Human Illness Caused by *E. coli* O157:H7 from Food and Non-food Sources

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Contents

Introduction
Epidemiology of <i>E. coli</i> O157:H72
Outbreak Data2
Reservoirs of E. coli O157:H7
Cattle—the primary reservoir
Other ruminants4
Other animals4
Transport Hosts
Routes of Human Infection5
Direct contact
Contaminated food
Contaminated water
Responses to E. coli O157:H7 Outbreaks
Surveillance
Animals6
Foods7
Human illness7
Regulations
Beef
Juice
Fresh produce
Drinking water10
Swimming pools and beaches
Fairs and petting zoos10
Industry Initiatives: Intervention Strategies10
Discussion and Summary11
Epidemiology11
Evaluation of current practices11
Recommendations
Interventions—Recent Improvements
Further Research
October 2006 Update
Figures
Tables
Reference List
Appendix

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INTRODUCTION

E. coli O157:H7 was first identified as a possible human pathogen in 1975 in a California patient with bloody diarrhea and was first associated with a foodborne (ground beef) outbreak of disease in 1982 (299;353). This serotype (defined by its O and H surface antigens) and some non-O157 serotypes of E. coli produce verocytotoxins, also called Shiga-like toxins because of their similarity to toxins produced by Shigella dysenteriae. These E. coli are called VTEC (verocytotoxin-producing E. coli), STEC (Shiga-toxin producing E. coli), and also EHEC (enterohemorrhagic E. coli) because of the symptoms they produce. Serotypes of VTEC bacteria may include different strains that differ in some virulence factors or other characteristics such as motility and sorbitol fermentation. It has been estimated that E. coli O157:H7 causes about 73,500 cases of illness and 60 deaths annually in the U.S. while non-O157 VTEC serotypes cause about 37,000 cases annually (73;254).

Epidemiological studies of foodborne outbreaks have indicated that fewer than 40 cells of E. coli O157:H7 can cause illness in some people (162;328;335). Waterborne outbreaks, involving both drinking water and pools and lakes, also indicate a very low infectious dose because of the great dilution factor in large quantities of water. Depending on their age, immune status, general health, dose of bacteria, and virulence factors in the bacteria, persons exposed to VTEC may experience mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) with kidney failure. Some cases, usually among young children and older people, are fatal. Children <5.5 years of age are more likely to develop HUS and some studies have indicated that antibiotics or antimotility agents increase risk for HUS (53;101). Other recent studies indicated that correlations of HUS with antibiotic use were not very strong (304;339). Patients with higher temperatures, higher white blood cell counts, and a longer periods of diarrhea appear to be more likely to develop HUS (181;339).

Although this literature review is focused on epidemiology of *E. coli* O157:H7, which is the primary serotype causing outbreaks of VTEC in the U.S., more than 200 different serotypes of *E. coli* produce shiga-like toxins and many have been implicated in outbreaks and cases of HUS in the U.S. and other countries (73;196;251). Verotoxinproducing *E. coli* can be sorted into 4 clonal groups according to different virulence factors and other characteristics that are encoded by genes on their chromosomes, plasmids, and phages. Many of these genes, including those encoding Shiga toxins, were apparently acquired from other organisms while some other functions, such as motility and sorbitol fermentation, were lost during the evolution of *E. coli* O157:H7 from avirulent ancestors. [Some recent European isolates of *E. coli* O157:H7 have been found to ferment sorbitol (43).] Approximately 25% of the *E. coli* O157:H7 genome is derived from bacteriophages indicating that these viruses were very important in the horizontal transfer of genes (80;213; 344;355). VTEC bacteria continue to evolve even during the course of infections as strains isolated from stool samples early in infection sometimes differ from those isolated later (195;240;355). Analyses by CDC indicate that 10.8% of *E. coli* O157:H7 tested in 2003 have become resistant to one or more antibiotics (85).

