environment and from manure piles at Minnesota (MN) county fairs from 2001 to 2002. A total of 2540 samples were collected from 28 dairy cattle farms (8 organic and 20 conventional), 17 calf pens (5 organic and 12 conventional), and 12 county fairs. STB were detected from 71 (3.2%) of 2208 fecal samples with 20 (71.4%) of 28 dairy farms having at least one positive animal sample. In samples collected from conventional farms, 41 (2.3%) of 1750 fecal samples were STB-positive and 13 (65%) of 20 farms had at least one positive animal. Thirty (6.6%) of 458 fecal samples from organic farms were STB-positive and 7 (87.5%) of 8 farms had at least one positive animal. STB was detected from 31 (17.4%) of 178 samples and 7 (58.3%) out of 12 manure piles at county fairs. A total of 43 STEC isolates were recovered and belonged to 26 different serotypes (19 O and 18 H types). Among STEC, 60.5% possessed only stx1, 30.2% stx2, and 9.3% both stx1 and stx2. The genes eae and hlyA were detected in more than 50% of the STEC isolates. STB can be found on most dairy cattle farms including organic and conventional herds and county fairs. The presence of these potentially pathogenic bacteria in county fairs may pose a risk to the public who have contact with cattle or their environment

CIDRAP. Diarrhaegenic Escherichia coli. 2006. University of Minnesota, Center for Infectious Disease Research and Policy.
Ref Type: Generic

Cote,C. and S.Quessy. 2005. "Persistence of Escherichia coli and Salmonella in surface soil following application of liquid hog manure for production of pickling cucumbers." *J.Food Prot.* 68:900-905.
Abstract: Liquid hog manure is routinely applied to farm land as a crop fertilizer. However, this practice raises food safety concerns, especially when manure is used on fruit and vegetable crops. The objectives of this project were to evaluate the persistence of Escherichia coli and Salmonella in surface soil after application of liquid hog manure to fields where pickling cucumbers were grown and to verify the microbiological quality of harvested cucumbers. Mineral fertilizers were replaced by liquid hog manure at various ratios in the production of pickling cucumbers in a 3-year field study. The experimental design was a randomized complete block comprising four replicates in sandy loam (years 1, 2, and 3) and loamy sand (year 3). Soil samples were taken at a depth of 20 cm every 2 weeks after June application of organic and inorganic fertilizers. Vegetable samples were also taken at harvest time. Liquid hog manure, soil, and vegetable (washed and unwashed) samples were analyzed for the presence of Salmonella and E. coli. An exponential decrease of E. coli populations was observed in surface soil after the application of manure. The estimated average time required to reach undetectable concentrations of E. coli in sandy loam varied from 56 to 70 days, whereas the absence of E. coli was estimated at 77 days in loamy sand. The maximal Salmonella persistence in soil was 54 days. E. coli and Salmonella were not detected in any vegetable samples

Davis,M.A., D.D.Hancock, D.H.Rice, D.R.Call, R.Digiacomo, M.Samadpour, and T.E.Besser. 2003. "Feedstuffs as a vehicle of cattle exposure to Escherichia coli O157:H7 and

Salmonella enterica." *Vet.Microbiol.* 95:199-210.
Abstract: Feed has been reported as a vehicle for transmission of Salmonella enterica in cattle and several lines of evidence suggest that feed can be a vehicle for transmitting Escherichia coli O157:H7 as well. To show whether microbial contamination of feeds could contribute to the populations of S. enterica and E. coli O157:H7 on a farm, we compared isolates from feed samples to bovine fecal isolates from the same farm using pulsed-field gel electrophoresis (PFGE). Four of 2365 component feed samples (0.2%) and 1 of 226 feed mill samples (0.4%) were positive for E. coli O157:H7. Twenty of 2405 (0.8%) component feed samples and none of 226 feed mill samples were positive for Salmonella. PFGE profiles from E. coli O157:H7 isolated from a component feed sample closely resembled that from a fecal isolate collected later from the same farm, and a similar observation was made of a Salmonella Tyhpimurium isolate from component feed on another farm. There were indistinguishable PFGE profiles from component feed Salmonella Tyhpimurium DT104 isolates and fecal isolates from the same farm. These results provide evidence for a role of cattle feed in transmission of E. coli O157:H7; S. enterica; cattle-bacteria

Diez-Gonzalez,F., T.R.Callaway, M.G.Kizoulis, and J.B.Russell. 1998. "Grain feeding and the dissemination of acid-resistant Escherichia coli from cattle." *Science*. 281:1666-1668.
Abstract: The gastric stomach of humans is a barrier to food-borne pathogens, but Escherichia coli can survive at pH 2.0 if it is grown under mildly acidic conditions. Cattle are a natural reservoir for pathogenic E. coli, and cattle fed mostly grain had lower colonic pH and more acid-resistant E. coli than cattle fed only hay. On the basis of numbers and survival after acid shock, cattle that were fed grain had 10(6)-fold more acid-resistant E. coli than cattle fed hay, but a brief period of hay feeding decreased the acid-resistant count substantially

Dodd,C.C., M.W.Sanderson, J.M.Sargeant, T.G.Nagaraja, R.D.Oberst, R.A.Smith, and D.D.Griffin. 2003. "Prevalence of Escherichia coli O157 in cattle feeds in Midwestern feedlots." *Appl.Environ.Microbiol.* 69:5243-5247.
Abstract: Comparisons of enrichment methods (with or without antibiotics and with or without a preenrichment step) using gram-negative (GN) broth or tryptic soy broth (TSB) were conducted with feeds inoculated with Escherichia coli O157:H7. TSB was more sensitive than GN broth, and TSB with a preenrichment step followed by TSB with antibiotics was more sensitive than plain TSB enrichment, in detecting E. coli O157 in inoculated feeds. Feed samples were collected from feed bunks from 54 feedlots to determine the prevalence of E. coli O157 in cattle feeds. TSB preenrichment followed by TSB with antibiotics and the standard GN broth enrichment were used for each feed sample. All samples underwent immunomagnetic separation and were plated onto sorbitol MacConkey agar with cefixime and potassium tellurite. Identification of E. coli O157 was based on indole production, positive latex agglutination for O157 antigen, API 20E test strip results, PCR for the eaeA gene, and the presence of at least one Shiga toxin. E. coli O157 was detected in 52 of 504 feed samples (10.3%) by using GN broth enrichment and in 46 of 504 feed samples (9.1%) by using TSB followed by TSB supplemented with cefixime and vancomycin. E. coli O157 was detected in 75 of 504 feed bunk samples (14.9%) by one or both methods. There was no correlation between E. coli O157 prevalence and generic coliform counts in feeds. The prevalence of E. coli O157 in cattle feed warrants further studies to increase our knowledge of the on-farm ecology of E. coli O157 in order to develop strategies to prevent food-borne disease in humans

Duncan,S.H., I.R.Booth, H.J.Flint, and C.S.Stewart. 2000. "The potential for the control of Escherichia coli O157 in farm animals." *Symp.Ser.Soc.Appl.Microbiol.*157S-165S.
Abstract: The presence of Escherichia coli O157 in the faeces of farm animals appears to provide a primary route for human infection, either through physical contact or by contamination of the food chain. Controlling the survival and proliferation of this

pathogen in the ruminant gut could offer a measure of protection in the short term, and ultimately complement alternative biotechnological based solutions. Normally, E. coli is greatly outnumbered in the ruminant gut by anaerobic bacteria, producers of weak acids inhibitory to the growth of this species. Withdrawal of feed prior to animal slaughter reduces the concentration of these acids in the gut and may be accompanied by the proliferation of E. coli. There are conflicting reports concerning the effects of changes in the ruminant diet upon faecal shedding of E. coli O157. It is contended that it is important to identify animal husbandry methods or feed additives that may be accompanied by an increased risk of proliferation of this pathogen. Greater understanding of the mechanisms involved in bacterial survival in the presence of weak acids, in the interactions between E. coli and other gut bacteria, and of the effects of some antibacterial plant secondary plant compounds on E. coli, could lead to the development of novel control methods

