References

Akiba,M., D.H.Rice, M.A.Davis, T.Masuda, T.Sameshima, M.Nakazawa, and D.D.Hancock. 2000. "A comparison of Escherichia coli O157 isolates from cattle in Japan and the USA by molecular biological methods." *Epidemiol.Infect.* 125:221-224.
Abstract: Escherichia coli O157 isolates from cattle in Japan (n = 91) and in the USA (n = 415) were compared by pulsed-field gel electrophoresis of endonuclease-cleaved genomic DNA, location of the stx genes and bacteriophage typing. Three isolates from cattle in Japan with high similarity to isolates from cattle in the USA were found. Isolates from cattle farms in Japan and the USA may share a common source

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

Alfano,J.R. and A.Collmer. 1996. "Bacterial Pathogens in Plants: Life up against the Wall." *Plant Cell*. 8:1683-1698.

Anderson,G.L., S.J.Kenney, P.D.Millner, L.R.Beuchat, and P.L.Williams. 2006. "Shedding of foodborne pathogens by Caenorhabditis elegans in compost-amended and unamended soil." *Food Microbiol.* 23:146-153.
Abstract: A study was done to characterize the shedding of foodborne pathogenic bacteria by Caenorhabditis elegans, evaluate the persistence of worm populations cocultured with foodborne pathogens, and determine if C. elegans disperses ingested pathogens in soil as a result of shedding. Escherichia. coli O157:H7, Salmonella enterica serotype Poona, and Listeria monocytogenes, as well as E. coli OP50, a non-pathogenic strain, were studied. Synchronous populations of C. elegans were fed for 24 h on confluent lawns of nalidixic acid-adapted bacteria. C. elegans shed viable cells of ingested bacteria on tryptic soy agar supplemented with nalidixic acid (50 microg ml(-1)) (TSAN) throughout a 5-h post-feeding period. C. elegans persisted for up to 10 days by feeding on bacteria that had been shed and grew on TSAN. Eggs harvested from C. elegans cultured on shed foodborne pathogens had the same level of viability as those collected from C. elegans grown on shed E. coli OP50. After 6-7 days, 78%, 64%, 64%, and 76% of eggs laid by C. elegans that had fed on E. coli O157:H7, S. Poona, L. monocytogenes, and E. coli OP50, respectively, were viable. Worms fed on E. coli O157:H7 were inoculated into soil and soil amended with turkey manure compost. Populations of C. elegans persisted in compost-amended soil for at least 7 days but declined in unamended soil. E. coli O157:H7 was detected at 4 and 6 days post inoculation in compost-amended and unamended soil, and in unamended soil inoculated with E. coli OP50. Populations of E. coli O157:H7 in soil amended with turkey manure

compost were significantly(alpha = 0.05) higher than those in unamended soil. Results indicate that C. elegans can act as a vector to disperse foodborne pathogens in soil, potentially resulting in increased risk of contaminating the surface of pre-harvest fruits and vegetables

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

Barak,J.D., L.C.Whitehand, and A.O.Charkowski. 2002. "Differences in attachment of Salmonella enterica serovars and Escherichia coli O157:H7 to alfalfa sprouts." *Appl.Environ.Microbiol.* 68:4758-4763.
Abstract: Numerous Salmonella enterica and Escherichia coli O157:H7 outbreaks have been associated with contaminated sprouts. We examined how S. enterica serovars, E. coli serotypes, and nonpathogenic bacteria isolated from alfalfa sprouts grow on and adhere to alfalfa sprouts. Growth on and adherence to sprouts were not significantly different among different serovars of S. enterica, but all S. enterica serovars grew on and adhered to alfalfa sprouts significantly better than E. coli O157:H7. E. coli O157:H7 was essentially rinsed from alfalfa sprouts with repeated washing steps, while 1 to 2 log CFU of S. enterica remained attached per sprout. S. enterica Newport adhered to 3-day-old sprouts as well as Pantoea agglomerans and 10-fold more than Pseudomonas putida and Rahnella aquatilis, whereas the growth rates of all four strains throughout seed sprouting were similar. S. enterica Newport and plant-associated bacteria adhered 10- to 1,000-fold more than E. coli O157:H7; however, three of four other E. coli serotypes, isolated from cabbage roots exposed to sewage water following a spill, adhered to sprouts better than E. coli O157:H7 and as well as the Pseudomonas and Rahnella strains. Therefore, attachment to alfalfa sprouts among E. coli serotypes is variable, and nonpathogenic strains of E. coli to be used as surrogates for the study of pathogenic E. coli may be difficult to identify and should be selected carefully, with knowledge of the biology being examined

Barak,J.D., L.Gorski, P.Naraghi-Arani, and A.O.Charkowski. 2005. "Salmonella enterica virulence genes are required for bacterial attachment to plant tissue." *Appl.Environ.Microbiol.* 71:5685-5691.
Abstract: Numerous Salmonella enterica food-borne illness outbreaks have been associated with contaminated vegetables, in particular sprouted seeds, and the incidence of reported contamination has steadily risen. In order to understand the physiology of S. enterica serovar Newport on plants, a screen was developed to identify transposon mutants that were defective in attachment to alfalfa sprouts. Twenty independent mutants from a pool of 6,000 were selected for reduced adherence to alfalfa sprouts. Sixty-five percentage of these mutants had insertions in uncharacterized genes. Among the characterized genes were strains with insertions in the intergenic

region between agfB, the surface-exposed aggregative fimbria (curli) nucleator, and agfD, a transcriptional regulator of the LuxR superfamily, and rpoS, the stationary-phase sigma factor. Both AgfD and RpoS have been reported to regulate curli and cellulose production and RpoS regulates other adhesins such as pili. The intergenic and rpoS mutants were reduced in initial attachment to alfalfa sprouts by 1 log unit compared to the wild type. Mutations of agfA, curli subunit, and agfB in S. enterica serovar Enteritidis differentially affected attachment to plant tissue. The agfA mutation was not reduced in ability to attach to or colonize alfalfa sprouts, whereas the agfB mutation was reduced. Thus, agfB alone can play a role in attachment of S. enterica to plant tissue. These results reveal that S. enterica genes important for virulence in animal systems are also required for colonization of plants, a secondary host that can serve as a vector of S. enterica from animal to animal

Barlow,R.S., K.S.Gobius, and P.M.Desmarchelier. 2006. "Shiga toxin-producing Escherichia coli in ground beef and lamb cuts: Results of a one-year study." *Int.J.Food Microbiol.* 111:1-5.
Abstract: Shiga toxin-producing Escherichia coli (STEC) have been associated with a broad spectrum of diarrhoeal syndromes. Some of these cases have been attributed to foods of bovine origin or other foods cross-contaminated by beef products or cow manure. The purpose of this study was to determine the pattern of STEC distribution in selected red meats over time. Samples of ground beef and lamb cuts were collected over a 52-week period from 31 different outlets and 25 g portions were assayed for STEC. STEC were isolated from 46/285 (16%) ground beef and 111/275 (40%) lamb samples using an stx PCR screen followed by colony hybridisation. All isolates were tested by PCR for additional STEC virulence markers with 95% of ground beef isolates shown to possess stx(2) and 80% of lamb cutlet isolates shown to possess stx(1) and stx(2). The enterohaemolysin gene (ehxA) was detected in 65% and 53% of ground beef and lamb isolates respectively. Putative enterohaemorrhagic E. coli (EHEC), i.e. STEC possessing the E. coli attaching and effacing gene (eae) were not isolated. The STEC isolates comprised 18 and 15 different serotypes from ground beef and lamb respectively. STEC of serotypes O157, O111 and O26 (common enterohaemorrhagic E. coli serotypes) were not isolated. Serotypes O174 and O91 were the most common serotypes isolated from ground beef samples and O128 and O91 the most common from lamb cutlet samples. The presence of STEC in retail red meats highlights the need for a clearer understanding of STEC in food and human illness to interpret the public health significance of these findings

Batz,M.B., M.P.Doyle, G.Morris, Jr., J.Painter, R.Singh, R.V.Tauxe, M.R.Taylor, and D.M.Lo Fo Wong. 2005. "Attributing illness to food." *Emerg.Infect.Dis.* 11:993-999.
Abstract: Identification and prioritization of effective food safety interventions require an understanding of the relationship between food and pathogen from farm to consumption. Critical to this cause is food attribution, the capacity to attribute cases of foodborne disease to the food vehicle or other source responsible for illness. A wide variety of food attribution approaches and data are used around the world, including the analysis of outbreak data, case-control studies, microbial subtyping and source tracking methods, and expert judgment, among others. The Food Safety Research Consortium sponsored the Food Attribution Data Workshop in October 2003 to discuss the virtues and limitations of these approaches and to identify future options for collecting food attribution data in the United States. We summarize workshop discussions and identify challenges that affect progress in this critical component of a risk-based approach to improving food safety

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

Besser,R.E., S.M.Lett, J.T.Weber, M.P.Doyle, T.J.Barrett, J.G.Wells, and P.M.Griffin. 1993. "An outbreak of diarrhea and hemolytic uremic syndrome from Escherichia coli O157:H7 in fresh-pressed apple cider." *JAMA*. 269:2217-2220.
Abstract: OBJECTIVE--Escherichia coli O157:H7 causes hemorrhagic colitis and the hemolytic uremic syndrome. In the fall of 1991, an outbreak of E coli O157:H7 infections in southeastern Massachusetts provided an opportunity to identify transmission by a seemingly unlikely vehicle. DESIGN--Case-control study to determine the vehicle of infection. New England cider producers were surveyed to assess production practices and determined the survival time of E coli O157:H7 organisms in apple cider. RESULTS--Illness was significantly associated with drinking one brand of apple cider. Thirteen (72%) of 18 patients but only 16 (33%) of 49 controls reported drinking apple cider in the week before illness began (odds ratio [OR], 8.3; 95% confidence interval [CI], 1.8 to 39.7). Among those who drank cider, 12 (92%) of 13 patients compared with two (13%) of 16 controls drank cider from cider mill A (lower 95% CI, 2.9; P < .01). This mill pressed cider in a manner similar to that used by other small cider producers: apples were not washed, cider was not pasteurized, and no preservatives were added. In the laboratory, E coli O157:H7 organisms survived for 20 days in unpreserved refrigerated apple cider. Addition of sodium benzoate 0.1% reduced survival to less than 7 days. CONCLUSIONS--Fresh-pressed, unpreserved apple cider can transmit E coli O157:H7 organisms, which cause severe infections. Risk of transmission can be reduced by washing and brushing apples before pressing, and preserving cider with sodium benzoate. Consumers can reduce their risk by only drinking cider made from apples that have been washed and brushed

Besser,T.E., D.D.Hancock, L.C.Pritchett, E.M.McRae, D.H.Rice, and P.I.Tarr. 1997. "Duration of detection of fecal excretion of Escherichia coli O157:H7 in cattle." *J.Infect.Dis.* 175:726-729.
Abstract: To better define the bovine reservoir of Escherichia coli O157:H7, cattle were tested monthly by bacteriologic culture for fecal excretion of E. coli. E. coli O157:H7 was isolated from feces of 56 cattle sampled an average of 6.98 times (2-12 samples). By broth enrichment culture, immunomagnetic separation, or both, 35 cattle had 1 positive sample, 12 had 2, 7 had 3, and 1 each had 4 and 5. Five cattle with > or = 2 positive samples were in a herd in which 5 pulsed-field gel electrophoretic (PFGE) types were simultaneously present; in 3 of these cattle, different PFGE types were detected in different samples. The duration of detected excretion E. coli O157:H7 by individual cattle in this study was <1 month in 35 (63%) of 56 cattle. Both serial and concurrent excretion of different E. coli O157:H7 strains by individual cattle was observed

Besser,T.E., B.L.Richards, D.H.Rice, and D.D.Hancock. 2001. "Escherichia coli O157:H7 infection of calves: infectious dose and direct contact transmission." *Epidemiol.Infect.*

127:555-560.
Abstract: Cattle are considered to be a reservoir host of Escherichia coli O157:H7 and contaminated foods of bovine origin are important vehicles of human infection. In this study, the susceptibility of calves to experimental E. coli O157:H7 infection following low oral exposures was determined. Two of 17 calves exposed to very low (< 300 c.f.u.) doses, and 3 of 4 calves exposed to low (< 10,000 c.f.u.) doses, subsequently excreted the challenge strains in their faeces. All calves (n = 12) sharing isolation rooms with calves that excreted the challenge strain in their faeces similarly began faecal excretion of the same strains within 21 days or less. The identity between the challenge strains and the strains excreted in calf faeces was confirmed by restriction digestion electrophoretic patterns using pulsed field gel electrophoresis. Calves shed E. coli O157:H7 in their faeces after very low dose exposures at concentrations ranging from < 30 to > 10(7) c.f.u./g, and for durations similar to the values previously reported for calves challenged by larger doses. The susceptibility of calves to infection following very low exposures or direct contact with infected calves has important implications for programmes for pre-harvest control of this agent

Beuchat,L.R. 1999. "Survival of enterohemorrhagic Escherichia coli O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant." *J.Food Prot.* 62:845-849.
Abstract: Bovine feces are a potential vehicle for transmitting enterohemorrhagic Escherichia coli O157:H7 to humans. A study was undertaken to determine survival characteristics of E. coli O157:H7 on iceberg lettuce using 0.1% peptone water and bovine feces as carriers for inocula. Four levels of inoculum, ranging from 10(0) to 10(5) CFU of E. coli O157:H7 per g of lettuce, were applied. Populations surviving on lettuce stored at 4 degrees C were monitored for up to 15 days. Regardless of the type of carrier, viable cells of E. coli O157:H7 were detected on lettuce after 15 days, even when the initial inoculum was 10(0) to 10(1) CFU/g. Spray treatments of lettuce with 200 ppm chlorine solution or deionized water were equally effective in killing or removing E. coli O157:H7 from lettuce. Holding lettuce for 5 min after spray treatment was not more effective in reducing populations than holding for 1 min before rinsing with water. Prevention of contamination of lettuce with bovine feces that may harbor E. coli O157:H7 as well as other infectious microorganisms is essential to minimizing the risk of illness. The development of sanitizers more efficacious than chlorine for the removal of pathogens from raw fruits and vegetable is needed

Beuchat,L.R. 2002. "Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables." *Microbes.Infect.* 4:413-423.
Abstract: Outbreaks of human infections associated with consumption of raw fruits and vegetables have occurred with increased frequency during the past decade. Factors contributing to this increase may include changes in agronomic and processing practices, an increase in per capita consumption of raw or minimally processed fruits and vegetables, increased international trade and distribution, and an increase in the number of immuno-compromised consumers. A general lack of efficacy of sanitizers in removing or killing pathogens on raw fruits and vegetables has been attributed, in part, to their inaccessibility to locations within structures and tissues that may harbor pathogens. Understanding the ecology of pathogens and naturally occurring microorganisms is essential before interventions for elimination or control of growth can be devised

Beuchat,L.R., J.H.Ryu, B.B.Adler, and M.D.Harrison. 2006. "Death of Salmonella, Escherichia coli O157:H7, and Listeria monocytogenes in shelf-stable, dairy-based, pourable salad dressings." *J.Food Prot.* 69:801-814.
Abstract: The objectives of this study were to determine the death rates of Salmonella, Escherichia coli O157:H7, and Listeria monocytogenes in three commercially manufactured full-fat ranch salad dressings, three reduced-fat ranch salad dressings,

two full-fat blue cheese salad dressings, and two reduced-fat blue cheese salad dressings and to affirm the expectation that these dressings do not support the growth of these pathogens. The respective initial pH values of the four types of shelf-stable, dairy-based, pourable dressings were 2.87 to 3.72, 2.82 to 3.19, 3.08 to 3.87, and 2.83 to 3.49, respectively. Dressings were inoculated with low (2.4 to 2.5 log CFU/g) and high (5.3 to 5.9 log CFU/g) populations of separate five-strain mixtures of each pathogen and stored at 25 degrees C for up to 15 days. Regardless of the initial inoculum population, all test pathogens rapidly died in all salad dressings. Salmonella was undetectable by enrichment (<1 CFU/25-ml sample in three replicate trials) in all salad dressings within 1 day, and E. coli O157:H7 and L. monocytogenes were reduced to undetectable levels by enrichment between 1 and 8 days and 2 and 8 days, respectively. E. coli O157:H7 was not detected in 4 of the 10 salad dressings stored for 2 or more days and 9 of the 10 dressings stored for 6 or more days after inoculation. L. monocytogenes was detected in 9 of the 10 salad dressings stored for 3 days but in only one dressing, by enrichment, at 6 days, indicating that it had the highest tolerance among the three pathogens to the acidic environment imposed by the dressings. Overall, the type of dressing (i.e., ranch versus blue cheese) and level of fat in the dressings did not have a marked effect on the rate of inactivation of pathogens. Total counts and populations of lactic acid bacteria and yeasts and molds remained low or undetectable (<1.0 log CFU/ml) throughout the 15-day storage period. Based on these observations, shelf-stable, dairy-based, pourable ranch and blue cheese salad dressings manufactured by three companies and stored at 25 degrees C do not support the growth of Salmonella, E. coli O157:H7, and L. monocytogenes and should not be considered as potentially hazardous foods (time-temperature control for safety foods) as defined by the U.S. Food and Drug Administration Food Code

Boehme,S., G.Werner, I.Klare, R.Reissbrodt, and W.Witte. 2004. "Occurrence of antibiotic-resistant enterobacteria in agricultural foodstuffs." *Mol.Nutr.Food Res.* 48:522-531.
Abstract: Antibiotic-resistant bacteria or their corresponding resistance determinants are known to spread from animals to humans via the food chain. We screened 20 vegetable foods for antibiotic-resistant coliform bacteria and enterococci. Isolates were directly selected on antibiotic-containing selective agar (color detection). Thirteen "common vegetables" (tomato, mushrooms, salad) possessed 10(4)-10(7) cfu/g vegetable of coliform bacteria including only few antibiotic-resistant variants (0-10(5) cfu/g). All seven sprout samples showed a some orders of magnitude higher contamination with coliform bacteria (10(7)-10(9) cfu/g) including a remarkable amount of resistant isolates (up to 10(7) cfu/g). Multiple resistances (up to 9) in single isolates were more common in sprout isolates. Resistant bacteria did not originate from sprout seeds. The most common genera among 92 isolates were: 25 Enterobacter spp. (19 E. cloacae), 22 Citrobacter spp. (8 C. freundii), and 21 Klebsiella spp. (9 K. pneumoniae). Most common resistance phenotypes were: tetracycline (43%), streptomycin (37%), kanamycin (26%), chloramphenicol (29%), co-trimoxazol (9%), and gentamicin (4%). The four gentamicin-resistant isolates were investigated in molecular details. Only three (chloramphenicol) resistant, typical plant-associated enterococci were isolated from overnight enrichment cultures. In conclusion, a contribution of sprouts contaminated with multiresistant, Gram-negative enterobacteria to a common gene pool among human commensal and pathogenic bacteria cannot be excluded

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

Brandl,M.T., A.F.Haxo, A.H.Bates, and R.E.Mandrell. 2004. "Comparison of survival of Campylobacter jejuni in the phyllosphere with that in the rhizosphere of spinach and radish plants." *Appl.Environ.Microbiol.* 70:1182-1189.
Abstract: Campylobacter jejuni has been isolated previously from market produce and has caused gastroenteritis outbreaks linked to produce. We have tested the ability of this human pathogen to utilize organic compounds that are present in leaf and root exudates and to survive in the plant environment under various conditions. Carbon utilization profiles revealed that C. jejuni can utilize many organic acids and amino acids available on leaves and roots. Despite the presence of suitable substrates in the phyllosphere and the rhizosphere, C. jejuni was unable to grow on lettuce and spinach leaves and on spinach and radish roots of plants incubated at 33 degrees C, a temperature that is conducive to its growth in vitro. However, C. jejuni was cultured from radish roots and from the spinach rhizosphere for at least 23 and 28 days, respectively, at 10 degrees C. This enteric pathogen also persisted in the rhizosphere of spinach for prolonged periods of time at 16 degrees C, a temperature at which many cool-season crops are grown. The decline rate constants of C. jejuni populations in the spinach and radish rhizosphere were 10- and 6-fold lower, respectively, than on healthy spinach leaves at 10 degrees C. The enhanced survival of C. jejuni in soil and in the rhizosphere may be a significant factor in its contamination cycle in the environment and may be associated with the sporadic C. jejuni incidence and campylobacteriosis outbreaks linked to produce

Brandl,M.T. 2006. "Fitness of human enteric pathogens on plants and implications for food safety." *Annu.Rev.Phytopathol.* 44:367-392.
Abstract: The continuous rise in the number of outbreaks of foodborne illness linked to fresh fruit and vegetables challenges the notion that enteric pathogens are defined mostly by their ability to colonize the intestinal habitat. This review describes the epidemiology of produce-associated outbreaks of foodborne disease and presents recently acquired knowledge about the behavior of enteric pathogens on plants, with an emphasis on Salmonella enterica, Escherichia coli O157:H7, and Listeria monocytogenes. The growth and survival of enteric pathogens on plants are discussed in the light of knowledge and concepts in plant microbial ecology, including epiphytic fitness, the physicochemical nature of plant surfaces, biofilm formation, and microbe-microbe and plant-microbe interactions. Information regarding the various stresses that affect the survival of enteric pathogens and the molecular events that underlie their interactions in the plant environment provides a good foundation for assessing their role in the infectious dose of the pathogens when contaminated fresh produce is the vehicle of illness

Buchko,S.J., R.A.Holley, W.O.Olson, V.P.Gannon, and D.M.Veira. 2000. "The effect of different grain diets on fecal shedding of Escherichia coli O157:H7 by steers." *J.Food Prot.* 63:1467-1474.
Abstract: Three groups of six yearling steers (three rumen fistulated plus three nonfistulated) fed one of three different grain diets (85% cracked corn, 15% whole cottonseed and 70% barley, or 85% barley) were inoculated with 10(10) CFU of Escherichia coli O157:H7 strain 3081, and the presence of the inoculated strain was followed in the rumen fluid and feces for a 10-week period. E. coli O157:H7 was rapidly eliminated from the rumen of the animals on all three diets but persisted in the feces of some animals up to 67 days after inoculation, suggesting that the bovine hindgut is the site of E. coli O157:H7 persistence. A significant difference existed in the levels of E. coli O157:H7 shed by the animals among diets on days 5, 7, 49, and 63 after inoculation ($P < 0.05$). No significant difference was found between the levels shed among diets on days 9 through 42 and on day 67 ($P > 0.05$). The number of animals that were culture positive for E. coli O157:H7 strain 3081 during the 10-week period was significantly higher for the barley fed group (72 of 114 samplings) as opposed to the corn fed group (44 of 114 samplings) ($P < 0.005$) and the cottonseed and barley fed group (57 of 114 samplings) ($P < 0.05$). The fecal pH of the animals fed the corn diet was significantly lower ($P < 0.05$) than the fecal pH of the animals fed the cottonseed and barley and barley diets, likely resulting in a less suitable environment for E. coli O157:H7 in the hindgut of the corn fed animals. E. coli O157:H7 strain 3081 was present in 3 of 30 (corn, 1 of 10; cottonseed, 1 of 10; barley, 1 of 10) animal drinking water samples, 3 of 30 (corn, 1 of 10; cottonseed, 0 of 10; barley, 2 of 10) water trough biofilm swabs, 5 of 30 (corn, 0 of 10; cottonseed, 2 of 10; barley, 3 of 10) feed samples, and 30 of 30 manure samples taken from the pens during the entire experimental period. Mouth swabs of the steers were also culture positive for E. coli O157:H7 strain 3081 in 30 of 180 samples (corn, 7 of 60; cottonseed, 4 of 60; barley, 19 of 60) taken during the 10-week period. Minimizing environmental dissemination of E. coli O157:H7 in conjunction with diet modification may reduce numbers of E. coli O157:H7-positive cattle

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

Burt,S.A. and R.D.Reinders. 2003. "Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7." *Lett.Appl.Microbiol.* 36:162-167.
Abstract: AIMS: To quantify the antibacterial properties of five essential oils (EO) on a non-toxigenic strain of Escherichia coli O157:H7 in the presence and absence of a stabilizer and an emulsifier and at three different temperatures. METHODS AND RESULTS: Five EOs known to exhibit antibacterial properties were screened by disc diffusion assay and the most active were selected for further study in microdilution colorimetric assays. Oregano (Origanum vulgare) and thyme (Thymus vulgaris; light and red varieties) EO had the strongest bacteriostatic and bactericidal properties, followed by bay (Pimenta racemosa) and clove bud (Eugenia caryophyllata synonym: Syzygium aromaticum) EO. Oregano oil was colicidal at 625 microl l(-1) at 10, 20 and 37 degrees C. The addition of 0.05% (w/v) agar as stabilizer reinforced the antibacterial properties, particularly at 10 degrees C, whereas 0.25% (w/v) lecithin reduced antibacterial activity. Scanning electron micrographs showed extensive morphological changes to treated cells. CONCLUSIONS: Oregano and thyme EO possess significant in vitro colicidal and colistatic properties, which are exhibited in a broad temperature range and substantially improved by the addition of agar as stabilizer. Bay and clove bud EO are less active.

Lecithin diminished antibacterial properties. The bactericidal concentration of oregano EO irreversibly damaged E. coli O157:H7 cells within 1 min. SIGNIFICANCE AND IMPACT OF THE STUDY: Oregano and light thyme EO, particularly when enhanced by agar stabilizer, may be effective in reducing the number or preventing the growth of E. coli O157:H7 in foods

Chapman,P.A., C.A.Siddons, A.T.Gerdan Malo, and M.A.Harkin. 1997. "A 1-year study of Escherichia coli O157 in cattle, sheep, pigs and poultry." *Epidemiol.Infect.* 119:245-250. Abstract: Samples of rectal faeces were collected immediately after slaughter from 400 cattle each month for a 1-year period and from 1000 each of sheep, pigs and poultry over the same period. Samples were examined for Escherichia coli O157 by enrichment culture in buffered peptone water with vancomycin, cefixime and cefsulodin followed by immunomagnetic separation and culture of magnetic particles onto cefixime tellurite sorbitol MacConkey agar. E. coli O157 was isolated from 752 (15.7%) of 4800 cattle, 22 (2.2%) of 1000 sheep and from 4 (0.4%) of 1000 pigs, but not from any of 1000 chickens. Of the cattle sampled. 1840 (38.4%) were prime beef animals, 1661 (34.6%) were dairy animals being culled and the status could not be determined for the other 1299 (27%) animals. E. coli O157 was found in 246 (13.4%) of the 1840 beef cattle and 268 (16.1%) of the 1661 dairy cattle. The monthly prevalence of E. coli O157 in cattle was 4.8-36.8% and was at its highest in spring and late summer. Seventeen of the 22 isolates from sheep were also made over the summer period. All E. coli O157 isolates from sheep and 749 (99.6%) of the 752 E. coli O157 isolates from cattle were verocytotoxigenic as determined by Vero cell assay and DNA hybridization, eaeA gene positive, contained a 92 kb plasmid and were thus typical of strains causing infections in man. In contrast isolates from pigs were non-toxigenic, eaeA gene negative and did not contain a 92 kb plasmid and would, therefore, be unlikely to be a source of infection for man

Chapman,P.A., C.A.Siddons, J.Manning, and C.Cheetham. 1997. "An outbreak of infection due to verocytotoxin-producing Escherichia coli O157 in four families: the influence of laboratory methods on the outcome of the investigation." *Epidemiol.Infect.* 119:113-119. Abstract: Three members of family A, who had diarrhoea on 20 October, lived on a small arable farm which had 10 cattle. Manure from the animals was used to fertilize the ground for growing potatoes which were then offered for retail sale, unwashed, directly from the farm. The mother from family B bought potatoes, which were covered with manure, from family A in early November and over the subsequent 10 days she became ill with diarrhoea and her daughter and son both became ill with bloody diarrhoea. The mother from family C visited family B while the daughter from the latter family was symptomatic; the mother developed diarrhoea several days later. The mother and two sons from family D visited family B while the son from the latter family was symptomatic; the first son developed bloody diarrhoea 6 days later which progressed to development of haemolytic-uraemic syndrome. Direct culture of faecal samples onto cefixime rhamnose sorbitol MacConkey agar failed to isolate E. coli O157 from any of the symptomatic patients, and direct culture onto cefixime tellurite sorbitol MacConkey agar isolated the organism from only one patient. In contrast, a combination of isolation of E. coli O157 by immunomagnetic separation and detection of E. coli O157-specific secretory IgA, suggested E. coli O157 infection in all eight symptomatic patients, but not in any of the family members who were not ill. Two children who excreted the organism for 60 and 89 days respectively were the only two patients who did not develop a secretory IgA response. E. coli O157 was not isolated from potatoes from the farm and faecal samples from the farm animals were not available for examination. The study illustrates the need to use the most sensitive methods available during the investigation and follow up of cases of E. coli O157 infection

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

Chapman,P.A., C.A.Siddons, A.T.Cerdan Malo, and M.A.Harkin. 2000. "A one year study of Escherichia coli O157 in raw beef and lamb products." *Epidemiol.Infect.* 124:207-213.
Abstract: Between April 1996 and March 1997 we examined 5093 samples of raw beef and lamb products for the presence of E. coli O157. Samples were purchased from 81 small butchers' shops in south Yorkshire. In March 1997 we also examined five samples of dried mint for the presence of E. coli O157. Strains of E. coli O157 were isolated by enrichment culture in modified buffered peptone water followed by immunomagnetic separation and culture of magnetic beads onto cefixime tellurite sorbitol MacConkey agar. Strains were characterized by phage typing, toxin genotyping and plasmid analysis. Strains of E. coli O157 were isolated from 72 (1.4%) of 5093 samples; it was isolated from 36 (1.1%) of 3216 samples of beef products and from 29 (2.9%) samples of lamb products. The highest prevalence was found in lamb sausages and lamb burgers where E. coli O157 was isolated from 3 (4.1%) of 73 and 18 (3.7%) of 484 samples respectively. Strains of E. coli O157 were isolated most frequently during early summer. Strains of E. coli O157 were also isolated from 2 of 5 samples of dried mint although we did not determine how the mint had become contaminated. All isolates of E. coli O157 were Verocytotoxin-producing as determined by both Vero cell assay and DNA hybridization for the genes encoding Verocytotoxin and all were positive for the eaeA gene. A combination of phage typing, toxin genotyping and plasmid profile subdivided the 72 strains of E. coli isolated into 20 different subtypes, of which 18 were indistinguishable from strains isolated previously from cattle and sheep; of these 18 strains, 8 were indistinguishable from strains isolated from human cases of infection during the study period

Chapman,P.A., A.T.Cerdan Malo, M.Ellin, R.Ashton, and Harkin. 2001. "Escherichia coli O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK." *Int.J.Food Microbiol.* 64:139-150.
Abstract: A 1 year study of Escherichia coli O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products from retail butchers' shops was performed in the Sheffield area. Each month, samples of rectal faeces were collected immediately after slaughter from 400 cattle and 600 sheep, and 400-430 samples of raw meat products were purchased from butchers' shops. Meat samples were also obtained from 1500 beef and 1500 lamb carcasses. All samples were

examined for E. coli O157 by enrichment culture, immunomagnetic separation and culture of magnetic particles onto cefixime tellurite sorbitol MacConkey agar. Raw meat products were also examined for numbers of generic E. coli by a standard membrane culture method. E. coli O157 was isolated from 620 (12.9%) of 4800 cattle, 100 (7.4%) of 7200 sheep, 21 (1.4%) of 1500 beef carcasses, 10 (0.7%) of 1500 lamb carcasses and from 22 (0.44%) of 4983 raw meat products. E. coli O157 was isolated more frequently from lamb products (0.8%) than from beef products (0.4%). Numbers of generic E. coli in meat products reached seasonal peaks in July and August with counts of > 10(4)/g occurring more frequently in lamb products (50.8 and 42.4%, respectively) than in beef products (19.3 and 23.8%, respectively). The majority of E. coli O157 strains, from animals, carcasses and meat samples, were isolated during the summer. Most were verocytotoxigenic as determined by Vero cell assay and DNA hybridisation, eaeA gene positive and contained a 92 kb plasmid. The isolates were compared with 66 isolates from human cases over the same period. A combination of phage type, toxin genotype and plasmid analysis allowed subdivision of all the E. coli O157 isolates into 96 subtypes. Of these subtypes, 53 (55%) were isolated only from bovine faecal samples. However, 61 (92%) of the 66 isolates from humans belonged to 13 subtypes which were also found in the animal population

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

Cho,S., J.B.Bender, F.Diez-Gonzalez, C.P.Fossler, C.W.Hedberg, J.B.Kaneene, P.L.Ruegg, L.D.Warnick, and S.J.Wells. 2006. "Prevalence and characterization of Escherichia coli O157 isolates from Minnesota dairy farms and county fairs." *J.Food Prot.* 69:252-259.

