

References

- 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.
Ref ID: 131
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
- Cobbold, R.N., D.H.Rice, M.Szymanski, D.R.Call, and D.D.Hancock. 2004. "Comparison of shiga-toxigenic *Escherichia coli* prevalences among dairy, feedlot, and cow-calf herds in Washington State." *Appl.Environ.Microbiol.* 70:4375-4378.
Ref ID: 109
Abstract: Shiga-toxigenic *Escherichia coli* (STEC) strains were isolated from 7.4% of 1,440 fecal and farm environmental samples. Shiga toxin gene and STEC prevalences were significantly associated with animal production type and season. A range of serogroups were identified. Nine percent of isolates possessed all three principal virulence markers: stx(2), eae, and ehx
- 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.
Ref ID: 79
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.
Ref ID: 137
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

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.

Ref ID: 87

Abstract: OBJECTIVE: To describe shiga-toxigenic *Escherichia coli* 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 (n = 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

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.

Ref ID: 167

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 5.3×10^4 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

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.

Ref ID: 144

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

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.

Ref ID: 157

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

Hancock, D.D., T.E. Besser, D.H. Rice, E.D. Ebel, D.E. Herriott, and L.V. Carpenter. 1998. "Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA." *Prev. Vet. Med.* 35:11-19.

Ref ID: 115

Abstract: Samples from cattle, other domestic and wild animals, flies, feeds, and water-troughs were collected from 12 cattle farms and tested for *Escherichia coli* O157. *E. coli* O157 was isolated from bovine fecal samples on all 12 farms with a within herd prevalence ranging from 1.1% to 6.1%. *E. coli* O157 was also found in 1 of 90 (1.1%) equine fecal samples, 2 of 65 (3.1%) canine fecal samples, 1 of 200 pooled bird samples

(0.5%), 2 of 60 pooled fly samples (3.3%), and 10 of 320 (3.1%) water-trough sample sets (biofilm and water). No *E. coli* O157 were isolated from 300 rodents, 33 cats, 34 assorted wildlife, or 335 cattle feed samples. Indistinguishable pulsed-field gel electrophoresis patterns of XbaI digested chromosomal DNA and Shiga toxin types were observed for bovine and water-trough isolates from two farms and for one equine and two bovine isolates from one farm

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.

Ref ID: 45

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.

Ref ID: 9

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

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.

Ref ID: 160

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

Mukherjee,A., D.Speh, E.Dyck, and F.Diez-Gonzalez. 2004. "Preharvest evaluation of coliforms, Escherichia coli, Salmonella, and Escherichia coli O157:H7 in organic and conventional produce grown by Minnesota farmers." *J.Food Prot.* 67:894-900.

Ref ID: 76

Abstract: Microbiological analyses of fresh fruits and vegetables produced by organic and conventional farmers in Minnesota were conducted to determine the coliform count and the prevalence of Escherichia coli, Salmonella, and E. coli O157:H7. A total of 476 and 129 produce samples were collected from 32 organic and 8 conventional farms, respectively. The samples included tomatoes, leafy greens, lettuce, green peppers, cabbage, cucumbers, broccoli, strawberries, apples, and seven other types of produce. The numbers of fruits and vegetables was influenced by their availability at participating farms and varied from 11 strawberry samples to 108 tomato samples. Among the organic farms, eight were certified by accredited agencies and the rest reported the use of organic practices. All organic farms used aged or composted animal manure as fertilizer. The average coliform counts in both organic and conventional produce were 2.9 log most probable number per g. The percentages of E. coli-positive samples in conventional and organic produce were 1.6 and 9.7%, respectively. However, the E. coli prevalence in certified organic produce was 4.3%, a level not statistically different from that in conventional samples. Organic lettuce had the largest prevalence of E. coli (22.4%) compared with other produce types. Organic samples from farms that used manure or compost aged less than 12 months had a prevalence of E. coli 19 times greater than that of farms that used older materials. Serotype O157:H7 was not detected in any produce samples, but Salmonella was isolated from one organic lettuce and one organic green pepper. These results provide the first microbiological assessment of organic fruits and vegetables at the farm level

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.

Ref ID: 2

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

Mukherjee,A., S.Cho, J.Scheffel, S.Jawahir, K.Smith, and F.Diez-Gonzalez. 2006. "Soil survival of Escherichia coli O157:H7 acquired by a child from garden soil recently fertilized with cattle manure." *J.Appl.Microbiol.* 101:429-436.

Ref ID: 94

Abstract: AIMS: This investigation was conducted to determine the survival of a naturally occurring Escherichia coli O157:H7 in garden soil linked to a sporadic case of E. coli O157 infection in Minnesota. METHODS AND RESULTS: The presence and viability of E. coli O157:H7 was monitored in manure-contaminated garden soil for several weeks. Bacterial isolates were characterized using PCR and pulsed-field gel electrophoresis (PFGE). Isolates obtained from the patient and the garden plots during this investigation had indistinguishable PFGE patterns and had the same virulence factors (stx1, stx2, eaeA, ehxA). The E. coli O157:H7 levels obtained from the garden plots declined gradually for a period of 2 months, and on day 69 only one garden plot of four had detectable levels of pathogen. All plots were negative on day 92. The rate of decline in the soil samples stored at 4 degrees C was faster compared with soil samples that remained in ambient conditions, and in refrigerated storage E. coli O157:H7 could not be detected after 10 days. CONCLUSIONS: E. coli O157:H7 strains can survive on manure-amended soil for more than 2 months, and this survival could be reduced by low temperature. SIGNIFICANCE AND IMPACT OF THE STUDY: This is one of the few reports that have investigated the survival of a proven virulent strain in naturally contaminated soil samples. This case stresses the importance of avoiding the use of raw cattle manure to amend soil for cultivation of foods, including soils in residential garden plots

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.

Ref ID: 77

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

Solomon, E.B., S. Yaron, and K.R. Matthews. 2002. "Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization." *Appl. Environ. Microbiol.* 68:397-400.

Ref ID: 159

Abstract: The transmission of *Escherichia coli* O157:H7 from manure-contaminated soil and irrigation water to lettuce plants was demonstrated using laser scanning confocal microscopy, epifluorescence microscopy, and recovery of viable cells from the inner tissues of plants. *E. coli* O157:H7 migrated to internal locations in plant tissue and was thus protected from the action of sanitizing agents by virtue of its inaccessibility. Experiments demonstrate that *E. coli* O157:H7 can enter the lettuce plant through the root system and migrate throughout the edible portion of the plant