Dunn,J.R., J.E.Keen, R.Del Vecchio, T.E.Wittum, and R.A.Thompson. 2004. "Escherichia coli O157:H7 in a cohort of weaned, preconditioned range beef calves." *J.Food Prot.* 67:2391-2396.
Abstract: Escherichia coli O157:H7 (EC O157) is an important cause of foodborne disease. Cattle are reservoirs for the bacteria and are implicated in transmission to humans. Prevalence data in prefeedlot calves are limited. With the use of sensitive methods, a cohort of weaned beef calves (n = 408) was sampled before and after preconditioning to estimate fecal point prevalence and describe changes in EC O157 fecal shedding. EC O157 isolates were confirmed and characterized by PCR and pulsed-field gel electrophoresis. Calves from 29 cow-calf farms were commingled at three preconditioning sites and placed on a transition ration containing oxytetracycline (200 g/ton) for 45 days. Initial animal-level fecal point prevalence was 2.5% (95% confidence interval, 1 to 5) with a herd-level prevalence of 17.2% (95% confidence interval, 6 to 36). Point prevalence following the preconditioning feeding period was 0%. An unexpected finding in our study was EC O157 isolates that were Shiga toxin-deficient. Pulsed-field gel electrophoresis subtypes of EC O157 were unique in epidemiologically unlinked herds, except one herd that had two unique subtypes. We expected, but observed, neither increased fecal shedding in the cohort nor horizontal transmission of unique EC O157 subtypes. The absence of fecal shedding following the 45-day feeding period might be attributable to seasonal influences, inhibitory concentrations of oxytetracycline in the transition ration, or transient colonization that ended before sampling. EC O157 is apparently widely dispersed at low prevalence in U.S. prefeedlot, weaned calves

Dunn,J.R., J.E.Keen, D.Moreland, and T.Alex. 2004. "Prevalence of Escherichia coli O157:H7 in white-tailed deer from Louisiana." *J.Wildl.Dis.* 40:361-365.
Abstract: Escherichia coli O157:H7 (EC O157) is an important zoonosis. White-tailed deer (Odocoileus virginianus) have been implicated in transmission of this bacterium to humans and have been suggested as reservoirs that might affect carriage in cattle populations. Our study objectives were to estimate prevalence of EC O157 in feces of hunter-harvested deer and to describe fecal shedding patterns in a captive herd sampled over 1 yr. Prevalence of EC O157 in hunter-harvested deer was 0.3% (n = 338). In August 2001, EC O157 was detected in one of 55 deer (1.8%) from the captive herd. Prevalence over the 1-yr period was 0.4% (n = 226). Escherichia coli O157:H7 was rarely isolated from hunter-harvested deer during the winter. We could not describe a seasonal shedding pattern based on one positive sample in the captive herd. These data do not support a prominent role of deer as a reservoir for EC O157 for cattle or humans

Dunn,J.R., J.E.Keen, and R.A.Thompson. 2004. "Prevalence of Shiga-toxigenic Escherichia coli O157:H7 in adult dairy cattle." *J.Am.Vet.Med.Assoc.* 224:1151-1158.
Abstract: OBJECTIVE: To describe shiga-toxigenic Escherichia coil O157:H7 (STEC

O157:H7) fecal shedding prevalence, seasonal fecal shedding patterns, and site-specific prevalence from the oral cavity, skin, and feces of dairy cattle. DESIGN: Cross-sectional study. ANIMALS: Adult dairy cattle from 13 herds in Louisiana. PROCEDURE: Samples were cultured for STEC O157 by use of sensitive and specific techniques, including selective broth enrichment, immunomagnetic separation, monoclonal antibody-based O:H enzyme immunoassay serotyping, and polymerase chain reaction virulence gene characterization. Point estimates and 95% confidence intervals were calculated for fecal shedding prevalence as well as site-specific prevalence from the oral cavity, skin, and feces. Logistic regression was used to assess seasonal variation and differences at various stages of lactation with respect to fecal shedding of STEC O157 in cattle sampled longitudinally. RESULTS: Summer prevalence in herds in = 13) was 38.5%, with a cow-level prevalence of 6.5%. Among positive herds, prevalence ranged from 3% to 34.6%. Samples from 3 of 5 herds sampled quarterly over 1 year yielded positive results for STEC O157. In herds with STEC O157, an increase in cow-level prevalence was detected during spring (13.3%) and summer (10.5%), compared with values for fall and winter. Site-specific prevalences of STEC O157:H7 from oral cavity, skin, and fecal samples were 0%, 0.7%, and 25.2%, respectively. CONCLUSIONS AND CLINICAL RELEVANCE: Our data indicated that STEC O157:H7 was commonly isolated from dairy cows in Louisiana, seasonally shed, and isolated from the skin surface but not the oral cavity of cows

Entry,J.A., A.B.Leytem, and S.Verwey. 2005. "Influence of solid dairy manure and compost with and without alum on survival of indicator bacteria in soil and on potato." *Environ.Pollut.* 138:212-218.

Abstract: We measured Escherichia coli, Enterococcus spp. and fecal coliform numbers in soil and on fresh potato skins after addition of solid dairy manure and dairy compost with and without alum (Al(2)(SO(4))(3)) treatment 1, 7, 14, 28, 179 and 297 days after application. The addition of dairy compost or solid dairy manure at rates to meet crop phosphorus uptake did not consistently increase E. coli and Enterococcus spp. and fecal coliform bacteria in the soil. We did not detect E. coli in any soil sample after the first sampling day. Seven, 14, 28, 179 and 297 days after solid dairy waste and compost and alum were applied to soil, alum did not consistently affect Enterococcus spp. and fecal coliform bacteria in the soil. We did not detect E. coli in any soil, fresh potato skin or potato wash-water at 214 days after dairy manure or compost application regardless of alum treatment. Dairy compost or solid dairy manure application to soil at rates to meet crop phosphorus uptake did not consistently increase Enterococcus spp. and fecal coliform numbers in bulk soil. Solid dairy manure application to soil at rates to meet crop phosphorus uptake, increased Enterococcus spp. and fecal coliform numbers in potato rhizosphere soil. However, fresh potato skins had higher Enterococcus spp. and fecal coliform numbers when solid dairy manure was added to soil compared to compost, N and P inorganic fertilizer and N fertilizer treatments. We did not find any E. coli, Enterococcus or total coliform bacteria on the exterior of the tuber, within the peel or within a whole baked potato after microwave cooking for 5 min

Erickson,M.C., M.Islam, C.Sheppard, J.Liao, and M.P.Doyle. 2004. "Reduction of Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis in chicken manure by larvae of the black soldier fly." *J.Food Prot.* 67:685-690.