Non-O157:H7 strains vary in their ability to cause severe human illness and outbreaks but there is evidence that many are associated with cattle and other ruminants as is E. coli O157:H7 (295). During 2004, 110 non-O157 VTEC infections were identified in the ten states surveyed by FoodNet with serogroups O111, O103, and O26 most frequently detected (84). Outbreaks in the U.S. include: a 1994 outbreak in Montana associated with milk (serogroup O104) (245), a 1999 outbreak in Connecticut associated with lake water (serogroup O121) (235), a 1999 outbreak in Texas associated with a salad (serogroup O111) (72), a 2000 outbreak in Washington associated with punch (serogroup O103), a 2001 outbreak in South Dakota in a day care (serogroup O111), and a 2001 outbreak in Minnesota associated with lake water (serogroup O26) (243). In Australia, E. coli O157:H7 is not as commonly isolated in cases of bloody diarrhea and HUS as are other serotypes. Serogroup O111 was identified in a 1995 outbreak associated with sausage (271) and a 2003 outbreak thought to result from person to person transfer (106). Other Australian outbreaks most likely resulting from contact with cattle were caused by serotype O86:H27 (247) and O48:H21 (154). Non-O157 VTEC have also caused outbreaks in several European countries and Argentina (10;155;192;257;310;354).

EPIDEMIOLOGY OF E. coli O157:H7

Outbreak Data

E. coli O157:H7 has been isolated from ill people around the world. It tends to be reported more often from more developed countries but this may be an artifact caused by the paucity of sophisticated diagnostic laboratories in developing countries. Appendix 1 lists, chronologically, published details of 207 reported outbreaks of *E. coli* O157:H7. Undoubtedly, other outbreaks have occurred but details have not been published in accessible journals. A review of data on outbreaks of infectious intestinal disease in the UK from 1992 to 2003 pointed out that there were 1763 outbreaks reported to authorities but only 55 of these were presented in peer-reviewed journals. Of 45 VTEC outbreaks reported to National Surveillance Program, only 7 were described in the literature (*261*).

FoodNet data indicate that *E. coli* O157:H7 causes significantly more cases of sporadic infections than cases linked to an outbreak (*84*). For example, in 2004, only 9% of 402 confirmed cases of infection with *E. coli* O157:H7 were associated with outbreaks (www.cdc.gov/foodnet/annual/2004/report.pdf). Sporadic infections appear to be associated with some of the same factors that cause outbreaks: undercooked hamburgers and exposure to farms and cattle. Some sporadic infections are also associated with use of immunosuppressive medications and dining at table service restaurants (*197*).

Vehicles of infection, suspected or confirmed, have been identified for most outbreaks listed in the appendix. Figures 1 and 2 depict the relative importance of different vehicles in terms of number of outbreaks and cases. Table 1 lists outbreaks with >100 cases. Of the eight largest outbreaks, four were associated with meat, three with drinking water, and the largest outbreak to date, in Japan, was associated with radish sprouts shipped from one farm.

Importance of different vehicles of infection has changed somewhat over time. Table 2 lists the first recognized outbreaks for different vehicles of infection starting with the first outbreak in 1982 linked to ground beef. Figure 3 shows this data on a timeline. During the 1980s most outbreaks of *E. coli* O157:H7 were associated with inadequately cooked hamburgers and unpasteurized milk (80;176;178;305). Some later outbreaks have been traced to other dairy products such as cheese and yogurt (24;172;248). In most cases, dairy products were made from unpasteurized milk (121); in others, there was a problem with postpasteurization contamination (342).

Increasingly, contaminated water has been reported as a source of human infection. This includes drinking water sources contaminated with animal feces and also contaminated lake and pool water used for swimming and playing (*112*). Fruits and vegetables have also been cited as vehicles for human infection with *E. coli* O157:H7. They have presumably been exposed to untreated manure in the environment or else foods have been washed or irrigated with contaminated water. In a 2005 letter, FDA stated that it was aware of 18 outbreaks of *E. coli* O157:H7 associated with lettuce and one associated with spinach in the U.S. since 1995. (www.cfsan.fda.gov/~dms/prodltr2.html) A large outbreak in Japan, affecting more than 12,000 persons, was associated with contaminated radish sprouts (145;241), and other outbreaks have been associated with contaminated fruit juices, melon, and salad greens (3;59;105;162;294).