Abstract: Samples were collected from 26 organic and conventional farms and 12 county fairs in Minnesota during 2001 and 2002 to identify the presence of Escherichia coli O157. Immunomagnetic separation was used for isolation of E. coli O157. Isolates were further characterized by the presence of virulence marker genes (stx1, stx2, eaeA, E-hly, katP, etpD, and espP), antimicrobial susceptibility profiles, and genotypes. During 2001, E. coli O157 was isolated from 16 (5.2%) of 305 fecal samples and from 7 (36.8%) of 19 farms. During 2002, E. coli O157 was isolated from 6 (4.5%) of 132 fecal samples from weaned calves at 4 (23.5%) of 17 farms. During 2001 and 2002, cattle manure samples were collected from 12 county fairs, and E. coli O157 was isolated from 19 (11%) of 178 samples and 9 (75%) of 12 county fairs. Among 40 E. coli O157 isolates, 17 isolates (43%) had both the stx1 and stx2 genes, and 21 strains (53%) had the stx2 gene only. Thirteen percent of O157 isolates were resistant to tetracycline, and 25% were resistant

to sulfadimethoxine. Heterogeneity of E. coli O157 strains was demonstrated by the presence of 22 different pulsed-field gel electrophoresis (PFGE) patterns. Four PFGE patterns matched those of isolates previously found in humans. The presence of E. coli O157 at county fairs suggests the potential for transmission to the public, who may 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

Cieslak,P.R., S.J.Noble, D.J.Maxson, L.C.Empey, O.Ravenholt, G.Legarza, J.Tuttle, M.P.Doyle, T.J.Barrett, J.G.Wells, A.M.McNamara, and P.M.Griffin. 1997. "Hamburger-associated Escherichia coli O157:H7 infection in Las Vegas: a hidden epidemic." *Am.J.Public Health*. 87:176-180.
Abstract: OBJECTIVES: This study sought to determine whether a multistate fast food hamburger-associated outbreak of Escherichia coli O157:H7 infection involved Las Vegas residents as well and, if so, why public health officials had not detected it. METHODS: A matched case-control study was conducted among persons with bloody diarrhea and their healthy meal companions. Hamburger production, distribution, and cooking methods were reviewed. Unused hamburger patties were cultured, and E. coli O157:H7 isolates were characterized. Local laboratory stool culture practices were reviewed. RESULTS: Fifty-eight cases of bloody diarrhea were identified. Illness was associated with eating regular hamburgers (matched odds ratio [OR] = 9.0, 95% confidence interval [CI] = 1.02,433.4), but 25% of ill persons reported eating only jumbo hamburgers. Regular and jumbo hamburger patties yielded E. coli O157:H7 indistinguishable from the lone clinical isolate. No local laboratory cultured routinely for E. coli O157:H7 until after the outbreak. CONCLUSIONS: A large outbreak of E. coli O157:H7 infections escaped timely notice in Las Vegas because local laboratories did not culture for this pathogen. Health officials should encourage laboratories to screen at least all bloody stools on sorbitol-MacConkey medium

Clavero,M.R., L.R.Beuchat, and M.P.Doyle. 1998. "Thermal inactivation of Escherichia coli O157:H7 isolated from ground beef and bovine feces, and suitability of media for enumeration." *J.Food Prot.* 61:285-289.
Abstract: Rates of thermal inactivation of five strains of Escherichia coli O157:H7 isolated from ground beef implicated in outbreaks of hemorrhagic colitis and five strains isolated from bovine feces were determined. Ground beef (22% fat, 10 g), inoculated with individual test strains at populations ranging from 6.85 to 7.40 log10 CFU g-1 of beef, was formed into patties (0.3 cm thick and 8.0 cm in diameter) and sealed in polyethylene bags. For each strain and treatment temperature (54.4, 58.9, 62.8, 65.6, or 68.3 degrees C), 6 bags were simultaneously immersed into a recirculating water bath. Viable cells in patties heated for various lengths of time were enumerated by plating diluted samples on sorbitol MacConkey agar supplemented with 4-methylumbelliferyl-beta-D-glucuronide (MSMA) and modified eosin methylene blue (MEMB) agar. Regardless of strain or treatment temperature, higher numbers of E. coli O157:H7 cells were generally recovered on MEMB agar than on MSMA, indicating the inferiority of MSMA as a recovery medium for quantitative determination of E. coli O157:H7 cells in heat-processed ground beef. Significantly ($P < or = 0.05$) higher D values when enumeration was done using MEMB agar compared with MSMA. Mean D values for combined strain data at 54.4, 58.9, 62.8, and 65.6 degrees C from cultures on MEMB agar were 123.90, 6.47, 0.62, and 0.20 min, respectively, whereas D values of 25.5, 5.21, and 0.18 min were obtained at the same temperatures from cultures on MSMA. Results suggest that cooking ground beef patties to an internal temperature of 68.3 degrees C for 40 s will inactivate at least 99.99% of E. coli O157:H7 cells; z values of 4.0 and 5.1 degrees C were calculated from mean D values obtained from MEMB agar and MSMA, respectively, as recovery media. Differences in D values existed

among strains but rates of thermal inactivation do not appear to be correlated with the sources of the isolates

Cobbold,R.N. and P.M.Desmarchelier. 2004. "In vitro studies on the colonization of bovine colonic mucosa by Shiga-toxigenic Escherichia coli (STEC)." *Epidemiol.Infect.* 132:87-94.
Abstract: This study investigated host-related factors that influence intestinal colonization by Shiga-toxigenic E. coli (STEC). A quantitative colonization assay was developed to comparatively measure attachment of STEC to bovine colonic tissues maintained in vitro. No differences were determined in colonization susceptibility between tissues derived from weaning calves and adult cattle, or for tissues from cattle fed grain and forage-based rations. Substrate conditions designed to represent various intra-enteric environments were tested for their effect on STEC/mucosal interaction. Under conditions corresponding to a well-fed ruminant (high volatile fatty acid and lactate concentrations, low pH), significantly less STEC colonized the mucosal surface of colonic biopsies. These results may help explain why fasted, poorly or intermittently fed cattle and pre-ruminant calves excrete STEC to a greater degree. Studies on the ecology of STEC within the ruminant gut help identify mechanisms to reduce their threat to public health

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.
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

Conedera,G., P.A.Chapman, S.Marangon, E.Tisato, P.Dalvit, and A.Zuin. 2001. "A field survey of Escherichia coli O157 ecology on a cattle farm in Italy." *Int.J.Food Microbiol.* 66:85-93.
Abstract: A field survey was performed in a heifer raising operation in Northern Italy to study the introduction, maintenance and dissemination of Escherichia coli O157 in the herd and to identify possible control measures at the farm level. Rectal swabs from two different groups of animals (surveys 1 and 2) were tested for E. coli O157 by an immunomagnetic separation technique. In survey 1, a group of female calves (341 animals initially) introduced from 30 dairy herds during April 1996 to March 1997 were tested for E. coli O157 on arrival from the original herd when housed in individual hutches, 2-3 days after completion of weaning (which was associated with grouping) and 2 months after weaning. No statistically significant difference between excretion rates (3.8%, 4.2%, 4.4%, respectively) was found. Calves from which E. coli O157 was isolated on arrival came from 6 of the 30 dairy herds. Strains isolated during survey 1 belonged to seven different pulsed field gel electrophoresis (PFGE) profiles. In survey 2, a group of young animals aged, at the beginning of the study, between 2 1/2 and 7 1/2 months (median = 124 days) was tested monthly for E. coli O157 for 11-15 months from May 1996 to July 1997. The group included 92 animals for 11 months and then gradually decreased to 59 animals. Overall, E. coli O157, belonging to six different PFGE profiles, were isolated from 138 (10.7%) of 1293 rectal swabs. Monthly excretion rates ranged from 2.7% to 23.7%, with summer peaks in both years. Fifty-nine (64.1%) of the 92 heifers were positive at least once: of these 59 animals, 22 (37.3%) were positive on only one occasion, 23 (39%) were positive on two occasions and 14 (23.7%) were positive on three or more occasions. From two heifers positive on 9 out of the 15 sampling visits, strains with the same PFGE profile were isolated, respectively, on seven and eight occasions while strains with only one band difference were isolated on the remaining occasions. E. coli O157 was also isolated from 6 of 16 samples of bedding, two of two samples of slurry and one of five samples from water troughs collected during survey 2

Cooley,M.B., W.G.Miller, and R.E.Mandrell. 2003. "Colonization of Arabidopsis thaliana with Salmonella enterica and enterohemorrhagic Escherichia coli O157:H7 and competition by Enterobacter asburiae." *Appl.Environ.Microbiol.* 69:4915-4926.
Abstract: Enteric pathogens, such as Salmonella enterica and Escherichia coli O157:H7, have been shown to contaminate fresh produce. Under appropriate conditions, these bacteria will grow on and invade the plant tissue. We have developed Arabidopsis thaliana (thale cress) as a model system with the intention of studying plant responses to human pathogens. Under sterile conditions and at 100% humidity, S. enterica serovar Newport and E. coli O157:H7 grew to 10(9) CFU g(-1) on A. thaliana roots and to 2 x 10(7) CFU g(-1) on shoots. Furthermore, root inoculation led to contamination of the entire plant, indicating that the pathogens are capable of moving on or within the plant in the absence of competition. Inoculation with green fluorescent protein-labeled S. enterica and E. coli O157:H7 showed invasion of the roots at lateral root junctions. Movement was eliminated and invasion decreased when nonmotile mutants of S. enterica were used. Survival of S. enterica serovar Newport and E. coli O157:H7 on soil-grown plants declined as the plants matured, but both pathogens were detectable for at least 21 days. Survival of the pathogen was reduced in unautoclaved soil and amended soil, suggesting competition from indigenous epiphytes from the soil. Enterobacter asburiae was isolated from soil-grown A. thaliana and shown to be effective at suppressing epiphytic growth of both pathogens under gnotobiotic conditions. Seed and chaff harvested from contaminated plants were occasionally contaminated. The rate of recovery of S. enterica and E. coli O157:H7 from seed varied from undetectable to 19% of the seed pools tested, depending on the method of inoculation. Seed contamination by these pathogens was undetectable in the presence of the competitor, Enterobacter asburiae. Sampling of 74 pools of chaff indicated a strong correlation between contamination of the chaff and seed (P = 0.025). This suggested that contamination of the seed occurred directly from contaminated chaff or by invasion of the flower or silique. However, contaminated seeds were not sanitized by extensive washing and chlorine treatment, indicating that some of the bacteria reside in a protected niche on the seed surface or under the seed coat

Cooley,M.B., D.Chao, and R.E.Mandrell. 2006. "Escherichia coli O157:H7 survival and growth on lettuce is altered by the presence of epiphytic bacteria." *J.Food Prot.* 69:2329-2335.
Abstract: Escherichia coli O157:H7 can survive in low numbers in soil and on plants. Occasionally, conditions may occur in the field that lead to contamination of produce. Survival of enteric pathogens in the field is controlled to a certain extent by complex interactions with indigenous soilborne and seedborne epiphytes. Identifying these interactions may assist in developing strategies to improve produce safety. Two epiphytes were isolated from pathogen-contaminated plants that interact differently with E. coli O157:H7. Wausteria paucula enhanced the survival of E. coli O157:H7 six-fold on lettuce foliage grown from coinoculated lettuce seed. In contrast, Enterobacter asburiae decreased E. coli O157:H7 survival 20- to 30-fold on foliage. Competition also occurred in the rhizosphere and in plant exudate. This competition may be the result of E. asburiae utilization of several of the carbon and nitrogen substrates typically present in exudate and also used by E. coli O157:H7. Hence, competition observed on the plant may involve one or more nutrients provided by the plant. In contrast, a different mechanism may exist between E. coli O157:H7 and W. paucula since commensalism was only observed on foliage, not in the rhizosphere or plant exudate. Good agricultural practices that encourage the growth of competing bacteria, like E. asburiae, may reduce the incidence of produce contamination

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

Crump,J.A., C.R.Braden, M.E.Dey, R.M.Hoekstra, J.M.Rickelman-Apisa, D.A.Baldwin, S.J.De Fijter, S.F.Nowicki, E.M.Koch, T.L.Bannerman, F.W.Smith, J.P.Sarisky, N.Hochberg, and P.S.Mead. 2003. "Outbreaks of Escherichia coli O157 infections at multiple county agricultural fairs: a hazard of mixing cattle, concession stands and children." *Epidemiol.Infect.* 131:1055-1062.
Abstract: Escherichia coli O157 infections cause an estimated 60 deaths and 73 000 illnesses annually in the United States. A marked summer peak in incidence is largely unexplained. We investigated an outbreak of E. coli O157 infections at an agricultural fair in Ohio and implicated consumption of beverages made with fairground water and sold by a geographically localized group of vendors who were all on the same branch of the fairground water distribution system. To examine county fair attendance as a risk factor for infection, we conducted two further epidemiological studies. In the first, we enhanced surveillance for E. coli O157 infections in 15 Northeast Ohio counties during the 2000 agricultural fair season and showed increased risk of E. coli O157 infection among fair attendees. In the second study, we examined Ohio Public Health Laboratory Information Service (PHLIS) data for 1999 using a time-varying covariate proportional hazards model and demonstrated an association between agricultural fairs and E. coli O157 infections, by county. Agricultural fair attendance is a risk factor for E. coli O157 infection in the United States and may contribute to the summer peak in incidence. Measures are needed to reduce transmission of enteric pathogens at agricultural fairs

Daniels,N.A., L.MacKinnon, S.M.Rowe, N.H.Bean, P.M.Griffin, and P.S.Mead. 2002. "Foodborne disease outbreaks in United States schools." *Pediatr.Infect.Dis.J.* 21:623-628.
Abstract: BACKGROUND: The objective of this study was to describe the epidemiology of foodborne disease outbreaks in schools and to identify where preventive measures could be targeted. METHODS: Reports by state and local health departments of foodborne disease outbreaks occurring in primary and secondary schools, colleges and universities from January 1, 1973, through December 31, 1997, were reviewed. Data from ill persons identified through foodborne outbreak investigations and subsequently reported to the Centers for Disease Control and Prevention in the Foodborne Outbreak Surveillance System were examined. The number and size of foodborne disease outbreaks, as well as the etiologic agents, food vehicles of transmission, site of food preparation and contributing factors associated with outbreaks were also examined. RESULTS: From 1973 through 1997, states and local health departments reported 604 outbreaks of foodborne disease in schools. The median number of school outbreaks annually was 25 (range, 9 to 44). In 60% of the outbreaks an etiology was not determined, and in 45% a specific food vehicle of transmission was not determined. Salmonella was the most commonly identified pathogen, accounting for 36% of outbreak reports with a known etiology. Specific food vehicles of transmission were epidemiologically identified in 333 (55%) of the 604 outbreaks. The most commonly

implicated vehicles were foods containing poultry (18.6%), salads (6.0%), Mexican-style food (6.0%), beef (5.7%) and dairy products excluding ice cream (5.0%). The most commonly reported food preparation practices that contributed to these school-related outbreaks were improper food storage and holding temperatures and food contaminated by a food handler. CONCLUSIONS: Strengthening food safety measures in schools would better protect students and school staff from outbreaks of foodborne illness. Infection control policies, such as training and certification of food handlers in the proper storage and cooking of foods, meticulous hand washing and paid sick leave for food handlers with gastroenteritis, could make meals safer for American students

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

DeFrancesco,K.A., R.N.Cobbold, D.H.Rice, T.E.Besser, and D.D.Hancock. 2004. "Antimicrobial resistance of commensal Escherichia coli from dairy cattle associated with recent multi-resistant salmonellosis outbreaks." *Vet.Microbiol.* 98:55-61.
Abstract: The use of antimicrobial drugs in livestock is suspected to contribute to bacterial antimicrobial resistance (AR) development. Dairy farms experiencing recent outbreaks of salmonellosis involving multi-resistant (MR) Salmonella strains were compared to control farms with respect to AR among bovine commensal E. coli isolates. For most antimicrobials tested, the percentage of AR E. coli isolated from salmonellosis-affected farms was significantly higher than that from control farms. Calf E. coli from both case and control farms had greater levels of AR than cow isolates. Commensal E. coli isolates from case farms and calves tended to more frequently be MR. These data are consistent with the existence of higher antimicrobial selection pressure on farms with recent salmonellosis outbreaks, however, the directionality of the relationship remains to be elucidated. An improved understanding of the epidemiology of AR bacteria in livestock production, both at the herd and molecular level, is essential to mitigate risk to public health and food safety

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

DiLorenzo,N., F.Diez-Gonzalez, and A.DiCostanzo. 2006. "Effects of feeding polyclonal antibody preparations on ruminal bacterial populations and ruminal pH of steers fed high-grain diets." *J.Anim Sci.* 84:2178-2185.
Abstract: Three experiments with factorial arrangements of treatments were designed to test the efficacy of avian-derived polyclonal antibody preparations (PAP) against Streptococcus bovis (PAP-Sb) or Fusobacterium necrophorum (PAP-Fn) in reducing ruminal counts of target bacteria in beef steers supplemented or not with feed additives (300 mg of monensin/d and 90 mg of tylosin/d; MT). Feeding increasing doses of PAP-Sb in Exp. 1 or a single dose in Exp. 2 reduced S. bovis counts in a cubic fashion (P = 0.014). In Exp. 1 and 2, inclusion of MT in the diet had no effect (P > 0.05) on ruminal S. bovis counts. In Exp. 2, ruminal pH was increased (P < 0.05) by feeding PAP-Sb, MT, and PAP-Sb plus MT. Ruminal F. necrophorum counts were reduced by feeding PAP-Fn (P = 0.002) and MT (P < 0.001). Reduction in ruminal F. necrophorum counts was greater (P = 0.008) when feeding MT alone than when feeding PAP-Fn and MT together. In Exp. 3, ruminal S. bovis counts were not affected (P = 0.64) by PAP-Fn. Ruminal pH was not affected (P = 0.61) by feeding PAP-Fn, and the total anaerobic bacterial count was not affected (P > 0.05) by either PAP-Sb or PAP-Fn in Exp. 1 or Exp. 3. In conclusion, PAP of avian origin and against S. bovis or F. necrophorum were effective in reducing target ruminal bacterial populations. These PAP could be effective in preventing the deleterious effects associated with these bacteria, and possibly in enhancing animal performance

Dingman,D.W. 1994. "Inhibitory Effects of Turf Pesticides on Bacillus popilliae and the Prevalence of Milky Disease." *Appl.Environ.Microbiol.* 60:2343-2349.
Abstract: Fourteen pesticides (fungicides, herbicides, and insecticides) were tested to determine whether they had deleterious effects on the bioinsecticide Bacillus popilliae, the causal agent of milky disease. All of these pesticides reduced levels of spore viability, spore germination, and/or vegetative cell growth when they were tested over a range of concentrations from 0 to 1,000 ppm of active ingredient, and the fungicides had the greatest detrimental effects. As determined by tests in water, the level of spore viability was significantly reduced by chlorothalonil, iprodione, (2,4-dichlorophenoxy)acetic acid plus 2-(2,4-dichlorophenoxy)propionic acid, and 2-[(4-chloro-o-tolyl)oxy]propionic acid plus (2,4-dichlorophenoxy)acetic acid. In tests performed with iprodione, loss of viability was evident at concentrations less than the concentration calculated to result from recommended use. Tests performed in soil demonstrated that triadimefon, chlorothalonil, (2,4-dichlorophenoxy)acetic acid plus 2-(2,4-dichlorophenoxy)propionic acid, and pendimethalin at concentrations resulting from recommended rates of application reduced spore titers. Spore germination did not occur in the continued presence of 2-[(4-chloro-otolyl)oxy]propionic acid plus (2,4-dichlorophenoxy)acetic acid, isofenphos, and chlordane, whereas exposure of spores to triadimefon or pendimethalin for 2 days stimulated germination. The tests to determine effects on spore germination were inconclusive for all other pesticides. Triadimefon, chlorothalonil, iprodione, pendimethalin, and chlorpyrifos at concentrations less than the concentrations recommended for use inhibited vegetative cell growth of B. popilliae, and chlordane at a concentration that was twice the concentration expected to result from the recommended rate of application repressed cell growth. My data support the hypothesis that use of synthetic pesticides can contribute to a low incidence of milky disease in white grubs

Dixon,B. 2005. "Panic pending over farming waste." *Lancet Infect.Dis.* 5:397.

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

Doyle,M.P. and J.L.Schoeni. 1987. "Isolation of Escherichia coli O157:H7 from retail fresh meats and poultry." *Appl.Environ.Microbiol.* 53:2394-2396.
Abstract: A total of 896 samples of retail fresh meats and poultry was assayed for Escherichia coli serogroup O157:H7 by a hydrophobic grid membrane filter-immunoblot procedure developed specifically to isolate the organism from foods. The procedure involves several steps, including selective enrichment, filtration of enrichment culture through hydrophobic grid membrane filters, incubation of each filter on nitrocellulose paper on selective agar, preparation of an immunoblot (by using antiserum to E. coli O157:H7 culture filtrate) of each nitrocellulose paper, selection from the filters of colonies which corresponded to immunopositive sites on blots, screening of isolates by a Biken test for precipitin lines from metabolites and antiserum to E. coli O157:H7 culture filtrate, and confirmation of isolates as Vero cell cytotoxic E. coli O157:H7 by biochemical, serological, and Vero cell cytotoxicity tests. E. coli O157:H7 was isolated from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples. One of 14 pork samples and 5 of 17 beef samples contaminated with the organism were from Calgary, Alberta, Canada, grocery stores, whereas all other contaminated samples were from Madison, Wis., retail outlets. This is the first report of the isolation of E. coli O157:H7 from food other than ground beef, and results indicate that the organism is not a rare contaminant of fresh meats and poultry

Doyle,M.P. 1991. "Escherichia coli O157:H7 and its significance in foods." *Int.J.Food Microbiol.* 12:289-301.
Abstract: Escherichia coli O157:H7 was conclusively identified as a pathogen in 1982 following its association with two food-related outbreaks of an unusual gastrointestinal illness. The organism is now recognized as an important cause of foodborne disease, with outbreaks reported in the U.S.A., Canada, and the United Kingdom. Illness is generally quite severe, and can include three different syndromes, i.e., hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Most outbreaks have been associated with eating undercooked ground beef or, less frequently, drinking raw milk. Surveys of retail raw meats and poultry revealed E. coli O157:H7 in 1.5 to 3.5% of ground beef, pork, poultry, and lamb. Dairy cattle, especially young animals, have been identified as a reservoir. The organism is typical of most E. coli, but does possess distinguishing characteristics. For example, E. coli O157:H7 does not ferment sorbitol within 24 h, does not possess beta-glucuronidase activity, and does not grow well or at all at 44-45.5 degrees C. The organism has no unusual heat resistance; heating ground beef sufficiently to kill typical strains of salmonellae will also kill E. coli O157:H7. The mechanism of pathogenicity has not been fully elucidated, but

clinical isolates produce one or more verotoxins which are believed to be important virulence factors. Little is known about the significance of pre-formed verotoxins in foods. The use of proper hygienic practices in handling foods of animal origin and proper heating of such foods before consumption are important control measures for the prevention of E. coli O157:H7 infections

Doyle,M.P. 2000. "Reducing foodborne disease: what are the priorities?" *Nutrition*. 16:647-649.

Doyle,M.P. and M.C.Erickson. 2006. "Reducing the carriage of foodborne pathogens in livestock and poultry." *Poult.Sci.* 85:960-973.
Abstract: Several foodborne pathogens, including Salmonella species and campylobacters, are common contaminants in poultry and livestock. Typically, these pathogens are carried in the animal's intestinal tract asymptomatically; however, they can be shed in feces in large populations and be transmitted by other vectors from feces to animals, produce, or humans. A wide array of interventions has been developed to reduce the carriage of foodborne pathogens in poultry and livestock, including genetic selection of animals resistant to colonization, treatments to prevent vertical transmission of enteric pathogens, sanitation practices to prevent contamination on the farm and during transportation, elimination of pathogens from feed and water, feed and water additives that create an adverse environment for colonization by the pathogen, and biological treatments that directly or indirectly inactivate the pathogen within the host. To successfully reduce the carriage of foodborne pathogens, it is likely that a combination of intervention strategies will be required

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

Eriksson,E., E.Nerbrink, E.Borch, A.Aspan, and A.Gunnarsson. 2003. "Verocytotoxin-producing Escherichia coli O157:H7 in the Swedish pig population." *Vet.Rec.* 152:712-717.
Abstract: Verocytotoxin-producing Escherichia coli O157:H7 (VTEC O157:H7) was detected in two of 2446 individual faecal samples collected from pigs slaughtered at five Swedish slaughterhouses, indicating a prevalence of 0.08 per cent, with a 95 per cent confidence interval from 0 to 0.16 per cent Four Swedish VTEC O157:H7-positive farms which kept ruminants and pigs were studied by repeated faecal sampling; VTEC O157:H7 was isolated from the ruminants and pigs on all the farms and the same strains were present in the pigs and the ruminants. On one of the farms, the organism persisted in the pig population for 11 months. On all four farms, management practices which might have influenced the isolation rate in pigs were identified. A group of young VTEC

O157:H7-positive pigs was moved from one of the VTEC O157:H7-positive farms to a fattening herd where there were no ruminants. The number of VTEC O157:H7-positive faecal samples decreased gradually and after nine weeks the pigs were all negative; at slaughter none of the pigs was VTEC O157:H7-positive

Eriksson,E., A.Aspan, A.Gunnarsson, and I.Vagsholm. 2005. "Prevalence of verotoxin-producing Escherichia coli (VTEC) 0157 in Swedish dairy herds." *Epidemiol.Infect.* 133:349-358.
Abstract: A prevalence study of verotoxin-producing Escherichia coli O157 (VTEC O157) was performed in 371 randomly selected dairy herds distributed throughout Sweden. Faecal and manure samples were collected and analysed by immunomagnetic separation and culturing. Data were recorded for each herd regarding herd size, age of sampled animals and whether, in addition to cattle, the farm kept other animals. VTEC O157 was isolated from 33 (8.9%) of the 371 investigated herds. The prevalence was higher (23.3%) in Halland county than in the rest of Sweden (P > 0.01). Halland was also the county in Sweden that during the study period had the highest incidence of human VTEC O157 cases. VTEC O157 was not detected on any farm in northern Sweden. Identified risk factors, in the multivariate analyses, for herds being VTEC O157 positive were herd size, geographical localization, presence of pigs on the farm and median age of sampled animals

Farzan,A., R.M.Friendship, C.E.Dewey, K.Warriner, C.Poppe, and K.Klotins. 2006. "Prevalence of Salmonella spp. on Canadian pig farms using liquid or dry-feeding." *Prev.Vet.Med.* 73:241-254.
Abstract: The objective of this study was to determine whether the shedding and antibody titre to Salmonella was lower for pig herds provided liquid-feed compared to those on traditional dry rations. Twenty liquid-feeding farms and 61 dry-feeding farms were selected. The amount of antibodies to Salmonella in sera from 15 finisher pigs on each of 80 Ontario swine farms was analyzed by means of enzyme-linked immunosorbent assay (ELISA). In addition, the presence of Salmonella on the 20 liquid-feeding farms and 21 of the dry-feeding farms was assessed by culture of 15 fecal samples taken directly from finisher pigs and five pooled pen-fecal samples at each farm. A cut-off of OD% 10 was used. The Salmonella sero-prevalence differed between the two groups of farms. At least one pig tested sero-positive on 98% of the dry-feeding farms and 84% of the liquid-feeding farms (P<0.05). A multi-variable mixed linear regression model with the farm as a random variable and farm factors as the fixed effects was fitted. Crude optical density (OD) of the individual pig was considered as the continuous dependent variable. Dry-feeding and antimicrobial daily usage was associated with crude OD (P<0.05). In addition, crude OD increased with increasing herd size (P<0.05). Salmonella was isolated from 25 out of 420 fecal samples (6%) from dry-feeding farms compared to three out of 400 samples (0.8%) from liquid-feeding farms. Eight of the dry-feeding farms (38%) tested positive compared to only three of the liquid-feeding farms (15%). Salmonella was also recovered from the pen environment on five dry-feeding farms but were not isolated from the facilities using liquid-feeding. Salmonella Typhimurium was isolated from four farms in the dry-feed group and on one farm with liquid-feeding. The one S. Typhimurium isolate from the liquid-feeding farm exhibited no antimicrobial resistance, but those from dry-feeding farms were resistant to four or more antimicrobial agents. The results of the logistic regression, with farm as a random effect showed that dry-feeding [OR=2.7 (1.1-15.1)] and continuous flow system [OR=2.3 (1.2-12.7)] increased risk of finding Salmonella in the individual pig. These findings indicate that liquid-feeding and all-in all-out management of the grower-finisher barns can reduce the Salmonella prevalence

Fekete,P.Z., G.Schneider, F.Olasz, G.Blum-Oehler, J.H.Hacker, and B.Nagy. 2003. "Detection of a plasmid-encoded pathogenicity island in F18+ enterotoxigenic and verotoxigenic Escherichia coli from weaned pigs." *Int.J.Med.Microbiol.* 293:287-298.
Abstract: Most virulence genes of enterotoxigenic Escherichia coli (ETEC) are located

on plasmids. The gene for heat-stable enterotoxin I (sta) is part of the transposon Tn1681, and the heat-stable enterotoxin II (stb) gene was described to be part of the transposon Tn4521. In the studies presented here, we describe the linkage of the sta and stb genes on an approximately 10-kb fragment designated as toxin-specific locus (TSL). The TSL has been isolated from the 120-kb virulence plasmid pTC of the porcine ETEC strain 2173 that produces F18 fimbriae. The nucleotide sequence of the TSL fragment was determined. Sequences in the flanking regions of the sta gene indicated the presence of Tn1681, but--unexpectedly--flanking sequences of the neighbouring stb gene were completely different from those of Tn4521. The 10-kb TSL is part of a 40-kb fragment that contains the replication origin of pTC. This 40-kb fragment was mobilised into plasmid pACYC177 and the nucleotide sequence of the bordering fragments was determined. The 40-kb fragment was flanked by IS10 elements at both ends, indicating the existence of a new 40-kb pathogenicity island (PAI) in strain 2173. Several F4(K88)+ ETEC and F18+ ETEC as well as F18+ E. coli strains producing enterotoxins and verotoxin-2 (ETEC/VTEC) from weaned pigs of different geographical origin were tested for the flanking regions by PCR to see if they belong to the "Tn4521-like" or the "pTC-like" stb type. It turned out that the Tn4521-like stb-type was characteristic of F4(K88)+ ETEC, while the pTC-like stb type was present in most F18+ ETEC and F18+ ETEC/VTEC, although polymorphism was observed both in the K88 and F18 groups. These results suggest the existence and worldwide spread of a new plasmid-encoded pathogenicity island in porcine post weaning ETEC and ETEC/VTEC strains producing F18 fimbriae

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

Fischer,J.R., T.Zhao, M.P.Doyle, M.R.Goldberg, C.A.Brown, C.T.Sewell, D.M.Kavanaugh, and C.D.Bauman. 2001. "Experimental and field studies of Escherichia coli O157:H7 in white-tailed deer." *Appl.Environ.Microbiol.* 67:1218-1224.
Abstract: Studies were conducted to evaluate fecal shedding of Escherichia coli O157:H7 in a small group of inoculated deer, determine the prevalence of the bacterium in free-ranging white-tailed deer, and elucidate relationships between E. coli O157:H7 in wild deer and domestic cattle at the same site. Six young, white-tailed deer were orally administered 10(8) CFU of E. coli O157:H7. Inoculated deer were shedding E. coli O157:H7 by 1 day postinoculation (DPI) and continued to shed decreasing numbers of the bacteria throughout the 26-day trial. Horizontal transmission to an uninoculated deer was demonstrated. Although E. coli O157:H7 bacteria were recovered from the gastrointestinal tracts of deer necropsied from 4 to 26 DPI, attaching and effacing lesions were not apparent in any deer. Results are similar to those of inoculation studies in calves and sheep. In field studies, E. coli O157 was not detected in 310 fresh deer fecal samples collected from the ground. It was detected in feces, but not in meat, from 3 of 469 free-ranging deer in 1997. In 1998, E. coli O157 was not detected in 140 deer at the single positive site found in 1997; however, it was recovered from 13 of 305 dairy

and beef cattle at the same location. Isolates of E. coli O157:H7 from deer and cattle at this site differed with respect to pulsed-field gel electrophoresis patterns and genes encoding Shiga toxins. The low overall prevalence of E. coli O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of E. coli O157:H7 in the southeastern United States. However, there may be individual locations where deer sporadically harbor the bacterium, and venison should be handled with the same precautions recommended for beef, pork, and poultry

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. 2000. "Leaching of Escherichia coli O157:H7 in diverse soils under various agricultural management practices." *Appl.Environ.Microbiol.* 66:877-883.
Abstract: Application of animal manures to soil as crop fertilizers is an important means for recycling the nitrogen and phosphorus which the manures contain. Animal manures also contain bacteria, including many types of pathogens. Manure pathogen levels depend on the source animal, the animal's state of health, and how the manure was stored or treated before use. Rainfall may result in pathogen spread into soil by runoff from stored or unincorporated manure or by leaching through the soil profile. Steady rainfall consisting of 16.5 mm h(-1) was applied to 100-mm disturbed soil cores that were treated with manure and inoculated with Escherichia coli O157:H7 strain B6914. The level of B6914 in leachate was near the inoculum level each hour for 8 h, as was the level of B6914 at several soil depths after 24 h, indicating that there was a high rate of growth. Bacterial movement through three different types of soil was then compared by using disturbed (tilled) and intact (no-till) soil cores and less intense rainfall consisting of 25.4 mm on 4 consecutive days and then four more times over a 17-day period. Total B6914 levels exceeded the inoculum levels for all treatments except intact clay loam cores. B6914 levels in daily leachate samples decreased sharply with time, although the levels were more constant when intact sandy loam cores were used. The presence of manure often increased total B6914 leachate and soil levels in intact cores but had the opposite effect on disturbed soil cores. Ammonia and nitrate levels correlated with B6914 and total coliform levels in leachate. We concluded that tillage practice, soil type, and method of pathogen delivery affect but do not prevent vertical E. coli O157:H7 and coliform transport in soil and that soluble nitrogen may enhance transport

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

Garber,L.P., S.J.Wells, D.D.Hancock, M.P.Doyle, J.Tuttle, J.A.Shere, and T.Zhao. 1995. "Risk factors for fecal shedding of Escherichia coli O157:H7 in dairy calves." *J.Am.Vet.Med.Assoc.* 207:46-49.
Abstract: A case-control study was conducted to determine risk factors for fecal shedding of Escherichia coli O157:H7 (ECO) in dairy calves. Three herds previously found to lack calves that shed ECO in their feces were selected for each herd previously found to have calves that shed ECO. Fecal samples from 965 calves on 64 farms were tested for ECO by microbial culture. Sample prevalence of ECO in calves less than 8 weeks old was 1.4% and in calves 8 weeks or older was 4.8%. Calves were 3 times more likely to shed ECO after weaning than before weaning. Shedding of ECO was associated with grouping calves before weaning; feeding whole cottonseed was negatively associated with ECO shedding. The change in results between testing periods illustrated that an individual herd's status cannot be defined by a single testing of a small sample of cattle

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

Guan,T.T., G.Blank, and R.A.Holley. 2005. "Survival of pathogenic bacteria in pesticide solutions and on treated tomato plants." *J.Food Prot.* 68:296-304.
Abstract: The ability of Salmonella, Escherichia coli O157:H7, Listeria monocytogenes, and Shigella to survive or grow in pesticide solutions (Ambush 240EC, Benlate T-N-G, Bravo 500, Botran 75WP, Captan 80WDG, Parasol, and Vendex 50W) used by the horticultural industry was examined. In the laboratory, individual cultures were inoculated at 4 log CFU/ml in pesticides diluted with sterile saline to the lowest recommended spray concentrations. During 21 degrees C incubation for < or =96 h, bacterial survivors in the samples and a control consisting of saline were enumerated either by agar surface plating or hydrophobic grid membrane filtration. Most formulations tested were somewhat inhibitory to the pathogenic bacteria. All inoculated bacteria survived or grew in Bravo 500. Among bacteria tested, Salmonella spp. were best able to survive and Listeria spp. were least able to survive in pesticide solutions. When the incubation temperature or pesticide concentration was increased, survival of Salmonella varied depending on the type of formulation. In the field, when a bacterial cocktail containing E. coli O157:H7 and Salmonella Enteritidis was added to Bravo 500 at 6 log CFU/ml, both organisms were recovered from leaves and fruit skins of sprayed tomato plants after the recommended 1 day-to-harvest interval. E. coli and Salmonella survived longer on tomato leaves when sprayed in saline (at least 26 and 56 days, respectively) than when sprayed in Bravo 500 (>45 h and <15 days, respectively). While Salmonella serovars Typhimurium and Heidelberg grew in the fungicide Bravo, and Enteritidis grew in the insecticide Vendex within 96 h at 21 degrees C in the laboratory, pathogen growth in other pesticide formulations did not occur. Higher temperature (< or =30 degrees C) or doubling pesticide concentrations had either no or a negative effect on Salmonella Heidelberg survival. Use of unexpired pesticide formulations may have contributed to the reduced bacterial survival and growth found in the laboratory and during the field trials with Bravo

Guber,A.K., D.R.Shelton, and Y.A.Pachepsky. 2005. "Effect of manure on Escherichia coli attachment to soil." *J.Environ.Qual.* 34:2086-2090.
Abstract: Attachment of bacteria to soil is an important component of bacterial fate and transport. Escherichia coli are commonly used as indicators of fecal contamination in the environment. Despite the fact that E. coli are derived exclusively from feces or manure, effect of the presence of manure colloids on bacteria attachment to agricultural soils was never directly studied. The objective of this work was to evaluate the magnitude of the effect of manure on E. coli attachment to soil. Escherichia coli attachment to soil was studied in batch experiments with samples of loam and sandy clay loam topsoil that were taken in Pennsylvania and Maryland. Escherichia coli cells were added to the water-manure suspensions containing 0, 20, and 40 g L(-1) of filtered liquid bovine manure, which subsequently were equilibrated with air-dry sieved soil in different soil to suspension ratios. The Langmuir isotherm equation was fitted to data. Manure dramatically affected E. coli attachment to soil. Attachment isotherms were closer to linear without manure and were strongly nonlinear in the presence of manure. The

maximum E. coli attachment occurred in the absence of manure. Increasing manure content generally resulted in decreased attachment

Hanajima,D., K.Kuroda, Y.Fukumoto, and K.Haga. 2006. "Effect of addition of organic waste on reduction of Escherichia coli during cattle feces composting under high-moisture condition." *Bioresour.Technol.* 97:1626-1630.
Abstract: To ensure Escherichia coli reduction during cattle feces composting, co-composting with a variety of organic wastes was examined. A mixture of dairy cattle feces and shredded rice straw (control) was blended with organic wastes (tofu residue, rice bran, rapeseed meal, dried chicken feces, raw chicken feces, or garbage), and composted using a bench-scale composter under the high-moisture condition (78%). The addition of organic waste except chicken feces brought about maximum temperatures of more than 55 degrees C and significantly reduced the number of E. coli from 10(6) to below 10(2)CFU/g-wet after seven days composting, while in the control treatment, E. coli survived at the same level as that of raw feces. Enhancements of the thermophilic phase and E. coli reduction were related to the initial amount of easily digestible carbon in mass determined as BOD. BOD value more than 166.2 mg O2/DMg brought about significant E. coli reduction

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

Hancock,D.D., T.E.Besser, D.H.Rice, D.E.Herriott, and P.I.Tarr. 1997. "A longitudinal study of Escherichia coli O157 in fourteen cattle herds." *Epidemiol.Infect.* 118:193-195.
Abstract: Escherichia coli O157 shedding in 14 cattle herds was determined by faecal culture at intervals of approximately 1 month for up to 13 months. The overall prevalence was 1.0% (113/10832 faecal samples) and 9 of the 14 herds were detected as positive. Herds positive 2 years previously (n = 5) had a higher prevalence of positive cattle (median = 1.9%) than herds which had been negative on a previous sampling (n = 8, median = 0.2%). Weaned heifers had a higher prevalence (1.8%) than did unweaned calves (0.9%) or adults (0.4%). For all herds the highest prevalence occurred in the summer months, which resulted in most of the positive faecal samples being collected on a minority of sampling visits

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.
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

Hancock,D.D., T.E.Besser, C.Gill, and C.H.Bohach. 1999. "Cattle, hay and E. coli." *Science*. 284:51-53.