Abstract: Green fluorescent protein-labeled Escherichia coli O157:H7 and Salmonella enterica serovar Enteritidis were inoculated at 10(7) CFU/g into cow, hog, or chicken manure. Ten- or 11-day-old soldier fly larvae (Hermetia illucens L.) (7 to 10 g) were added to the manure and held at 23, 27, or 32 degrees C for 3 to 6 days. Soldier fly larvae accelerated inactivation of E. coli O157:H7 in chicken manure but had no effect in cow manure and enhanced survival in hog manure. The initial pH values of the hog and chicken manure were 6.0 to 6.2 and 7.4 to 8.2, respectively, and it is surmised that these conditions affected the stability of the larval antimicrobial system. Reductions of E. coli O157:H7 populations in chicken manure by larvae were affected by storage

temperature, with greater reductions in samples held for 3 days at 27 or 32 degrees C than at 23 degrees C. Pathogen inactivation in chicken manure by larvae was not affected by the indigenous microflora of chicken manure, because Salmonella Enteritidis populations in larvae-treated samples were approximately 2.5 log lower than control samples without larvae when either autoclaved or nonautoclaved chicken manure was used as the contaminated medium during 3 days of storage. Extending the storage time to 6 days, larvae again accelerated the reduction in Salmonella Enteritidis populations in chicken manure during the first 4 days of storage; however, larvae became contaminated with the pathogen. After 2 days of feeding on contaminated manure, Salmonella Enteritidis populations in larvae averaged 3.3 log CFU/g. Populations decreased to 1.9 log CFU/g after 6 days of exposure to contaminated chicken manure; however, the absence of feeding activity by the maggots in later stages of storage may be responsible for the continued presence of Salmonella Enteritidis in larvae. Transfer of contaminated larvae to fresh chicken manure restored feeding activity but led to cross-contamination of the fresh manure

Fenlon,D.R., I.D.Ogden, A.Vinten, and I.Svoboda. 2000. "The fate of Escherichia coli and E. coli O157 in cattle slurry after application to land." *Symp.Ser.Soc.Appl.Microbiol.*149S-156S.
Abstract: The fate of both faecal Escherichia coli and E. coli O157 in slurry following application to arable and grass plots on a clay loam soil was studied. Slurry (5% dry matter) containing 5.3 x 10(4) ml(-1) E. coli and 30 E. coli O157 100 ml(-1) was spread in early March. Initially, almost all E. coli were retained in the upper layers of the soil. Escherichia coli numbers steadily declined to less than 1% of those applied by day 29, and E. coli O157 were only detected in the soil and on the grass for the first week after application. There was some transport of bacteria to deeper layers of the soil, but this was approximately 2% of the total; transport to drains over the same period was mainly associated with rainfall events and amounted to approximately 7% of applied E. coli. However, there were indications that periods of heavy rainfall could cause significant losses of E. coli by both leaching and run-off. Experimental studies showed that E. coli O157 on grass, which was subsequently ensiled in conditions allowing aerobic spoilage, could multiply to numbers exceeding 10(6) g(-1) in the silage

FoodNet. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food --- 10 States, United States, 2005. MMWR 55, 392-395. 6 A.D. Centers for Disease Control.
Ref Type: Generic

Franz,E., A.D.van Diepeningen, O.J.de Vos, and A.H.van Bruggen. 2005. "Effects of cattle feeding regimen and soil management type on the fate of Escherichia coli O157:H7 and salmonella enterica serovar typhimurium in manure, manure-amended soil, and lettuce." *Appl.Environ.Microbiol.* 71:6165-6174.
Abstract: Survival of the green fluorescent protein-transformed human pathogens Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium was studied in a laboratory-simulated lettuce production chain. Dairy cows were fed three different roughage types: high-digestible grass silage plus maize silage (6:4), low-digestible grass silage, and straw. Each was adjusted with supplemental concentrates to high and low crude protein levels. The pathogens were added to manure, which was subsequently mixed (after 56 and 28 days for E. coli O157:H7 and Salmonella serovar Typhimurium, respectively) with two pairs of organically and conventionally managed loamy and sandy soil. After another 14 days, iceberg lettuce seedlings were planted and then checked for pathogens after 21 days of growth. Survival data were fitted to a logistic decline function (exponential for E. coli O157:H7 in soil). Roughage type significantly influenced the rate of decline of E. coli O157:H7 in manure, with the fastest decline in manure from the pure straw diet and the slowest in manure from the diet of grass silage plus maize silage. Roughage type showed no effect on the rate of decline of Salmonella serovar Typhimurium, although decline was significantly faster in the manure derived from straw

than in the manure from the diet of grass silage plus maize silage. The pH and fiber content of the manure were significant explanatory factors and were positively correlated with the rate of decline. With E. coli O157:H7 there was a trend of faster decline in organic than in conventional soils. No pathogens were detected in the edible lettuce parts. The results indicate that cattle diet and soil management are important factors with respect to the survival of human pathogens in the environment

Frenzen,P.D., A.Drake, and F.J.Angulo. 2005. "Economic cost of illness due to Escherichia coli O157 infections in the United States." *J.Food Prot.* 68:2623-2630.
Abstract: The Centers for Disease Control and Prevention (CDC) has estimated that Shiga toxin-producing Escherichia coli O157 (0157 STEC) infections cause 73,000 illnesses annually in the United States, resulting in more than 2,000 hospitalizations and 60 deaths. In this study, the economic cost of illness due to O157 STEC infections transmitted by food or other means was estimated based on the CDC estimate of annual cases and newly available data from the Foodborne Diseases Active Surveillance Network (FoodNet) of the CDC Emerging Infections Program. The annual cost of illness due to O157 STEC was $405 million (in 2003 dollars), including $370 million for premature deaths, $30 million for medical care, and $5 million in lost productivity. The average cost per case varied greatly by severity of illness, ranging from $26 for an individual who did not obtain medical care to $6.2 million for a patient who died from hemolytic uremic syndrome. The high cost of illness due to O157 STEC infections suggests that additional efforts to control this pathogen might be warranted

Gagliardi,J.V. and J.S.Karns. 2002. "Persistence of Escherichia coli O157:H7 in soil and on plant roots." *Environ.Microbiol.* 4:89-96.
Abstract: Soil microcosms were inoculated with Escherichia coli O157:H7 to test persistence in fallow soil, on roots of cover crops and in presence of manure. In fallow soils, E. coli O157:H7 persisted for 25-41 days, on rye roots for 47-96 days and on alfalfa roots, in a silt loam soil, for 92 days whereas on other legumes persistence ranged from 25-40 days, similar to fallow soil. Manure did not seem to affect the persistence of E. coli O157:H7 in these soils. Indigenous and manure-applied coliform populations often decreased faster when E. coli O157:H7 was applied, indicating possible competition between microflora. Coliform populations in microcosms not inoculated with E. coli O157:H7 decreased more slowly or increased. Microbial community analyses showed little effect for E. coli O157:H7 inoculation or addition of manure. Microbial community metabolic activity was enhanced from rye roots after 14 days and by 63 days from alfalfa roots. Microbial community lactose utilization increased over time on rye roots in all soils and on alfalfa roots in a silt loam soil when E. coli O157:H7 was inoculated. Lactose utilization also increased for uninoculated rye roots, soil around rye roots and in some fallow soils. Our data suggest that clay increases persistence and activity of E. coli O157:H7 and other coliforms. In frozen soil stored for over 500 days, E. coli O157:H7 was viable in 37% of tested samples. In summary, E. coli O157:H7 persisted longer and activity was enhanced with some cover crops in these soils due to plant roots, the presence of clay and freezing

Galland,J.C., D.R.Hyatt, S.S.Crupper, and D.W.Acheson. 2001. "Prevalence, antibiotic susceptibility, and diversity of Escherichia coli O157:H7 isolates from a longitudinal study of beef cattle feedlots." *Appl.Environ.Microbiol.* 67:1619-1627.
Abstract: Prevalence, antibiotic susceptibility, and genetic diversity were determined for Escherichia coli O157:H7 isolated over 11 months from four beef cattle feedlots in southwest Kansas. From the fecal pat (17,050) and environmental (7,134) samples collected, 57 isolates of E. coli O157:H7 were identified by use of bacterial culture and latex agglutination (C/LA). PCR showed that 26 isolates were eaeA gene positive. Escherichia coli O157:H7 was identified in at least one of the four feedlots in 14 of the 16 collections by C/LA and in 9 of 16 collections by PCR, but consecutive positive collections at a single feedlot were rare. Overall prevalence in fecal pat samples was low