More recently a number of outbreaks have occurred among children visiting farms and petting zoos where they come into direct contact with animals carrying *E. coli* O157:H7 and their environment (*158*). Outbreaks at county fairs may also result from airborne dispersion of bacteria in buildings used to show animals (*57*;*345*). Finally, direct person-toperson infection occurs particularly among children and their caregivers, such as in day care facilities and also within families (*205*;*357*).

Reservoirs of E. coli O157:H7

Understanding the epidemiology of this organism requires a knowledge of where these bacteria live and grow in nature (their reservoir) and of how humans come into contact them. Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections. Other ruminants known to harbor these bacteria include sheep, goats, and deer. STEC bacteria are occasionally isolated from other animals but it is believed that the bacteria are present as transients and that the animals acquired these bacteria from meat, foods, or water contaminated by fecal material from ruminants (*80*). STEC bacteria usually do not cause illness in animals—with a few exceptions such as diarrhea in calves (*194*).

Cattle-the primary reservoir

Cattle are probably the most important ultimate source of infections for humans. Of the outbreaks listed in Appendix 1, 64 appear to be associated directly with cattle. These include 32 associated with beef, 10 with "meat," and 22 with dairy products from cows. In addition there were 15 outbreaks associated with contact with animals at farms or petting zoos and 5 outbreaks linked to exposure to mud, dust or other environmental sources around farm fields and buildings where farm animals are shown. (Some of these animal-associated outbreaks may have been due to infected sheep or goats.) Many other outbreaks associated with contaminated water and fresh produce may be indirectly associated with cattle.

VTEC have been detected in calves, dairy cows and beef cattle worldwide. Prevalence of these bacteria in cattle and their excreta appears to vary seasonally as well as with the age of the animals and other factors but is generally <10%. Examination of naturally and experimentally infected calves and cattle demonstrated that most *E. coli* O157:H7 adhere to mucosal epithelium in a short 5 cm long region just proximal from the recto-anal junction. As a result, *E. coli* O157:H7 is present predominantly on the surface of the cow pats (223;255).

Evidence has accumulated indicating that some infected animals may shed *E. coli* at much higher levels than others. Analyses of 440 *E. coli*-positive fecal pats in Scotland revealed that *E. coli* O157:H7 levels ranged from <100 cfu/g (in about 75% of pats) to a high of about 9×10^5 cfu/g in the most contaminated pat. Data on cattle from more than 900 farms suggested that the 20% of animals that are most infectious are responsible for approximately 80% of transmission of this pathogen on the farms. It is not presently known if these "super-shedders" are genetically more hospitable to *E. coli* O157:H7 (*232;233*). Generally, shedding of *E. coli* O157:H7 is higher during warm months and higher in calves just after weaning than earlier or later in life (*80;149*).

Other ruminants

Sheep are another significant source of *E. coli* O157:H7 for human infection and these bacteria have been detected in meat (48;93;94) and in animals from several countries (62-64;90;166;167;179;199;236; 259). Monitoring of a flock of sheep for 16 months revealed that the animals shed E. coli O157:H7 only during the summer, several different strains were shed by sheep in a flock at a single time, and the strains shed by different sheep changed over time (209). Diet may affect shedding of E. coli (208). Sheep may shed as many as 10^4 cfu of *E. coli* O157:H7 per gram feces (265) and these bacteria were found to survive for 15 weeks in manure in a field associated with an outbreak of E. coli O157:H7 among scouts at a camp (264). Infected lambs may have been associated with outbreaks involving visits to farms or petting zoos.