Hara-Kudo,Y., H.Watanabe, and H.Konuma. 2005. "Differences in survival of Escherichia coli O157:H7 under various conditions that re-enact the cooking of lunches implicated in an outbreak of haemorrhagic diarrhoea." *Epidemiol.Infect.* 133:1043-1048.
Abstract: Two elementary schools were served lunches that were cooked in the same kitchen. An outbreak of Escherichia coli O157:H7 occurred at one school where the dishes that were prepared for the school were lukewarm and kept for 33 min at an average temperature of 45 degrees C before serving. However, no outbreak occurred at the other school where dishes were hot and were kept for 60 min at an average temperature of 50 degrees C before serving. In a series of experiments on the survival of E. coli O157:H7 in the liquid portion of similarly prepared food, the population of E. coli O157:H7 was reduced by 10-3 by heating at 50 degrees C for 60 min and by only 10-1 by heating at 45 degrees C for 40 min. Further, E. coli O157:H7 survived at 45 degrees C for 40 min but not at 50 degrees C for 60 min at pH 4.0 with a 4.0% salt concentration that was similar to that of the liquid part of the food. These results indicate that pH and salt concentration of cooked food markedly affect the survival of E. coli O157:H7 and help to explain the occurrence of the disease outbreak at only one of the schools

Harmon,B.G., C.A.Brown, S.Tkalcic, P.O.Mueller, A.Parks, A.V.Jain, T.Zhao, and M.P.Doyle. 1999. "Fecal shedding and rumen growth of Escherichia coli O157:H7 in fasted calves." *J.Food Prot.* 62:574-579.
Abstract: Nine weaned calves aged from 8 to 12 weeks were fitted with rumen cannulas and were inoculated by cannula with 10(10) CFU of a five-strain mixture of nalidixic acid-resistant Escherichia coli O157:H7. Six calves were fasted for 48 h on days 15 and 16 and days 22 and 23 after inoculation. Samples of rumen contents and feces were obtained daily to enumerate E. coli O157:H7 populations and to determine rumen volatile fatty acid (VFA) concentrations and rumen pH. Fasting resulted in a marked decrease in rumen VFA concentrations from a mean of 135 mmol/liter before the fast to a mean of 35 mmol/liter during the second day of the fast. However, there was no correlation between daily VFA concentration and daily rumen or fecal numbers of E. coli O157:H7 in any of the calves. Fasting generally had no significant effect on the rumen or fecal numbers of E. coli O157:H7. The exception was a single fasted calf that experienced a 3-log(10) CFU/g increase in fecal shedding during and after the first fast. Despite the consistent changes in VFA concentrations in fasted calves, the fluctuations in rumen numbers of E. coli O157:H7 in the rumen of fasted calves were minimal. At the end of the experiment, E. coli O157:H7 was detected in either the rumen or omasum in two of three control calves at necropsy and in either the rumen or reticulum in five of six fasted calves. E. coli O157:H7 was detected in the colon in two of three control calves and in six of six fasted calves at necropsy. These results suggest that in cattle already shedding E. coli O157:H7, feed withdrawal and the associated changes in rumen pH and VFA concentrations have little effect on fecal shedding and rumen proliferation of E. coli O157:H7

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

Hill,D.D., W.E.Owens, and P.B.Tchounwou. 2005. "Prevalence of selected bacterial infections associated with the use of animal waste in Louisiana." *Int.J.Environ.Res.Public Health*. 2:84-93.
Abstract: Human health is a major concern when considering the disposal of large quantities of animal waste. Health concerns could arise from exposure to pathogens and excess nitrogen associated with this form of pollution. The objective was to collect and analyze health data related to selected bacterial infections associated with the use of animal waste in Louisiana. An analysis of adverse health effects has been conducted based on the incidence/prevalence rates of campylobacteriosis, E. coli O157:H7 infection, salmonellosis and shigellosis. The number of reported cases increased during the summer months. Analysis of health data showed that reported disease cases of E. coli O157:H7 were highest among Caucasian infants in the 0-4 year old age category and in Caucasian children in the 5-9 year old age category. Fatalities resulting from salmonellosis are low and increases sharply with age. The number of reported cases of shigellosis was found to be higher in African American males and females than in Caucasians. The high rate of identification in the younger population may result from the prompt seeking of medical care, as well as the frequent ordering of stool examination when symptoms become evident among this group of the population. The association with increasing age and fatality due to salmonellosis could be attributed to declining health and weaker immune systems often found in the older population. It is concluded that both animal waste and non-point source pollution may have a significant impact on human health

Himathongkham,S., H.Riemann, S.Bahari, S.Nuanualsuwan, P.Kass, and D.O.Cliver. 2000. "Survival of Salmonella typhimurium and Escherichia coli O157:H7 in poultry manure and manure slurry at sublethal temperatures." *Avian Dis.* 44:853-860.
Abstract: Exponential inactivation was observed for Salmonella typhimurium and Escherichia coli O157:H7 in poultry manure with decimal reduction times ranging from half a day at 37 C to 1-2 wk at 4 C. There was no material difference in inactivation rates between S. typhimurium and E. coli O157:H7. Inactivation was slower in slurries made by mixing two parts of water with one part of manure; decimal reduction times (time required for 90% destruction) ranged from 1-2 days at 37 C to 6-22 wk at 4 C. Escherichia coli O157:H7 consistently exhibited slightly slower inactivation than S. typhimurium. Log decimal reduction time for both strains was a linear function of storage temperature for manure and slurries. Chemical analysis indicated that accumulation of free ammonia in poultry manure was an important factor in inactivation of the pathogens. This finding was experimentally confirmed for S. typhimurium by adding ammonia directly to peptone water or to bovine manure, which was naturally low in ammonia, and adjusting pH to achieve predetermined levels of free ammonia

Hora,R., M.Kumar, L.Garcia, B.Schumacher, J.Odumeru, and K.Warriner. 2005. "Spatial distribution of Salmonella, Escherichia coli O157:H7, and other bacterial populations in commercial and laboratory-scale sprouting mung bean beds." *J.Food Prot.* 68:2510-2518.
Abstract: The reliability of testing spent irrigation water to assess the microbiological status of sprouting mung bean beds has been investigated. In commercial trials, the distribution of opportunistic contaminants within 32 bean sprout beds (25 kg of mung

beans per bin) was assessed 48 h after germination. The prevalence of generic Escherichia coli, thermotolerant coliforms, and Aeromonas in sprouts (n = 288) was 5, 11, and 39%, respectively, and 57, 70, and 79% in the corresponding spent irrigation water samples (n = 96). Contamination was heterogeneously distributed within the seedbed. In laboratory trials, beans inoculated with a five-strain cocktail of either Salmonella or E. coli O157:H7 (10(3) to 10(4) CFU/g) were introduced (1 g/500 g of noninoculated seeds) at defined locations (top, middle, or base), and the beans were then sprouted for 48 h. When seeds inoculated with pathogens were introduced at the base or top of the seedbed, the pathogens were typically restricted to these sites and resulted in 44% of the spent irrigation water samples returning false-negative results. Introducing inoculated beans into the middle or at the presoak stage enhanced the distribution of both pathogens within the subsequent sprout bed and resulted in comparable levels recovered in spent irrigation water. The study demonstrated that even though screening a single sample of spent irrigation water is more reliable than testing sprouts directly, it does not provide an accurate assessment of the microbiological status of sprouting mung bean beds. Such limitations may be addressed by ensuring that bean batches are mixed prior to use and by taking spent irrigation water samples from multiple sites at the latter stages of the sprouting process

Hora,R., K.Warriner, B.J.Shelp, and M.W.Griffiths. 2005. "Internalization of Escherichia coli O157:H7 following biological and mechanical disruption of growing spinach plants." *J.Food Prot.* 68:2506-2509.
Abstract: The internalization and persistence of a bioluminescent Escherichia coli O157:H7 Ph1 was investigated in growing spinach plants that had been either biologically or mechanically damaged. In control (undamaged) plants cultivated in soil microcosms inoculated with E. coli O157:H7 Phl, the bacterium was recovered from surface-sterilized root tissue but not from leaves. Mechanical disruption of the seminal root and root hairs of the plants did not result in the internalization of the pathogen into the aerial leaf tissue. When imprints of the root tissue were made on plates of tryptic soy agar plus ampicillin, no colonies of E. coli O157:H7 were observed around damaged tissue. The roots of growing plants were exposed to the northern root-knot nematode, Meloidogyne hapla, in the presence of E. coli O157:H7. Although this treatment caused knot formation on the roots, it did not enhance the internalization of the bacterium into the plant vascular system. Coinoculation of intact leaves with E. coli O157:H7 and the phytopathogen Pseudomonas syringae DC3000 resulted in localized necrosis, but the persistence of the human pathogen was not affected. The mechanical disruption of roots does not result in the internalization of E. coli O157:H7 into the aerial tissue of spinach, and there does not appear to be any effect of P. syringae in terms of enhancing the persistence of E. coli O157:H7 in spinach leaves

Hussein,H.S. and T.Sakuma. 2005. "Shiga toxin-producing Escherichia coli: pre- and postharvest control measures to ensure safety of dairy cattle products." *J.Food Prot.* 68:199-207.
Abstract: The large number of cases of human illness caused by Shiga toxin-producing Escherichia coli (STEC) worldwide has raised safety concerns for foods of bovine origin. These human illnesses include diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Severe cases end with chronic renal failure, chronic nervous system deficiencies, and death. Over 100 STEC serotypes, including E. coli O157:H7, are known to cause these illnesses and to be shed in cattle feces. Thus, cattle are considered reservoirs of these foodborne pathogens. Because beef and dairy products were responsible for a large number of STEC outbreaks, efforts have been devoted to developing and implementing control measures that assure safety of foods derived from dairy cattle. These efforts should reduce consumers' safety concerns and support a competitive dairy industry at the production and processing levels. The efficacy of control measures both before harvest (i.e., on-farm management practices) and after harvest (i.e., milk processing and meat

packing) for decreasing the risk of STEC contamination of dairy products was evaluated. The preharvest measures included sanitation during milking and management practices designed to decrease STEC prevalence in the dairy herd (i.e., animal factors, manure handling, drinking water, and both feeds and feeding). The postharvest measures included the practices or treatments that could be implemented during processing of milk, beef, or their products to eliminate or minimize STEC contamination

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

Ibekwe,A.M., S.K.Papiernik, J.Gan, S.R.Yates, C.H.Yang, and D.E.Crowley. 2001. "Impact of fumigants on soil microbial communities." *Appl.Environ.Microbiol.* 67:3245-3257.
Abstract: Agricultural soils are typically fumigated to provide effective control of nematodes, soilborne pathogens, and weeds in preparation for planting of high-value cash crops. The ability of soil microbial communities to recover after treatment with fumigants was examined using culture-dependent (Biolog) and culture-independent (phospholipid fatty acid [PLFA] analysis and denaturing gradient gel electrophoresis [DGGE] of 16S ribosomal DNA [rDNA] fragments amplified directly from soil DNA) approaches. Changes in soil microbial community structure were examined in a microcosm experiment following the application of methyl bromide (MeBr), methyl isothiocyanate, 1,3-dichloropropene (1,3-D), and chloropicrin. Variations among Biolog fingerprints showed that the effect of MeBr on heterotrophic microbial activities was most severe in the first week and that thereafter the effects of MeBr and the other fumigants were expressed at much lower levels. The results of PLFA analysis demonstrated a community shift in all treatments to a community dominated by gram-positive bacterial biomass. Different 16S rDNA profiles from fumigated soils were quantified by analyzing the DGGE band patterns. The Shannon-Weaver index of diversity, H, was calculated for each fumigated soil sample. High diversity indices were maintained between the control soil and the fumigant-treated soils, except for MeBr (H decreased from 1.14 to 0.13). After 12 weeks of incubation, H increased to 0.73 in the MeBr-treated samples. Sequence analysis of clones generated from unique bands showed the presence of taxonomically unique clones that had emerged from the MeBr-treated samples and were dominated by clones closely related to Bacillus spp. and Heliothrix oregonensis. Variations in the data were much higher in the Biolog assay than in the PLFA and DGGE assays, suggesting a high sensitivity of PLFA analysis and DGGE in monitoring the effects of fumigants on soil community composition and structure. Our results indicate

that MeBr has the greatest impact on soil microbial communities and that 1,3-D has the least impact

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

Ingham,S.C., R.K.Wadhera, M.A.Fanslau, and D.R.Buege. 2005. "Growth of Salmonella serovars, Escherichia coli O157:H7, and Staphylococcus aureus during thawing of whole chicken and retail ground beef portions at 22 and 30 degrees C." *J.Food Prot.* 68:1457-1461.
Abstract: Food regulatory agencies advise against thawing frozen meat and poultry at room temperature. In this study, whole chickens (1,670 g) and ground beef (453 and 1,359 g) were inoculated with Salmonella serovars, Escherichia coli O157:H7, and Staphylococcus aureus on the surface (all products) and in the center (ground beef). After freezing at -20 degrees C for 24 h, products were thawed at 22 or 30 degrees C for 9 h. Pathogen growth was predicted using product time and temperature data and growth values from the U.S. Department of Agriculture Agricultural Research Service Pathogen Modeling Program 7.0 predictive models of pathogen growth. No pathogen growth was predicted for whole chicken or 1,359 g of ground beef thawed at 30 degrees C or 453 g of ground beef thawed at 22 degrees C. Growth (< or = 5 generations) was predicted for 453 g of ground beef at 30 degrees C. Inoculation study data corroborated the predictions. No growth occurred on whole chickens or 1,359-g portions of ground beef thawed at 30 degrees C for 9 h. Pathogen numbers increased an average of 0.2 to 0.5 log on the surface of 453-g ground beef portions thawed for 9 h at 22 or 30 degrees C. Our results suggest that thawing > or = 1,670 g of whole chicken at < or = 30 degrees C for < or = 9 h and thawing >453 g ground beef portions at < or = 22 degrees C for < or = 9 h are not particularly hazardous practices. Thawing smaller portions at higher temperatures and/or for longer times cannot be recommended, however. Use of values derived from the Pathogen Modeling Program 7.0 model provided realistic predictions of pathogen growth during thawing of frozen ground beef and chicken

Ingham,S.C., M.A.Fanslau, R.A.Engel, J.R.Breuer, J.E.Breuer, T.H.Wright, J.K.Reith-Rozelle, and J.Zhu. 2005. "Evaluation of fertilization-to-planting and fertilization-to-harvest

intervals for safe use of noncomposted bovine manure in Wisconsin vegetable production." *J.Food Prot.* 68:1134-1142.
Abstract: Fresh bovine manure was mechanically incorporated into loamy sand and silty clay loam Wisconsin soils in April 2004. At varying fertilization-to-planting intervals, radish, lettuce, and carrot seeds were planted; crops were harvested 90, 100, 110 or 111, and 120 days after manure application. As an indicator of potential contamination with fecal pathogens, levels of Escherichia coli in the manure-fertilized soil and presence of E. coli on harvested vegetables were monitored. From initial levels of 4.0 to 4.2 log CFU/g, E. coli levels in both manure-fertilized soils decreased by 2.4 to 2.5 log CFU/g during the first 7 weeks. However, E. coli was consistently detected from enriched soil samples through week 17, perhaps as a result of contamination by birds and other wildlife. In the higher clay silty clay loam soil, the fertilization-to-planting interval affected the prevalence of E. coli on lettuce but not on radishes and carrots. Root crop contamination was consistent across different fertilization-to-harvest intervals in silty clay loam, including the National Organic Program minimum fertilization-to-harvest interval of 120 days. However, lettuce contamination in silty clay loam was significantly ($P < 0.10$) affected by fertilization-to-harvest interval. Increasing the fertilization-to-planting interval in the lower clay loamy sand soil decreased the prevalence of E. coli on root crops. The fertilization-to-harvest interval had no clear effect on vegetable contamination in loamy sand. Overall, these results do not provide grounds for reducing the National Organic Program minimum fertilization-to-harvest interval from the current 120-day standard

Iniguez,A.L., Y.Dong, H.D.Carter, B.M.Ahmer, J.M.Stone, and E.W.Triplett. 2005. "Regulation of enteric endophytic bacterial colonization by plant defenses." *Mol.Plant Microbe Interact.* 18:169-178.
Abstract: Bacterial endophytes reside within the interior of plants without causing disease or forming symbiotic structures. Some endophytes, such as Klebsiella pneumoniae 342 (Kp342), enhance plant growth and nutrition. Others, such as Salmonella enterica serovar Typhimurium (S. typhimurium), are human pathogens that contaminate raw produce. Several lines of evidence are presented here to support the hypothesis that plant defense response pathways regulate colonization by endophytic bacteria. An ethylene-insensitive mutant of Medicago truncatula is hypercolonized by Kp342 compared to the parent genotype. Addition of ethylene, a signal molecule for induced systemic resistance in plants, decreased endophytic colonization in Medicago spp. This ethylene-mediated inhibition of endophytic colonization was reversed by addition of the ethylene action inhibitor, 1-methylcyclopropene. Colonization of Medicago spp. by S. typhimurium also was affected by exogenous ethylene. Mutants lacking flagella or a component of the type III secretion system of Salmonella pathogenicity island 1 (TTSS-SPI1) colonize the interior of Medicago spp. in higher numbers than the wild type. Arabidopsis defense response-related genotypes indicated that only salicylic acid (SA)-independent defense responses contribute to restricting colonization by Kp342. In contrast, colonization by S. typhimurium is affected by both SA-dependent and -independent responses. S. typhimurium mutants further delineated these responses, suggesting that both flagella and TTSS-SPI1 effectors can be recognized. Flagella act primarily through SA-independent responses (compromising SA accumulation still affected colonization in the absence of flagella). Removal of a TTSS-SPI1 effector resulted in hypercolonization regardless of whether the genotype was affected in either SA-dependent or SA-independent responses. Consistent with these results, S. typhimurium activates the promoter of PR1, a SA-dependent pathogenesis-related gene, while S. typhimurium mutants lacking the TTSS-SPI1 failed to activate this promoter. These observations suggest approaches to reduce contamination of raw produce by human enteric pathogens and to increase the number of growth-promoting bacteria in plants

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

Islam,M., J.Morgan, M.P.Doyle, and X.Jiang. 2004. "Fate of Escherichia coli O157:H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber." *J.Food Prot.* 67:574-578.
Abstract: Studies were done to determine the fate of Escherichia coli O157:H7 in manure compost-amended soil and on carrots and green onions grown in an environmentally controlled growth chamber. Commercial dairy cattle manure compost was inoculated with a five-strain mixture of green fluorescent protein-labeled E. coli O157:H7 at 10(7) CFU g(-1) and mixed with unsterilized Tifton sandy loam soil at a ratio of 1:5. Baby carrot or green onion seedlings were planted into the manure compost-amended soil in pots, and soil samples surrounding the plant, edible carrot roots and onion bulb samples, and soil immediately beneath the roots were assayed for E. coli O157:H7 in triplicate at weekly intervals for the first 4 weeks, and every 2 weeks for the remainder of the plant growth cycle (up to 3 months). E. coli O157:H7 cell numbers decreased within 64 days by 3 log CFU/g in soil and soil beneath the roots of green onions and by more than 2 log CFU/g on onions. E. coli O157:H7 survived better during the production of carrots, with a 2.3-log CFU/g reduction in soil and a 1.7-log CFU g reduction on carrots within 84 days. These results indicate that the type of plant grown is an important factor influencing the survival of E. coli O157:H7 both on the vegetable and in the soil in which the vegetable is grown

Islam,M., J.Morgan, M.P.Doyle, S.C.Phatak, P.Millner, and X.Jiang. 2004. "Fate of Salmonella enterica serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water." *Appl.Environ.Microbiol.* 70:2497-2502.
Abstract: Three different types of compost, PM-5 (poultry manure compost), 338 (dairy cattle manure compost), and NVIRO-4 (alkaline-pH-stabilized dairy cattle manure compost), and irrigation water were inoculated with an avirulent strain of Salmonella enterica serovar Typhimurium at 10(7) CFU g(-1) and 10(5) CFU ml(-1), respectively, to

determine the persistence of salmonellae in soils containing these composts, in irrigation water, and also on carrots and radishes grown in these contaminated soils. A split-plot block design plan was used for each crop, with five treatments (one without compost, three with each of the three composts, and one without compost but with contaminated water applied) and five replicates for a total of 25 plots for each crop, with each plot measuring 1.8 x 4.6 m. Salmonellae persisted for an extended period of time, with the bacteria surviving in soil samples for 203 to 231 days, and were detected after seeds were sown for 84 and 203 days on radishes and carrots, respectively. Salmonella survival was greatest in soil amended with poultry compost and least in soil containing alkaline-pH-stabilized dairy cattle manure compost. Survival profiles of Salmonella on vegetables and soil samples contaminated by irrigation water were similar to those observed when contamination occurred through compost. Hence, both contaminated manure compost and irrigation water can play an important role in contaminating soil and root vegetables with salmonellae for several months

Jablasone,J., K.Warriner, and M.Griffiths. 2005. "Interactions of Escherichia coli O157:H7, Salmonella typhimurium and Listeria monocytogenes plants cultivated in a gnotobiotic system." *Int.J.Food Microbiol.* 99:7-18.
Abstract: The growth and persistence of Escherichia coli O157:H7, Salmonella typhimurium and Listeria monocytogenes on a diverse range of plant types over extended cultivation periods was studied. When introduced on the seed of carrot, cress, lettuce, radish, spinach and tomato all the pathogens became rapidly established shortly after germination, attaining cell densities of the order of 5.5-6.5 log cfu/g. In general, Es. coli O157:H7 and L. monocytogenes became established and persisted at significantly higher levels on seedlings (9 days post-germination) than Salmonella. Es. coli O157:H7 became internalized in cress, lettuce, radish and spinach seedlings but was not recovered within the tissues of mature plants. Internalization of Salmonella was also observed in lettuce and radish but not cress or spinach seedlings. In contrast, L. monocytogenes did not internalize within seedlings but did persist on the surface of plants throughout the cultivation period. Co-inoculation of isolates recovered from the rhizosphere of plants did not significantly affect the numbers or persistence of human pathogens. The only exception was with Enterobacter cloacae, which reduced Es. coli O157:H7 Ph1 and L. monocytogenes levels by ca. 1 log cfu/g on lettuce. With the bioluminescent phenotype of Es. coli O157:H7 Ph1, it was demonstrated that the human pathogen became established on the roots of growing plants. Scanning electron micrographs of root seedlings suggested that Es. coli O157:H7 Ph1 preferentially colonized the root junctions of seedlings. It is proposed that such colonization sites enhanced the persistence of Es. coli O157:H7 on plants and facilitated internalization within developing seedlings. The results suggest that the risk associated with internalized human pathogens in salad vegetables at harvest is low. Nevertheless, the introduction of human pathogens at an early stage of plant development could enhance their persistence in the rhizosphere. The implications of the study with regards to on-farm food safety initiatives are discussed

Janisiewicz,W.J., W.S.Conway, and B.Leverentz. 1999. "Biological control of postharvest decays of apple can prevent growth of Escherichia coli O157:H7 in apple wounds." *J.Food Prot.* 62:1372-1375.
Abstract: Fresh cells of the antagonist Pseudomonas syringae at 2.4 x 10(8) CFU/ml inoculated into wounds of 'Golden Delicious' apple prevented Escherichia coli O157:H7 (concentrations ranging from 2.4 x 10(5) to 2.4 x 10(7) CFU/ml) from growing in the wounds. This occurred when the two microorganisms were co-inoculated or inoculation with E. coli O157:H7 was conducted 1 or 2 days after inoculation with the antagonist. In similar tests, application of the commercial formulation of this antagonist prevented the growth of E. coli O157:H7 in wounds when inoculated 1 or 2 days after application of the antagonist. Populations of E. coli O157:H7 in wounds treated with water (control) before inoculation with this pathogen increased approximately 2 log units during the first 48 h

after inoculation. These results indicate that biocontrol agents developed for controlling storage decays of fruits may have the additional benefit of preventing the growth of foodborne pathogens in freshly wounded tissue of intact and fresh-cut fruits

Janisiewicz,W.J., W.S.Conway, M.W.Brown, G.M.Sapers, P.Fratamico, and R.L.Buchanan. 1999. "Fate of Escherichia coli O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies." *Appl.Environ.Microbiol.* 65:1-5.
Abstract: Pathogenic Escherichia coli O157:H7, as well as nonpathogenic strains ATCC 11775 and ATCC 23716, grew exponentially in wounds on Golden Delicious apple fruit. The exponential growth occurred over a longer time period on fruit inoculated with a lower concentration of the bacterium than on fruit inoculated with a higher concentration. The bacterium reached the maximum population supported in the wounds regardless of the initial inoculum concentrations. Populations of E. coli O157:H7 in various concentrations of sterilized apple juice and unsterilized cider declined over time and declined more quickly in diluted juice and cider. The decline was greater in the unsterilized cider than in juice, which may have resulted from the interaction of E. coli O157:H7 with natural populations of yeasts that increased with time. Experiments on the transmission of E. coli by fruit flies, collected from a compost pile of decaying apples and peaches, were conducted with strain F-11775, a fluorescent transformant of nonpathogenic E. coli ATCC 11775. Fruit flies were easily contaminated externally and internally with E. coli F-11775 after contact with the bacterium source. The flies transmitted this bacterium to uncontaminated apple wounds, resulting in a high incidence of contaminated wounds. Populations of the bacterium in apple wounds increased significantly during the first 48 h after transmission. Further studies under commercial conditions are necessary to confirm these findings

Jiang,X. and M.P.Doyle. 1999. "Fate of Escherichia coli O157:H7 and Salmonella Enteritidis on currency." *J.Food Prot.* 62:805-807.
Abstract: The fate of foodborne pathogens Escherichia coli O157:H7 and Salmonella Enteritidis on coin surfaces was determined at room temperature (25 degrees C). A five-strain mixture of E. coli O157:H7 or Salmonella Enteritidis of approximately 5 x 10(4) CFU was applied to the surfaces of sterile U.S. coins (pennies, nickels, dimes, and quarters) and to the surfaces of two control substrata (Teflon and glass coverslips). During storage at room temperature, E. coli O157:H7 survived for 7, 9, and 11 days on the surfaces of pennies, nickels, and dimes and quarters, respectively. However, the pathogen died off within 4 to 7 days on both the Teflon and glass surfaces. Salmonella Enteritidis survived for 1, 2, 4, and 9 days on the surfaces of pennies, nickels, quarters, and dimes, respectively. Unlike E. coli O157:H7, survival of Salmonella Enteritidis was greatest on both Teflon and glass coverslips, with more than 100 cells per substratum detected at the 17th day of storage. Results indicate that coins could serve as potential vehicles for transmitting both E. coli O157:H7 and Salmonella Enteritidis

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

Jiang,X., J.Morgan, and M.P.Doyle. 2003. "Fate of Escherichia coli O157:H7 during composting of bovine manure in a laboratory-scale bioreactor." *J.Food Prot.* 66:25-30.
Abstract: Inactivation profiles of Escherichia coli O157:H7 in inoculated bovine manure-based compost ingredients were determined by composting these ingredients in a bioreactor under controlled conditions. A 15-liter bioreactor was constructed to determine the fate of E. coli O157:H7 and changes in pH, moisture content, temperature, and aerobic mesophilic and thermophilic bacterial counts during composting. Fresh cow manure, wheat straw, cottonseed meal, and ammonium sulfate were combined to obtain a moisture content of ca. 60% and a carbon/nitrogen ratio of 29:1. The compost ingredients were held in the bioreactor at a constant external temperature of 21 or 50 degrees C. Self-heating of the ingredients due to microbial activity occurred during composting, with stratified temperatures occurring within the bioreactor. At an external temperature of 21 degrees C, self-heating occurred for 0 to 3 days, depending on the location within the bioreactor. E. coli O157:H7 populations increased by 1 to 2 log10 CFU/g during the initial 24 h of composting and decreased by ca. 3.5 log10 CFU/g near the bottom of the bioreactor and by ca. 2 log10 CFU/g near the middle and at the top during 36 days of composting. At an external temperature of 50 degrees C. E. coli O157:H7 was inactivated rapidly (by ca. 4.9 log10 CFU/g at the top of the bioreactor, by 4.0 log10 CFU/g near the middle, and by 5.9 log10 CFU/g near the bottom) within 24 h of composting. When inoculated at an initial level of ca. 10(7) CFU/g. E. coli O157:H7 survived for 7 days but not for 14 days at all three sampling locations, as indicated by either direct plating or enrichment culture. At the top of the bioreactor a relatively constant moisture content of 60% was maintained, whereas the moisture content near the bottom decreased steadily to 37 to 45% over 14 days of composting. The pH of the composting mixture decreased to ca. 6 within 1 to 3 days and subsequently increased to 8 to 9. Results obtained in this study indicate that large populations (10(4) to 10(7) CFU g) of E coli O157:H7 survived for 36 days during composting in a bioreactor at an external temperature of 21 degrees C but were inactivated to undetectable levels after 7 to 14 days when the external temperature of the bioreactor was 50 degrees C. Hence, manure contaminated with large populations (e.g., 10(7) CFU/g) of E. coli O157:H7 should be composted for more than 1 week, and preferably for 2 weeks, when held at a minimum temperature of 50 degrees C

Jiang,X., M.Islam, J.Morgan, and M.P.Doyle. 2004. "Fate of Listeria monocytogenes in bovine manure-amended soil." *J.Food Prot.* 67:1676-1681.
Abstract: The survival and growth of Listeria monocytogenes in soil amended with bovine manure was studied under different environmental conditions of temperature, nutrients, and soil microflora. Autoclaved soil was compared with unautoclaved soil for assessing the influence of competitive soil microflora on the survival of L.

monocytogenes. Initial L. monocytogenes cell numbers of 5 to 6 log CFU/g survived for up to 43, 43, and 14 days in manure-amended autoclaved soil at 5, 15, and 21 degrees C, respectively. In manure-amended unautoclaved soil, the pathogen was detectable for up to 43, 21, and 21 days at 5, 15, and 21 degrees C, respectively. L. monocytogenes was inactivated more rapidly in autoclaved soil amended with manure at a manure/soil ratio of 1:10 than in the more dilute (1:100) manure in soil samples at both 15 and 21 degrees C. However, in manure-amended unautoclaved soil, L. monocytogenes survived longer in samples with ratios of 1:10 than in the more dilute (1:100) manure-amended soil. The persistence of L. monocytogenes for several weeks in manure-amended soil suggests listeriae could be transmitted through soil to fresh produce or to shoes, clothing, and hands of field workers, especially during the cold months

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

Johannessen,G.S., G.B.Bengtsson, B.T.Heier, S.Bredholt, Y.Wasteson, and L.M.Rorvik. 2005. "Potential uptake of Escherichia coli O157:H7 from organic manure into crisphead lettuce." *Appl.Environ.Microbiol.* 71:2221-2225.
Abstract: To investigate the potential transfer of Escherichia coli O157:H7 from contaminated manure to fresh produce, lettuce seedlings were transplanted into soil fertilized with bovine manure which had been inoculated with approximately 10(4) CFU g(-1) E. coli O157:H7. The lettuce was grown for approximately 50 days in beds in climate-controlled rooms in a greenhouse. As the bacterium was not detected in the edible parts of the lettuce, the outer leaves of the lettuce, or the lettuce roots at harvest it was concluded that transmission of E. coli O157:H7 from contaminated soil to lettuce did not occur. The pathogen persisted in the soil for at least 8 weeks after fertilizing but was not detected after 12 weeks. Indigenous E. coli was detected only sporadically on the lettuce at harvest, and enterococci were not detected at all. The numbers of enterococci declined more rapidly than those of E. coli in the soil. Pseudomonas fluorescens, which inhibited growth of E. coli O157:H7 in vitro, was isolated from the rhizosphere

Johannessen,G.S., C.E.James, H.E.Allison, D.L.Smith, J.R.Saunders, and A.J.McCarthy. 2005. "Survival of a Shiga toxin-encoding bacteriophage in a compost model." *FEMS Microbiol.Lett.* 245:369-375.
Abstract: Bacteriophages that carry the Shiga toxin gene (stx) represent an additional hazard in cattle manure-based fertilizers in that their survival could lead to toxigenic conversion of Escherichia coli and other bacteria post-composting. A Stx-phage in which

the Shiga toxin (stx(2)) gene was inactivated by insertion of a chloramphenicol resistance gene was used in combination with a rifampicin-resistant E. coli host where RecA is constitutively activated so that all infectious phage particles could be enumerated by plaque assay. PCR-based confirmation methods and the additional application of a host enrichment protocol ensured that very low numbers of surviving bacteriophage could be detected and unequivocally identified. Stx-bacteriophage numbers declined rapidly over the first 48 h and none could be detected after 3 days. The host enrichment method was applied after 6 days and no bacteriophages were recovered. While addition of fresh E. coli cells at intervals after the compost temperature had reduced below 40 degrees C demonstrated that E. coli growth could be supported in the compost, Stx-phages or their lysogens were never detected. Here, we demonstrate that composting animal manure for 40 days during which a temperature of >60 degrees C is maintained for at least 5 days is effective at removing both E. coli and a model infectious Stx-encoding bacteriophage