(0.26% by C/LA, and 0.08% by PCR). No detectable differences in prevalence or antibiotic resistance were found between isolates collected from home pens and those from hospital pens, where antibiotic use is high. Resistant isolates were found for six of the eight antibiotics that could be used to treat E. coli infections in food animals, but few isolates were multidrug resistant. The high diversity of isolates as measured by random amplification of polymorphic DNA and other characteristics indicates that the majority of isolates were unique and did not persist at a feedlot, but probably originated from incoming cattle. The most surprising finding was the low frequency of virulence markers among E. coli isolates identified initially by C/LA as E. coli O157:H7. These results demonstrate that better ways of screening and confirming E. coli O157:H7 isolates are required for accurate determination of prevalence

Garber,L., S.Wells, L.Schroeder-Tucker, and K.Ferris. 1999. "Factors associated with fecal shedding of verotoxin-producing Escherichia coli O157 on dairy farms." *J.Food Prot.* 62:307-312.
Abstract: Fecal samples were collected from 4,361 dairy cows on 91 dairy operations between 26 February and 8 July 1996. Fecal samples were cultured for Escherichia coli O157, and positive isolates were probed for verotoxin-producing genes. A total of 52 (1.2%) fecal samples on 22 (24.2%) operations were positive for verotoxin-producing E. coli O157. Herds in which samples were collected on or after 1 May 1996 were significantly more likely to test positive than herds sampled before that date (odds ratio = 7.7). Herds maintained on farms on which alleyways were flushed with water to remove manure were 8.0 times more likely to have samples test positive for verotoxin-producing E. coli O157 than were herds maintained on farms cleaned by use of other methods of manure removal

Gilbert,R.A., N.Tomkins, J.Padmanabha, J.M.Gough, D.O.Krause, and C.S.McSweeney. 2005. "Effect of finishing diets on Escherichia coli populations and prevalence of enterohaemorrhagic E. coli virulence genes in cattle faeces." *J.Appl.Microbiol.* 99:885-894.
Abstract: AIM: To determine the effect of different carbohydrate-based finishing diets on fermentation characteristics and the shedding of Escherichia coli and enterohaemorrhagic E. coli (EHEC) virulence genes in cattle faeces. METHODS AND RESULTS: The size of faecal E. coli populations and fermentation characteristics were ascertained in three experiments where cattle were maintained on a range of finishing diets including high grain, roughage, and roughage + molasses (50%) diets. Increased E. coli numbers, decreased pH and enhanced butyrate and lactate fermentation pathways were associated with grain diets, whereas roughage and roughage + molasses diets resulted in decreased concentrations of ehxA, eaeA and stx(1) genes, this trend remaining at lairage. In one experiment, faecal E. coli numbers were significantly lower in animals fed roughage and roughage + molasses, than animals fed grain (4.5, 5.2 and 6.3 mean log10 g(-1) digesta respectively). In a second experiment, faecal E. coli numbers were 2 log lower in the roughage and roughage + molasses diets compared with grain-fed animals prior to lairage (5.6, 5.5 and 7.9 mean log10 g(-1) digesta respectively) this difference increasing to 2.5 log at lairage. CONCLUSIONS: The type of dietary carbohydrate has a significant effect on E. coli numbers and concentration of EHEC virulence genes in faeces of cattle. SIGNIFICANCE AND IMPACT OF THE STUDY: The study provides a better understanding of the impact finishing diet and commercial lairage management practices may have on the shedding of E. coli and EHEC virulence factors, thus reducing the risk of carcass contamination by EHEC

Hancock,D.D., T.E.Besser, M.L.Kinsel, P.I.Tarr, D.H.Rice, and M.G.Paros. 1994. "The prevalence of Escherichia coli O157.H7 in dairy and beef cattle in Washington State." *Epidemiol.Infect.* 113:199-207.
Abstract: Escherichia coli O157.H7 was found in 10 of 3570 (0.28%) faecal samples

from dairy cattle in 5 of 60 herds (8.3%). Several tentative associations with manure handling and feeding management practices on dairy farms were identified. Faecal/urine slurry samples, bulk milk samples, and milk filters from dairy herds were negative for E. coli O157.H7. E. coli O157.H7 was also isolated from 10 of 1412 (0.71%) faecal samples from pastured beef cattle in 4 of 25 (16%) herds. The prevalence of E. coli O157.H7 excretion in feedlot beef cattle was 2 of 600 (0.33%). The identification of cattle management practices associated with colonization of cattle by E. coli O157.H7 suggests the possibility that human E. coli O157.H7 exposure may be reduced by cattle management procedures

Herriott,D.E., D.D.Hancock, E.D.Ebel, L.V.Carpenter, D.H.Rice, and T.E.Besser. 1998. "Association of herd management factors with colonization of dairy cattle by Shiga toxin-positive Escherichia coli O157." *J.Food Prot.* 61:802-807.
Abstract: Management factors in 36 Pacific Northwest dairy herds were evaluated for their association with the prevalence of Shiga toxin-positive Escherichia coli O157 (E. coli O157) in dairy cattle. The within-herd prevalence of E. coli O157 was estimated by bacteriological culture of fecal pat samples, collected monthly for 6 months (approximately 60 per visit), from heifer cattle. During the first visit to each farm, a management questionnaire was administered that covered a broad range of animal husbandry practices. On each subsequent visit, a brief questionnaire was administered to detect changes in management practices. A significantly higher prevalence of E. coli O157 was noted in herds that fed corn silage to heifers compared to herds that did not feed corn silage. More tentative associations of E. coli O157 prevalence were observed for weaning method, protein level of calf starter, feeding of ionophores in heifer rations, feeding of grain screens to heifers, and feeding of animal by-products to cows

Hutchison,M.L., L.D.Walters, S.M.Avery, F.Munro, and A.Moore. 2005. "Analyses of livestock production, waste storage, and pathogen levels and prevalences in farm manures." *Appl.Environ.Microbiol.* 71:1231-1236.
Abstract: Survey results describing the levels and prevalences of zoonotic agents in 1,549 livestock waste samples were analyzed for significance with livestock husbandry and farm waste management practices. Statistical analyses of survey data showed that livestock groups containing calves of <3 months of age, piglets, or lambs had higher prevalences and levels of Campylobacter spp. and Escherichia coli O157 in their wastes. Younger calves that were still receiving milk, however, had significantly lower levels and prevalence of E. coli O157. Furthermore, when wastes contained any form of bedding, they had lowered prevalences and levels of both pathogenic Listeria spp. and Campylobacter spp. Livestock wastes generated by stock consuming a diet composed principally of grass were less likely to harbor E. coli O157 or Salmonella spp. Stocking density did not appear to influence either the levels or prevalences of bacterial pathogens. Significant seasonal differences in prevalences were detected in cattle wastes; Listeria spp. were more likely to be isolated in March to June, and E. coli O157 was more likely to be found in May and June. Factors such as livestock diet and age also had significant influence on the levels and prevalences of some zoonotic agents in livestock wastes. A number of the correlations identified could be used as the basis of a best-practice disposal document for farmers, thereby lowering the microbiological risks associated with applying manures of contaminated livestock to land