Goats are another reservoir of *E. coli* O157:H7 and other verotoxin producing *E. coli* (63;124;199) and outbreaks of human disease have been linked to cheese made from unpasteurized goats' milk (135) and to petting zoos with goats (116;166). Following an oral dose of *E. coli* O157:H7, attaching and effacing lesions, similar to those seen in cattle, develop in the colon and recto-anal junction of goats (210).

Deer are present in significant numbers in some environments also used by cattle, sheep and goats. Since they are also ruminants, deer may serve as a reservoir for *E. coli* O157:H7 and their droppings may contaminate some fresh fruits and vegetables such as apples (*105;139*). Numerous studies in the U.S. and abroad have documented the presence of *E. coli* O157:H7 and other verotoxin-producing *E. coli* in wild deer (*46;129;139;211;296;298;307;349*). *E. coli* O157:H7 has also been detected in some farmed deer (91) and in raw deer meat (253;336). Several cases of human infection with *E. coli* O157:H7 have been traced to contaminated deer meat (202;288).

Other animals

E. coli O157:H7 has been detected in numerous other animals but none of them are considered a significant source of human infection. In one case, a farmer handling a horse infected with *E. coli* O157:H7 subsequently developed an infection with the same bacterial strain (*89*). VTEC bacteria have been detected in several domestic and wild animals including horses (*50*;*161*;*280*), dogs (*62*;*63*;*161*;*198*), rats (*102*;*103*), an opossum (*297*) and cats (*62*) and in a few zoo animals including monkeys and lemurs (*50*) and an orangutan (*64*).

Swine have been found, in several studies, to be infected with *E. coli* O157:H7 but usually only a small percentage (0.4–14%) of animals test positive (62;63;68;95;168;191;198;198;199;203;269;313; 369). Experimentally infected pigs shed these bacteria in their feces for at least two months (108).

Rabbits, both wild individuals and animals being raised commercially on some farms, were reported to harbor enterohemorrhagic *E. coli* (150;214;286). Fecal pellets collected in the summer from wild rabbits on four of six UK farms that harbored VTECshedding cattle tested positive for VTEC, including *E. coli* O157:H7. Rabbit fecal pellets collected in winter tested negative (309).

Poultry meat sometimes has *E. coli* O157:H7 on its surface and these bacteria do persist in the ceca of experimentally infected chicks for as long as 11 months (80;312;326). It was recently reported that 26 of 720 cloacal swab samples from living layer hens in Italian intensive management layer hen farms tested positive for *E. coli* O157:H7 (123). There are a few reports of the isolation of *E. coli* O157:H7 in chicken feces (281;313) and turkey feces (168).

Shellfish in contaminated waters are known to concentrate some pathogens such as *Cryptosporidium*. *E. coli* can be detected in sewage and the possibility exists that pathogenic strains such as *E. coli* O157:H7 could be present in water contaminated by sanitary sewer overflow or runoff from farm fields. Although there has been one recent report of several strains of STEC, including *E. coli* O157:H7, isolated from shellfish collected from coastal areas of France (*156*), it appears that *E. coli* O157:H7 does not significantly contaminate shellfish as yet (*227*).

Transport Hosts

Birds are thought to be possible transport hosts for *E. coli* O157:H7. Some wild birds harbor these bacteria, and pigeons, for example, might spread these bacteria around a farm environment. *E. coli* O157:H7 has been isolated from gulls (347), a rook (relative of crows) (133), and pigeons (159;246;311;316). Experimentally infected pigeons continue to shed these bacteria for about two weeks (103). However, a recent study in Colorado suggests that pigeons may not be a major route of transmission of *E. coli* O157:H7. None of the *E. coli* isolated from 406 pigeon samples collected at dairies produced shiga-like toxins (276).