Johnson,J.Y., J.E.Thomas, T.A.Graham, I.Townshend, J.Byrne, L.B.Selinger, and V.P.Gannon. 2003. "Prevalence of Escherichia coli O157:H7 and Salmonella spp. in surface waters of southern Alberta and its relation to manure sources." *Can.J.Microbiol.* 49:326-335.
Abstract: The Oldman River watershed in southern Alberta, Canada, is an extensively irrigated region in which intensive agricultural practices have flourished. Concern over water quality in the basin has been expressed because of high levels of enteric disease indigenous to the region. To address these concerns, we conducted a 2-year study to estimate the prevalence of Escherichia coli O157:H7 and Salmonella spp. in surface water within the basin. This study is the first of its kind to identify E. coli O157:H7 repeatedly in surface water collected from a Canadian watershed. Prevalence of E. coli O157:H7 and Salmonella spp. in water samples was 0.9% (n = 1,483) and 6.2% (n = 1,429), respectively. While data examined at a regional level show a relationship between high livestock density and high pathogen levels in southern Alberta, statistical analysis of point source data indicates that predicted manure output from bovine, swine, and poultry feeding operations was not directly associated with either Salmonella spp. or E. coli O157:H7 prevalence. However, geography and weather variables, which are likely to influence bacterial runoff, were not considered in this model. We also postulate that variations in time, amount, and frequency of manure application onto agricultural lands may have influenced levels of surface-water contamination with these bacterial pathogens

Johnston,L.M. and L.A.Jaykus. 2004. "Antimicrobial resistance of Enterococcus species isolated from produce." *Appl.Environ.Microbiol.* 70:3133-3137.
Abstract: The purpose of this study was to characterize the antibiotic resistance profiles of Enterococcus species isolated from fresh produce harvested in the southwestern United States. Among the 185 Enterococcus isolates obtained, 97 (52%) were Enterococcus faecium, 38 (21%) were Enterococcus faecalis, and 50 (27%) were other Enterococcus species. Of human clinical importance, E. faecium strains had a much higher prevalence of resistance to ciprofloxacin, tetracycline, and nitrofurantoin than E. faecalis. E. faecalis strains had a low prevalence of resistance to antibiotics used to treat E. faecalis infections of both clinical and of agricultural relevance, excluding its intrinsic resistance patterns. Thirty-four percent of the isolates had multiple-drug-resistance patterns, excluding intrinsic resistance. Data on the prevalence and types of antibiotic resistance in Enterococcus species isolated from fresh produce may be used to describe baseline antibiotic susceptibility profiles associated with Enterococcus spp. isolated from the environment. The data collected may also help elucidate the role of foods in the transmission of antibiotic-resistant strains to human populations

Johnston,L.M., L.A.Jaykus, D.Moll, M.C.Martinez, J.Anciso, B.Mora, and C.L.Moe. 2005. "A field study of the microbiological quality of fresh produce." *J.Food Prot.* 68:1840-1847.
Abstract: The Centers for Disease Control and Prevention has reported that foodborne

disease outbreaks associated with fruits and vegetables increased during the past decade. This study was conducted to characterize the routes of microbial contamination in produce and to identify areas of potential contamination from production through postharvest handling. We report here the levels of bacterial indicator organisms and the prevalence of selected pathogens in produce samples collected from the southern United States. A total of 398 produce samples (leafy greens, herbs, and cantaloupe) were collected through production and the packing shed and assayed by enumerative tests for total aerobic bacteria, total coliforms, total Enterococcus, and Escherichia coli. These samples also were analyzed for Salmonella, Listeria monocytogenes, and E. coli O157:H7. Microbiological methods were based on methods recommended by the U.S. Food and Drug Administration. For all leafy greens and herbs, geometric mean indicator levels ranged from 4.5 to 6.2 log CFU/g (aerobic plate count); less than 1 to 4.3 log CFU g (coliforms and Enterococcus); and less than 1 to 1.5 log CFU/g (E. coli). In many cases, indicator levels remained relatively constant throughout the packing shed, particularly for mustard greens. However, for cilantro and parsley, total coliform levels increased during the packing process. For cantaloupe, microbial levels significantly increased from field through packing, with ranges of 6.4 to 7.0 log CFU/g (aerobic plate count); 2.1 to 4.3 log CFU/g (coliforms); 3.5 to 5.2 log CFU/g (Enterococcus); and less than 1 to 2.5 log CFU/g (E. coli). The prevalence of pathogens for all samples was 0, 0, and 0.7% (3 of 398) for L. monocytogenes, E. coli O157:H7, and Salmonella, respectively. This study demonstrates that each step from production to consumption may affect the microbial load of produce and reinforces government recommendations for ensuring a high-quality product

Johnston,L.M., L.A.Jaykus, D.Moll, J.Anciso, B.Mora, and C.L.Moe. 2006. "A field study of the microbiological quality of fresh produce of domestic and Mexican origin." *Int.J.Food Microbiol.* 112:83-95.

Abstract: Produce is responsible for an increasingly larger proportion of foodborne disease outbreaks. In particular, the globalization of the food supply may introduce new food safety risks and allow widespread distribution of contaminated food, particularly produce. The objectives of this study were to: (i) compare the overall quality of domestic and Mexican produce throughout the packing process; (ii) examine changes in microbiological quality of both domestic and Mexican produce at each stage of production and processing; and (iii) evaluate the prevalence of select pathogens on fresh produce, including leafy green, herbs, melons, and vegetables. Furthermore, we also sought to characterize the antibiotic resistance profiles of Enterococcus faecium and Enterococcus faecalis strains isolated from fresh produce. A total of 466 produce and matching environmental swab samples was collected from various locations in packing sheds in the southern US from November 2002 through December 2003. These samples were assayed by enumerative tests for total aerobic bacteria (APC), total coliforms, total Enterococcus, and E. coli. Produce samples were also analyzed for the presence of Salmonella, Listeria monocytogenes, Shigella, and E. coli O157:H7. A total of 112 E. faecium and E. faecalis isolates were further screened for antibiotic resistance using a panel of seventeen antibiotics. Overall, the microbiological quality of fresh produce ranged from 4.0 to 7.9 log(10) CFU/g (APC); less than 1.0 log(10) to 4.5 log(10) CFU/g (coliforms); less than 1.0 log(10) to 4.0 log(10) CFU/g (E. coli); and less than 1.0 log(10) to 5.4 log(10) CFU/g (Enterococcus). No Salmonella, Shigella, or E. coli O157:H7 were detected from the 466 25-g produce samples tested. However, three domestic cabbage samples were found to be positive for L. monocytogenes. Of the Enterococcus isolates, E. faecium had a higher degree of resistance to antibiotics in general, while Enterococcus spp. isolated from Mexican produce had a higher degree of antibiotic resistance when compared to strains isolated from produce samples of domestic origin. Despite increased attention to the role of imported produce in foodborne disease, this study does not support the assumption that domestic produce is of higher microbial quality than Mexican produce

Jonsson,M.E., A.Aspan, E.Eriksson, and I.Vagsholm. 2001. "Persistence of verocytotoxin-producing Escherichia coli O157:H7 in calves kept on pasture and in calves kept indoors during the summer months in a Swedish dairy herd." *Int.J.Food Microbiol.* 66:55-61.
Abstract: In 1997, a Swedish dairy farm was implicated in a human case of verocytotoxigenic Escherichia coli (VTEC) infection. The bacterium was found in a faecal sample from the human case and in faecal samples from cattle on the farm. Subtyping with pulsed field gel electrophoresis (PFGE) showed that the isolates were identical. The farm was further studied to assess the occurrence and the epidemiology of the agent at the farm level. The objective of this part of the study presented here was to examine the persistence of VTEC O157:H7 in calves that were kept on pasture and indoors, respectively, during the summer. Twelve calves in the herd, with one positive faecal sample each of VTEC O157:H7 in April 1999, were followed by faecal sampling during the summer months. Six calves were kept indoors and six were kept on pasture. Faecal samples from each calf were collected once a month on five occasions from April to September. Bacterial examination was performed with immunomagnetic separation (IMS) and cultivation on CT-SMAC. PCR was used to test for the presence of genes encoding for verocytotoxin (VT), intimin (eaeA), enterohemorrhagic E. coli-hemolysin (EHEC-Hly) and the flagellar antigen H7. PFGE was used for genotyping the isolates. The faecal samples from the calves kept on pasture were negative during the whole period. It is possible that the faecal samples had bacterial counts lower than the detection limits for our procedure, or that the faecal samples were free from the bacteria at the time of sampling. This suggests that calves on pasture may be less exposed to the bacteria or that they clear themselves. In the pen group, there were between one and six culture positive individuals per sampling occasion. One of the calves that was housed indoors was positive in faecal culture on four consecutive samplings

Karmali,M.A. 2004. "Infection by Shiga toxin-producing Escherichia coli: an overview." *Mol.Biotechnol.* 26:117-122.
Abstract: Shiga toxin-producing Escherichia coli (STEC), especially of serotype O157:H7, cause a zoonotic food or waterborne enteric illness that is often associated with large epidemic outbreaks as well as the hemolytic uremic syndrome (HUS), the leading cause of acute renal failure in children. After ingestion, STEC colonize enterocytes of the large bowel with a characteristic attaching and effacing pathology, which is mediated by components of a type III secretion apparatus encoded by the LEE pathogenicity island. Shiga toxins are translocated from the bowel to the circularoty system and transported by leukocytes to capillary endothelial cells in renal glomeruli and other organs. After binding to the receptor globotriaosylceramide on target cells, the toxin is internalized by receptor-mediated endocytosis and interacts with the subcellular machinery to inhibit protein synthesis. This leads to pathophysiological changes that result in HUS. Specific therapeutic or preventive strategies are presently not available. The recent sequencing of genomes of two epidemic E. coli O157 strains has revealed novel pathogenicity islands which will likely provide new insights into the virulence of these bacteria

Keen,J.E., T.E.Wittum, J.R.Dunn, J.L.Bono, and L.M.Durso. 2006. "Shiga-toxigenic Escherichia coli O157 in agricultural fair livestock, United States." *Emerg.Infect.Dis.* 12:780-786.
Abstract: Agricultural fairs exhibiting livestock are increasingly implicated in human Shiga-toxigenic Escherichia coli O157:H7 (STEC O157:H7) outbreaks. To estimate livestock STEC O157:H7 prevalence at US fairs, we collected 2,919 fecal specimens at 29 county fairs in 2 states and at 3 state fairs in 2002. Fly pools were also collected. STEC O157:H7 was isolated from livestock at 31 (96.9%) of 32 fairs, including 11.4% of 1,407 cattle, 1.2% of 1,102 swine, 3.6% of 364 sheep and goats, and 5.2% of 154 fly pools. Cattle, swine, and flies at some fairs shared indistinguishable STEC O157:H7 isolate subtypes. In 2003, a total of 689 ambient environmental samples were collected at 20 fairgrounds 10-11 months after 2002 livestock sampling while fairgrounds were

livestock-free. Four beef barn environmental samples at 3 fairgrounds yielded STEC O157:H7. These data suggest that STEC O157 is common and transmissible among livestock displayed at agricultural fairs and persists in the environment after the fair

Keene,W.E., K.Hedberg, D.E.Herriott, D.D.Hancock, R.W.McKay, T.J.Barrett, and D.W.Fleming. 1997. "A prolonged outbreak of Escherichia coli O157:H7 infections caused by commercially distributed raw milk." *J.Infect.Dis.* 176:815-818.
Abstract: A protracted outbreak of Escherichia coli O157:H7 infections was caused by consumption of unpasteurized ("raw") milk sold at Oregon grocery stores. Although it never caused a noticeable increase in reported infections, the outbreak was recognized because of routine follow-up interviews. Six of 16 Portland-area cases reported between December 1992 and April 1993 involved people who drank raw milk from dairy A. By pulsed-field gel electrophoresis (PFGE), E. coli O157:H7 isolates from these cases and from the dairy A herd were homologous (initially, 4 of 132 animals were E. coli O157:H7-positive). Despite public warnings, new labeling requirements, and increased monitoring of dairy A, retail sales and dairy-associated infections continued until June 1994 (a total of 14 primary cases). Seven distinguishable PFGE patterns in 3 homology groups were identified among patient and dairy herd E. coli O157:H7 isolates. Without restrictions on distribution, E. coli O157:H7 outbreaks caused by raw milk consumption can continue indefinitely, with infections occurring intermittently and unpredictably

Keene,W.E., E.Sazie, J.Kok, D.H.Rice, D.D.Hancock, V.K.Balan, T.Zhao, and M.P.Doyle. 1997. "An outbreak of Escherichia coli O157:H7 infections traced to jerky made from deer meat." *JAMA*. 277:1229-1231.
Abstract: OBJECTIVE: To investigate a 1995 outbreak of Escherichia coli O157:H7 infections and to assess the safety of meat dehydration methods. DESIGN: Survey subsequent to routine surveillance report, environmental investigations, and laboratory experimentation. SETTING: Oregon community. PARTICIPANTS: Members of an extended household and their social contacts with confirmed or presumptive E coli O157:H7 infections. RESULTS: A total of 6 confirmed and 5 presumptive cases were identified. Homemade venison jerky was implicated as the source of transmission. E coli O157:H7 with the same distinctive, pulsed-field gel electrophoresis pattern seen in the case isolates was recovered from leftover jerky, uncooked meat from the same deer, a saw used to dismember the carcass, and fragments of the deer hide. In a subsequent survey, E coli O157:H7 was recovered from 3 (9%) of 32 deer fecal pellets collected in nearby forest land. In the laboratory, inoculated venison was dried at several time and temperature combinations, ranging up to 10 hours at 62.8 degrees C. Viable organisms were recovered under all conditions tested. CONCLUSIONS: Deer can be colonized by E coli O157:H7 and can be a source of human infections. Conditions necessary to ensure the safety of dried meat deserve further review. Game should be handled with the same caution indicated for commercially slaughtered meat

Kenney,S.J., S.L.Burnett, and L.R.Beuchat. 2001. "Location of Escherichia coli O157:H7 on and in apples as affected by bruising, washing, and rubbing." *J.Food Prot.* 64:1328-1333.
Abstract: Confocal scanning laser microscopy (CSLM) was used to determine the location of Escherichia coli O157:H7 cells on the surface and in tissue of bruised Red Delicious cv. apples. Undamaged and bruised apples were inoculated by immersing in a suspension of E. coli O157:H7 cells transformed with a plasmid that encodes for the production of a green fluorescent protein. Apples were then washed in 0.1% (wt/vol) peptone water and/or rubbed with a polyester cloth and examined to determine if these treatments removed or introduced cells into lenticels, cutin, and cracks on the skin surface. Optical slices of the apples obtained using CSLM were examined to determine the depth at which colonization or attachment of cells occurred. Populations of E. coli O157:H7 on the surface of apples were determined to assess the effectiveness of washing and rubbing in physically removing cells. The location of cells on or in undamaged and bruised areas of apples that were not washed or rubbed did not differ

significantly. However, washing apples resulted in an approximate 2-log reduction in CFU of E. coli O157:H7 per cm2 of apple surface. On unwashed apples, cells were detected at depths up to 30 microm below the surface. No E. coli O157:H7 cells were detected at locations more than 6 microm below the surface of washed apples. Cells that remained on the surface of rubbed apples appeared to be sealed within naturally occurring cracks and crevices in waxy cutin platelets. These cells may be protected from disinfection and subsequently released when apples are eaten or pressed for cider production

Kenney,S.J., G.L.Anderson, P.L.Williams, P.D.Millner, and L.R.Beuchat. 2005. "Persistence of Escherichia coli O157:H7, Salmonella Newport, and Salmonella Poona in the gut of a free-living nematode, Caenorhabditis elegans, and transmission to progeny and uninfected nematodes." *Int.J.Food Microbiol.* 101:227-236.
Abstract: A study was undertaken to determine the persistence of Escherichia coli O157:H7 and salmonellae in the gut of a free-living nematode, Caenorhabditis elegans, as affected by temperature and relative humidity and to determine if infected worms transmit Salmonella enterica serotype Newport to progeny and uninfected worms. Worms were fed cells of a non-pathogenic strain of E. coli (OP50), E. coli O157:H7, S. enterica serotype Newport, and S. enterica serotype Poona, followed by incubating at 4, 20, or 37 degrees C for up to 5 days. Initial populations of ingested pathogens significantly increased by up to 2.93 log(10) cfu/worm within 1 day at 20 degrees C on K agar and remained constant for an additional 4 days. When worms were placed on Bacto agar, populations of ingested pathogens remained constant at 4 degrees C, decreased significantly at 20 degrees C, and increased significantly at 37 degrees C within 3 days. Worms fed E. coli OP50 or S. Newport were incubated at 4 or 20 degrees C at relative humidities of 33%, 75%, or 98% to determine survival characteristics of ingested bacteria. Fewer cells of the pathogens survived incubation at 33% relative humidity compared to higher relative humidities. Populations of ingested E. coli OP50 and S. Newport decreased by up to 1.65 and 3.44 log(10) cfu/worm, respectively, in worms incubated at 20 degrees C and 33% relative humidity. Placement together on K agar of adult worms, labeled with green fluorescent protein (gfp) in the pharynx area, that had ingested gfp-labeled S. Newport and uninfected wild type worms resulted in transfer of the pathogen to gut of wild type worms. S. Newport was isolated from C. elegans two generations removed from exposure to the pathogen. Results of these studies show that C. elegans may serve as a temporary reservoir of foodborne pathogens, and could perhaps be a vector for contaminating preharvest fruits and vegetables, thus potentially increasing the risk of enteric infections associated with consumption of raw produce

Kenney,S.J., G.L.Anderson, P.L.Williams, P.D.Millner, and L.R.Beuchat. 2006. "Migration of Caenorhabditis elegans to manure and manure compost and potential for transport of Salmonella newport to fruits and vegetables." *Int.J.Food Microbiol.* 106:61-68.
Abstract: A study was done to determine if a free-living, bacterivorous nematode, Caenorhabditis elegans, migrates to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with Salmonella Newport. Movement of the worm to lettuce, strawberries, and carrots was also studied. C. elegans moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of C. elegans in test materials were not affected by the presence of S. newport. Bovine manure and bovine manure compost inoculated with S. newport (8.6 log10 CFU/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with C. elegans. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20 degrees C for up to 10 days. In the system using soil inoculated with C. elegans, S. newport initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was

detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when C. elegans was not present in the soil. Results indicate that C. elegans has the potential for transporting S. newport in soil to the surface of preharvest fruits and vegetables in contact with soil

Khachatryan,A.R., D.D.Hancock, T.E.Besser, and D.R.Call. 2004. "Role of calf-adapted Escherichia coli in maintenance of antimicrobial drug resistance in dairy calves." *Appl.Environ.Microbiol.* 70:752-757.
Abstract: The prevalence of antimicrobial drug-resistant bacteria is typically highest in younger animals, and prevalence is not necessarily related to recent use of antimicrobial drugs. In dairy cattle, we hypothesize that antimicrobial drug-resistant, neonate-adapted bacteria are responsible for the observed high frequencies of resistant Escherichia coli in calves. To explore this issue, we examined the age distribution of antimicrobial drug-resistant E. coli from Holstein cattle at a local dairy and conducted an experiment to determine if low doses of oxytetracycline affected the prevalence of antimicrobial drug-resistant E. coli. Isolates resistant to tetracycline (>4 microg/ml) were more prevalent in <3-month-old calves (79%) compared with lactating cows (14%). In an experimental trial where calves received diets supplemented with or without oxytetracycline, the prevalence of tetracycline-resistant E. coli was slightly higher for the latter group (P = 0.039), indicating that drug use was not required to maintain a high prevalence of resistant E. coli. The most common resistance pattern among calf E. coli isolates included resistance to streptomycin (>12 microg/ml), sulfadiazine (>512 microg ml), and tetracycline (>4 microg/ml) (SSuT), and this resistance pattern was most prevalent during the period when calves were on milk diets. To determine if prevalence was a function of differential fitness, we orally inoculated animals with nalidixic acid-resistant strains of SSuT E. coli and susceptible E. coli. Shedding of SSuT E. coli was significantly greater than that of susceptible strains in neonatal calves (P < 0.001), whereas there was no difference in older animals (P = 0.5). These data support the hypothesis that active selection for traits linked to the SSuT phenotype are responsible for maintaining drug-resistant E. coli in this population of dairy calves

Kim,J.F. and J.R.Alfano. 2002. "Pathogenicity islands and virulence plasmids of bacterial plant pathogens." *Curr.Top.Microbiol.Immunol.* 264:127-147.

King,J.C., R.E.Black, M.P.Doyle, K.L.Fritsche, B.H.Halbrook, O.A.Levander, S.N.Meydani, W.A.Walker, and C.E.Woteki. 2000. "Foodborne illnesses and nutritional status: a statement from an American Society for Nutritional Sciences Working Group." *J.Nutr.* 130:2613-2617.

Kuhnert,P., P.Boerlin, and J.Frey. 2000. "Target genes for virulence assessment of Escherichia coli isolates from water, food and the environment." *FEMS Microbiol.Rev.* 24:107-117.
Abstract: The widespread species Escherichia coli includes a broad variety of different types, ranging from highly pathogenic strains causing worldwide outbreaks of severe disease to avirulent isolates which are part of the normal intestinal flora or which are well characterized and safe laboratory strains. The pathogenicity of a given E. coli strain is mainly determined by specific virulence factors which include adhesins, invasins, toxins and capsule. They are often organized in large genetic blocks either on the chromosome ('pathogenicity islands'), on large plasmids or on phages and can be transmitted horizontally between strains. In this review we summarize the current knowledge of the virulence attributes which determine the pathogenic potential of E. coli strains and the methodology available to assess the virulence of E. coli isolates. We also focus on a recently developed procedure based on a broad-range detection system for E. coli-specific virulence genes that makes it possible to determine the potential pathogenicity and its nature in E. coli strains from various sources. This makes it possible to determine the pathotype of E. coli strains in medical diagnostics, to assess

the virulence and health risks of E. coli contaminating water, food and the environment and to study potential reservoirs of virulence genes which might contribute to the emergence of new forms of pathogenic E. coli

Kuhnert,P., C.R.Dubosson, M.Roesch, E.Homfeld, M.G.Doherr, and J.W.Blum. 2005. "Prevalence and risk-factor analysis of Shiga toxigenic Escherichia coli in faecal samples of organically and conventionally farmed dairy cattle." *Vet.Microbiol.* 109:37-45.
Abstract: Cattle are a natural reservoir for Shiga toxigenic Escherichia coli (STEC), however, no data are available on the prevalence and their possible association with organic or conventional farming practices. We have therefore studied the prevalence of STEC and specifically O157:H7 in Swiss dairy cattle by collecting faeces from approximately 500 cows from 60 farms with organic production (OP) and 60 farms with integrated (conventional) production (IP). IP farms were matched to OP farms and were comparable in terms of community, agricultural zone, and number of cows per farm. E. coli were grown overnight in an enrichment medium, followed by DNA isolation and PCR analysis using specific TaqMan assays. STEC were detected in all farms and O157:H7 were present in 25% of OP farms and 17% of IP farms. STEC were detected in 58% and O157:H7 were evidenced in 4.6% of individual faeces. Multivariate statistical analyses of over 250 parameters revealed several risk-factors for the presence of STEC and O157:H7. Risk-factors were mainly related to the potential of cross-contamination of feeds and cross-infection of cows, and age of the animals. In general, no significant differences between the two farm types concerning prevalence or risk for carrying STEC or O157:H7 were observed. Because the incidence of human disease caused by STEC in Switzerland is low, the risk that people to get infected appears to be small despite a relatively high prevalence in cattle. Nevertheless, control and prevention practices are indicated to avoid contamination of animal products

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

Lahti,E., M.Eklund, P.Ruutu, A.Siitonen, L.Rantala, P.Nuorti, and T.Honkanen-Buzalski. 2002. "Use of phenotyping and genotyping to verify transmission of Escherichia coli O157:H7 from dairy farms." *Eur.J.Clin.Microbiol.Infect.Dis.* 21:189-195.
Abstract: A total of 80 human infections by Escherichia coli O157:H7 were documented in Finland in 1997 and 1998. Most were sporadic and their sources undetermined. Five cases not associated with one another, one of which led to secondary transmission within a family, could be traced to five different dairy farms. These five case patients (age range 2-17 years, median age 3 years) were hospitalised with bloody diarrhoea; two of them developed haemolytic uraemic syndrome. All nine human isolates obtained were sorbitol negative, carried the verocytotoxin 2 and eae genes, and produced

verocytotoxin and enterohaemolysin. The phage and pulsed-field gel electrophoresis types of the human and bovine isolates from the corresponding farms were indistinguishable. The cattle (20-70 animals per farm) were monitored for up to 2 years after the human cases. The proportion of cattle excreting the type that caused the human infections varied from 3.2 to 66.7% when sampled soon after the human cases, and from 0.0 to 5.3% about a year or so later. On most of the farms, the animals excreted the pathogen intermittently. On one farm, Escherichia coli O157 isolates with other characteristics were also occasionally isolated. Although the infections were traced back to the farms, it could not be established whether the source was unpasteurised milk or direct or indirect contact with cattle. The results of this study emphasise the need for special recommendations for children visiting or living on a farm to prevent these infections

Lahti,E., O.Ruoho, L.Rantala, M.L.Hanninen, and T.Honkanen-Buzalski. 2003. "Longitudinal study of Escherichia coli O157 in a cattle finishing unit." *Appl.Environ.Microbiol.* 69:554-561.
Abstract: In a longitudinal study in a Finnish cattle finishing unit we investigated excretion and sources of Escherichia coli O157 in bulls from postweaning until slaughter. Three groups of 31 to 42 calves were sampled in a calf transporter before they entered the farm and four to seven times at approximately monthly intervals at the farm. All calves sampled in the livestock transporter were negative for E. coli O157 on arrival, whereas positive animals were detected 1 day later. During the fattening period the E. coli O157 infection rate varied between 0 and 38.5%. The animals were also found to be shedding during the cold months. E. coli O157 was isolated from samples taken from water cups, floors, and feed passages. E. coli O157 was detected in 9.7 to 38.9% of the fecal samples taken at slaughter, while only two rumen samples and one carcass surface sample were found to be positive. E. coli O157 was isolated from barn surface samples more often when the enrichment time was 6 h than when the enrichment time was 24 h ($P < 0.0001$). Fecal samples taken at the abattoir had lower counts (< or = 0.4 MPN/g) than fecal samples at the farm ($P < 0.05$). E. coli O157 was isolated more often from 10-g fecal samples than from 1-g fecal samples ($P < 0.0001$). Most farm isolates belonged to one pulsed-field gel electrophoresis (PFGE) genotype (79.6%), and the rest belonged to closely related PFGE genotypes. In conclusion, this study indicated that the finishing unit rather than introduction of new cattle was the source of E. coli O157 at the farm and that E. coli O157 seemed to persist well on barn surfaces

Lanz,R., P.Kuhnert, and P.Boerlin. 2003. "Antimicrobial resistance and resistance gene determinants in clinical Escherichia coli from different animal species in Switzerland." *Vet.Microbiol.* 91:73-84.
Abstract: Antimicrobial susceptibility testing was performed on a total of 581 clinical Escherichia coli isolates from diarrhea and edema disease in pigs, from acute mastitis in dairy cattle, from urinary tract infections in dogs and cats, and from septicemia in laying hens collected in Switzerland between 1999 and 2001. Among the 16 antimicrobial agents tested, resistance was most frequent for sulfonamides, tetracycline, and streptomycin. Isolates from swine presented significantly more resistance than those from the other animal species. The distribution of the resistance determinants for sulfonamides, tetracycline, and streptomycin was assessed by hybridization and PCR in resistant isolates. Significant differences in the distribution of resistance determinants for tetracycline (tetA, tetB) and sulfonamides (sulII) were observed between the isolates from swine and those from the other species. Resistance to sulfonamides could not be explained by known resistance mechanisms in more than a quarter of the sulfonamide-resistant and sulfonamide-intermediate isolates from swine, dogs and cats. This finding suggests that one or several new resistance mechanisms for sulfonamides may be widespread among E. coli isolates from these animal species. The integrase gene (intI) from class I integrons was detected in a large proportion of resistant isolates

in association with the sulI and aadA genes, thus demonstrating the importance of integrons in the epidemiology of resistance in clinical E. coli isolates from animals

Lau,M.M. and S.C.Ingham. 2001. "Survival of faecal indicator bacteria in bovine manure incorporated into soil." *Lett.Appl.Microbiol.* 33:131-136.
Abstract: AIMS: Survival of Escherichia coli and enterococci was evaluated in bovine manure incorporated into two Wisconsin soils. METHODS AND RESULTS: Silty clay loam (SCL) and loamy sand (LS) were mixed with fresh bovine manure, exposed daily to 10 h at 22 degrees C/14 h at 9 degrees C, and watered weekly for 12 weeks. Escherichia coli numbers increased 1-2 log cfu g(-1), then decreased < 1 and about 2 log cfu g(-1) in SCL and LS, respectively. Enterococci numbers rose less and then declined faster than those of E. coli. Watering intervals of 3, 7 and 14 days were evaluated in weeks 13-19, but did not affect the slow decline in numbers of E. coli or enterococci. CONCLUSION: Escherichia coli and enterococci may survive at least 19 weeks at 9-21 degrees C in bovine manure/soil, with E. coli surviving better. SIGNIFICANCE AND IMPACT OF THE STUDY: Quantification of E. coli or enterococci in late spring/early summer soil may be useful in indicating recent application of bovine manure

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, and D.D.Hancock. 2001. "Cattle water troughs as reservoirs of Escherichia coli O157." *Appl.Environ.Microbiol.* 67:3053-3057.
Abstract: Environmental survival of Escherichia coli O157 may play an important role in the persistence and dissemination of this organism on farms. The survival of culturable and infectious E. coli O157 was studied using microcosms simulating cattle water troughs. Culturable E. coli O157 survived for at least 245 days in the microcosm sediments. Furthermore, E. coli O157 strains surviving more than 6 months in contaminated microcosms were infectious to a group of 10-week-old calves. Fecal excretion of E. coli O157 by these calves persisted for 87 days after challenge. Water trough sediments contaminated with feces from cattle excreting E. coli O157 may serve as a long-term reservoir of this organism on farms and a source of infection for cattle

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

LeJeune,J.T., D.D.Hancock, and T.E.Besser. 2006. "Sensitivity of Escherichia coli O157 detection in bovine feces assessed by broth enrichment followed by immunomagnetic separation and direct plating methodologies." *J.Clin.Microbiol.* 44:872-875.
Abstract: In order to more precisely predict food safety risks, the fecal presence of food-borne pathogens among animals at slaughter must be correctly determined. Quantification of Escherichia coli O157 is also desirable. In two separate experiments, detection and enumeration of a nalidixic acid-resistant strain of E. coli O157 in bovine feces was assessed by culture on MacConkey agar supplemented with nalidixic acid (MACnal) and compared to overnight broth enrichment followed by immunomagnetic separation (IMS) and to direct plating of dilutions of bovine feces onto sorbitol MacConkey agar containing cefixime and tellurite (SMACct). The sensitivity of detection of E. coli O157 by both direct plating and IMS was highly dependent upon the initial concentration of the target organism in the sample. Sensitivity of detection by IMS was poor below 100 CFU/g but was better, and not affected by initial E. coli O157 numbers, above this concentration. Sensitivity of detection of E. coli O157 in bovine feces at low initial concentrations is very poor for both direct plating and IMS. Direct plating of dilutions of bovine feces on SMACct can be used to determine the magnitude of fecal E. coli excretion among cattle excreting greater than 100 CFU/g. Among positive samples identified by direct plating on SMACct, the direct counts of E. coli O157:H7 were highly correlated with the estimates obtained with the MACnal plates ($r = 0.88$, $P < 0.001$). Because the majority of cattle excrete less than 10(2) CFU E. coli O157/g feces, most studies, including those using IMS methods, probably grossly underestimate the prevalence of E. coli O157 in cattle

Leveau,J.H. and S.E.Lindow. 2001. "Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere." *Proc.Natl.Acad.Sci.U.S.A*. 98:3446-3453.
Abstract: We report here the construction, characterization, and application of a bacterial bioreporter for fructose and sucrose that was designed to monitor the availability of these sugars to microbial colonizers of the phyllosphere. Plasmid pP(fruB)-gfp[AAV] carries the Escherichia coli fruB promoter upstream from the gfp[AAV] allele that codes for an unstable variant of green fluorescent protein (GFP). In Erwinia herbicola, this plasmid brings about the accumulation of GFP fluorescence in response to both fructose and sucrose. Cells of E. herbicola (pP(fruB)-gfp[AAV]) were sprayed onto bean plants, recovered from leaves at various time intervals after inoculation, and analyzed individually for GFP content by quantitative analysis of digital microscope images. We observed a positive correlation between single-cell GFP accumulation and ribosomal content as determined by fluorescence in situ hybridization, indicating that foliar growth

of E. herbicola occurred at the expense of fructose and/or sucrose. One hour after inoculation, nearly all bioreporter cells appeared to be actively engaged in fructose consumption. This fraction dropped to approximately 11% after 7 h and to approximately 1% a day after inoculation. This pattern suggests a highly heterogeneous availability of fructose to individual E. herbicola cells as they colonize the phyllosphere. We estimated that individual cells were exposed to local initial fructose abundances ranging from less than 0.15 pg fructose to more than 4.6 pg

Lin,C.M., S.S.Moon, M.P.Doyle, and K.H.McWatters. 2002. "Inactivation of Escherichia coli O157:H7, Salmonella enterica serotype enteritidis, and Listeria monocytogenes on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat." *J.Food Prot.* 65:1215-1220.
Abstract: Iceberg lettuce is a major component in vegetable salad and has been associated with many outbreaks of foodborne illnesses. In this study, several combinations of lactic acid and hydrogen peroxide were tested to obtain effective antibacterial activity without adverse effects on sensory characteristics. A five-strain mixture of Escherichia coli O157:H7, Salmonella enterica serotype Enteritidis, and Listeria monocytogenes was inoculated separately onto fresh-cut lettuce leaves, which were later treated with 1.5% lactic acid plus 1.5% hydrogen peroxide (H2O2) at 40 degrees C for 15 min, 1.5% lactic acid plus 2% H2O2 at 22 degrees C for 5 min, and 2% H2O2 at 50 degrees C for 60 or 90 s. Control lettuce leaves were treated with deionized water under the same conditions. A 4-log reduction was obtained for lettuce treated with the combinations of lactic acid and H2O2 for E. coli O157:H7 and Salmonella Enteritidis, and a 3-log reduction was obtained for L. monocytogenes. However, the sensory characteristics of lettuce were compromised by these treatments. The treatment of lettuce leaves with 2% H2O2 at 50 degrees C was effective not only in reducing pathogenic bacteria but also in maintaining good sensory quality for up to 15 days. A < or = 4-log reduction of E. coli O157:H7 and Salmonella Enteritidis was achieved with the 2% H2O2 treatment, whereas a 3-log reduction of L. monocytogenes was obtained. There was no significant difference (P > 0.05) between pathogen population reductions obtained with 2% H2O2 with 60- and 90-s exposure times. Hydrogen peroxide residue was undetectable (the minimum level of sensitivity was 2 ppm) on lettuce surfaces after the treated lettuce was rinsed with cold water and centrifuged with a salad spinner. Hence, the treatment of lettuce with 2% H2O2 at 50 degrees C for 60 s is effective in initially reducing substantial populations of foodborne pathogens and maintaining high product quality

Lin,C.M., F.M.Wu, H.K.Kim, M.P.Doyle, B.S.Michael, and L.K.Williams. 2003. "A comparison of hand washing techniques to remove Escherichia coli and caliciviruses under natural or artificial fingernails." *J.Food Prot.* 66:2296-2301.
Abstract: Compared with other parts of the hand, the area beneath fingernails harbors the most microorganisms and is most difficult to clean. Artificial fingernails, which are usually long and polished, reportedly harbor higher microbial populations than natural nails. Hence, the efficacy of different hand washing methods for removing microbes from natural and artificial fingernails was evaluated. Strains of nonpathogenic Escherichia coli JM109 and feline calicivirus (FCV) strain F9 were used as bacterial and viral indicators, respectively. Volunteers with artificial or natural nails were artificially contaminated with ground beef containing E. coli JM109 or artificial feces containing FCV. Volunteers washed their hands with tap water, regular liquid soap, antibacterial liquid soap, alcohol-based hand sanitizer gel, regular liquid soap followed by alcohol gel, or regular liquid soap plus a nailbrush. The greatest reduction of inoculated microbial populations was obtained by washing with liquid soap plus a nailbrush, and the least reduction was obtained by rubbing hands with alcohol gel. Lower but not significantly different (P > 0.05) reductions of E. coli and FCV counts were obtained from beneath artificial than from natural fingernails. However, significantly (P < or = 0.05) higher E. coli and FCV counts were recovered from hands with artificial nails than from natural nails before and

after hand washing. In addition, microbial cell numbers were correlated with fingernail length, with greater numbers beneath fingernails with longer nails. These results indicate that best practices for fingernail sanitation of food handlers are to maintain short fingernails and scrub fingernails with soap and a nailbrush when washing hands

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

Mahon,B.E., P.M.Griffin, P.S.Mead, and R.V.Tauxe. 1997. "Hemolytic uremic syndrome surveillance to monitor trends in infection with Escherichia coli O157:H7 and other shiga toxin-producing E. coli." *Emerg.Infect.Dis.* 3:409-412.