Ingham,S.C., J.A.Losinski, M.P.Andrews, J.E.Breuer, J.R.Breuer, T.M.Wood, and T.H.Wright. 2004. "Escherichia coli contamination of vegetables grown in soils fertilized with noncomposted bovine manure: garden-scale studies." *Appl.Environ.Microbiol.* 70:6420-6427.
Abstract: In this study we tested the validity of the National Organic Program (NOP) requirement for a > or =120-day interval between application of noncomposted manure and harvesting of vegetables grown in manure-fertilized soil. Noncomposted bovine manure was applied to 9.3-m2 plots at three Wisconsin sites (loamy sand, silt loam, and

silty clay loam) prior to spring and summer planting of carrots, radishes, and lettuce. Soil and washed (30 s under running tap water) vegetables were analyzed for indigenous Escherichia coli. Within 90 days, the level of E. coli in manure-fertilized soil generally decreased by about 3 log CFU/g from initial levels of 4.2 to 4.4 log CFU/g. Low levels of E. coli generally persisted in manure-fertilized soil for more than 100 days and were detected in enriched soil from all three sites 132 to 168 days after manure application. For carrots and lettuce, at least one enrichment-negative sample was obtained < or =100 days after manure application for 63 and 88% of the treatments, respectively. The current > or =120-day limit provided an even greater likelihood of not detecting E. coli on carrots (> or =1 enrichment-negative result for 100% of the treatments). The rapid maturation of radishes prevented conclusive evaluation of a 100- or 120-day application-to-harvest interval. The absolute absence of E. coli from vegetables harvested from manure-fertilized Wisconsin soils may not be ensured solely by adherence to the NOP > or =120-day limit. Unless pathogens are far better at colonizing vegetables than indigenous E. coli strains are, it appears that the risk of contamination for vegetables grown in Wisconsin soils would be elevated only slightly by reducing the NOP requirement to > or =100 days

Islam,M., M.P.Doyle, S.C.Phatak, P.Millner, and X.Jiang. 2004. "Persistence of enterohemorrhagic Escherichia coli O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water." *J.Food Prot.* 67:1365-1370.
Abstract: Outbreaks of enterohemorrhagic Escherichia coli O157:H7 infections associated with lettuce and other leaf crops have occurred with increasing frequency in recent years. Contaminated manure and polluted irrigation water are probable vehicles for the pathogen in many outbreaks. In this study, the occurrence and persistence of E. coli O157:H7 in soil fertilized with contaminated poultry or bovine manure composts or treated with contaminated irrigation water and on lettuce and parsley grown on these soils under natural environmental conditions was determined. Twenty-five plots, each 1.8 by 4.6 m, were used for each crop, with five treatments (one without compost, three with each of the three composts, and one without compost but treated with contaminated water) and five replication plots for each treatment. Three different types of compost, 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. Pathogen concentrations were 10(7) CFU/g of compost and 10(5) CFU/ml of water. Contaminated compost was applied to soil in the field as a strip at 4.5 metric tons per hectare on the day before lettuce and parsley seedlings were transplanted in late October 2002. Contaminated irrigation water was applied only once on the plants as a treatment in five plots for each crop at the rate of 2 liters per plot 3 weeks after the seedlings were transplanted. E. coli O157:H7 persisted for 154 to 217 days in soils amended with contaminated composts and was detected on lettuce and parsley for up to 77 and 177 days, respectively, after seedlings were planted. Very little difference was observed in E. coli O157:H7 persistence based on compost type alone. E. coli O157:H7 persisted longer (by > 60 days) in soil covered with parsley plants than in soil from lettuce plots, which were bare after lettuce was harvested. In all cases, E. coli O157:H7 in soil, regardless of source or crop type, persisted for > 5 months after application of contaminated compost or irrigation water

Jiang,X., J.Morgan, and M.P.Doyle. 2002. "Fate of Escherichia coli O157:H7 in manure-amended soil." *Appl.Environ.Microbiol.* 68:2605-2609.
Abstract: Escherichia coli O157:H7 cells survived for up to 77, >226, and 231 days in manure-amended autoclaved soil held at 5, 15, and 21 degrees C, respectively. Pathogen populations declined more rapidly in manure-amended unautoclaved soil under the same conditions, likely due to antagonistic interactions with indigenous soil microorganisms. E. coli O157:H7 cells were inactivated more rapidly in both autoclaved and unautoclaved soils amended with manure at a ratio of 1 part manure to 10 parts soil

at 15 and 21 degrees C than in soil samples containing dilute amounts of manure. The manure-to-soil ratio, soil temperature, and indigenous microorganisms of the soil appear to be contributory factors to the pathogen's survival in manure-amended soil

Jiang,X., J.Morgan, and M.P.Doyle. 2003. "Thermal inactivation of Escherichia coli O157:H7 in cow manure compost." *J.Food Prot.* 66:1771-1777.
Abstract: Rates of inactivation of a five-strain mixture of green fluorescent protein-labeled Escherichia coli O157:H7 in autoclaved and unautoclaved commercial cow manure compost with a moisture content of ca. 38% were determined at temperatures of 50, 55, 60, 65, and 70 degrees C. Trypticase soy agar with ampicillin was determined to be the best medium for the enumeration of heat-injured and uninjured cells of green fluorescent protein-labeled E. coli O157:H7. The results obtained in this study revealed that in autoclaved compost, E. coli O157:H7 reductions of ca. 4 log CFU g occurred within 8 h, 3 h, 15 min, 2 min, and < 1 min at 50, 55, 60, 65, and 70 degrees C, respectively. At 65 and 70 degrees C, considerably less time was required to kill the pathogen in unautoclaved compost than in autoclaved compost. Decimal reduction times (D-values) for autoclaved compost at 50, 55, 60, 65, and 70 degrees C were 137, 50.3, 4.1, 1.8, and 0.93 min, respectively, and D-values for unautoclaved compost at 50, 55, and 60 degrees C were 135, 35.4, and 3.9 min, respectively. Considerable tailing was observed for inactivation curves, especially at 60, 65, and 70 degrees C. These results are useful for identifying composting conditions that will reduce the risk of the transmission of E. coli O157:H7 to foods produced in the presence of animal fecal waste

Johannessen,G.S., R.B.Froseth, L.Solemdal, J.Jarp, Y.Wasteson, and M.Rorvik. 2004. "Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce." *J.Appl.Microbiol.* 96:787-794.
Abstract: AIM: To investigate the bacteriological quality, and the occurrence of selected pathogenic bacteria from organically grown Iceberg lettuce fertilized with bovine manure in the form of compost, firm manure and slurry in a 2-year field trial. METHODS AND RESULTS: Samples of soil, fertilizer, fertilized soil, seedlings and lettuce were analysed for aerobic plate counts (APC), thermotolerant coliform bacteria (TCB), Escherichia coli, E. coli O157:H7, Salmonella spp. and Listeria monocytogenes. No difference in bacteriological quality could be shown in lettuce at harvest, however, APC varied significantly from year to year in the study. The various treatments gave significantly different APC and numbers of TCB isolated from fertilized soil. Escherichia coli O157:H7 was isolated from firm manure and slurry, and soils fertilized with the respective fertilizers the second year, but were not recovered from the lettuce. CONCLUSIONS: No difference in bacteriological quality could be detected in lettuce at harvest after application of various types of manure-based fertilizers grown under Norwegian conditions. SIGNIFICANCE AND IMPACT OF THE STUDY: The results may indicate that the use of manure does not have considerable influence on the bacteriological quality of organic lettuce. However, others have suggested that there is a risk by using manure. There is a need for more research in the field

Lahti,E., M.Keskimaki, L.Rantala, P.Hyvonen, A.Siitonen, and T.Honkanen-Buzalski. 2001. "Occurrence of Escherichia coli O157 in Finnish cattle." *Vet.Microbiol.* 79:239-251.
Abstract: Bovine faecal samples were collected during June-December 1997 at 14 major abattoirs slaughtering cattle in Finland. Escherichia coli O157 was isolated from 19 of the 1448 samples (1.31%) after enrichment and immunomagnetic separation (IMS). The positive faecal isolates originated from 16 farms and eight abattoirs. The occurrence of E. coli O157 was highest in July (8/204; 3.92%) and September (6/244; 2.46%). No E. coli O157 was detected in November and December, nor from the faecal samples from the northernmost region where cattle density is low. All of the isolates carried the eae gene and showed the enterohaemolytic phenotype. All except one were motile and had the flagella antigen H7. Seventeen of the isolates were positive for stx(2) gene and one carried both the stx(1) and stx(2) genes. Of the 17 isolates with stx genes, 16 were

verocytotoxin-positive in a reversed passive latex agglutination test after polymyxin extraction but only eight without extraction. The isolates belonged to 10 different pulsed-field gel electrophoresis (PFGE) patterns. The most common PFGE pattern (1.42) was detected in eight isolates (42.1%). Four PFGE patterns (1.1; 1.6; 1.12; 1.14) were identical with those isolated from humans in Finland, suggesting that at least some human E. coli O157 infections may be of bovine origin

LeJeune,J.T., T.E.Besser, N.L.Merrill, D.H.Rice, and D.D.Hancock. 2001. "Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle." *J.Dairy Sci.* 84:1856-1862.
Abstract: The microbial quality of livestock drinking water was evaluated in 473 cattle water troughs located at 99 different cattle operations. The mean log10-transformed coliform and Escherichia coli concentrations per milliliter of trough water were 1.76 +/- 1.25 (SD) and 0.98 +/- 1.06 (SD), respectively. The degree of E. coli contamination was positively associated with the proximity of the water trough to the feedbunk, protection of the trough from direct sunlight, lower concentrations of protozoa in the water, and warmer weather. Salmonella sp. were isolated from 2/235 (0.8%) troughs and shigatoxigenic-E. coli O157 was recovered from 6/473 (1.3%) troughs. Four experimental microcosms simulating cattle water troughs were used to further evaluate the effects of protozoal populations on the survival of E. coli O157 in cattle water troughs. Escherichia coli O157 of bovine fecal origin proliferated in all microcosms. Reduction of protozoal populations by treatment with cycloheximide was associated with increased persistence of E. coli O157 concentrations in the microcosms. Water troughs are a major source of exposure of cattle to enteric bacteria, including a number of foodborne pathogens, and this degree of bacterial contamination appeared to be associated with potentially controllable factors

LeJeune,J.T., T.E.Besser, D.H.Rice, J.L.Berg, R.P.Stilborn, and D.D.Hancock. 2004. "Longitudinal study of fecal shedding of Escherichia coli O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover." *Appl.Environ.Microbiol.* 70:377-384.
Abstract: Identification of the sources and methods of transmission of Escherichia coli O157:H7 in feedlot cattle may facilitate the development of on-farm control measures for this important food-borne pathogen. The prevalence of E. coli O157:H7 in fecal samples of commercial feedlot cattle in 20 feedlot pens between April and September 2000 was determined throughout the finishing feeding period prior to slaughter. Using immunomagnetic separation, E. coli O157:H7 was isolated from 636 of 4,790 (13%) fecal samples in this study, with highest prevalence earliest in the feeding period. No differences were observed in the fecal or water trough sediment prevalence values of E. coli O157:H7 in 10 pens supplied with chlorinated drinking water supplies compared with nonchlorinated water pens. Pulsed-field gel electrophoresis of XbaI-digested bacterial DNA of the 230 isolates obtained from eight of the pens revealed 56 unique restriction endonuclease digestion patterns (REDPs), although nearly 60% of the isolates belonged to a group of four closely related genetic subtypes that were present in each of the pens and throughout the sampling period. The other REDPs were typically transiently detected, often in single pens and on single sample dates, and in many cases were also closely related to the four predominant REDPs. The persistence and predominance of a few REDPs observed over the entire feeding period on this livestock operation highlight the importance of the farm environment, and not necessarily the incoming cattle, as a potential source or reservoir of E. coli O157:H7 on farms

Lung,A.J., C.M.Lin, J.M.Kim, M.R.Marshall, R.Nordstedt, N.P.Thompson, and C.I.Wei. 2001. "Destruction of Escherichia coli O157:H7 and Salmonella enteritidis in cow manure composting." *J.Food Prot.* 64:1309-1314.
Abstract: Application of cow manure and composted manure in agricultural practice could potentially cause contamination of foodstuffs with pathogenic bacteria such as

Salmonella Enteritidis and Escherichia coli O157:H7. In this study, rifampicin-resistant (RifR) E. coli O157:H7 and Salmonella Enteritidis at a level of 7 log CFU/g of raw compost feed were used to determine the effect of a bench-scale composting system on their survival. RifR E. coli O157:H7 was not detected after 72 h of composting at 45 degrees C, and RifR Salmonella Enteritidis was not detected after 48 h. The use of selective media for enrichment failed to recover in the composting samples held at 45 degrees C for 96 h. However, the pathogens showed no change in bacterial numbers when the composting system was held at room temperature. Thus, properly composted manure can be safely used in food crop production while minimizing the likelihood of microbial contamination

Lynn,T.V., D.D.Hancock, T.E.Besser, J.H.Harrison, D.H.Rice, N.T.Stewart, and L.L.Rowan. 1998. "The occurrence and replication of Escherichia coli in cattle feeds." *J.Dairy Sci.* 81:1102-1108.
Abstract: Sixty-three of 209 (30.1%) samples of cattle feed that were collected from multiple commercial sources and from farms were found to contain Escherichia coli. However, none of the feed samples examined were culture-positive for E. coli O157. Replication of fecal E. coli, including E. coli O157, was demonstrated in a variety of feeds at temperatures that were similar to those found on farms in summer months. Fresh mixed rations containing corn silage were sampled from 16 dairies. Rations from 12 of these dairies were found to contain E. coli, and the rations from 5 dairies had concentrations of E. coli that were greater than 1000 cfu/g. The ability of experimental mixed rations to support the replication of E. coli was correlated with the concentration of organic acids in the corn silage that was used in the ration. Widespread contamination of cattle feeds with E. coli and the ability of E. coli to replicate in feeds suggest that feeds are a potentially important factor in the ecology of organisms that can be transmitted from feces to mouth, such as E. coli O157

McGee,P., D.J.Bolton, J.J.Sheridan, B.Earley, G.Kelly, and N.Leonard. 2002. "Survival of Escherichia coli O157:H7 in farm water: its role as a vector in the transmission of the organism within herds." *J.Appl.Microbiol.* 93:706-713.
Abstract: AIMS: The study aimed to investigate the survival characteristics of Escherichia coli O157:H7 in farm water (FW), and in sterile distilled municipal water (SDW), stored outdoors under field conditions, with or without the addition of faeces (1% w/v), in a farmyard shed and the laboratory at 15 degrees C. METHODS AND RESULTS: Water samples were inoculated with E. coli O157:H7 at 10(3) and 10(6) ml(-1), and sampled over a 31-day period. In FW stored outdoors in a field, E. coli O157:H7 survived for 14 days at temperatures <15 degrees C, at both inoculation levels, while in the laboratory at 15 degrees C, the organism was still detectable at low levels (<1 log10 cfu ml(-1)) after 31 days. The addition of bovine faeces to water outdoors (1% w/v) resulted in survival for 24 days. In SDW inoculated at 10(6) ml(-1) and stored in the laboratory (15 degrees C), only a 2.5 log reduction was observed after 31 days, while the organism could not be detected after 17 days in the field. Preliminary screening of water samples stored outdoors isolated a bacterium which exhibited antimicrobial activity towards E. coli O157:H7. CONCLUSIONS: The survival of E. coli O157:H7 observed in this study illustrates the potential of farm water to act as a vehicle in the transfer of the organism across a herd. SIGNIFICANCE AND IMPACT OF THE STUDY: The difficulty in extrapolating results from controlled laboratory situations to on-farm conditions is also highlighted in this study

McSweeney,C.S., R.A.Gilbert, D.O.Krause, J.Padmanabha, and S.E.Denman. 2004. "Effect of diet on E. coli populations in the faeces of cattle." *Asia Pac.J.Clin.Nutr.* 13:S27.
Abstract: Background - A study on enterohaemorrhagic Escherichia coli (EHEC) contamination of beef carcasses at slaughter concluded that faecal and carcase levels of EHEC are positively correlated and that there was a role for control of EHEC in live cattle. In this current study we examined the effect of dietary inclusion of molasses