Mak,P.P., B.H.Ingham, and S.C.Ingham. 2001. "Validation of apple cider pasteurization treatments against Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes." *J.Food Prot.* 64:1679-1689.
Abstract: Time and temperature pasteurization conditions common in the Wisconsin cider industry were validated using a six-strain cocktail of Escherichia coli O157:H7 and acid-adapted E. coli O157:H7 in pH- and degrees Brix-adjusted apple cider. Strains employed were linked to outbreaks (ATCC 43894 and 43895, C7927, and USDA-FSIS-380-94) or strains engineered to contain the gene for green fluorescent protein (pGFP ATCC 43894 and pGFP ATCC 43889) for differential enumeration. Survival of Salmonella spp. (CDC 0778. CDC F2833, and CDC H0662) and Listeria monocytogenes (H0222, F8027, and F8369) was also evaluated. Inoculated cider of pH 3.3 or 4.1 and 11 or 14 degrees Brix was heated under conditions ranging from 60 degrees C for 14 s to 71.1 degrees C for 14 s. A 5-log reduction of nonadapted and acid-adapted E. coli O157:H7 was obtained at 68.1 degrees C for 14 s. Lower

temperatures, or less time at 68.1 degrees C, did not ensure a 5-log reduction in E. coli O157:H7. A 5-log reduction was obtained at 65.6 degrees C for 14 s for Salmonella spp. L. monocytogenes survived 68.1 degrees C for 14 s, but survivors died in cider within 24 h at 4 degrees C. Laboratory results were validated with a surrogate E coli using a bench-top plate heat-exchange pasteurizer. Results were further validated using fresh unpasteurized commercial ciders. Consumer acceptance of cider pasteurized at 68.1 degrees C for 14 s (Wisconsin recommendations) and at 71.1 degrees C for 6 s (New York recommendations) was not significantly different. Hence, we conclude that 68.1 degrees C for 14 s is a validated treatment for ensuring adequate destruction of E. coli O157:H7, Salmonella spp., and L. monocytogenes in apple cider

McCluskey,B.J., D.H.Rice, D.D.Hancock, C.J.Hovde, T.E.Besser, S.Gray, and R.P.Johnson. 1999. "Prevalence of Escherichia coli O157 and other Shiga-toxin-producing E. coli in lambs at slaughter." *J.Vet.Diagn.Invest*. 11:563-565.

McGee,P., D.J.Bolton, J.J.Sheridan, B.Earley, and N.Leonard. 2001. "The survival of Escherichia coli O157:H7 in slurry from cattle fed different diets." *Lett.Appl.Microbiol.* 32:152-155.
Abstract: AIMS: The survival characteristics of Escherichia Coli O157:H7 were investigated in bovine slurry from cattle fed two different diets: (i) silage and (ii) silage + concentrates. METHODS AND RESULTS: Slurry samples collected from freshly-agitated tanks were inoculated at a level of log10 6.0 cfu g(-1) and stored in the laboratory at 10 degrees C. Over a 12 week storage period, a 3.5 and 5.5 log reduction was observed in slurry from cattle fed a silage and silage plus concentrate diet, respectively. CONCLUSIONS: The persistence of E. coli O157:H7 in slurry over a 3 month storage period indicates its potential for transmitting the organism back into the environment. SIGNIFICANCE AND IMPACT OF THE STUDY: The discussion concludes however, that despite pathogen survival in slurry, it may not represent a major source of transmission in the farm environment

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

McGee,P., L.Scott, J.J.Sheridan, B.Earley, and N.Leonard. 2004. "Horizontal transmission of Escherichia coli O157:H7 during cattle housing." *J.Food Prot.* 67:2651-2656.
Abstract: Ruminant livestock, particularly cattle, is considered the primary reservoir of Escherichia coli O157:H7. This study examines the transmission of E. coli O157:H7

within groups of cattle during winter housing. Holstein Friesian steers were grouped in six pens of five animals. An animal inoculated with and proven to be shedding a marked strain of E. coli O157: H7 was introduced into each pen. Fecal (rectal swabs) and hide samples (900 cm2 from the right rump) were taken from the 36 animals throughout the study. Water, feed, and gate or partition samples from each pen were also examined. Within 24 h of introducing the inoculated animals into the pens, samples collected from the drinking water, pen barriers, and animal hides were positive for the pathogen. Within 48 h, the hides of 20 (66%) of 30 cohort animals from the six pens were contaminated with E. coli O157:H7. The first positive fecal samples from the noninoculated cohort animals were detected 3 days after the introduction of the inoculated steers. During the 23 days of the study, 15 of 30 cohort animals shed the marked E. coli O157:H7 strain in their feces on at least one occasion. Animal behavior in the pens was monitored during a 12-h period using closed circuit television cameras. The camera footage showed an average of 13 instances of animal grooming in each pen per hour. The study suggests that transmission of E. coli O157:H7 between animals may occur following ingestion of the pathogen at low levels and that animal hide may be an important source of transmission

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.Finelli, M.A.Lambert-Fair, D.Champ, J.Townes, L.Hutwagner, T.Barrett, K.Spitalny, and E.Mintz. 1997. "Risk factors for sporadic infection with Escherichia coli O157:H7." *Arch.Intern.Med.* 157:204-208.
Abstract: BACKGROUND: Little is known about risk factors for sporadic infection with Escherichia coli O157:H7. In response to a sharp increase in reported cases in New Jersey during July 1994, we conducted a case-control study to identify principal sources of infection and contributing practices. METHODS: Standardized questionnaires were used to evaluate (1) potential exposures of case patients and matched controls and (2) knowledge, attitudes, and practices of food preparers in case and control households. Patient isolates were subtyped by pulsed-field gel electrophoresis. RESULTS: Patients with E coli O157:H7 infection (N = 23; median age, 9 years; 55% female) were more likely than healthy controls to have eaten a hamburger in the week preceding illness (matched odds ratio, undefined; P < .001); 80% of the hamburgers eaten by ill persons were prepared at home. Food preparers in case households were less likely than those in control households to report washing their hands (odds ratio, 8.5; P < .005) and work surfaces (odds ratio, 10.5; P < .05) after handling raw ground beef. Pulsed-field gel electrophoresis yielded 17 unique subtypes among the 23 patient isolates, indicating multiple sources of infection. CONCLUSIONS: Hamburgers prepared at home are an important source of sporadic E coli O157:H7 infections. We estimate that adequate hand washing by food preparers could have prevented 34% of E coli O157:H7 infections in the study population

Mead,P.S. and P.M.Griffin. 1998. "Escherichia coli O157:H7." *Lancet*. 352:1207-1212.
Abstract: Escherichia coli O157 was first identified as a human pathogen in 1982. One of several Shiga toxin-producing serotypes known to cause human illness, the organism probably evolved through horizontal acquisition of genes for Shiga toxins and other virulence factors. E. coli O157 is found regularly in the faeces of healthy cattle, and is transmitted to humans through contaminated food, water, and direct contact with infected people or animals. Human infection is associated with a wide range of clinical illness, including asymptomatic shedding, non-bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome, and death. Since laboratory practices vary, physicians need to know whether laboratories in their area routinely test for E. coli O157 in stool specimens. Treatment with antimicrobial agents remains controversial: some studies suggest that treatment may precipitate haemolytic uraemic syndrome, and other studies suggest no effect or even a protective effect. Physicians can help to prevent E. coli O157 infections by counselling patients about the hazards of consuming undercooked ground meat or unpasteurised milk products and juices, and about the importance of handwashing to prevent the spread of diarrhoeal illness, and by informing public-health authorities when they see unusual numbers of cases of bloody diarrhoea or haemolytic uraemic syndrome

Mead,P.S., L.Slutsker, P.M.Griffin, and R.V.Tauxe. 1999. "Food-related illness and death in the united states reply to dr. hedberg." *Emerg.Infect.Dis.* 5:841-842.

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

Meals,D.W. and D.C.Braun. 2006. "Demonstration of methods to reduce E. coli runoff from dairy manure application sites." *J.Environ.Qual.* 35:1088-1100.
Abstract: Contamination by bacteria is a leading cause of impairment in U.S. waters, particularly in areas of livestock agriculture. We evaluated the effectiveness of several practices in reducing Escherichia coli levels in runoff from fields receiving liquid dairy (Bos taurus) manure. Runoff trials were conducted on replicated hay and silage corn (Zea mays L.) plots using simulated rainfall. Levels of E. coli in runoff were approximately 10(4) to 10(6) organisms per 100 mL, representing a significant pollution potential. Practices tested were: manure storage, delay between manure application and rainfall, manure incorporation by tillage, and increased hayland vegetation height. Storage of manure for 30 d or more consistently and dramatically lowered E. coli counts in our experiments, with longer storage providing greater reductions. Manure E. coli declined by > 99% after approximately 90 d of storage. On average, levels of E. coli in runoff were 97% lower from plots receiving 30-d-old and > 99% lower from plots receiving 90-d-old manure than from plots where fresh manure was applied. Runoff from hayland and cornland plots where manure was applied 3 d before rainfall contained approximately 50% fewer E. coli than did runoff from plots that received manure 1 d before rainfall. Hayland vegetation height alone did not significantly affect E. coli levels in runoff, but interactions with rainfall delay and manure age were observed. Manure incorporation alone did not significantly affect E. coli levels in cornland plot runoff, but incorporation could reduce bacteria export by reducing field runoff and interaction with rainfall delay was observed. Extended storage that avoids additions of fresh manure, combined with application several days before runoff, incorporation on tilled land, and higher vegetation on hayland at application could substantially reduce microorganism loading from agricultural land

Mechie,S.C., P.A.Chapman, and C.A.Siddons. 1997. "A fifteen month study of Escherichia coli O157:H7 in a dairy herd." *Epidemiol.Infect.* 118:17-25.
Abstract: A dairy herd associated with Escherichia coli O157 infection in humans was studied for the 15 months following the outbreak to examine seasonal, age and management factors affecting faecal excretion of the organism and to determine the mode and frequency of milk contamination with the organism. Between May 1993 and July 1994, 28 visits were made to the farm to collect a total of 3593 rectal swabs from cows, heifers and calves and 329 milk samples. E. coli O157:H7 was isolated from 153 (4.3%) of 3593 bovine rectal swabs. The maximum prevalence at any one visit was 14% in lactating cows, 40% in non-lactating cows, 56% in calves and 68% in heifers. The prevalence in lactating cows, which was significantly lower than in the other groups, peaked during May-July 1993 and again briefly after the cattle were housed during November 1993 and then again during May 1994. Excretion rates of E. coli O157:H7 in lactating cows were highest during the first month after calving, falling during lactation and rising to another peak at 7 months postpartum. Between November 1993 and May 1994 there was no evidence of excretion in any group. Eighty-seven (74%) of the animals which excreted E. coli O157:H7 did so on only one occasion but 23 (32%) of 73 cows and heifers and 7 (16%) of 44 calves which excreted the organism did so on more than one occasion. E. coli O157:H7 was not isolated from milk taken from the bulk tank but it was isolated from individual milk samples (one milk jar and one fore-milk) from two animals previously shown to be faecal excretors of the organism. All isolates of E. coli O157:H7 obtained were of the same phage type, toxin genotype and plasmid profile

Meng,J., S.Zhao, M.P.Doyle, and S.W.Joseph. 1998. "Antibiotic resistance of Escherichia coli O157:H7 and O157:NM isolated from animals, food, and humans." *J.Food Prot.* 61:1511-1514.

Abstract: Antibiotic resistance was determined for 118 E. coli O157:H7 and 7 O157:NM isolates from animals, foods, and humans. Among the 125 isolates, 30 (24%) were resistant to at least one antibiotic and 24 (19%) were resistant to three or more antibiotics. Cattle isolates had the highest rate (34%) of antibiotic resistance. The seven resistant food isolates were all from ground beef. The most frequent resistance type overall was streptomycin-sulfisoxazole-tetracycline, which accounted for over 70% of the resistant strains. Two E. coli O157:NM isolates from cattle were resistant to six antibiotics: ampicillin, kanamycin, sulfisoxazole, streptomycin, tetracycline, and ticarcillin. Streptomycin was the most common antibiotic to which E. coli O157:H7 and O157:NM were resistant (29 out of 30 isolates), followed by tetracycline (26 isolates). This study suggests that E. coli O157:H7 and O157:NM have developed resistance to antibiotics. Research is needed to define mechanisms of antibiotic resistance in E. coli O157:H7 and to minimize the development of resistance

Molbak,K., P.S.Mead, and P.M.Griffin. 2002. "Antimicrobial therapy in patients with Escherichia coli O157:H7 infection." *JAMA*. 288:1014-1016.

Morrow,A.L. and J.M.Rangel. 2004. "Human milk protection against infectious diarrhea: implications for prevention and clinical care." *Semin.Pediatr.Infect.Dis.* 15:221-228.
Abstract: Breastfeeding is the major strategy for prevention of morbidity and mortality resulting from diarrhea in the first few years of life. Health-system and community based interventions have been shown to increase the prevalence of breastfeeding and reduce the incidence of diarrhea and associated healthcare costs in infancy. The protective effect of breastfeeding is attributable to a complex of acquired and innate factors unique to human milk that have anti-infective, anti-inflammatory, and immunoregulatory functions, including secretory antibodies, oligosaccharides, glycoconjugates, lactoferrin, leukocytes, cytokines, and other agents. The American Academy of Pediatrics recommends exclusive breastfeeding until the infant is approximately 6 months of age, with timely introduction of complementary foods and continued breastfeeding to a year, or longer if desired. The number of deaths of children that could be prevented worldwide each year if these breastfeeding recommendations were followed has been estimated to be more than 1 million

Muirhead,R.W., R.P.Collins, and P.J.Bremer. 2006. "Numbers and transported state of Escherichia coli in runoff direct from fresh cowpats under simulated rainfall." *Lett.Appl.Microbiol.* 42:83-87.
Abstract: AIMS: To investigate the number of Escherichia coli in runoff derived directly from fresh cowpats and to determine if the E. coli are attached to dense particles, in flocs or as individual cells. METHODS AND RESULTS: Three cowpats were collected monthly from the same farm for 13 months and the number of E. coli in them estimated. A rainfall simulator was used to generate runoff from the individual cowpats, which was fractioned to determine the transported state of any E. coli present. The number of E. coli in the cowpat runoff was highly variable and was strongly correlated with the number of E. coli in the cowpat. Only a small percentage (approx. 8%) of the E. coli in runoff were attached to dense (>1.3 g ml(-1)) particles and there was no evidence of flocculation of the cells. CONCLUSIONS: Escherichia coli in runoff from cowpats are transported predominantly as individual cells. SIGNIFICANCE AND IMPACT OF THE STUDY: Mitigation strategies to reduce the number of faecal bacteria in overland flow from agricultural land need to be designed to trap single bacterial cells

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.
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.
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.Scheftel, 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.
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

Murinda,S.E., L.T.Nguyen, T.L.Landers, F.A.Draughon, A.G.Mathew, J.S.Hogan, K.L.Smith, D.D.Hancock, and S.P.Oliver. 2004. "Comparison of Escherichia coli Isolates from humans, food, and farm and companion animals for presence of Shiga toxin-producing E. coli virulence markers." *Foodborne.Pathog.Dis.* 1:178-184.
Abstract: The objective of this study was to characterize Escherichia coli isolates from dairy cows/feedlots, calves, mastitis, pigs, dogs, parrot, iguana, human disease, and food products for prevalence of Shiga toxin-producing E. coli (STEC) virulence markers. The rationale of the study was that, isolates of the same serotypes that were obtained from different sources and possessed the same marker profiles, could be cross-species transmissible. Multiplex polymerase chain reaction (PCR) was used to detect presence of genes encoding Shiga toxin 1 and 2 (stx1 and stx2), H7 flagella (flicC), enterohemolysin (hly) and intimin (eaeA) in E. coli isolates (n = 400). Shiga toxin-producing isolates were tested for production of Shiga toxins (Stx1 and Stx2 and enterohemolysin. Of the E. coli O157:H7/H- strains, 150 of 164 (mostly human, cattle, and food) isolates were stx+. Sixty-five percent of O157 STEC produced both Stx1 and Stx2; 32% and 0.7% produced Stx2 or Stx1, respectively. Ninety-eight percent of O157 STEC had sequences for genes encoding intimin and enterohemolysin. Five of 20 E. coli O111, 4 of 14 O128 and 4 of 10 O26 were stx+ . Five of 6 stx+ O26 and O111 produced Stx1, however, stx+ O128 were Stx-negative. Acid resistance (93.3%) and tellurite resistance (87.3%) were common attributes of O157 STEC, whereas, non-O157 stx+ strains exhibited 38.5% and 30.8% of the respective resistances. stx-positive isolates were mostly associated with humans and cattle, whereas, all isolates from mastitis (n = 105), and pigs, dogs, parrot and iguanas (n = 48) were stx-negative. Multiplex PCR was an effective tool for characterizing STEC pathogenic profiles and distinguished STEC O157:H7 from other STEC. Isolates from cattle and human disease shared similar toxigenic profiles, whereas isolates from other disease sources had few characteristics in common with the former isolates. These data suggest interspecies transmissibility of certain serotypes, in particular, STEC O157:H7, between humans and cattle

Nagels,J.W., R.J.Davies-Colley, A.M.Donnison, and R.W.Muirhead. 2002. "Faecal contamination over flood events in a pastoral agricultural stream in New Zealand." *Water Sci.Technol.* 45:45-52.
Abstract: Faecal bacterial dynamics during flood events were studied in the Topehaehae Stream near Morrinsville, New Zealand, in a catchment used for grazing dairy and beef cattle. During the rising limb of a natural flood event, E. coli bacterial concentration rose by more than 2 orders of magnitude and peaked at 41,000 cfu/100 mL. E. coli correlated closely with turbidity over the flood event, and both variables peaked close to the time of maximum flow acceleration rather than peak flow. An artificial flood on the same stream, created by releasing water from a supply reservoir during fine weather with no wash-in from the catchment, produced a broadly similar pattern of faecal contamination (peak E. coli = 12,500 cfu/100 mL). This and other evidence suggests that direct deposition of faecal matter by cattle in the stream channel may be of similar or greater importance than wash-in from land. The flood experiments have been useful for constructing a

model of faecal bacterial yields, and they imply that exclusion of livestock from stream channels may appreciably improve water quality

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

Natvig,E.E., S.C.Ingham, B.H.Ingham, L.R.Cooperband, and T.R.Roper. 2002. "Salmonella enterica serovar Typhimurium and Escherichia coli contamination of root and leaf vegetables grown in soils with incorporated bovine manure." *Appl.Environ.Microbiol.* 68:2737-2744.
Abstract: Bovine manure, with or without added Salmonella enterica serovar Typhimurium (three strains), was incorporated into silty clay loam (SCL) and loamy sand (LS) soil beds (53- by 114-cm surface area, 17.5 cm deep) and maintained in two controlled-environment chambers. The S. enterica serovar Typhimurium inoculum was 4 to 5 log CFU/g in manure-fertilized soil. The conditions in the two environmental chambers, each containing inoculated and uninoculated beds of manure-fertilized soil, simulated daily average Madison, Wis., weather conditions (hourly temperatures, rainfall, daylight, and humidity) for a 1 March or a 1 June manure application and subsequent vegetable growing seasons ending 9 August or 28 September, respectively. Core soil samples were taken biweekly from both inoculated and uninoculated soil beds in each chamber. Radishes, arugula, and carrots were planted in soil beds, thinned, and harvested. Soils, thinned vegetables, and harvested vegetables were analyzed for S. enterica serovar Typhimurium and Escherichia coli (indigenous in manure). After the 1 March manure application, S. enterica serovar Typhimurium was detected at low levels in both soils on 31 May, but not on vegetables planted 1 May and harvested 12 July from either soil. After the 1 June manure application, S. enterica serovar Typhimurium was detected in SCL soil on 7 September and on radishes and arugula planted in SCL soil on 15 August and harvested on 27 September. In LS soil, S. enterica serovar Typhimurium died at a similar rate (P >or= 0.05) after the 1 June manure application and was less often detected on arugula and radishes harvested from this soil compared to the SCL soil. Pathogen levels on vegetables were decreased by washing. Manure application in cool (daily average maximum temperature of <10 degrees C) spring conditions is recommended to ensure that harvested vegetables are not contaminated with S. enterica serovar Typhimurium. Manure application under warmer (daily average maximum temperature >20 degrees C) summer conditions is not recommended when vegetable planting is done between the time of manure application and late summer. A late fall manure application will not increase the risk of contaminating vegetables planted the next spring, since further experiments showed that repeated freeze-thaw cycles were detrimental to the survival of S. enterica serovar Typhimurium and E. coli in manure-fertilized soil. The number of indigenous E. coli in soil was never significantly lower (P < 0.05) than that of S. enterica serovar Typhimurium, suggesting its usefulness as an indicator organism for evaluating the risk of vegetable contamination with manure-borne S. enterica serovar Typhimurium

Nicholson,F.A., S.J.Groves, and B.J.Chambers. 2005. "Pathogen survival during livestock manure storage and following land application." *Bioresour.Technol.* 96:135-143.
Abstract: This paper reports the first year results of field experiments to determine the survival times of pathogens in livestock manures during storage and following land application, using viable count methods. E. coli O157, Salmonella and Campylobacter survived in stored slurries and dirty water for up to three months, with Listeria surviving for up to three months. In contrast, all these pathogens survived for less than one month in solid manure heaps where temperatures greater than 55 degrees C were obtained. Following manure spreading to land, E. coli O157, Salmonella and Campylobacter generally survived in the soil for up to one month after application to both the sandy arable and clay loam grassland soils, whereas Listeria commonly survived for more than

one month. These data are being used to develop guidelines on the management of manures to minimize the risks of pathogen transfer from animal manures to the human food chain

Ogden,L.D., D.R.Fenlon, A.J.Vinten, and D.Lewis. 2001. "The fate of Escherichia coli O157 in soil and its potential to contaminate drinking water." *Int.J.Food Microbiol.* 66:111-117.
Abstract: The survival and transport of Escherichia coli and E. coli O157 after cattle slurry application were studied on drained plots in both grassland and arable stubble at three sites in Scotland. Leaching losses were between 0.2% and 10% of total E. coli and were dependent on rainfall. Recovery of E. coli in grass and soil declined with approximately first order kinetics. Residual numbers, in excess of background declined more slowly. The pattern was similar for both grass and arable plots. Laboratory incubations of soil cores, with applied slurry containing E. coli and E. coli O157 were performed in soils with different moisture contents at two temperatures for clay loam and sandy loam soils. Both E. coli populations were measured over a 4-week period. Using a dual population approach, the die off of the susceptible pool was linear with a half-life of 3-4 days, and was faster at the higher temperature and lowest moisture content. The resistant pool was not strongly affected by temperature or moisture and had a half-life for die off of between 18 and 24 days. After a 4-week period, < 100 cfu g/soil of E. coli and E. coli O157 remained. The die off rate of E. coli O157 was the same or slightly faster than that of the commensal E. coli population, indicating that the field behaviour of E. coli O157 can be studied by monitoring the total population of E. coli applied with slurry. The risk of significant pollution of water by E. coli is highest immediately after application of slurry, and the first increments of drainflow carry significant concentrations. Thereafter, the risk of pollution is very low. If weather conditions are dry after application on well-drained sandy soils, it is unlikely that any significant losses of organisms to drains will occur. Such data can be used to control and minimise the risk of E. coli O157 contaminating drinking water

Oliver,D.M., L.Heathwaite, P.M.Haygarth, and C.D.Clegg. 2005. "Transfer of Escherichia coli to water from drained and undrained grassland after grazing." *J.Environ.Qual.* 34:918-925.
Abstract: The aim of this study was to determine the load of Escherichia coli transferred via drainage waters from drained and undrained pasture following a grazing period. Higher concentrations (ranging between 10(4) and 10(3) colony forming units [CFU] g(-1)) of E. coli persisted in soil for up to 60 d beyond the point where cattle were removed from the plots, but these eventually declined in the early months of spring to concentrations less than 10(2) CFU g(-1). The decline reflects the combined effect of cell depletion from the soil store through both wash-out and die-off of E. coli. No difference (P > 0.05) was observed in E. coli loads exported from drained and undrained plots. Similarly, no difference (P > 0.05) was observed in E. coli concentrations in drainage waters of mole drain flow and overland plus subsurface interflow. Intermittent periods of elevated discharge associated with storm events mobilized E. coli at higher concentrations (e.g., in excess of 400 CFU mL(-1)) than observed during low flow conditions (often <25 CFU mL(-1)). The combination of high discharge and cell concentrations resulted in the export of E. coli loads from drained and undrained plots exceeding 10(6) CFU L(-1) s(-1). The results highlight the potential for drained land to export E. coli loads comparable with those transferred from undrained pasture

Park,G.W. and F.Diez-Gonzalez. 2003. "Utilization of carbonate and ammonia-based treatments to eliminate Escherichia coli O157:H7 and Salmonella Typhimurium DT104 from cattle manure." *J.Appl.Microbiol.* 94:675-685.
Abstract: AIMS: The objective of this study was to investigate alkaline treatments of cattle manure to kill coliforms, Escherichia coli O157:H7 and Salmonella Typhimurium DT104 based on their inhibition by carbonate ion and ammonia. METHODS AND RESULTS: Pure cultures of S. Typhimurium DT104 and E. coli O157:H7 strains were treated with sodium carbonate and ammonia to determine threshold inhibitory

concentrations. Fresh cattle manure samples were inoculated with the same strains and their survival was determined after addition of sodium hydroxide, ammonium sulphate, sodium carbonate and/or urea. Control of CO and NH3 concentrations in manure by pH adjustment to 9.5 with sodium hydroxide to more than 5 and 30 mmol l-1, respectively, killed more than 106 cells g-1 in 7 days. Addition of sodium carbonate enhanced the killing effect of NaOH by increasing the CO and NH3 concentrations. Addition of 100 mmol l-1 urea, produced high levels of CO and NH3 and decreased all bacterial counts by at least 106 cells g-1 after 7 days. CONCLUSIONS: Reduction of food-borne pathogens in manure can be achieved by a combination of high concentrations of CO and NH3 which are pH-dependent parameters. SIGNIFICANCE AND IMPACT OF STUDY: Addition of urea could provide a simple manure treatment by combining both antimicrobial factors

Paros,M., P.I.Tarr, H.Kim, T.E.Besser, and D.D.Hancock. 1993. "A comparison of human and bovine Escherichia coli O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage lambda-restriction fragment length polymorphism profile." *J.Infect.Dis.* 168:1300-1303.
Abstract: Foods of bovine origin have been linked to human disease outbreaks caused by Escherichia coli O157:H7 and may be linked to the more common sporadic cases as well. In this study, E. coli O157:H7 from the bovine reservoir (22 isolates: 12 from dairy and 10 from beef breed cows) and from human patients (50 isolates from sporadic human infections) were compared using Shiga-like toxin genotypes, plasmid profiles, and DNA restriction fragment length polymorphisms identified with a bacteriophage lambda probe (lambda-RFLP). Twenty-three lambda-RFLP profiles, 4 Shiga-like toxin genotypes, and 8 plasmid profiles were identified among the isolates tested. Together the typing methods distinguished 43 strains, of which 3 were isolated from both humans (5 isolates) and cattle (6 isolates; 5 from dairy herds). These data demonstrate the value of lambda-RFLP as a means of strain identification for E. coli O157:H7

Parveen,S., J.Lukasik, T.M.Scott, M.L.Tamplin, K.M.Portier, S.Sheperd, K.Braun, and S.R.Farrah. 2006. "Geographical variation in antibiotic resistance profiles of Escherichia coli isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida." *J.Appl.Microbiol.* 100:50-57.
Abstract: AIMS: The aim of this study was to assess geographical variation in multiple antibiotic resistance (MAR) profiles of livestock Escherichia coli as well as to evaluate the ability of MAR profiles to differentiate sources of faecal pollution. METHODS AND RESULTS: More than 2000 E. coli isolates were collected from water retention ponds and manure of swine, poultry, beef and dairy farms in south, central and north Florida, and analysed for MAR using nine antibiotics. There were significant differences in antibiotic resistance of E. coli by season and livestock type for more than one antibiotic, but regional differences were significant only for ampicillin. Over the three regions, discriminant analysis using MAR profiles correctly classified 27% of swine, 49% of poultry, 56% of beef and 51% of dairy isolates. CONCLUSIONS: Regional variations in MAR combined with moderate discrimination success suggest that MAR profiles of E. coli may only be marginally successful in identifying sources of faecal pollution. SIGNIFICANCE AND IMPACT OF THE STUDY: This study demonstrates the existence of regional and seasonal differences in MAR profiles as well as the limited ability of MAR profiles to discriminate among livestock sources

Paunio,M., R.Pebody, M.Keskimaki, M.Kokki, P.Ruutu, S.Oinonen, V.Vuotari, A.Siitonen, E.Lahti, and P.Leinikki. 1999. "Swimming-associated outbreak of Escherichia coli O157:H7." *Epidemiol.Infect.* 122:1-5.
Abstract: In 1997 the first outbreak of Escherichia coli O157:H7 infections involving 14 cases occurred in Finland. A case was defined as a resident of Alavus with an episode of diarrhoea between 5 and 17 July 1997, and from whom E. coli O157:H7 was isolated from stool. The investigation included case searching and a population-based case

control study. Five primary and eight symptomatic secondary cases of E. coli O157:H7 illness were detected. In the 10 days before the outbreak, all 5 primary patients (aged 3-8 years), but only 6 of 32 population controls from the same age range (Fisher's test, P < 0.001) and 4 of 10 sibling controls (P < 0.05) had visited (but had not necessarily bathed in) a shallow beach popular among young children. Four out of 5 primary cases had remained within 5 m of the beach while swimming and had swallowed lake water compared to 1 of 5 population controls. These analytical epidemiologic findings incriminated fresh lake water as the vehicle of E. coli O157:H7 transmission

Pearce,M.C., C.Jenkins, L.Vali, A.W.Smith, H.I.Knight, T.Cheasty, H.R.Smith, G.J.Gunn, M.E.Woolhouse, S.G.Amyes, and G.Frankel. 2004. "Temporal shedding patterns and virulence factors of Escherichia coli serogroups O26, O103, O111, O145, and O157 in a cohort of beef calves and their dams." *Appl.Environ.Microbiol.* 70:1708-1716.
Abstract: This study investigated the shedding of Escherichia coli O26, O103, O111, O145, and O157 in a cohort of beef calves from birth over a 5-month period and assessed the relationship between shedding in calves and shedding in their dams, the relationship between shedding and scouring in calves, and the effect of housing on shedding in calves. Fecal samples were tested by immunomagnetic separation and by PCR and DNA hybridization assays. E. coli O26 was shed by 94% of calves. Over 90% of E. coli O26 isolates carried the vtx(1), eae, and ehl genes, 6.5% carried vtx(1) and vtx(2), and one isolate carried vtx(2) only. Serogroup O26 isolates comprised seven pulsed-field gel electrophoresis (PFGE) patterns but were dominated by one pattern which represented 85.7% of isolates. E. coli O103 was shed by 51% of calves. Forty-eight percent of E. coli O103 isolates carried eae and ehl, 2% carried vtx(2), and none carried vtx(1). Serogroup O103 isolates comprised 10 PFGE patterns and were dominated by two patterns representing 62.5% of isolates. Shedding of E. coli O145 and O157 was rare. All serogroup O145 isolates carried eae, but none carried vtx(1) or vtx(2). All but one serogroup O157 isolate carried vtx(2), eae, and ehl. E. coli O111 was not detected. In most calves, the temporal pattern of E. coli O26 and O103 shedding was random. E. coli O26 was detected in three times as many samples as E. coli O103, and the rate at which calves began shedding E. coli O26 for the first time was five times greater than that for E. coli O103. For E. coli O26, O103, and O157, there was no association between shedding by calves and shedding by dams within 1 week of birth. For E. coli O26 and O103, there was no association between shedding and scouring, and there was no significant change in shedding following housing