(simple sugars), grain (starch) and roughage (structural carbohydrate) on the shedding of E. coli in cattle faeces. Enterohaemorrhagic E. coli (EHEC) virulence factors [shiga toxin genes, stx1 and stx2; accessory virulence factors, intimin (eaeA) and plasmid-encoded enterohemolysin (hlyA)] in cattle faeces were also investigated. Objective - To determine firstly, whether roughage and/or molasses based diets reduce the population of E. coli and EHEC virulence factors compared with grain based feedlot diets, and secondly, if commercial lairage management practices promote or diminish these responses. Design - Thirty Brahman cross steers (mean LW +/- sem) 329+/-3.2kg, were initially fed a high grain (80%) diet. The cattle were then allocated into 3 groups of 10 animals and fed ad libitum (a) 50% molasses, 28% Rhodes grass (Chloris gayana) hay, 15.0% whole cotton seed, 4.5% cotton seed meal, 1.5% urea and 1% mineral vitamin premix (M+R); (b) 80% sorghum, 5% peanut shells, 5.5% cotton seed meal (G); and (c) Rhodes grass plus 20g urea/kg DM (R). A fresh faecal sample (100g) was collected from each animal on the baseline grain diet, on 2 separate days during the final week of each dietary treatment (PL), and just prior to slaughter at lairage (L). A multiplex PCR method was used to quantify the virulence genes stx1 and stx2, eaeA and hlyA in faeces. Outcomes - Prior to lairage, faecal E. coli numbers were two logs lower (8.1 vs 5.6 log10/g digesta) in the R and R+M diets compared with G fed animals and this difference increased to 2.5 logs at lairage. Analysis of the concentration of EHEC virulence factors in faeces indicated a marked decrease in hlyA, eaeA and stx1 genes in the R and R+M diets and this trend remained at lairage. VFA patterns were similar in the roughage and molasses diets whereas increased E. coli numbers, decreased pH and enhanced butyrate and lactate fermentation pathways were associated with the grain diet. This would indicate a shift in the microbial population of the hindgut. Cluster analysis of predominant E. coli serotypes isolated from faeces from each of the three dietary treatment groups showed that the R and R+M groups were similar, but quite distinctive from populations isolated from grain fed animals. Conclusions - This study indicates that the type of dietary carbohydrate has a significant effect on the E. coli community structure and therefore may determine the level of pathogenic serotypes. Future work is focussed on developing detection methods for quantification of putative EHEC populations in response to diet. These detection methods will be used to determine whether diets based on R or R+M combinations, which have low fermentable carbohydrate reaching the hindgut, have the potential to reduce EHEC populations

Mead,P.S., L.Slutsker, V.Dietz, L.F.McCaig, J.S.Bresee, C.Shapiro, P.M.Griffin, and R.V.Tauxe. 1999. "Food-related illness and death in the United States." *Emerg.Infect.Dis.* 5:607-625.
Abstract: To better quantify the impact of foodborne diseases on health in the United States, we compiled and analyzed information from multiple surveillance systems and other sources. We estimate that foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. Known pathogens account for an estimated 14 million illnesses, 60, 000 hospitalizations, and 1,800 deaths. Three pathogens, Salmonella, Listeria, and Toxoplasma, are responsible for 1,500 deaths each year, more than 75% of those caused by known pathogens, while unknown agents account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3,200 deaths. Overall, foodborne diseases appear to cause more illnesses but fewer deaths than previously estimated

Mukherjee,A., D.Speh, A.T.Jones, K.M.Buesing, and F.Diez-Gonzalez. 2006. "Longitudinal microbiological survey of fresh produce grown by farmers in the upper midwest." *J.Food Prot.* 69:1928-1936.
Abstract: Microbiological analyses of fruits and vegetables produced by farms in Minnesota and Wisconsin were conducted to determine coliform and Escherichia coli counts and the prevalence of E. coli, Salmonella, and E. coli O157:H7. During the 2003 and 2004 harvest seasons, 14 organic farms (certified by accredited organic agencies), 30 semiorganic farms (used organic practices but not certified), and 19 conventional farms were sampled to analyze 2,029 preharvest produce samples (473 organic, 911

semiorganic, and 645 conventional). Produce varieties included mainly lettuces, leafy greens, cabbages, broccoli, peppers, tomatoes, zucchini, summer squash, cucumber, and berries. Semiorganic and organic farms provided the majority of leafy greens and lettuces. Produce samples from the three farm types had average coliform counts of 1.5 to 2.4 log most probable number per g. Conventional produce had either significantly lower or similar coliform populations compared with the semiorganic and organic produce. None of the produce samples collected during the 2 years of this study were contaminated with Salmonella or E. coli O157:H7. E. coli contamination was detected in 8% of the samples, and leafy greens, lettuces, and cabbages had significantly higher E. coli prevalence than did all the other produce types in both years for the three farm types. The prevalence of E. coli contamination by produce type was not significantly different between the three farm types during these 2 years, with the exception of organic leafy greens, in which E. coli prevalence was one-third that of semiorganic leafy greens in 2003. These results indicate that the preharvest microbiological quality of produce from the three types of farms was very similar during these two seasons and that produce type appears to be more likely than farm type to influence E. coli contamination

NARMS. National Antibiotic Resistance Monitoring System: Enteric Bacteria. 2000. NARMS, CDC.
Ref Type: Generic

Rangel,J.M., P.H.Sparling, C.Crowe, P.M.Griffin, and D.L.Swerdlow. 2005. "Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982-2002." *Emerg.Infect.Dis.* 11:603-609.
Abstract: Escherichia coli O157:H7 causes 73,000 illnesses in the United States annually. We reviewed E. coli O157 outbreaks reported to Centers for Disease Control and Prevention (CDC) to better understand the epidemiology of E. coli O157. E. coli O157 outbreaks (>or=2 cases of E. coli O157 infection with a common epidemiologic exposure) reported to CDC from 1982 to 2002 were reviewed. In that period, 49 states reported 350 outbreaks, representing 8,598 cases, 1,493 (17%) hospitalizations, 354 (4%) hemolytic uremic syndrome cases, and 40 (0.5%) deaths. Transmission route for 183 (52%) was foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 31 (9%) waterborne, 11 (3%) animal contact, and 1 (0.3%) laboratory-related. The food vehicle for 75 (41%) foodborne outbreaks was ground beef, and for 38 (21%) outbreaks, produce

Renter,D.G., J.M.Sargeant, S.E.Hygnstorm, J.D.Hoffman, and J.R.Gillespie. 2001. "Escherichia coli O157:H7 in free-ranging deer in Nebraska." *J.Wildl.Dis.* 37:755-760.
Abstract: In order to determine the prevalence and distribution of the human pathogen, Escherichia coli O157:H7, in free-ranging deer, hunters were asked to collect and submit fecal samples from deer harvested during a regular firearm season (14-22 November 1998). Prior to the season, 47% of the hunters with permits in the southeastern Nebraska (USA) study area indicated a willingness to participate in the study. Approximately 25% of successful hunters in the area submitted deer fecal samples. Escherichia coli O157:H7 was cultured from four (0.25%) of 1,608 total samples submitted. All of the fecal samples that were properly identified (1,426) and all that were positive for E. coli O157:H7 were from white-tailed deer (Odocoileus virginianus). We were unable to detect a statistically significant geographic distribution pattern of E. coli O157:H7. The presence of E. coli O157:H7 in the feces of free-ranging deer has implications not only for hunters, consumers of venison, and others in contact with deer or deer feces, but also for the development of strategies aimed at reducing and/or controlling this pathogen in water sources and domestic livestock