Petersen,A., J.P.Christensen, P.Kuhnert, M.Bisgaard, and J.E.Olsen. 2006. "Vertical transmission of a fluoroquinolone-resistant Escherichia coli within an integrated broiler operation." *Vet.Microbiol.* 116:120-128.
Abstract: The epidemiology of an enrofloxacin-resistant Escherichia coli clone was investigated during two separate outbreaks of colibacillosis in the Danish broiler production. In total five flocks were reported affected by the outbreaks. Recorded first-week mortalities were in the range of 1.7-12.7%. The clone was first isolated from dead broilers and subsequently demonstrated in samples from associated hatchers and the parent flock with its embryonated eggs, suggesting a vertical transmission from the parents. The second outbreak involved two broiler flocks unrelated to the affected flocks from the first outbreak. However, the clone could not be demonstrated in the associated parent flock. Furthermore, samplings from grand-parent flocks were negative for the outbreak clone. The clonality was evaluated by plasmid profiling and pulsed-field gel electrophoresis. None of the recognized virulence factors were demonstrated in the outbreak clone by microarray and PCR assay. The molecular background for the fluoroquinolone-resistance was investigated and point mutations in gyrA and parC leading to amino-acid substitutions in quinolone-resistance determining regions of GyrA and ParC were demonstrated. Vertical transmission of enrofloxacin-resistant E. coli from healthy parents resulting in high first-week mortality in the offspring illustrates the

potential of the emergence and spreading of fluoroquinolone-resistant bacteria in animal husbandry, even though the use of fluoroquinolones is restricted

Phillips,C.A. and M.A.Harrison. 2005. "Comparison of the microflora on organically and conventionally grown spring mix from a California processor." *J.Food Prot.* 68:1143-1146.
Abstract: Considerable speculation has occurred concerning the potential for higher numbers of foodborne pathogens on organically grown produce compared with produce not grown organically. The microflora composition of spring mix or mesclun, a mixture of multiple salad ingredients, grown either by organic or conventional means was determined. Unwashed or washed spring mix was obtained from a commercial California fresh-cut produce processor who does not use manure in their cultivation practices. Fifty-four samples of each type of product were supplied over a 4-month period. Analysis included enumeration of total mesophiles, psychrotrophs, coliforms, generic Escherichia coli, lactic acid bacteria, yeasts, and molds. In addition, spring mix was analyzed for the presence of Salmonella and Listeria monocytogenes. The mean populations of mesophilic and psychrotrophic bacteria, yeasts, molds, lactic acid bacteria, and coliforms on conventionally grown spring mix were not statistically different (P > 0.05) from respective mean populations on organically grown spring mix. The mean population of each microbial group was significantly higher on unwashed spring mix compared with the washed product. Of the 14 samples found to contain E. coli, eight were from nonwashed conventional spring mix, one was from washed conventional spring mix, and four were from nonwashed organic spring mix. Salmonella and L. monocytogenes were not detected in any of the samples analyzed

Prysor,W.A., P.Roberts, L.M.Avery, K.Killham, and D.L.Jones. 2006. "Earthworms as vectors of Escherichia coli O157:H7 in soil and vermicomposts." *FEMS Microbiol.Ecol.* 58:54-64.
Abstract: Survival and movement of Escherichia coli O157:H7 in both soil and vermicompost is of concern with regards to human health. Whilst it is accepted that E. coli O157:H7 can persist for considerable periods in soils, it is not expected to survive thermophilic composting processes. However, the natural behavior of earthworms is increasingly utilized for composting (vermicomposting), and the extent to which earthworms promote the survival and dispersal of the bacterium within such systems is unknown. The faecal material produced by earthworms provides a ready supply of labile organic substrates to surrounding microbes within soil and compost, thus promoting microbial activity. Earthworms can also cause significant movement of organisms through the channels they form. Survival and dispersal of E. coli O157:H7 were monitored in contaminated soil and farmyard manure subjected to earthworm digestion over 21 days. Our findings lead to the conclusion that anecic earthworms such as Lumbricus terrestris may significantly aid vertical movement of E. coli O157 in soil, whereas epigeic earthworms such as Dendrobaena veneta significantly aid lateral movement within compost. Although the presence of earthworms in soil and compost may aid proliferation of E. coli O157 in early stages of contamination, long-term persistence of the pathogen appears to be unaffected

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

Reid,S.D., C.J.Herbelin, A.C.Bumbaugh, R.K.Selander, and T.S.Whittam. 2000. "Parallel evolution of virulence in pathogenic Escherichia coli." *Nature*. 406:64-67.
Abstract: The mechanisms underlying the evolution and emergence of new bacterial pathogens are not well understood. To elucidate the evolution of pathogenic Escherichia coli strains, here we sequenced seven housekeeping genes to build a phylogenetic tree and trace the history of the acquisition of virulence genes. Compatibility analysis indicates that more than 70% of the informative sites agree with a single phylogeny, suggesting that recombination has not completely obscured the remnants of ancestral chromosomes. On the basis of the rate of synonymous substitution for E. coli and Salmonella enterica (4.7 x 10(-9) per site per year), the radiation of clones began about 9 million years ago and the highly virulent pathogen responsible for epidemics of food poisoning, E. coli O157:H7, separated from a common ancestor of E. coli K-12 as long as 4.5 million years ago. Phylogenetic analysis reveals that old lineages of E. coli have acquired the same virulence factors in parallel, including a pathogenicity island involved in intestinal adhesion, a plasmid-borne haemolysin, and phage-encoded Shiga toxins. Such parallel evolution indicates that natural selection has favoured an ordered acquisition of genes and the progressive build-up of molecular mechanisms that increase virulence

Reinders,R.D., M.F.Weber, L.J.Lipman, J.Verhoeff, and P.G.Bijker. 2001. "Control of VTEC in Dutch livestock and meat production." *Int.J.Food Microbiol.* 66:79-83.
Abstract: The Dutch government and the meat industry, recognising VTEC as having important public health, meat quality and economic implications, have taken a number of initiatives within the last 5 years to control VTEC in livestock and meat. These initiatives, brought together last year in a 'Masterplan VTEC', include short-, middle- and long-term priorities. Short-term priorities include advice on interventions in the cases of an outbreak of VTEC associated with a cattle herd, the implementation of handbooks for Good Manufacturing Practice (GMP) in slaughterhouses and deboning plants, and the execution of an action programme on zero-tolerance to faecal contamination of carcasses. Mid-term activities include surveillance of the occurrence of VTEC and other enteropathogens in livestock and meat, and the investigations of VTEC population dynamics in dairy farms, transportation and farm hygiene. In the longer term, this programme aims to produce a system of Integrated Quality Assurance, consolidating effective measures to control VTEC in Dutch livestock and meat, and integrating emerging means for control and prevention

Reinders,R.D., S.Biesterveld, and P.G.Bijker. 2001. "Survival of Escherichia coli O157:H7 ATCC 43895 in a model apple juice medium with different concentrations of proline and caffeic acid." *Appl.Environ.Microbiol.* 67:2863-2866.
Abstract: The effects of proline and caffeic acid on the survival of Shiga toxin-producing Escherichia coli (STEC) O157:H7 strain ATCC 43895 in a model apple juice medium were studied. It is hypothesized that the inhibitory effect of caffeic acid may explain why almost all outbreaks of STEC O157:H7 infections linked to apple juice or cider have occurred in October or November

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

Renter,D.G. and J.M.Sargeant. 2002. "Enterohemorrhagic Escherichia coli O157: epidemiology and ecology in bovine production environments." *Anim Health Res.Rev.* 3:83-94.
Abstract: Enterohemorrhagic Escherichia coli, particularly the O157(:H7) serogroup, has become a worldwide public health concern. Since cattle feces are often implicated as the source of E. coli O157 in human infections, considerable resources have been devoted to defining the epidemiology and ecology of E. coli O157 in cattle environments so that control might begin at the farm level. Diagnostic limitations and the complexity of often interrelated microbial, animal, herd, environmental and production factors have hindered the determination of the epidemiology, ecology and subsequent farm-level control of E. coli O157. The widespread distribution of E. coli O157, the transitory nature of fecal shedding, multiple potential environmental sources, lack of species specificity, and age-, feed- and time-related differences in cattle prevalence are documented. However, the significance and/or role of these factors in the epidemiology and ecology of E. coli O157 is still unclear. Cattle are a major source of E. coli O157, but it may be simplistic to believe that most herds are relatively closed systems with small percentages of cattle serving as true reservoirs. Practical on-farm control may require explicit definitions of the seemingly complex system(s) and the microbial, animal, herd, environmental and production factors involved in themultiplication, maintenance and transmission of E. coli O157

Renter,D.G., J.M.Sargeant, R.D.Oberst, and M.Samadpour. 2003. "Diversity, frequency, and persistence of Escherichia coli O157 strains from range cattle environments." *Appl.Environ.Microbiol.* 69:542-547.
Abstract: Genetic diversity, isolation frequency, and persistence were determined for Escherichia coli O157 strains from range cattle production environments. Over the 11-month study, analysis of 9,122 cattle fecal samples, 4,083 water source samples, and 521 wildlife fecal samples resulted in 263 isolates from 107 samples presumptively considered E. coli O157 as determined by culture and latex agglutination. Most isolates (90.1%) were confirmed to be E. coli O157 by PCR detection of intimin and Shiga toxin genes. Pulsed-field gel electrophoresis (PFGE) of XbaI-digested preparations revealed 79 unique patterns (XbaI-PFGE subtypes) from 235 typeable isolates confirmed to be E. coli O157. By analyzing up to three isolates per positive sample, we detected an average of 1.80 XbaI-PFGE subtypes per sample. Most XbaI-PFGE subtypes (54 subtypes) were identified only once, yet the seven most frequently isolated subtypes represented over one-half of the E. coli O157 isolates (124 of 235 isolates). Recurring XbaI-PFGE subtypes were recovered from samples on up to 10 sampling occasions and up to 10 months apart. Seven XbaI-PFGE subtypes were isolated from both cattle feces and water sources, and one of these also was isolated from the feces of a wild opossum (Didelphis sp.). The number of XbaI-PFGE subtypes, the variable frequency and persistence of subtypes, and the presence of identical subtypes in cattle feces, free-flowing water sources, and wildlife feces indicate that the complex molecular epidemiology of E. coli O157 previously described for confined cattle operations is also evident in extensively managed range cattle environments

Renter,D.G., J.M.Sargeant, and L.L.Hungerford. 2004. "Distribution of Escherichia coli O157:H7 within and among cattle operations in pasture-based agricultural areas." *Am.J.Vet.Res.* 65:1367-1376.

Abstract: OBJECTIVE: To determine the distribution of Escherichia coli O157:H7 in pasture-based cattle production areas. SAMPLE POPULATION: Two 100-km2 agricultural areas consisting of 207 pasture, 14 beef-confinement, and 3 dairy locations within 24 cattle operations. PROCEDURE: 13,726 samples from cattle, wildlife, and water sources were obtained during an 11-month period. Escherichia coli O157:H7 was identified by use of culture and polymerase chain reaction assays and characterized by pulsed-field gel electrophoresis (PFGE). RESULTS: Odds of recovering E coli O157:H7 from feeder-aged cattle were > 4 times the odds for cow-calf or dairy cattle. There was no difference in prevalence for pastured versus confined cattle after controlling for production age group. Number of samples collected (37 to 4,829), samples that yielded E coli O157:H7 (0 to 53), and PFGE subtypes (0 to 48) for each operation varied and were highly correlated. Although most PFGE subtypes were only detected once, 17 subtypes were detected on more than 1 operation. Ten of 12 operations at which E coli O157:H7 was detected had at least 1 subtype that also was detected on another operation. We did not detect differences in the probability of having the same subtype for adjacent operations, nonadjacent operations in the same study area, or operations in the other study area. CONCLUSIONS AND CLINICAL RELEVANCE: Strategies aimed at controlling E coli O157:H7 and specific subtypes should account for the widespread distribution and higher prevalence in feeder-aged cattle regardless of production environment and the fact that adjacent and distant cattle operations can have similar subtypes

Renter,D.G., S.L.Checkley, J.Campbell, and R.King. 2004. "Shiga toxin-producing Escherichia coli in the feces of Alberta feedlot cattle." *Can.J.Vet.Res.* 68:150-153.
Abstract: Shiga toxin-producing Escherichia coli (STEC) are a public health concern. Bacterial culture techniques commonly used to detect E. coli O157:H7 will not detect other STEC serotypes. Feces from cattle and other animals are a source of O157:H7 and other pathogenic serotypes of STEC. The objective of this study was to estimate the pen-level prevalence of Shiga toxins and selected STEC serotypes in pre-slaughter feedlot cattle. Composite fecal samples were cultured and a polymerase chain reaction (PCR) was used to detect genes for Shiga toxins (stx1 and stx2) and genes for O157:H7, O111:H8, and O26:H11 serotypes. Evidence of Shiga toxins was found in 23 pens (92%), O157:H7 in 2 (8%), O111:H8 in 5 (20%), and O26:H11 in 20 (80%) of the 25 pens investigated. Although pen-level prevalence estimates for Shiga toxins and non-O157 serotypes seem high relative to O157:H7, further effort is required to determine the human health significance of non-O157 serotypes of STEC in feedlot cattle

Rice,D.H., D.D.Hancock, R.L.Vetter, and T.E.Besser. 1996. "Escherichia coli O157 infection in a human linked to exposure to infected livestock." *Vet.Rec.* 138:311.

Rice,D.H., K.M.McMenamin, L.C.Pritchett, D.D.Hancock, and T.E.Besser. 1999. "Genetic subtyping of Escherichia coli O157 isolates from 41 Pacific Northwest USA cattle farms." *Epidemiol.Infect.* 122:479-484.
Abstract: Escherichia coli O157 (n = 376) from 41 cattle farms were subtyped using pulsed field gel electrophoresis of endonuclease cleaved chromosomal DNA. Cleavage with XbaI resulted in 81 subtypes. Fifty-one isolates from subtypes found in more than one herd, or in herds on multiple sample collection dates were compared using the endonuclease NotI, resulting in 23 additional subtypes. Up to 11 XbaI subtypes were found per farm with up to 7 subtypes/farm identified from a single date. Indistinguishable subtypes (both XbaI and NotI) were found to persist on 4 farms for 6-24 months. Five subtypes were found on more than one farm separated by up to 640 km. Dairy farms where cattle had moved onto the farm had a similar number of subtypes as farms with no movement of cattle, and feedlots had more subtypes than dairy farms. These data indicate that there is a mechanism for multiple herd exposure to specific subtypes, there are multiple sources of exposure for cattle on farms, and on-farm reservoirs other than cattle may exist

Rice,D.H., D.D.Hancock, and T.E.Besser. 2003. "Faecal culture of wild animals for Escherichia coli O157:H7." *Vet.Rec.* 152:82-83.

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

Russell,J.B., F.Diez-Gonzalez, and G.N.Jarvis. 2000. "Potential effect of cattle diets on the transmission of pathogenic Escherichia coli to humans." *Microbes.Infect.* 2:45-53.
Abstract: Grain feeding seems to promote the growth and acid resistance of Escherichia coli in fattening beef cattle, and acid-resistant E. coli are more likely to survive the human gastric stomach. When cattle were fed hay for only five days, the number and acid resistance of E. coli decreased dramatically

Russell,J.B. and J.L.Rychlik. 2001. "Factors that alter rumen microbial ecology." *Science*. 292:1119-1122.
Abstract: Ruminant animals and ruminal microorganisms have a symbiotic relationship that facilitates fiber digestion, but domestic ruminants in developed countries are often fed an abundance of grain and little fiber. When ruminants are fed fiber-deficient rations, physiological mechanisms of homeostasis are disrupted, ruminal pH declines, microbial ecology is altered, and the animal becomes more susceptible to metabolic disorders and, in some cases, infectious disease. Some disorders can be counteracted by feed additives (for example, antibiotics and buffers), but these additives can alter the composition of the ruminal ecosystem even further

Sage,J.R. and S.C.Ingham. 1998. "Survival of Escherichia coli O157:H7 after freezing and thawing in ground beef patties." *J.Food Prot.* 61:1181-1183.
Abstract: Survival of Escherichia coli O157:H7 strains QA 326, and ATCC 43889, 43894, and 43895 after freezing (-20 degrees C, 24 h) and thawing (4 degrees C for 12 h, 23 degrees C for 3 h, or microwave heating of 700 W for 120 s) in ground beef patties was determined by reference most probable number (MPN), hydrophobic grid membrane

filter SD-39 agar, and sorbitol MacConkey agar (SMA) spread-plating methods. Populations decreased from 0.62 to 2.52 log10 CFU/g, with the extent varying significantly by strain. Strain QA 326 populations almost always decreased the most, up to 1.87 log10 CFU/g more than the least sensitive strain. Microwave heating was the most lethal thawing treatment for strain QA 326, and 4 degrees C thawing was the most lethal treatment for strain ATCC 43894. Thawing treatments varied in relative lethality for the other two strains. For strain QA 326 (4 degrees C and microwave thaw treatments) and strain ATCC 43889 (4 and 23 degrees C thawing), the enumeration method significantly affected a population decrease. The SD-39 agar method best recovered strain QA 326 while the SD-39 agar method and the reference MPN method best recovered strain ATCC 43889 after 4 and 23 degrees C thawing, respectively. The greatest difference in population decrease measured by any two methods was 0.58 log10 CFU/g. Results showed (i) a wide range in freeze-thaw sensitivity among E. coli O157:H7 strains, (ii) no thawing method had consistently and significantly greater lethality, and (iii) the reference MPN, SD-39 agar, and SMA methods differed little in ability to enumerate E. coli O157:H7

Sanchez,S., M.D.Lee, B.G.Harmon, J.J.Maurer, and M.P.Doyle. 2002. "Animal issues associated with Escherichia coli O157:H7." *J.Am.Vet.Med.Assoc.* 221:1122-1126.

Sanderson,M.W., T.E.Besser, J.M.Gay, C.C.Gay, and D.D.Hancock. 1999. "Fecal Escherichia coli O157:H7 shedding patterns of orally inoculated calves." *Vet.Microbiol.* 69:199-205.
Abstract: To assess the duration of fecal shedding upon initial infection, the duration of shedding after subsequent re-infection and the effects of dietary restriction and antibiotic treatment on shedding recrudescence, four, one-week-old calves were orally inoculated on three separate occasions with 5x10(8) cfu of Escherichia coli O157:H7 strain 86-24 Nal-R. Fecal shedding was followed by serial culture three times weekly. Following the first inoculation, the calves shed E. coli O157:H7 in their feces for a mean of 30 days, with a range of 20 to 43 days. Following the second and third inoculations, the calves shed E. coli O157:H7 in their feces for 3-8 days. In each of the three inoculations, feed was withheld from the calves for 24 h after they had become fecal culture negative. Two calves resumed shedding, one for 1 day and the other for 4 days, after food was withheld after the third inoculation, but not in the first two inoculations. In the third inoculation, one calf resumed shedding for one day after treatment with oxytetracycline. No E. coli O157:H7 strain 86-24 Nal-R was found in the calves at necropsy. These calves did not exhibit persistent low-level shedding, and did not appear to be persistently colonized with E. coli O157:H7

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

Schamberger,G.P. and F.Diez-Gonzalez. 2002. "Selection of recently isolated colicinogenic Escherichia coli strains inhibitory to Escherichia coli O157:H7." *J.Food Prot.* 65:1381-1387.
Abstract: Escherichia coli strains were screened for their ability to inhibit E. coli O157:H7. An initial evaluation of 18 strains carrying previously characterized colicins determined that only colicin E7 inhibited all of the E. coli O157:H7 strains tested. A total of 540 strains that had recently been isolated from humans and nine different animal species (cats, cattle, chickens, deer, dogs, ducks, horses, pigs, and sheep) were tested by a flip-plating technique. Approximately 38% of these strains were found to inhibit noncolicinogenic E. coli K12 strains. The percentage of potentially colicinogenic E. coli per animal species ranged from 14% for horse isolates to 64% for sheep strains. Those isolates that inhibited E. coli K12 were screened against E. coli O157:H7, and 42 strains were found to be capable of inhibiting all 22 pathogenic strains tested. None of these 42 strains produced bacteriophages, and only 24 isolates inhibited serotype O157:H7 in liquid culture. The inhibitory activity of these strains was completely eliminated by treatment with proteinase K. When mixtures of these 24 colicinogenic strains were grown in anaerobic continuous culture, the four-strain E. coli O157:H7 population was reduced at a rate of 0.25 log10 cells per ml per h, which was fivefold faster than the washout rate. Two strains originally isolated from cat feces (F16) and human feces (H30) were identified by repetitive sequences polymerase chain reaction as the predominant isolates in continuous cultures. The results of this work indicate that animal species other than cattle can be sources of anti-O157 colicinogenic strains, and these results also lead to the identification of at least two isolates that could potentially be used in preharvest control strategies

Schamberger,G.P., R.L.Phillips, J.L.Jacobs, and F.Diez-Gonzalez. 2004. "Reduction of Escherichia coli O157:H7 populations in cattle by addition of colicin E7-producing E. coli to feed." *Appl.Environ.Microbiol.* 70:6053-6060.
Abstract: A cattle trial using artificially inoculated calves was conducted to determine the effect of the addition of colicinogenic Escherichia coli strains capable of producing colicin E7 (a 61-kDa DNase) to feed on the fecal shedding of serotype O157:H7. The experiment was divided into three periods. In period 1, which lasted 24 days, six calves were used as controls, and eight calves received 10(7) CFU of E. coli (a mixture of eight colicinogenic E. coli strains) per g of feed. Both groups were orally inoculated with nalidixic acid-resistant E. coli O157:H7 strains 7 days after the treatment started. In periods 2 and 3, the treatment and control groups were switched, and the colicinogenic E. coli dose was increased 10-fold. During period 3, which lasted as long as period 1, both groups were reinoculated with E. coli O157:H7. The numbers of E. coli O157:H7 were consistently greater in the control groups during the three periods, but comparisons within each time period determined a statistically significant ($P < 0.05$) difference only at day 21 of period 1. However, when the daily average counts were compared between the period 1 control group and the period 3 treatment group that included the same six animals, an overall reduction of 1.1 log(10) CFU/g was observed, with a maximum decrease of 1.8 log(10) CFU/g at day 21 (overall statistical significance, $P = 0.001$). Serotype O157:H7 was detected in 44% of the treatment group's intestinal tissue samples and in 64% of those from the control group ($P < 0.04$). These results indicated that the daily addition of 10(8) CFU of colicin E7-producing E. coli per gram of feed could reduce the fecal shedding of serotype O157:H7

Schamberger,G.P. and F.Diez-Gonzalez. 2005. "Assessment of resistance to colicinogenic Escherichia coli by E. coli O157:H7 strains." *J.Appl.Microbiol.* 98:245-252.
Abstract: AIMS: To assess a collection of 96 Escherichia coli O157:H7 strains for their resistance potential against a set of colicinogenic E. coli developed as a probiotic for use

in cattle. METHODS AND RESULTS: Escherichia coli O157:H7 strains were screened for colicin production, types of colicins produced, presence of colicin resistance and potential for resistance development. Thirteen of 14 previously characterized colicinogenic E. coli strains were able to inhibit 74 serotype O157:H7 strains. Thirteen E. coli O157:H7 strains were found to be colicinogenic and 11 had colicin D genes. PCR products for colicins B, E-type, Ia/Ib and M were also detected. During in vitro experiments, the ability to develop colicin resistance against single-colicin producing E. coli strains was observed, but rarely against multiple-colicinogenic strains. The ability of serotype O157:H7 strains to acquire colicin plasmids or resistance was not observed during a cattle experiment. CONCLUSIONS: Escherichia coli O157:H7 has the potential to develop single-colicin resistance, but simultaneous resistance against multiple colicins appears to be unlikely. Colicin D is the predominant colicin produced by colicinogenic E. coli O157:H7 strains. SIGNIFICANCE AND IMPACT OF THE STUDY: The potential for resistance development against colicin-based strategies for E. coli O157:H7 control may be very limited if more than one colicin type is used

Schouten,J.M., M.Bouwknegt, A.W.van de Giessen, K.Frankena, M.C.De Jong, and E.A.Graat. 2004. "Prevalence estimation and risk factors for Escherichia coli O157 on Dutch dairy farms." *Prev.Vet.Med.* 64:49-61.
Abstract: To estimate the prevalence of Escherichia coli O157 on Dutch dairy herds, faecal samples were collected once from 678 randomly selected dairy farms in the period October 1996-December 2000. Samples were cultured for E. coli O157. Thirty-eight isolates were tested for virulence genes (eae, VT1 and VT2). A questionnaire about farm characteristics was taken from the farm manager, resulting in variables that could be analysed to identify and quantify factors associated with presence of E. coli O157. In total, 49 of the 678 herds (7.2%) showed at least one positive pooled sample. E. coli O157 was not isolated from herds sampled in December-April in consecutive years (except for one isolate found in March, 2000). VT- and eae-genes were found in 37 and 38 isolates, respectively. Logistic regression was performed on variables obtained from the questionnaire, comparing E. coli O157-positive herds to negative herds. To account for season, a sine function was included in the logistic regression as an offset variable. In the final model, the presence of at least one pig at the farm (OR = 3.4), purchase of animals within the last 2 years before sampling (OR = 1.9), supply of maize (OR = 0.29) to the cows, and sampling a herd in the year 1999 or 2000 (compared to sampling in 1998; OR = 2.1 and 2.9, respectively) had associations with the presence of E. coli O157

Schouten,J.M., A.W.van de Giessen, K.Frankena, M.C.De Jong, and E.A.Graat. 2005. "Escherichia coli O157 prevalence in Dutch poultry, pig finishing and veal herds and risk factors in Dutch veal herds." *Prev.Vet.Med.* 70:1-15.
Abstract: In the period October 1996 through December 2000, a total of 7163 pooled faecal samples of laying hen and broiler flocks, finishing-pig herds and veal herds were examined for the presence of Salmonella spp., Campylobacter spp. and verocytotoxin-producing Escherichia coli O157 as part of a national monitoring programme in The Netherlands. Isolates were tested for eae and VT genes. Risk factors for Dutch veal herds were quantified. For all herd/flock types, faecal samples were cultured for E. coli O157. Of broiler flocks, laying flocks and finishing pig herds, respectively, 1.7%, 0.5% and 0.4% were E. coli O157 positive. In total, 42 of the 454 veal herds (9.3%) showed at least one positive pooled sample. E. coli O157-positive herds were compared (with logistic regression) to negative herds, regarding variables obtained from the questionnaire taken from the farm manager. To account for season, a sine function was included in the logistic regression as offset variable. In the final model, 'pink-veal production' (compared to white-veal production), 'group housing of the sampled herd' (compared to individual housing), 'more than one stable present' (compared to one stable present), 'hygienic measures regarding visitors' (compared to no hygienic measures), 'interval arrival-sampling of a herd of >20 weeks' (compared to <

or =10 weeks), and 'presence of other farms within 1 km' (compared to no presence of farms <1 km) showed associations (P<0.05) with the presence of E. coli O157. These results need careful interpretation; they should be considered as indications for further (experimental or cohort-based) research rather then causal associations

Schouten,J.M., E.A.Graat, K.Frankena, A.W.van de Giessen, W.K.van der Zwaluw, and M.C.De Jong. 2005. "A longitudinal study of Escherichia coli O157 in cattle of a Dutch dairy farm and in the farm environment." *Vet.Microbiol.* 107:193-204.
Abstract: From July 1999 till November 2000, a longitudinal study was conducted on a dairy farm in The Netherlands to study within herd prevalence and types of verocytotoxin producing Escherichia coli (VTEC) of serogroup O157 over time, and determine environmental reservoirs and possible transmission routes. Faeces, blood, milk and environmental samples were collected 14 times with intervals varying from 4 to 10 weeks during the study period. Faecal samples were selectively cultured for Escherichia coli O157. Isolates were tested by PCR for the most common virulence genes, VTI, VTII and eae, and typed by pulsed field gel electrophoresis. In total, 71 isolates were obtained, of which 49 from dairy cows, 8 from young stock, 5 from other animals and 9 from the environment. Positive samples were all detected in summer and early fall. VT- and eae-genes were found in all tested isolates, except in one. DNA typing showed that three clusters of O157 isolates could be identified. One of these clusters contained samples of two shedding seasons, indicating persistence on the farm during winter and spring. Repeated measures analysis of variance showed that cows with O157 VTEC infection had higher daily milk production in the period preceding sampling (p = 0.0055). There was no significant association between the results of the LPS-ELISA on serum samples from dairy cows and their O157 status

Schroeder,C.M., J.Meng, S.Zhao, C.Debroy, J.Torcolini, C.Zhao, P.F.McDermott, D.D.Wagner, R.D.Walker, and D.G.White. 2002. "Antimicrobial resistance of Escherichia coli O26, O103, O111, O128, and O145 from animals and humans." *Emerg.Infect.Dis.* 8:1409-1414.
Abstract: Susceptibilities to fourteen antimicrobial agents important in clinical medicine and agriculture were determined for 752 Escherichia coli isolates of serotypes O26, O103, O111, O128, and O145. Strains of these serotypes may cause urinary tract and enteric infections in humans and have been implicated in infections with Shiga toxin-producing E. coli (STEC). Approximately 50% of the 137 isolates from humans were resistant to ampicillin, sulfamethoxazole, cephalothin, tetracycline, or streptomycin, and approximately 25% were resistant to chloramphenicol, trimethoprim-sulfamethoxazole, or amoxicillin-clavulanic acid. Approximately 50% of the 534 isolates from food animals were resistant to sulfamethoxazole, tetracycline, or streptomycin. Of 195 isolates with STEC-related virulence genes, approximately 40% were resistant to sulfamethoxazole, tetracycline, or streptomycin. Findings from this study suggest antimicrobial resistance is widespread among E. coli O26, O103, O111, O128, and O145 inhabiting humans and food animals

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

Schubert,S., A.Rakin, H.Karch, E.Carniel, and J.Heesemann. 1998. "Prevalence of the "high-pathogenicity island" of Yersinia species among Escherichia coli strains that are pathogenic to humans." *Infect.Immun.* 66:480-485.
Abstract: The fyuA-irp gene cluster contributes to the virulence of highly pathogenic Yersinia (Yersinia pestis, Yersinia pseudotuberculosis, and Yersinia enterocolitica 1B). The cluster encodes an iron uptake system mediated by the siderophore yersiniabactin and reveals features of a pathogenicity island. Two evolutionary lineages of this "high pathogenicity island" (HPI) can be distinguished on the basis of DNA sequence comparison: a Y. pestis group and a Y. enterocolitica group. In this study we demonstrate that the HPI of the Y. pestis evolutionary group is disseminated among species of the family Enterobacteriaceae which are pathogenic to humans. It prevails in enteroaggregative Escherichia coli and in E. coli blood culture isolates (93 and 80%, respectively), but is rarely found in enteropathogenic E. coli, enteroinvasive E. coli, and enterotoxigenic E. coli isolates. In contrast, the HPI was absent from enterohemorrhagic E. coli, Shigella, and Salmonella enterica strains investigated. Polypeptides encoded by the fyuA, irp1, and irp2 genes located on the HPI could be detected in E. coli strains pathogenic to humans. However, these E. coli strains showed a reduced sensitivity to the bacteriocin pesticin, whose uptake is mediated by the FyuA receptor. Escherichia strains do not possess the hms gene locus thought to be a part of the HPI of Y. pestis. Deletions of the juA-irp gene cluster affecting solely the fyuA part of the HPI were identified in 3% of the E. coli strains tested. These results suggest horizontal transfer of the HPI between Y. pestis and some pathogenic E. coli strains

Schuenzel,K.M. and M.A.Harrison. 2002. "Microbial antagonists of foodborne pathogens on fresh, minimally processed vegetables." *J.Food Prot.* 65:1909-1915.
Abstract: On many types of raw or minimally processed foods, the bacterial microbiota is often composed of mixed species. The activities of one bacterial species may influence the growth and activities of others that are present. The objective of this project was to evaluate the microbial composition of fresh and minimally processed vegetables to determine if naturally occurring bacteria on produce are competitive with or antagonistic to potentially encountered pathogens. Naturally occurring bacteria were obtained from ready-to-eat salad vegetables on four occasions to allow for seasonal variation. Minimally processed vegetables were sampled at various stages in their processing from raw vegetables to packaged products. Some portions were analyzed microbiologically within 24 h, while other portions were stored refrigerated and analyzed after 72 h. Microbiological analysis was conducted for bacterial enumeration and to obtain isolates. An agar spot method was used to screen isolates for antimicrobial activity against Staphylococcus aureus ATCC 27664, Escherichia coli O157:H7 E009, Listeria monocytogenes LCDC 81-861, and Salmonella Montevideo. Of the 1,180 isolates screened for inhibitory activity, 37 (3.22%) were found to have various degrees of inhibitory activity against at least one test pathogen. Many isolates showed inhibitory activity against all four pathogens. The isolates with the most extensive inhibition were removed from finished lettuce piece shreds. Of the 37 inhibitory isolates, 34 (91.9%)

were gram negative. All isolates with inhibitory activity are able to multiply at both 4 and 10 degrees C

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

Scott,L., P.McGee, D.Minihan, J.J.Sheridan, B.Earley, and N.Leonard. 2006. "The characterisation of E. coli O157:H7 isolates from cattle faeces and feedlot environment using PFGE." *Vet.Microbiol.* 114:331-336.
Abstract: The objectives of this study were to investigate the diversity of Escherichia coli O157:H7 isolates obtained over a 3-month period from a cattle feedlot in order to assess the relationship between environmental and faecal isolates and to determine the pattern of transmission of E. coli O157:H7 between groups of cattle. Faecal samples were obtained from cattle housed in four adjacent feedlot pens at monthly intervals, with environmental pen samples collected simultaneously. All E. coli O157:H7 isolates obtained were examined by pulsed field gel electrophoresis (PFGE), polymerase chain reaction (PCR) to detect eaeA, ehxA, stx1 and stx2 genes and antibiotic sensitivity profiling. Ten isolates were subjected to acid shock to imitate conditions in the acidic cattle abomasum and assess the effect on PFGE profiles. E. coli O157:H7 was isolated from 69 faecal samples and 26 environmental samples. All isolates (n=95) carried the genes for eaeA, ehxA and stx2 and were sensitive to all antibiotics tested. The PFGE profiles of all isolates differed by no more than two bands and clustered within 80% similarity following dendrogram analysis. Acid shock had no effect on the subsequent PFGE patterns. A total of 8.7% (6/69) of cattle were shedding E. coli O157:H7 in the first month with faecal shedding increasing to 52% (36/69) by the third month of the study. A single isolate of E. coli O157:H7 may be passed rapidly through cattle pens, with the environment acting as a significant reservoir for transmission. PFGE is a useful tool for tracking the direct and indirect transmission of E. coli O157:H7 isolates on the farm

Seo,K.H. and J.F.Frank. 1999. "Attachment of Escherichia coli O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy." *J.Food Prot.* 62:3-9.
Abstract: Confocal scanning laser microscopy was used to observe the location of Escherichia coli O157:H7 on and within lettuce leaves. Sections of leaves (ca. 0.5 by 0.5 cm) were inoculated by submersion in a suspension of E. coli O157:H7 (ca. 10(7) to 10(8) CFU/ml) overnight at 7 degrees C. Fluorescein isothiocyanate-labeled antibody was used to visualize the attached bacteria. E. coli O157:H7 was found attached to the surface, trichomes, stomata, and cut edges. Three-dimensional volume reconstruction of interior portions of leaves showed that E. coli O157:H7 was entrapped 20 to 100 microm below the surface in stomata and cut edges. Agar plate culturing and microscopic

observation indicated that E. coli O157:H7 preferentially attached to cut edges, as opposed to the intact leaf surface. Dual staining with fluorescein isothiocyanate-labeled antibody and propidium iodide was used to determine viability of cells on artificially contaminated lettuce leaves after treatment with 20 mg/liter chlorine solution for 5 min. Many live cells were found in stomata and on cut edges following chlorine treatment. E. coli O157:H7 did not preferentially adhere to biofilm produced by Pseudomonas fluorescens on the leaf surface. In contrast to E. coli O157:H7, Pseudomonas adhered to and grew mainly on the intact leaf surface rather than on the cut edges

Sharp,J.C., L.D.Ritchie, J.Curnow, and T.M.Reid. 1994. "High incidence of haemorrhagic colitis due to Escherichia coli O157 in one Scottish town: clinical and epidemiological features." *J.Infect.* 29:343-350.
Abstract: Verotoxin-producing strains of Escherichia coli (VTEC), in particular serotype O157:H7, are now recognised as the major cause of haemorrhagic colitis and the haemolytic uraemic syndrome (HUS) in the U.K. and in North America, and increasingly so in other countries. Over a 3-year period (1989-1991), 16 cases of E. coli 0157 infection occurred in one town (Peterhead) in north-east Grampian. Four patients required admission to hospital, of whom three developed HUS. The bovine source of VTEC infection has now been clearly established with foodborne, waterborne, person-to-person and zoonotic transmission described. Despite extensive local enquiries, the source(s) of infection of the 16 cases in Peterhead was not established. Much still needs to be learned about the epidemiology, risk factors and long-term clinical sequelae of VTEC infection and HUS. Close collaboration between the medical and veterinary professions is of paramount importance in order to provide better understanding of the prevalence of E. coli O157 infection in cattle and the route(s) of transmission to humans

Sheng,H., M.A.Davis, H.J.Knecht, D.D.Hancock, J.Van Donkersgoed, and C.J.Hovde. 2005. "Characterization of a shiga toxin-, intimin-, and enterotoxin hemolysin-producing Escherichia coli ONT:H25 strain commonly isolated from healthy cattle." *J.Clin.Microbiol.* 43:3213-3220.
Abstract: Among bovine fecal and recto-anal mucosal swab samples cultured in our laboratory for Escherichia coli O157:H7, we frequently isolated E. coli organisms that were phenotypically similar to the O157:H7 serotype as non-sorbitol fermenting and negative for beta-glucuronidase activity but serotyped O nontypeable:H25 (ONT:H25). This study determined the prevalence and virulence properties of the E. coli ONT:H25 isolates. Among dairy and feedlot cattle (n = 170) sampled in Washington, Idaho, and Alberta, Canada, the percentage of animals culture positive for E. coli ONT:H25 ranged from 7.5% to 22.5%, compared to the prevalence of E. coli O157:H7 that ranged from 0% to 15%. A longitudinal 8-month study of dairy heifers (n = 40) showed that 0 to 15% of the heifers were culture positive for E. coli O157:H7, while 15 to 22.5% of the animals were culture positive for E. coli ONT:H25. As determined by a multiplex PCR, the E. coli ONT:H25 isolates carried a combination of virulence genes characteristic of the enterohemorrhagic E. coli, including intimin, translocated intimin receptor, Stx2, and hemolysin (eae-beta, tir, stx(2vh-a), and hly). E. coli ONT:H25 isolates from diverse geographic locations and over time were fingerprinted by separating XbaI-restricted chromosomal DNA by pulsed-field gel electrophoresis (PFGE) separation. Two strains of E. coli ONT:H25 were highly similar by PFGE pattern. Experimental inoculation of cattle showed that E. coli ONT:H25, like E. coli O157:H7, colonized the bovine recto-anal junction mucosa for more than 4 weeks following a single rectal application of bacteria

Skyberg,J.A., T.J.Johnson, J.R.Johnson, C.Clabots, C.M.Logue, and L.K.Nolan. 2006. "Acquisition of Avian Pathogenic Escherichia coli Plasmids by a Commensal E. coli Isolate Enhances Its Abilities To Kill Chicken Embryos, Grow in Human Urine, and Colonize the Murine Kidney." *Infect.Immun.* 74:6287-6292.
Abstract: We have found an avian pathogenic Escherichia coli (APEC) plasmid,

pAPEC-O2-ColV, which contains many of the genes associated with APEC virulence and also shows similarity in content to a plasmid and pathogenicity island of human uropathogenic E. coli (UPEC). To test the possible role of this plasmid in virulence, it was transferred by conjugation along with a large R plasmid, pAPEC-O2-R, into a commensal avian E. coli strain. The transconjugant was compared to recipient strain NC, UPEC strain HE300, and donor strain APEC O2 using various assays, including lethality for chicken embryos, growth in human urine, and ability to cause urinary tract infection in mice. The transconjugant killed significantly more chicken embryos than did the recipient. In human urine, APEC O2 grew at a rate equivalent to that of UPEC strain HE300, and the transconjugant showed significantly increased growth compared to the recipient. The transconjugant also significantly outcompeted the recipient in colonization of the murine kidney. These findings suggest that APEC plasmids, such as pAPEC-O2-ColV, contribute to the pathogenesis of avian colibacillosis. Moreover, since avian E. coli and their plasmids may be transmitted to humans, evaluation of APEC plasmids as possible reservoirs of urovirulence genes for human UPEC may be warranted

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

Sofos,J.N., S.L.Kochevar, G.R.Bellinger, D.R.Buege, D.D.Hancock, S.C.Ingham, J.B.Morgan, J.O.Reagan, and G.C.Smith. 1999. "Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants." *J.Food Prot.* 62:140-145.
Abstract: This study determined microbiological loads of beef carcasses at different stages during the slaughtering to chilling process in seven (four steer/heifer and three cow/bull) plants. Potential sources of contamination (feces, air, lymph nodes) were also tested. Each facility was visited twice, once in November through January (wet season) and again in May through June (dry season). Carcasses were sampled by aseptic excision of surface tissue (100 cm2) from the brisket, flank, and rump (30 samples each) after hide removal (pre-evisceration), after final carcass washing, and after 24-h carcass chilling. The samples were analyzed individually by standard procedures for aerobic plate counts (APC), total coliform counts (TCC), Escherichia coli biotype I counts (ECC), and presence of Salmonella. Incidence of Salmonella was higher on dry feces of older compared to younger animals, fresh feces of younger compared to older animals, and on cow/bull carcasses compared to steer/heifer carcasses. Most factors and their interactions had significant (P < or = 0.05) effects on the bacterial counts obtained. Depending on plant and season, APC, TCC, and ECC were < or =10(4), < or =10(2), and < or =10(1) CFU/cm2 in 46.7 to 93.3, 50.0 to 100.0, and 74.7 to 100.0% of the samples, respectively. TCC exceeded 10(3) CFU/cm2 in 2.5% (wet season) and 1.5% (dry season) of the samples. ECC exceeded 10(2) CFU/cm2 in 8.7%, 0.3%, and 1.5% of the pre-evisceration, final carcass-washing, and 24-h carcass-chilling samples, respectively, during the wet season; the corresponding numbers during the dry season were 3.5%, 2.2%, and 3.0%, respectively. These data should serve as a baseline for future comparisons in measuring the microbiological status of beef carcasses, as the new inspection requirements are implemented

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.
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

Solomon,E.B., C.J.Potenski, and K.R.Matthews. 2002. "Effect of irrigation method on transmission to and persistence of Escherichia coli O157:H7 on lettuce." *J.Food Prot.* 65:673-676.
Abstract: In this study, the transmission of Escherichia coli O157:H7 to lettuce plants through spray and surface irrigation was demonstrated. For all treatments combined, the number of plants testing positive following a single exposure to E. coli O157: H7 through spray irrigation (29 of 32 plants) was larger than the number testing positive following surface irrigation (6 of 32 plants). E. coli O157:H7 persisted on 9 of 11 plants for 20 days following spray irrigation with contaminated water. Immersion of harvested lettuce heads for 1 min in a 200 ppm chlorine solution did not eliminate all E. coli O157:H7 cells. The results of this study suggest that regardless of the irrigation method used, crops can become contaminated; therefore, the irrigation of food crops with water of unknown microbial quality should be avoided

Solomon,E.B., H.J.Pang, and K.R.Matthews. 2003. "Persistence of Escherichia coli O157:H7 on lettuce plants following spray irrigation with contaminated water." *J.Food Prot.* 66:2198-2202.
Abstract: Irrigation water collected at farms growing crops for human consumption was artificially contaminated with E. coli O157:H7 and used to irrigate lettuce plants. Plants in a growth chamber were spray irrigated either once or intermittently with water contaminated with 10(2) or 10(4) CFU of E. coli O157:H7 per ml and were then sampled over a 30-day period. Only plants exposed to 10(2) CFU/ml on day 1 did not harbor the pathogen at the end of the sampling period. All other treatments resulted in contaminated plants at harvest. Plants irrigated with 10(4) CFU/ml contained high levels (up to 5 log CFU/g) of the pathogen at harvest. The results obtained in this study underscore the assertion that spray irrigation (the application of water directly to plant leaves) is linked to the contamination of crops and suggest that repeated exposure increases the E. coli O157:H7 level on the plant

Solomon,E.B., B.A.Niemira, G.M.Sapers, and B.A.Annous. 2005. "Biofilm formation, cellulose production, and curli biosynthesis by Salmonella originating from produce, animal, and clinical sources." *J.Food Prot.* 68:906-912.
Abstract: The ability of 71 strains of Salmonella enterica originating from produce, meat, or clinical sources to form biofilms was investigated. A crystal violet binding assay demonstrated no significant differences in biofilm formation by isolates from any source when tested in any of the following three media: Luria-Bertani broth supplemented with 2% glucose, tryptic soy broth (TSB), or 1/20th-strength TSB. Incubation was overnight at 30 degrees C under static conditions. Curli production and cellulose production were monitored by assessing morphotypes on Luria-Bertani agar without salt containing Congo red and by assessing fluorescence on Luria-Bertani agar containing calcofluor, respectively. One hundred percent of the clinical isolates exhibited curli biosynthesis, and 73% demonstrated cellulose production. All meat-related isolates formed curli, and 84% produced cellulose. A total of 80% of produce-related isolates produced curli, but only 52% produced cellulose. Crystal violet binding was not statistically different between isolates representing the three morphotypes when grown in TSB; however, significant differences were observed when strains were cultured in the two other media tested. These data demonstrate that the ability to form biofilms is not dependent on the source of the test isolate and suggest a relationship between crystal violet binding and morphotype, with curli- and cellulose-deficient isolates being least effective in biofilm formation

Solomon,E.B. and K.R.Matthews. 2005. "Use of fluorescent microspheres as a tool to investigate bacterial interactions with growing plants." *J.Food Prot.* 68:870-873.

Abstract: Foodborne pathogens may exist as endophytes of growing plants. The internalization of Escherichia coli O157:H7 or other foodborne pathogens in growing lettuce plants may be independent of microbial factors. Mature lettuce plants were surface irrigated with E. coli O157:H7 or with FluoSpheres (fluorescent microspheres) and harvested 1, 3, and 5 days post-exposure. FluoSpheres were utilized as a bacterial surrogate. Microscopic examination of root, stem, and leaf tissue sections revealed that FluoSpheres were internalized into growing plants. Laser scanning confocal microscopy revealed that FluoSpheres were present within the root tissue and leaf stem tissue. The presence of FluoSpheres in internal portions of stem and leaf tissue suggests transport of the spheres from the root upward into the edible tissue. The level of uptake of FluoSpheres and E. coli O157:H7 was quantified using filtration. Numbers of FluoSpheres and E. coli O157:H7 cells in plant tissue were similar. The entry of E. coli O157:H7 into lettuce plants may be a passive event because the concentration of FluoSpheres was similar to that of the pathogen

Solomon,E.B. and K.R.Matthews. 2006. "Interaction of live and dead Escherichia coli O157:H7 and fluorescent microspheres with lettuce tissue suggests bacterial processes do not mediate adherence." *Lett.Appl.Microbiol.* 42:88-93.
Abstract: AIMS: The goal of this study was to determine whether any specific bacterial processes (biochemical or genetic) or cell surface moieties were required for the interaction between Escherichia coli O157:H7 and lettuce plant tissue. METHODS AND RESULTS: Escherichia coli O157:H7 and Fluospheres (fluorescent polystyrene microspheres) were used in experiments to investigate interactions with lettuce. Fluospheres were used as they are a non-biological material, of similar size and shape to a bacterial cell, but lack bacterial cell surface moieties and the ability to respond genetically. Live and glutaraldehyde-killed E. coli O157:H7 attached at levels of c. 5.8 log(10) cells per cm(2) following immersion of lettuce pieces into a suspension containing c. 8 log(10) CFU ml(-1). In a separate experiment, numbers of bacteria or Fluospheres associated with lettuce decreased by c. 1.5 log cm(-2) following a 1-min wash. Exposure times of 1 min, 1 h, or 6 h had little effect on the level of attachment for Fluospheres, and live or killed cells of E. coli O157:H7 to lettuce tissue. SIGNIFICANCE: These results indicate that bacterial processes and cell surface moieties are not required for the initial interaction of E. coli O157:H7 to lettuce plant tissue

Somarelli,J.A., J.C.Makarewicz, R.Sia, and R.Simon. 2006. "Wildlife identified as major source of Escherichia coli in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints." *J.Environ.Manage.*
Abstract: The presence of Escherichia coli in recreational and potable waters is a major concern to the general public as elevated levels of E. coli suggest the presence of pathogenic bacteria and viruses. Unfortunately, traditional microbial techniques do not allow specific identification of the source of E. coli. This reduces the ability to target management practices that reduce bacterial contamination. In the Finger Lakes region of western New York, USA, wildlife resides in relatively high densities on watersheds dominated by people and dairy farms, and as a result, the sources of fecal degradation of potable and recreational waters are often unknown. In the Conesus Lake watershed, the sources of microbial contamination were assessed using Rep-PCR molecular tools, a method of amplifying repetitive DNA sequences found throughout the E. coli genome to produce distinct fingerprints for a given ecotype. Molecular fingerprints of E. coli isolated from regional populations of cattle, humans, geese and deer were compared to E. coli isolated from stream water samples. Canonical discriminant function analysis indicated that the DNA fingerprints of the original source group isolates were correctly predicted 90.2% of the time. Since land use in the sub-watersheds was dominated by dairy and cash crop farms, it was expected that the majority of E. coli isolated would be identified as cows; however, an unexpectedly high percentage of isolates were identified as wildlife (geese and deer). Geese were the dominant source of E. coli (44.7-73.7% of the total sources) in four sub-watersheds followed by cows (10.5-21.1%), deer

(10.5-18.4%), humans (5.3-12.9%) and unidentifiable sources (0.0-11.8%). Management practices intended to decrease the number of cattle or the amount of manure spread in a sub-watershed were reflected in a decrease of E. coli ecotypes associated with dairy cows

Strachan,N.J., M.P.Doyle, F.Kasuga, O.Rotariu, and I.D.Ogden. 2005. "Dose response modelling of Escherichia coli O157 incorporating data from foodborne and environmental outbreaks." *Int.J.Food Microbiol.* 103:35-47.
Abstract: A human dose response model for Escherichia coli O157 would enable prediction of risk of infection to humans following exposure from either foodborne or environmental pathways. However, due to the severe nature of the disease, volunteer human dose response studies cannot be carried out. Surrogate models from Shigella fed to humans and E. coli O157 to rabbits have been utilised but are significantly different to one another. In addition data obtained by animal exposure may not be representative for human beings. An alternative approach to generating and validating a dose response model is to use quantitative data obtained from actual human outbreaks. This work collates outbreak data obtained from global sources and these are fitted using exponential and beta-Poisson models. The best fitting model was found to be the beta-Poisson model using a beta-binomial likelihood and the authors favour the exact version of this model. The confidence levels in this model encompass a previously published Shigella dose response model. The potential incorporation of this model into QMRAs is discussed together with applications of the model to help explain foodborne outbreaks

Taylor,D.E., M.Rooker, M.Keelan, L.K.Ng, I.Martin, N.T.Perna, N.T.Burland, and F.R.Blattner. 2002. "Genomic variability of O islands encoding tellurite resistance in enterohemorrhagic Escherichia coli O157:H7 isolates." *J.Bacteriol.* 184:4690-4698.
Abstract: Strains of Escherichia coli causing enterohemorrhagic colitis belonging to the O157:H7 lineage are reported to be highly related. Fifteen strains of E. coli O157:H7 and 1 strain of E. coli O46:H(-) (nonflagellated) were examined for the presence of potassium tellurite resistance (Te(r)). Te(r) genes comprising terABCDEF were shown previously to be part of a pathogenicity island also containing integrase, phage, and urease genes. PCR analysis, both conventional and light cycler based, demonstrated that about one-half of the Te(r) E. coli O157:H7 strains (6 of 15), including the Sakai strain, which has been sequenced, carried a single copy of the Te(r) genes. Five of the strains, including EDL933, which has also been sequenced, contained two copies. Three other O157:H7 strains and the O46:H(-) strain did not contain the Te(r) genes. In strains containing two copies, the Te(r) genes were associated with the serW and serX tRNA genes. Five O157:H7 strains resembled the O157 Sakai strain whose sequence contained one copy, close to serX, whereas in one isolate the single copy was associated with serW. There was no correlation between Te(r) and the ability to produce Shiga toxin ST1 or ST2. The Te(r) MIC for most strains, containing either one or two copies, was 1,024 micro g/ml, although for a few the MIC was intermediate, 64 to 128 micro g/ml, which could be increased to 512 micro g/ml by pregrowth of strains in subinhibitory concentrations of potassium tellurite. Reverse transcriptase PCR analysis confirmed that in most strains Te(r) was constitutive but that in the rest it was inducible and involved induction of terB and terC genes. Only the terB, -C, -D, and -E genes are required for Te(r). The considerable degree of homology between the ter genes on IncH12 plasmid R478, which originated in Serratia marcescens, and pTE53, from an E. coli clinical isolate, suggests that the pathogenicity island was acquired from a plasmid. This work demonstrates diversity among E. coli O157:H7 isolates, at least as far as the presence of Te(r) genes is concerned

Thunberg,R.L., T.T.Tran, R.W.Bennett, R.N.Matthews, and N.Belay. 2002. "Microbial evaluation of selected fresh produce obtained at retail markets." *J.Food Prot.* 65:677-682.
Abstract: The microbial quality of five types of fresh produce obtained at the retail level

was determined by standard quantitative techniques. These techniques included aerobic plate count (APC), total coliform counts, Escherichia coli counts, and yeast and mold counts. Three different methods were used to determine total coliform counts, which consisted of MacConkey agar plate counts, Colicomplete most probable number counts, and Petrifilm E. coli (EC) plate counts. The mean APCs for sprouts, lettuce, celery, cauliflower, and broccoli were 8.7, 8.6, 7.5, 7.4. and 6.3 log10 CFU/g, respectively. MacConkey agar counts indicated that 89 to 96% of the APCs consisted of gram-negative bacteria. Yeast and mold counts were in a range expected of fresh produce. Fresh produce was also analyzed for human pathogens. Samples were analyzed for Staphylococcus spp., Bacillus spp., Salmonella spp., Listeria spp., and Campylobacter spp. One isolate of Staphylococcus was found to be enterotoxigenic, and one species of Bacillus was also toxigenic. Neither Salmonella spp. nor Campylobacter spp. were detected in any of the produce samples. A variety of Listeria spp., including Listeria monocytogenes, were found in fresh produce

Tkalcic,S., C.A.Brown, B.G.Harmon, A.V.Jain, E.P.Mueller, A.Parks, K.L.Jacobsen, S.A.Martin, T.Zhao, and M.P.Doyle. 2000. "Effects of diet on rumen proliferation and fecal shedding of Escherichia coil O157:H7 in calves." *J.Food Prot.* 63:1630-1636.
Abstract: Calves inoculated with Escherichia coli O157:H7 and fed either a high-roughage or high-concentrate diet were evaluated for rumen proliferation and fecal shedding of E. coli O157:H7. Calves fed the high-roughage diet had lower mean rumen volatile fatty acid concentrations and higher rumen pH values than did calves fed the high-concentrate diet. Despite these differences in rumen conditions, the calves fed the high-roughage diet did not have greater rumen populations of E. coli O157: H7 and did not exhibit increased or longer fecal shedding compared with the calves fed the high-concentrate diet. Two calves shedding the highest mean concentrations of E. coli O157:H7 were both fed the high-concentrate diet. There was a significant ($P < 0.05$) positive correlation between fecal shedding and rumen volatile fatty acid concentration in calves fed a high-concentrate diet. The effects of diet on E. coli O157:H7 proliferation and acid resistance were investigated using an in vitro rumen fermentation system. Rumen fluid collected from steers fed a high-roughage diet, but not from steers fed a high-concentrate diet, supported the proliferation of E. coli O157:H7. Rumen fluid from steers fed a high-concentrate diet rapidly induced acid resistance in E. coli O157:H7. The impact of diet on fecal shedding of E. coli O157:H7 is still unclear and may depend on dietary effects on fermentation in the colon and on diet-induced changes in the resident microflora. However, rapid development of acid tolerance by E. coli O157:H7 in the rumens of calves fed high-concentrate diets, allowing larger populations to survive passage through the acidic abomasum to proliferate in the colon, may be one factor that influences fecal shedding in cattle on feed

Tkalcic,S., T.Zhao, B.G.Harmon, M.P.Doyle, C.A.Brown, and P.Zhao. 2003. "Fecal shedding of enterohemorrhagic Escherichia coli in weaned calves following treatment with probiotic Escherichia coli." *J.Food Prot.* 66:1184-1189.
Abstract: The fecal shedding and pathogenicity of enterohemorrhagic E. coli (EHEC) O26:H11, EHEC O111:NM, and EHEC O157:H7 in weaned calves (8 to 10 weeks of age) were compared with and without treatment with a three-strain mixture of probiotic bacteria (competitive-exclusion E. coli). Three groups of 12 calves were each perorally given a five-strain mixture of one of the EHEC serotypes (10(10) CFU of total bacteria per calf). Seventy-two hours later, six calves from each group were each administered 10(10) CFU of probiotic bacteria. None of the EHEC serotypes caused significant clinical disease, although a few calves developed mild transient diarrhea or pyrexia. Gross or microscopic lesions attributable to EHEC were not detected in control or probiotic-treated calves at necropsy. For probiotic-treated calves given E. coli O157:H7 and for probiotic-treated calves given E. coli O111:NM, fecal shedding was reduced compared with that for untreated calves. For the probiotic-treated calves given E. coli O157:H7, the reductions in fecal shedding on days 8, 12, 14, 16, 20, 22, 28, and 30 after

peroral administration were statistically significant (P<0.05). For probiotic-treated calves given E. coli O111:NM, there were statistically significant reductions (P<0.05) in fecal shedding on days 6, 8, 10, and 12. In contrast, there was no reduction in fecal shedding for calves administered E. coli O26:H11 and treated with the probiotic bacteria. In fact, calves in both the treated and the nontreated groups continued to shed large populations of E. coli O26:H11 throughout the 32-day trial. At necropsy, E. coli O157:H7 was isolated from five of six untreated calves and from only two of six probiotic-treated calves. E. coli O111:NM was isolated from four of six untreated calves at necropsy and from two of six probiotic-treated calves. However, E. coli O26:H11 was isolated from five of six untreated calves and from all six probiotic-treated calves. The results obtained in this study indicate that probiotic E. coli substantially reduced or eliminated fecal shedding of E. coli O157:H7 and E. coli O111:NM 8 to 30 days and 6 to 12 days after the administration of the probiotic culture, respectively, and reduced the persistence of E. coli O157:H7 in the gastrointestinal tract at necropsy (31 to 33 days after the administration of the probiotic culture). The probiotic E. coli did not reduce fecal shedding or gastrointestinal persistence of E. coli O26:H11

Torres,A.G. and J.B.Kaper. 2002. "Pathogenicity islands of intestinal E. coli." *Curr.Top.Microbiol.Immunol.* 264:31-48.

Torres,A.G., C.Jeter, W.Langley, and A.G.Matthysse. 2005. "Differential binding of Escherichia coli O157:H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures." *Appl.Environ.Microbiol.* 71:8008-8015.
Abstract: Escherichia coli O157:H7 carried on plant surfaces, including alfalfa sprouts, has been implicated in food poisoning and outbreaks of disease in the United States. Adhesion to cell surfaces is a key component for bacterial establishment and colonization on many types of surfaces. Several E. coli O157:H7 surface proteins are thought to be important for adhesion and/or biofilm formation. Therefore, we examined whether mutations in several genes encoding potential adhesins and regulators of adherence have an effect on bacterial binding to plants and also examined the role of these genes during adhesion to Caco-2 cells and during biofilm formation on plastic in vitro. The genes tested included those encoding adhesins (cah, aidA1, and ompA) and mediators of hyperadherence (tdcA, yidE, waaI, and cadA) and those associated with fimbria formation (csgA, csgD, and lpfD2). The introduction of some of these genes (cah, aidA1, and csg loci) into an E. coli K-12 strain markedly increased its ability to bind to alfalfa sprouts and seed coats. The addition of more than one of these genes did not show an additive effect. In contrast, deletion of one or more of these genes in a strain of E. coli O157:H7 did not affect its ability to bind to alfalfa. Only the absence of the ompA gene had a significant effect on binding, and the plant-bacterium interaction was markedly reduced in a tdcA ompA double mutant. In contrast, the E. coli O157:H7 ompA and tdcA ompA mutant strains were only slightly affected in adhesion to Caco-2 cells and during biofilm formation. These findings suggest that some adhesins alone are sufficient to promote binding to alfalfa and that they may exist in E. coli O157:H7 as redundant systems, allowing it to compensate for the loss of one or more of these systems. Binding to the three types of surfaces appeared to be mediated by overlapping but distinct sets of genes. The only gene which appeared to be irreplaceable for binding to plant surfaces was ompA

Torres,A.G., X.Zhou, and J.B.Kaper. 2005. "Adherence of diarrheagenic Escherichia coli strains to epithelial cells." *Infect.Immun.* 73:18-29.

Turner,C. 2002. "The thermal inactivation of E. coli in straw and pig manure." *Bioresour.Technol.* 84:57-61.
Abstract: Livestock manure may contain pathogenic organisms which pose a risk to the health of animals or humans if the manure is not adequately treated or disposed of. One possible treatment method is composting. However to ensure that pathogen destruction

occurs, temperatures need to be sufficiently high throughout the heap to ensure that pathogens are inactivated. The temperature required to inactivate a marker organism, Escherichia coli 11943, has been investigated, and found to depend on substrate composition, moisture content and duration of incubation. Results show that temperatures in excess of 55 degrees C for 2 h are required for inactivation. Data are presented showing the levels of faecal coliforms in compost heaps where temperatures did not rise above mesophilic levels (35 degrees C where samples were taken)

Tuttle,J., T.Gomez, M.P.Doyle, J.G.Wells, T.Zhao, R.V.Tauxe, and P.M.Griffin. 1999. "Lessons from a large outbreak of Escherichia coli O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties." *Epidemiol.Infect.* 122:185-192.
Abstract: Between November 1992 and February 1993, a large outbreak of Escherichia coli O157:H7 infections occurred in the western USA and was associated with eating ground beef patties at restaurants of one fast-food chain. Restaurants that were epidemiologically linked with cases served patties produced on two consecutive dates; cultures of recalled ground beef patties produced on those dates yielded E. coli O157:H7 strains indistinguishable from those isolated from patients, confirming the vehicle of illness. Seventy-six ground beef patty samples were cultured quantitatively for E. coli O157:H7. The median most probable number of organisms was 1.5 per gram (range, < 0.3-15) or 67.5 organisms per patty (range, < 13.5-675). Correlation of the presence of E. coli O157:H7 with other bacterial indicators yielded a significant association between coliform count and the presence of E. coli O157:H7 (P = 0.04). A meat traceback to investigate possible sources of contamination revealed cattle were probably initially colonized with E. coli O157:H7, and that their slaughter caused surface contamination of meat, which once combined with meat from other sources, resulted in a large number of contaminated ground beef patties. Microbiological testing of meat from lots consumed by persons who became ill was suggestive of an infectious dose for E. coli O157:H7 of fewer than 700 organisms. These findings present a strong argument for enforcing zero tolerance for this organism in processed food and for markedly decreasing contamination of raw ground beef. Process controls that incorporate microbiological testing of meat may assist these efforts

Uhlich,G.A., P.H.Cooke, and E.B.Solomon. 2006. "Analyses of the red-dry-rough phenotype of an Escherichia coli O157:H7 strain and its role in biofilm formation and resistance to antibacterial agents." *Appl.Environ.Microbiol.* 72:2564-2572.
Abstract: In a previous study, we identified Congo red-binding and -nonbinding phase variants of Escherichia coli serotype O157:H7 strain ATCC 43895. The Congo red-binding variant, strain 43895OR, produced a dry, aggregative colony that was similar to the red, dry, and rough (rdar) phenotype characteristic of certain strains of Salmonella. In contrast, variant 43895OW produced a smooth and white colony morphology. In this study, we show that, similar to rdar strains of Salmonella enterica serovar Typhimurium, strain 43895OR forms large aggregates in broth cultures, firm pellicles at the air-medium interface on glass, and dense biofilms on glass and polystyrene. However, unlike S. enterica serovar Typhimurium, strain 43895OR does not stain positive for cellulose production. When strain 43895OR was fixed on agar, scanning electron microscopy showed cells expressing extracellular matrix (ECM) containing curli fibers. Strain 43895OW was devoid of any ECM or curli fibers on agar but showed expression of curli fibers during attachment to glass. Strain 43895OR produced >4-fold-larger amounts of biofilm than strain 43895OW on polystyrene, glass, stainless steel, and Teflon; formation was >3-fold higher in rich medium than in nutrient-limited medium. Biofilm-associated cells of both strains showed statistically greater resistance (P < 0.05) to hydrogen peroxide and quaternary ammonium sanitizer than their respective planktonic cells. This study shows that the rdar phenotype of E. coli O157:H7 strain 43895OR is important in multicellular growth, biofilm formation, and

resistance to sanitizers. However, the lack of cellulose production by strain 43895OR indicates important differences in the ECM composition compared to that of Salmonella

Ulinski,T., C.Lervat, B.Ranchin, Y.Gillet, D.Floret, and P.Cochat. 2005. "Neonatal hemolytic uremic syndrome after mother-to-child transmission of Escherichia coli O157." *Pediatr.Nephrol.* 20:1334-1335.
Abstract: About 90% of cases of hemorrhagic uremic syndrome (HUS) occur in early childhood and most frequently are preceded by bloody diarrhea due to shiga-like toxin (SLT) producing Escherichia coli. We report a case of a newborn girl presenting with bloody diarrhea on her 7th day of life. Acute renal failure, severe arterial hypertension and hemolytic anemia were detected and prompt peritoneal dialysis and antihypertensive therapy were required. The girl had several episodes of seizures, necessitating intravenous phenobarbital. Transfontanel ultrasonography 48 h after disease onset was normal, whereas, MRI investigation 10 days later revealed severe ischemic lesions with beginning cystic encephalopathy. Renal function recovered and only very moderate tubular dysfunction remained. Serum analysis of factor H, von Willebrand factor protease, homocystinemia, proteins C and S, and antithrombin III were all normal. Mutation analysis of factor V Leiden, factor II, and methyltetrahydrofolate-reductase were normal. E. coli 0157:H7 and SLT 2 were detected in the stool. SLT 2 was also found in the mother's stool. This is the first report of mother-to-child transmission of SLT-producing E. coli

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

Vali,L., K.A.Wisely, M.C.Pearce, E.J.Turner, H.I.Knight, A.W.Smith, and S.G.Amyes. 2004. "High-level genotypic variation and antibiotic sensitivity among Escherichia coli O157 strains isolated from two Scottish beef cattle farms." *Appl.Environ.Microbiol.* 70:5947-5954.
Abstract: Escherichia coli O157:H7 is a human pathogen that is carried and transmitted by cattle. Scotland is known to have one of the highest rates of E. coli O157 human infections in the world. Two hundred ninety-three isolates were obtained from naturally infected cattle and the environment on two farms in the Scottish Highlands. The isolates were typed by pulsed-field gel electrophoresis (PFGE) with XbaI restriction endonuclease enzyme, and 19 different variations in patterns were found. There was considerable genomic diversity within the E. coli O157 population on the two farms. The PFGE pattern of one of the observed subtypes matched exactly with that of a strain obtained from a Scottish patient with hemolytic-uremic syndrome. To examine the stability of an individual E. coli O157 strain, continuous subculturing of a strain was performed 110 times. No variation from the original PFGE pattern was observed. We found three indistinguishable subtypes of E. coli O157 on both study farms, suggesting common sources of infection. We also examined the antibiotic resistance of the isolated strains. Phenotypic studies demonstrated resistance of the strains to sulfamethoxazole (100%), chloramphenicol (3.07%), and at a lower rate, other antibiotics, indicating the preservation of antibiotic sensitivity in a rapidly changing population of E. coli O157

Vali,L., M.C.Pearce, K.A.Wisely, A.Hamouda, H.I.Knight, A.W.Smith, and S.G.Amyes. 2005. "Comparison of diversities of Escherichia coli O157 shed from a cohort of spring-born beef calves at pasture and in housing." *Appl.Environ.Microbiol.* 71:1648-1652.
Abstract: A cohort of spring-born beef calves demonstrated limited genetic and phenotypic diversity of Escherichia coli O157 when kept in a state of isolation. Despite this, there was a difference in the pulsed-field gel electrophoresis and phage types of isolates shed by cattle at pasture compared with those shed by the same cattle when weaned and housed

Van Donkersgoed,J., T.Graham, and V.Gannon. 1999. "The prevalence of verotoxins, Escherichia coli O157:H7, and Salmonella in the feces and rumen of cattle at processing." *Can.Vet.J.* 40:332-338.
Abstract: Fecal samples collected from cattle at processing during a 1-year period were tested for verotoxins (VT1, VT2), Escherichia coli O157:H7, and Salmonella. Verotoxins were detected in 42.6% (95% CI, 39.8% to 45.4%), E. coli O157:H7 in 7.5% (95% CI, 6.1% to 9.1%), and Salmonella in 0.08% (95% CI, 0.004% to 0.5%) of the fecal samples. In yearling cattle, the median within-lot prevalence (percentage of positive samples within a lot) was 40% (range, 0% to 100%) for verotoxins and 0% for E. coli O157:H7 (range, 0% to 100%) and Salmonella (range, 0% to 17%). One or more fecal samples were positive for verotoxins in 80.4% (95% CI, 72.8% to 86.4%) of the lots of yearling cattle, whereas E. coli O157:H7 were detected in 33.6% (95% CI, 26.0% to 42.0%) of the lots. In cull cows, the median within-lot prevalence was 50% (range, 0% to 100%) for verotoxins and 0% (range, 0% to 100%) for E. coli O157:H7 and Salmonella (range, 0% to 0%). Verotoxins were detected in one or more fecal samples from 78.0% (95% CI, 70.4% to 84.2%) of the lots of cull cows, whereas E. coli O157:H7 were detected in only 6.0% (95% CI, 3.0% to 11.4%) of the lots of cull cows. The prevalence of verotoxins in fecal samples was lower in yearling cattle than in cull cows, whereas the prevalence of E. coli O157:H7 in fecal samples was higher in yearling cattle than in cull cows. The prevalence of E. coli O157:H7 in fecal samples was highest in the summer months. Rumen fill, body condition score, sex, type of cattle (dairy, beef), and distance travelled to the plant were not associated with the fecal prevalence of verotoxins or E. coli O157:H7. The prevalence of verotoxins in fecal samples of cull cows was associated with the source of the cattle. It was highest in cows from the auction market (52%) and farm/ranch (47%) and lowest in cows from the feedlot (31%). In rumen samples, the prevalence of verotoxins was 6.4% (95% CI, 4.2% to 9.4%), and it was 0.8% (95% CI, 0.2% to 2.3%) for E. coli O157:H7, and 0.3% (95% CI, 0.007% to 1.5%) for Salmonella