Rice,E.W. and C.H.Johnson. 2000. "Short communication: survival of Escherichia coli O157:H7 in dairy cattle drinking water." *J.Dairy Sci.* 83:2021-2023.
Abstract: Cattle drinking water from two dairy farms was used in a study to determine the

survival characteristics of the bacterial pathogen Escherichia coli O157:H7 and wild-type E. coli. The E. coli O157:H7 inoculum consisted of a consortium of isolates obtained from dairy cattle. Fresh manure was used as the source for the wild-type E. coli. In the water source from farm 1 the pathogens were present at both 5 and 15 degrees C during the 16-d duration of the study. In the water source from farm 2, the pathogens were detected at 5 degrees C through d 8 and through d 4 at 15 degrees C. The fecal indicator, wild-type E. coli, was always present when the pathogens were present

Russell,J.B., F.Diez-Gonzalez, and G.N.Jarvis. 2000. "Invited review: effects of diet shifts on Escherichia coli in cattle." *J.Dairy Sci.* 83:863-873.
Abstract: Escherichia coli O157:H7 is a pathogenic bacterium that causes acute illness in humans, but mature cattle are not affected. E. coli O157:H7 can enter the human food supply from cattle via fecal contamination of beef carcasses at slaughter. Previous attempts to correlate the incidence of E. coli O157:H7 with specific diets or feeding management practices gave few statistically significant or consistent findings. However, recent work indicates that cattle diets may be changed to decrease fermentation acid accumulation in the colon. When fermentation acids accumulate in the colon and pH decreases, the numbers of acid-resistant E. coli increase; acid-resistant E. coli are more likely to survive the gastric stomach of humans. When cattle were fed hay for a brief period (<7 d), acid-resistant E. coli numbers declined dramatically. Other workers have shown that brief periods of hay feeding can also decrease the number of cattle shedding E. coli O157:H7, and a similar trend was observed if cattle were taken off feed and exposed to simulated transport. These observations indicate that cattle feeding management practices may be manipulated to decrease the risk of foodborne illness from E. coli, but further work will be needed to confirm these effects

Sargeant,J.M., J.R.Gillespie, R.D.Oberst, R.K.Phebus, D.R.Hyatt, L.K.Bohra, and J.C.Galland. 2000. "Results of a longitudinal study of the prevalence of Escherichia coli O157:H7 on cow-calf farms." *Am.J.Vet.Res.* 61:1375-1379.
Abstract: OBJECTIVE: To describe the frequency and distribution of Escherichia coli O157:H7 in the feces and environment of cow-calf herds housed on pasture. SAMPLE POPULATION: Fecal and water samples for 10 cow-calf farms in Kansas. PROCEDURE: Fecal and water samples were obtained monthly throughout a 1-year period (3,152 fecal samples from 2,058 cattle; 199 water samples). Escherichia coli O157:H7 in fecal and water samples was determined, using microbial culture. RESULTS: Escherichia coli O157:H7 was detected in 40 of 3,152 (1.3%) fecal samples, and 40 of 2,058 (1.9%) cattle had > or = 1 sample with E coli. Fecal shedding by specific cattle was transient; none of the cattle had E coli in more than 1 sample. Significant differences were not detected in overall prevalence among farms. However, significant differences were detected in prevalence among sample collection dates. Escherichia coli O157:H7 was detected in 3 of 199 (1.5%) water samples. CONCLUSIONS AND CLINICAL RELEVANCE: Implementing control strategies for E coli O157:H7 at all levels of the cattle industry will decrease the risk of this organism entering the human food chain. Devising effective on-farm strategies to control E coli O157:H7 in cow-calf herds will require an understanding of the epidemiologic characteristics of this pathogen

Schroeder,C.M., C.Zhao, C.Debroy, J.Torcolini, S.Zhao, D.G.White, D.D.Wagner, P.F.McDermott, R.D.Walker, and J.Meng. 2002. "Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food." *Appl.Environ.Microbiol.* 68:576-581.
Abstract: A total of 361 Escherichia coli O157 isolates, recovered from humans, cattle, swine, and food during the years 1985 to 2000, were examined to better understand the prevalence of antimicrobial resistance among these organisms. Based on broth microdilution results, 220 (61%) of the isolates were susceptible to all 13 antimicrobials tested. Ninety-nine (27%) of the isolates, however, were resistant to tetracycline, 93 (26%) were resistant to sulfamethoxazole, 61 (17%) were resistant to cephalothin, and

48 (13%) were resistant to ampicillin. Highest frequencies of resistance occurred among swine isolates (n = 70), where 52 (74%) were resistant to sulfamethoxazole, 50 (71%) were resistant to tetracycline, 38 (54%) were resistant to cephalothin, and 17 (24%) were resistant to ampicillin. Based on the presence of Shiga toxin genes as determined by PCR, 210 (58%) of the isolates were identified as Shiga toxin-producing E. coli (STEC). Among these, resistance was generally low, yet 21 (10%) were resistant to sulfamethoxazole and 19 (9%) were resistant to tetracycline. Based on latex agglutination, 189 (52%) of the isolates were identified as E. coli O157:H7, among which 19 (10%) were resistant to sulfamethoxazole and 16 (8%) were resistant to tetracycline. The data suggest that selection pressure imposed by the use of tetracycline derivatives, sulfa drugs, cephalosporins, and penicillins, whether therapeutically in human and veterinary medicine or as prophylaxis in the animal production environment, is a key driving force in the selection of antimicrobial resistance in STEC and non-STEC O157

Scott,L., P.McGee, J.J.Sheridan, B.Earley, and N.Leonard. 2006. "A comparison of the survival in feces and water of Escherichia coli O157:H7 grown under laboratory conditions or obtained from cattle feces." *J.Food Prot.* 69:6-11.
Abstract: Escherichia coli O157:H7 is an important foodborne pathogen that can cause hemorrhagic colitis and hemolytic uremic syndrome. Cattle feces and fecally contaminated water are important in the transmission of this organism on the farm. In this study, the survival of E. coli O157:H7 in feces and water was compared following passage through the animal digestive tract or preparation in the laboratory. Feces were collected from steers before and after oral inoculation with a marked strain of E. coli O157:H7. Fecal samples collected before cattle inoculation were subsequently inoculated with the marked strain of E. coli O157:H7 prepared in the laboratory. Subsamples were taken from both animal and laboratory-inoculated feces to inoculate 5-liter volumes of water. E. coli O157:H7 in feces survived up to 97 days, and survival was not affected by the method used to prepare the inoculating strain. E. coli O157:H7 survived up to 109 days in water, and the bacteria collected from inoculated cattle were detected up to 10 weeks longer than the laboratory-prepared culture. This study suggests that pathogen survival in low-nutrient conditions may be enhanced by passage through the gastrointestinal tract

Smith DeWaal,C., K.Barlow, and G.Hicks. Outbreak Alert! 2005. Washington, D.C., Center for Science in the Public Interest.
Ref Type: Generic

Valcour,J.E., P.Michel, S.A.McEwen, and J.B.Wilson. 2002. "Associations between indicators of livestock farming intensity and incidence of human Shiga toxin-producing Escherichia coli infection." *Emerg.Infect.Dis.* 8:252-257.
Abstract: The impact of livestock farming on the incidence of human Shiga toxin-producing Escherichia coli (STEC) infection was assessed by using several livestock density indicators (LDI) that were generated in a systematic approach. A total of 80 LDI were considered suitable proxy measures for livestock density. Multivariate Poisson regression identified several LDI as having a significant spatial association with the incidence of human STEC infection. The strongest associations with human STEC infection were the ratio of beef cattle number to human population and the application of manure to the surface of agricultural land by a solid spreader and by a liquid spreader. This study demonstrates the value of using a systematic approach in identifying LDI and other spatial predictors of disease