Van Donkersgoed,J., J.Berg, A.Potter, D.Hancock, T.Besser, D.Rice, J.LeJeune, and S.Klashinsky. 2001. "Environmental sources and transmission of Escherichia coli O157 in feedlot cattle." *Can.Vet.J.* 42:714-720.
Abstract: A study was conducted in 2 feedlots in southern Alberta to identify environmental sources and management factors associated with the prevalence and transmission of Escherichia coli O157:H7. Escherichia coli O157:H7 was isolated in preslaughter pens of cattle from feces (0.8%), feedbunks (1.7%), water troughs (12%), and incoming water supplies (4.5%), but not from fresh total mixed rations. Fresh total mixed rations did not support the growth of E. coli O157:H7 and E. coli from bovine feces following experimental inoculation. Within a feedlot, the feces, water troughs, and feedbunks shared a few indistinguishable subtypes of E. coli O157:H7. A few subtypes were repeatedly isolated in the same feedlot, and the 2 feedlots shared a few indistinguishable subtypes. The prevalence of E. coli O157:H7 in water troughs of preslaughter cattle in 1 feedlot was associated with season, maximum climatic temperatures the week before sampling; total precipitation the week before sampling, and coliform and E. coli counts in the water trough

Varma,J.K., K.D.Greene, M.E.Reller, S.M.DeLong, J.Trottier, S.F.Nowicki, M.DiOrio, E.M.Koch, T.L.Bannerman, S.T.York, M.A.Lambert-Fair, J.G.Wells, and P.S.Mead. 2003. "An

outbreak of Escherichia coli O157 infection following exposure to a contaminated building." *JAMA*. 290:2709-2712.
Abstract: CONTEXT: Infection with Escherichia coli O157 causes an estimated 70 000 diarrheal illnesses per year in the United States and can result in hemolytic-uremic syndrome and death. Environmental contamination with E coli O157 may be a public health problem. OBJECTIVES: To determine risk factors for E coli O157 infection during an outbreak investigation at a county fair and to evaluate environmental contamination as a possible cause of the outbreak. DESIGN, SETTING, AND PARTICIPANTS: Case-control study of 23 patients (median age, 15 years) and 53 age-matched controls who had attended the Lorain County, Ohio, fair between August 20 and August 26, 2001. Case-patients had laboratory-confirmed E coli O157 infection, hemolytic-uremic syndrome, or bloody diarrhea within 7 days of attending the fair; controls attended the fair and did not have diarrhea. MAIN OUTCOME MEASURES: Risk factors for infection and isolates of E coli O157 from environmental specimens. RESULTS: Six (26%) case-patients were hospitalized and 2 (9%) developed hemolytic-uremic syndrome. Case-patients were more likely than controls to have visited building A (a multipurpose community facility on the fairgrounds; matched odds ratio [MOR], 21.4 [95% confidence interval [CI], 2.7-170.7]). Among visitors to building A, illness was independently associated with attending a dance in the building (MOR, 7.5; 95% CI, 1.4-41.2), handling sawdust from the floor (MOR, 4.6; 95% CI, 1.1-20.0), or eating and/or drinking in the building (MOR, 4.5; 95% CI, 1.2-16.6). Twenty-four (44%) of 54 specimens collected from building A 6 weeks after the fair grew Shiga toxin-producing E coli O157. Isolates from sawdust, the rafters, and other surfaces were identical by molecular fingerprinting to patient isolates. Sawdust specimens collected 42 weeks after the fair also grew the same E coli O157 strain. CONCLUSIONS: Absence of evidence implicating specific food or beverage sources and the recovery of E coli O157 from the rafters suggest that airborne dispersion of bacteria contributed to the contamination. Because E coli O157 can survive in the environment for more than 10 months, humans may be at risk of infection long after an environment is initially contaminated

Venkitanarayanan,K.S., C.M.Lin, H.Bailey, and M.P.Doyle. 2002. "Inactivation of Escherichia coli O157:H7, Salmonella enteritidis, and Listeria monocytogenes on apples, oranges, and tomatoes by lactic acid with hydrogen peroxide." *J.Food Prot.* 65:100-105.
Abstract: The objective of this study was to develop a practical and effective method for inactivating or substantially reducing Escherichia coli O157:H7, Salmonella Enteritidis, and Listeria monocytogenes on apples, oranges, and tomatoes. Apples, oranges, and tomatoes were spot-inoculated with five-strain mixtures of E. coli O157:H7, Salmonella Enteritidis, and L. monocytogenes near the stem end and were submerged in sterile deionized water containing 1.5% lactic acid plus 1.5% hydrogen peroxide for 15 min at 40 degrees C. Inoculated samples treated with sterile deionized water at the same temperature and for the same duration served as controls. The bacterial pathogens on fruits subjected to the chemical treatment were reduced by >5.0 log10 CFU per fruit, whereas washing in deionized water decreased the pathogens by only 1.5 to 2.0 log10 CFU per fruit. Furthermore, substantial populations of the pathogens survived in the control wash water, whereas no E. coli O157:H7, Salmonella Enteritidis, or L. monocytogenes cells were detected in the chemical treatment solution. The sensory and qualitative characteristics of apples treated with the chemical wash solution were not adversely affected by the treatment. It was found that the treatment developed in this study could effectively be used to kill E. coli O157:H7, Salmonella Enteritidis, and L. monocytogenes on apples, oranges, and tomatoes at the processing or packaging level

Vernozy-Rozand,C., M.P.Montet, F.Lequerrec, E.Serillon, B.Tilly, C.Bavai, S.Ray-Gueniot, J.Bouvet, C.Mazuy-Cruchaudet, and Y.Richard. 2002. "Prevalence of verotoxin-producing Escherichia coli (VTEC) in slurry, farmyard manure and sewage sludge in France." *J.Appl.Microbiol.* 93:473-478.
Abstract: AIMS: The aims of the present study were to determine VTEC prevalence in

manure, slurry and sewage sludge in France and to characterize the VTEC strains isolated (virulence genes and serotype). METHODS AND RESULTS: Seven hundred and fifty-two samples from 55 farmyard manures, 136 bovine and porcine faeces, 114 slurries, 10 composts, and 437 samples from outflows of sewage wastewater treatment plants were analysed. Twenty-four percent contained isolates which were PCR positive for stx gene. Twenty-one VTEC strains were recovered from positive samples by colony hybridization: 76% of them were positive for stx(2) gene, 33% for stx(1) gene,and 19% for eae gene. One strain belonged to serotype O157:H7 and two others to serogroups O26 and O55, respectively. CONCLUSIONS: Some of the VTEC strains isolated from environments in France should be considered as potentially pathogenic for humans. SIGNIFICANCE AND IMPACT OF THE STUDY: Appropriate handling or use of manure, slurry and sewage sludge is necessary so that contamination of the environment and food by VTEC can be prevented

Vokes,S.A., S.A.Reeves, A.G.Torres, and S.M.Payne. 1999. "The aerobactin iron transport system genes in Shigella flexneri are present within a pathogenicity island." *Mol.Microbiol.* 33:63-73.
Abstract: Genes encoding the synthesis and transport of aerobactin, a hydroxamate siderophore associated with increased virulence of enteric bacteria, were mapped within a pathogenicity island in Shigella flexneri. The island, designated SHI-2 for Shigella pathogenicity island 2, was located downstream of selC, the site of insertion of pathogenicity islands in several other enteric pathogens. DNA sequence analysis revealed the presence of multiple insertion sequences upstream and downstream of the aerobactin genes and an integrase gene that was nearly identical to an int gene found in Escherichia coli O157:H7. SHI-2 sequences adjacent to selC were similar to sequences at the junction between selC and pathogenicity islands found in E. coli O157:H7 and in enteropathogenic E. coli, but the junctions between the island and downstream yic genes were variable. SHI-2 also encoded immunity to the normally plasmid-encoded colicins I and V, suggesting a common origin for the aerobactin genes in both S. flexneri and E. coli pColV. Polymerase chain reaction and Southern hybridization data indicate that SHI-2 is present in the same location in Shigella sonnei, but the aerobactin genes are not located within SHI-2 in Shigella boydii or enteroinvasive E. coli. Shigella dysenteriae type 1 strains do not produce aerobactin but do contain sequences downstream of selC that are homologous to SHI-2. The presence of the aerobactin genes on plasmids in E. coli pColV and Salmonella, on a pathogenicity island in S. flexneri and S. sonnei and in a different chromosomal location in S. boydii and some E. coli suggests that these virulence-enhancing genes are mobile, and they may constitute an island within an island in S. flexneri

Vold,L., J.B.Klungseth, H.Kruse, E.Skjerve, and Y.Wasteson. 1998. "Occurrence of shigatoxinogenic Escherichia coli O157 in Norwegian cattle herds." *Epidemiol.Infect.* 120:21-28.
Abstract: To investigate if there is a reservoir of Escherichia coli O157 in Norwegian cattle, faecal samples from 197 cattle herds were screened for E. coli O157 by the use of immunomagnetic separation (IMS) and PCR during the 1995 grazing season. Six E. coli O157:H-isolates were detected in two herds, one isolate in one and five in the other. The isolates carried the stx1, stx2, and eae genes, and a 90 MDa virulence plasmid. They were toxinogenic in a Vero cell assay. From 57 other herds, 137 faecal samples were positive for stx1 and/or stx2 genes detected by PCR run directly on IMS-isolated material. Among these samples, stx2 were the most widely distributed toxin encoding genes. No difference was found among milking cows and heifers in the rate of stx1 and or stx2 in positive samples

Wachtel,M.R., L.C.Whitehand, and R.E.Mandrell. 2002. "Prevalence of Escherichia coli associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater." *J.Food Prot.* 65:471-475.

Abstract: Preharvest contamination of field crops may have many sources, including feces, soil, and irrigation water. In March 2000, a sewage spill released unchlorinated tertiary-treated effluent into a creek used to irrigate commercial produce. A field of young cabbage transplants was irrigated with creek water as the contaminated water flowed past this land. Cabbage samples were taken from plots within this field, and Escherichia coli was isolated from the roots of these plants but not from the edible portion of the cabbage. No E. coli was isolated from water samples or from control samples taken from a nearby cabbage field watered with chlorinated municipal water. The cabbage field under study had not been fertilized with manure for at least 2 years prior to the contamination incident. Six different E. coli serotypes were identified, although none of them proved to be pathogenic. These serotypes were separated into five groups by a RiboPrinter; the resulting groups correlated well with the serotypes and the locations in the field from which these strains were isolated. We previously found that certain nonpathogenic E. coli strains displayed lower levels of adherence to lettuce seedling roots in a hydroponic adherence assay. The E. coli field strains displayed variable patterns of adherence to lettuce seedlings: strain MW421 showed significantly lower root and shoot adherence levels than did the other field strains, while strains MW423 and MW425 showed significantly higher root and shoot adherence levels. These data suggest that water quality is of paramount importance for the food safety of growing crops

Wachtel,M.R., J.L.McEvoy, Y.Luo, A.M.Williams-Campbell, and M.B.Solomon. 2003. "Cross-contamination of lettuce (Lactuca sativa L.) with Escherichia coli O157:H7 via contaminated ground beef." *J.Food Prot.* 66:1176-1183.
Abstract: A lettuce outbreak strain of E. coli O157:H7 was used to quantitate the pathogen's survival in ground beef and its transfer to hands, cutting board surfaces, and lettuce. Overnight storage of inoculated beef at 4 degrees C resulted in no pathogen growth, while room-temperature storage allowed multiplication. Hamburger patty formation allowed the transfer of bacteria to hands. Contaminated fingers subsequently transferred the pathogen to lettuce during handling. E. coli was transferred from hamburgers to cutting board surfaces; overnight storage of boards decreased the numbers of recoverable pathogens by approximately 1 log CFU. A 15-s water rinse failed to remove significant numbers of pathogens from cutting boards whether it was applied immediately after contamination or following overnight room-temperature storage. Three lettuce leaves were successively applied to a single contaminated cutting board area both immediately after contamination and after overnight room-temperature storage of contaminated boards. Another set of leaves was pressed onto boards immediately following contamination and was then stored overnight at 4 degrees C before pathogen enumeration. The numbers of pathogens transferred to the first pressed leaves were larger than those transferred to the second or third leaves. There were no significant differences in the numbers of pathogensrecovered from leaves pressed immediately after contamination whether pathogens were enumerated immediately or following overnight storage at 4 degrees C. However, fewer pathogens were transferred to leaves pressed to boards stored overnight at room temperature prior to contact with lettuce. Twenty-five lettuce pieces were successively pressed onto one area on a board containing 1.25 x 10(2) CFU of E. coli. Pathogens were transferred to 46% of the leaves, including the 25th exposed leaf

Wang,G., T.Zhao, and M.P.Doyle. 1996. "Fate of enterohemorrhagic Escherichia coli O157:H7 in bovine feces." *Appl.Environ.Microbiol.* 62:2567-2570.
Abstract: Dairy cattle have been identified as a principal reservoir of Escherichia coli O157:H7. The fate of this pathogen in bovine feces at 5, 22, and 37 degrees C was determined. Two levels of inocula (10(3) and 10(5) CFU/g) of a mixture of five nalidixic acid-resistant E. coli O157:H7 strains were used. E. coli O157:H7 survived at 37 degrees C for 42 and 49 days with low and high inocula, respectively, and at 22 degrees C for 49 and 56 days with low and high inocula, respectively. Fecal samples at both

temperatures had low moisture contents (about 10%) and water activities (< 0.5) near the end of the study. E. coli O157:H7 at 5 degrees C survived for 63 to 70 days, with the moisture content (74%) of feces remaining high through the study. Chromosomal DNA fingerprinting of E. coli O157:H7 isolates surviving near the completion of the study revealed that the human isolate strain 932 was the only surviving strain at 22 or 37 degrees C. All five strains were isolated near the end of incubation from feces held at 5 degrees C. Isolates at each temperature were still capable of producing both verotoxin 1 and verotoxin 2. Results indicate that E. coli O157:H7 can survive in feces for a long period of time and retain its ability to produce verotoxins. Hence, bovine feces are a potential vehicle for transmitting E. coli O157:H7 to cattle, food, and the environment. Appropriate handling of bovine feces is important to control the spread of this pathogen

Wang,G. and M.P.Doyle. 1998. "Survival of enterohemorrhagic Escherichia coli O157:H7 in water." *J.Food Prot.* 61:662-667.
Abstract: Several recent Escherichia coli O157:H7 outbreaks associated with both drinking and recreational water raise concerns about waterborne illness caused by this pathogen. The survival characteristics of a mixture of five nalidixic acid-resistant E. coli O157:H7 strains (10(3) CFU/ml) in filtered and autoclaved municipal water, in reservoir water, and in water from two recreational lakes were determined for a period of 91 days at 8, 15 or 25 degrees C. Greatest survival was in filtered autoclaved municipal water and least in lake water. Regardless of the water source, survival was greatest at 8 degrees C and least at 25 degrees C. E. coli O157:H7 populations decreased by 1 to 2 log10 by 91 days at 8 degrees C, whereas the pathogen was not detectable (> or 3 = log10 decrease) within 49 to 84 days at 25 degrees C in three of the four water sources. SDS-PAGE of surface antigens of surviving cells revealed that there was no major alteration in lipopolysaccharide pattern, but outer membrane protein composition did change. These studies indicate that E. coli O157:H7 is a hardy pathogen that can survive for long periods of time in water, especially at cold temperatures. However, direct viable counts of E. coli O157:H7 determined by acridine orange staining remained essentially the same for 12 weeks at 25 degrees C, whereas viable counts on tryptic soy agar plates decreased to undetectable levels within 12 weeks. Results suggest that E. coli O157:H7 can enter a viable but nonculturable (VBNC) state in water

Warriner,K., F.Ibrahim, M.Dickinson, C.Wright, and W.M.Waites. 2003. "Internalization of human pathogens within growing salad vegetables." *Biotechnol.Genet.Eng Rev.* 20:117-134.

Warriner,K., F.Ibrahim, M.Dickinson, C.Wright, and W.M.Waites. 2003. "Interaction of Escherichia coli with growing salad spinach plants." *J.Food Prot.* 66:1790-1797.
Abstract: In this study, the interaction of a bioluminescence-labeled Escherichia coli strain with growing spinach plants was assessed. Through bioluminescence profiles, the direct visualization of E. coli growing around the roots of developing seedlings was accomplished. Subsequent in situ glucuronidase (GUS) staining of seedlings confirmed that E. coli had become internalized within root tissue and, to a limited extent, within hypocotyls. When inoculated seeds were sown in soil microcosms and cultivated for 42 days, E. coli was recovered from the external surfaces of spinach roots and leaves as well as from surface-sterilized roots. When 20-day-old spinach seedlings (from uninoculated seeds) were transferred to soil inoculated with E. coli, the bacterium became established on the plant surface, but internalization into the inner root tissue was restricted. However, for seedlings transferred to a hydroponic system containing 10(2) or 10(3) CFU of E. coli per ml of the circulating nutrient solution, the bacterium was recovered from surface-sterilized roots, indicating that it had been internalized. Differences between E. coli interactions in the soil and those in the hydroponic system may be attributed to greater accessibility of the roots in the latter model. Alternatively, the presence of a competitive microflora in soil may have restricted root colonization by E. coli. The implications of this study's findings with regard to the microbiological safety of minimally processed vegetables are discussed

Warriner,K., S.Spaniolas, M.Dickinson, C.Wright, and W.M.Waites. 2003. "Internalization of bioluminescent Escherichia coli and Salmonella Montevideo in growing bean sprouts." *J.Appl.Microbiol.* 95:719-727.
Abstract: AIMS: Investigate the interaction of bioluminescent Escherichia coli and Salmonella Montevideo with germinating mung bean sprouts. METHODS AND RESULTS: E. coli or Salm. Montevideo introduced on mung beans became established both internally and externally on sprouts after the initial 24 h germinating period. In both cases the inoculated bacterium formed the predominant microflora on the sprouted beans throughout. From the bioluminescent profile of inoculated sprouting beans, bacterial growth was found to be in close proximity to the roots but not on the hypocotyls. Clumps (biofilms) of cells with low viability were observed within the grooves between epidermal cells on hypocotyls. Treatment with 20,000 ppm sodium hypochlorite removed the majority of bacteria from the surface of hypocotyls although nonviable single cells were occasionally observed. However, viable bacteria were recovered from the apoplastic fluid, and extracts of surface-sterilized sprouts indicating that the internal bacterial populations had been protected. This was confirmed using in situ beta-glucuronidase staining of surface-sterilized sprouts where cleaved enzyme substrate (by the action of internalized E. coli) was visualized within the plant vascular system. CONCLUSIONS: E. coli or Salmonella present on seeds become internalized within the subsequent sprouts and cannot be removed by postharvest biocidal washing. SIGNIFICANCE AND IMPACT OF THE STUDY: Mung bean production should be carefully controlled to prevent contamination occurring in order to minimize the health risk associated with raw bean sprouts

Wasteson,Y., G.S.Johannessen, T.Bruheim, A.M.Urdahl, K.O'Sullivan, and L.M.Rorvik. 2005. "Fluctuations in the occurrence of Escherichia coli O157:H7 on a Norwegian farm*." *Lett.Appl.Microbiol.* 40:373-377.
Abstract: AIM: To describe the distribution of Escherichia coli O157:H7 on a sporadically positive dairy farm and on possible contact farms over a one-year period. METHODS AND RESULTS: Environmental and faecal samples from all animals at the farm, and faecal samples from animals at contact farms were analysed for E. coli O157:H7 by immunomagnetic separation methods or VIDAS.Confirmed isolates were tested for cytotoxicity in the Vero cell assay and typed by PFGE. Escherichia coli O157:H7 (stx2 and eae) of the same PFGE type were isolated from cattle, sheep, hens and environmental samples at variable levels during summer and fall 2002, but were not detected in 2003. CONCLUSIONS: Escherichia coli O157:H7 had a widespread distribution on the farm investigated, but the original source of contamination could not be identified. The occurrence of this bacterium on the farm did not result in any detectable increase in gastrointestinal disease in the associated population. SIGNIFICANCE AND IMPACT OF THE STUDY: Despite a low endemic level of E. coli O157:H7 in the Norwegian cattle population, the growth and spread of this potentially important bacterium may occur

Williams,A.P., L.M.Avery, K.Killham, and D.L.Jones. 2005. "Persistence of Escherichia coli O157 on farm surfaces under different environmental conditions." *J.Appl.Microbiol.* 98:1075-1083.
Abstract: AIMS: To compare the persistence of Escherichia coli O157 on a variety of common faecally contaminated farmyard material surfaces (wood and steel) under different moisture and temperature regimes. METHODS AND RESULTS: Samples of field-conditioned farmyard materials (galvanized steel and wood) were cut into pieces and contaminated with fresh cattle faeces inoculated with nontoxigenic E. coli O157 (strain 3704). Thereafter, they were stored at four different environmental conditions; with temperature (5 and 20 degrees C) and moisture (moist or dry) as variables. Transfer of the pathogen to hands from the surfaces was also evaluated. Escherichia coli O157 numbers declined over time on all surfaces albeit at different rates according to the sample material and environmental conditions. Persistence was greatest on moist

wood samples under cooler temperatures with large population numbers remaining after 28 days. Desiccation of surfaces resulted in a more rapid decline in E. coli O157 populations under both temperature regimes. Substantial numbers of colonies may also potentially be transferred to human hands from the surfaces during brief contact. CONCLUSIONS: When environmental conditions are favourable, E. coli O157 may persist for considerable times on a range of surfaces. However, when exposed to higher temperatures and dehydration, survival is notably decreased. Overall, bacterial persistence was significantly greater on wood samples relative to steel. SIGNIFICANCE AND IMPACT OF THE STUDY: Escherichia coli O157 is a prevalent pathogen, common in ruminant faeces. Contact with contaminated faeces may lead to human infection, resulting in possible severe illness. Although our study used only one strain of bacteria, our findings indicates that E. coli O157 has the potential to persist for long periods of time on gates, stiles and other farmyard surfaces under a range of environmental conditions. These farmyard surfaces therefore pose a potential infection pathway particularly where there is a high risk of direct human contact (e.g. child petting zoos, open farms)

Wu,F.M., L.R.Beuchat, M.P.Doyle, V.Garrett, J.G.Wells, and B.Swaminathan. 2002. "Fate of Escherichia coli O157:H7 in coleslaw during storage." *J.Food Prot.* 65:845-847. Abstract: An outbreak of Escherichia coli O157:H7 infection associated with the consumption of coleslaw in several units of a restaurant chain prompted a study to determine the fate of the pathogen in two commercial coleslaw preparations (pH 4.3 and 4.5) held at 4, 11, and 21 degrees C for 3 days. At an initial population of 5.3 log10 CFU g of coleslaw, E. coli O157:H7 did not grow in either coleslaw stored at the three temperatures. Rather, the population of E. coli O157:H7 decreased by 0.1 to 0.5 log10 CFU/g within 3 days. The greatest reduction (0.4 and 0.5 log10 CFU/g) in population occurred at 21 degrees C, whereas only slight decreases (0.1 to 0.2 log10 CFU/g) occurred at 4 and 11 degrees C. A pH of 4.3 to 4.5 of coleslaw had little effect on reducing E. coli O157:H7 populations. Results suggest that the tolerance of E. coli O157:H7 to acid pH, not temperature abuse, is a major factor influencing the pathogen's fate in restaurant-prepared coleslaw

Yang,C.H. and D.E.Crowley. 2000. "Rhizosphere microbial community structure in relation to root location and plant iron nutritional status." *Appl.Environ.Microbiol.* 66:345-351. Abstract: Root exudate composition and quantity vary in relation to plant nutritional status, but the impact of the differences on rhizosphere microbial communities is not known. To examine this question, we performed an experiment with barley (Hordeum vulgare) plants under iron-limiting and iron-sufficient growth conditions. Plants were grown in an iron-limiting soil in root box microcosms. One-half of the plants were treated with foliar iron every day to inhibit phytosiderophore production and to alter root exudate composition. After 30 days, the bacterial communities associated with different root zones, including the primary root tips, nonelongating secondary root tips, sites of lateral root emergence, and older roots distal from the tip, were characterized by using 16S ribosomal DNA (rDNA) fingerprints generated by PCR-denaturing gradient gel electrophoresis (DGGE). Our results showed that the microbial communities associated with the different root locations produced many common 16S rDNA bands but that the communities could be distinguished by using correspondence analysis. Approximately 40% of the variation between communities could be attributed to plant iron nutritional status. A sequence analysis of clones generated from a single 16S rDNA band obtained at all of the root locations revealed that there were taxonomically different species in the same band, suggesting that the resolving power of DGGE for characterization of community structure at the species level is limited. Our results suggest that the bacterial communities in the rhizosphere are substantially different in different root zones and that a rhizosphere community may be altered by changes in root exudate composition caused by changes in plant iron nutritional status

Yang,C.H., D.E.Crowley, J.Borneman, and N.T.Keen. 2001. "Microbial phyllosphere populations are more complex than previously realized." *Proc.Natl.Acad.Sci.U.S.A*. 98:3889-3894.
Abstract: Phyllosphere microbial communities were evaluated on leaves of field-grown plant species by culture-dependent and -independent methods. Denaturing gradient gel electrophoresis (DGGE) with 16S rDNA primers generally indicated that microbial community structures were similar on different individuals of the same plant species, but unique on different plant species. Phyllosphere bacteria were identified from Citrus sinesis (cv. Valencia) by using DGGE analysis followed by cloning and sequencing of the dominant rDNA bands. Of the 17 unique sequences obtained, database queries showed only four strains that had been described previously as phyllosphere bacteria. Five of the 17 sequences had 16S similarities lower than 90% to database entries, suggesting that they represent previously undescribed species. In addition, three fungal species were also identified. Very different 16S rDNA DGGE banding profiles were obtained when replicate cv. Valencia leaf samples were cultured in BIOLOG EcoPlates for 4.5 days. All of these rDNA sequences had 97--100% similarity to those of known phyllosphere bacteria, but only two of them matched those identified by the culture independent DGGE analysis. Like other studied ecosystems, microbial phyllosphere communities therefore are more complex than previously thought, based on conventional culture-based methods

Zhao,T., M.P.Doyle, and R.E.Besser. 1993. "Fate of enterohemorrhagic Escherichia coli O157:H7 in apple cider with and without preservatives." *Appl.Environ.Microbiol.* 59:2526-2530.
Abstract: A strain of enterohemorrhagic Escherichia coli serotype O157:H7 isolated from a patient in an apple cider-related outbreak was used to study the fate of E. coli O157:H7 in six different lots of unpasteurized apple cider. In addition, the efficacy of two preservatives, 0.1% sodium benzoate and 0.1% potassium sorbate, used separately and in combination was evaluated for antimicrobial effects on the bacterium. Studies were done at 8 or 25 degrees C with ciders having pH values of 3.6 to 4.0. The results revealed that E. coli O157:H7 populations increased slightly (ca. 1 log10 CFU/ml) and then remained stable for approximately 12 days in lots inoculated with an initial population of 10(5) E. coli O157:H7 organisms per ml and held at 8 degrees C. The bacterium survived from 10 to 31 days or 2 to 3 days at 8 or 25 degrees C, respectively, depending on the lot. Potassium sorbate had minimal effect on E. coli O157:H7 populations, with survivors detected for 15 to 20 days or 1 to 3 days at 8 or 25 degrees C, respectively. In contrast, survivors in cider containing sodium benzoate were detected for only 2 to 10 days or less than 1 to 2 days at 8 or 25 degrees C, respectively. The highest rates of inactivation occurred in the presence of a combination of 0.1% sodium benzoate and 0.1% potassium sorbate. The use of 0.1% sodium benzoate, an approved preservative used by some cider processors, will substantially increase the safety of apple cider in terms of E. coli O157:H7, in addition to suppressing the growth of yeasts and molds

Zhao,T., M.P.Doyle, J.Shere, and L.Garber. 1995. "Prevalence of enterohemorrhagic Escherichia coli O157:H7 in a survey of dairy herds." *Appl.Environ.Microbiol.* 61:1290-1293.
Abstract: The prevalence of Escherichia coli O157:H7 in dairy herds is poorly understood, even though young dairy animals have been reported to be a host. From February to May 1993, 662 fecal samples from 50 control herds in 14 states, and from June to August 1993, 303 fecal samples from 14 case herds in 11 states were collected for isolation of E. coli O157:H7. Case herds were those in which E. coli O157:H7 was isolated from preweaned calves in a previous U.S. Department of Agriculture study, whereas control herds from which E. coli O157:H7 had not been isolated previously were randomly selected from the same states as case herds. Among the control herds, E. coli O157:H7 was isolated from 6 of 399 calves (1.5%) that were between 24 h old and the age of weaning and from 13 of 263 calves (4.9%) that were between the ages of

weaning and 4 months. Eleven of 50 control herds (22%) were positive. Among the case herds, E. coli O157:H7 was isolated from 5 of 171 calves (2.9%) that were between 24 h old and the age of weaning and from 7 of 132 calves (5.3%) that were between the ages of weaning and 4 months. Seven of 14 case herds (50%) were positive. Sixteen of 31 isolates were obtained by direct plating, with populations ranging from 10(3) to 10(5) CFU/g. Fifteen of 31 isolates were isolated by enrichment only. Nineteen of the isolates produced both verocytotoxin 1 (VT-1) and VT-2, whereas 12 produced VT-2 only

Zhao,T., M.P.Doyle, P.Zhao, P.Blake, and F.M.Wu. 2001. "Chlorine inactivation of Escherichia coli O157:H7 in water." *J.Food Prot.* 64:1607-1609.
Abstract: Six human isolates of Escherichia coli O157:H7 and E. coli (ATCC 11229) were used to determine the concentrations of free chlorine and exposure times required for inactivation. Free chlorine concentrations of 0.25, 0.5, 1.0, and 2.0 ppm at 23 degrees C were evaluated, with sampling times at 0, 0.5, 1.0, and 2.0 min. Results revealed that five of six E. coli O157:H7 isolates and the E. coli control strain were highly susceptible to chlorine, with >7 log10 CFU/ml reduction of each of these strains by 0.25 ppm free chlorine within 1 min. However, comparatively, one of the seven strains was unusually tolerant to chlorine at 23 degrees C for 1 min, with a 4-, 5.5-, 5.8-, and >5.8-log CFU/ml reduction at free chlorine concentrations (ppm) of 0.25, 0.5, 1.0, and 2.0. respectively. Based on these studies most isolates of E. coli O157:H7 have no unusual tolerance to chlorine; however, one strain was exceptional in being recovered after 1-min of exposure of 10(7) CFU/ml to 2.0 ppm of free chlorine. This isolate may be a useful reference strain for future studies on chlorine tolerance of E. coli O157:H7

Zhao,T., S.Tkalcic, M.P.Doyle, B.G.Harmon, C.A.Brown, and P.Zhao. 2003. "Pathogenicity of enterohemorrhagic Escherichia coli in neonatal calves and evaluation of fecal shedding by treatment with probiotic Escherichia coli." *J.Food Prot.* 66:924-930.
Abstract: The pathogenicity and fecal shedding of enterohemorrhagic Escherichia coli (EHEC) O26:H11, O111:NM, and O157:H7 were compared in calves (< 1 week of age) with or without prior treatment with probiotic bacteria (competitive exclusion E. coli). Three groups of 12 to 14 calves were used for these treatments. Half of the calves in each group were perorally administered 10(10) CFU of probiotic bacteria per calf, and, 2 days thereafter, 10(8) CFU of a five-strain mixture with one of the three EHEC serotypes per calf were administered to each calf. None of the EHEC serotypes caused clinical disease,and neither gross nor microscopic lesions attributable to EHEC were detected in control or probiotic-treated calves at necropsy. In calves administered E. coli O157:H7, fecal shedding was greatly reduced (> 6 log10 CFU/g) by 8 days after administration, and there was no significant difference (P > 0.05) in fecal shedding of E. coli O157:H7 between probiotic-treated and untreated control groups at that time. In contrast, control calves perorally administered E. coil of serotypes O111:NM or O26:H11 continued to shed substantial populations (10(2.1) to 10(6) CFU/g of feces and 10(2.5) to 10(4.9) CFU/g of feces, respectively) throughout 7 days postadministration of EHEC. In both groups administered either E. coli O111:NM or O26:H11, significantly less (P < 0.05) EHEC was isolated from feces at 7 days postadministration of EHEC and at necropsy from theprobiotic-treated group than from the untreated control group. Overall, neonatal calves shed in the feces from 1 to 7 days following peroral administration of EHEC greater populations of E. coli O111:NM and O26:H111 than E. coli O157:H7. In addition, treatment of calves with probiotic E. coli reduced fecal shedding of E. coli O111:NM and O26:H11 in most calves

Zhao,T., P.Zhao, J.W.West, J.K.Bernard, H.G.Cross, and M.P.Doyle. 2006. "Inactivation of enterohemorrhagic Escherichia coli in rumen content- or feces-contaminated drinking water for cattle." *Appl.Environ.Microbiol.* 72:3268-3273.
Abstract: Cattle drinking water is a source of on-farm Escherichia coli O157:H7 transmission. The antimicrobial activities of disinfectants to control E. coli O157:H7 in on-farm drinking water are frequently neutralized by the presence of rumen content and

manure that generally contaminate the drinking water. Different chemical treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate, and competing E. coli, were tested individually or in combination for inactivation of E. coli O157:H7 in the presence of rumen content. Chlorine (5 ppm), ozone (22 to 24 ppm at 5 degrees C), and competing E. coli treatment of water had minimal effects (<1 log CFU/ml reduction) on killing E. coli O157:H7 in the presence of rumen content at water-to-rumen content ratios of 50:1 (vol/wt) and lower. Four chemical-treatment combinations, including (i) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (treatment A); (ii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (treatment B); (iii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (treatment C); and (iv) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 100 ppm chlorine dioxide (treatment D); were highly effective (>3 log CFU/ml reduction) at 21 degrees C in killing E. coli O157:H7, O26:H11, and O111:NM in water heavily contaminated with rumen content (10:1 water/rumen content ratio [vol/wt]) or feces (20:1 water/feces ratio [vol/wt]). Among them, treatments A, B, and C killed >5 log CFU E. coli O157:H7, O26:H11, and O111:NM/ml within 30 min in water containing rumen content or feces, whereas treatment D inactivated approximately 3 to 4 log CFU/ml under the same conditions. Cattle given water containing treatment A or C or untreated water (control) ad libitum for two 7-day periods drank 15.2, 13.8, and 30.3 liters/day, respectively, and cattle given water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 liters/day. The amounts of water consumed for all water treatments were significantly different from that for the control, but there were no significant differences among the water treatments. Such treatments may best be applied periodically to drinking water troughs and then flushed, rather than being added continuously, to avoid reduced water consumption by cattle