Flies and beetles, including houseflies and filth flies of several species (7;161;199;249;289;316;332) and dung beetles (362), collected on farms with animals shedding *E. coli* O157:H7, contain detectable levels of these bacteria. These insects frequent fecal deposits and may be able to transfer these bacteria to foods, feed and water. In experiments with houseflies, *E. coli* O157:H7 survived and replicated in the mouthparts and crop of the flies for up to 4 days (204;308).

Fruit flies collected from a compost pile of decaying apples and peaches contaminated with *E. coli* contained these bacteria both internally and externally and were able to transfer them to wounds in uncontaminated apples. *E. coli* O157:H7 can grow rapidly in apple wounds. Fruit flies could contribute to widespread contamination of wounded apples that may be processed into cider (*186*).

Slugs are known vegetarian pests that frequently traverse leafy vegetables and may be present on these foods when harvested. Slugs ingest bacteria from the environment and also accumulate bacteria in the mucus surrounding their bodies. Some common gray field slugs collected on a farm in Scotland were found to carry the same pathogenic strain of *E. coli* as detected in feces from sheep grazing there. Slugs may travel 12 m or more per night so there is a potential for slugs to carry *E. coli* O157:H7 from manure to vegetables (*325*).

Routes of Human Infection

Various routes for human infection with *E. coli* O157:H7 were reviewed in a recent article on the epidemiology of outbreaks of this bacterium in the U.S. (1982–2002) (291). Studies in Canada and France demonstrated that the incidence of HUS and VTEC infection in humans is correlated with indicators of cattle density (*165;343*). *E. coli* O157:H7 in ruminant feces may be directly ingested by persons interacting or working with animals. Fecal material may contaminate meat during slaughter, may enter lakes or drinking water sources by action of rain or wind, and may be deposited on fruits and vegetables inadvertently or by use of manure for fertilization. In addition, some animals may transport these bacteria from a fecal source to drinking water or foods. All of

these routes are variations of a pattern "from turd to tongue" (278).

Direct contact

E. coli O157:H7 shed by infected animals may be spread to many surfaces in enclosures where ruminants are kept including the hides of other animals (*98*). Depending on moisture and humidity, these bacteria may persist on gates, stiles and other farm surfaces for more than four weeks (*356*). *E. coli* O157:H7 survives in cattle feces for up to 18 weeks at 15°C (*146*). This helps explain why a substantial number of people residing on dairy farms have evidence of current (stool cultures) or past (serologic status) infection with VTEC (*361*). Several outbreaks among children who visited farms or petting zoos resulted from direct exposure to these bacteria followed by inadequate handwashing.

Person-to-person spread of *E. coli* O157:H7 has been the primary mode of infection in many outbreaks in day cares, schools and hospitals, particularly where there have been lapses in hygiene (*55*;*278*;*293*). In many other outbreaks, some of the cases who consumed contaminated food or water passed the infection directly to others. Although a majority of children infected with *E. coli* O157:H7 shed these bacteria in their feces for only a few days, in more seriously ill children, cells of *E. coli* O157:H7 may be shed for 20–30 days or longer. VTEC bacteria may be present in stool samples even after children become asymptomatic (*195*).

Contaminated food

Beef, lamb, and mutton can be contaminated during slaughter and processing by exposure to feces or hides containing E. coli O157:H7. In a recent study in the Midwest, more than 45% of over 330 carcasses tested during July-August contained detectable levels of E. coli O157:H7 (134). Prevalence of these bacteria on carcasses was 43% at pre-evisceration (immediately after hide removal), 18% at postevisceration (after evisceration, splitting and trimming), and 2% after postprocessing (after antimicrobial treatments including hot water and organic acid washes and steam pasteurization). The initial high level of contamination was greatly reduced during processing, suggesting that sanitary procedures within these plants were effective. However, it was also true that some carcass samples, from lots in which no preslaughter hide or fecal samples contained E. coli O157:H7, were found to test positive for these bacteria. This suggests that cross-contamination can occur in processing plants.

Milk from dairy cows, sheep, and goats may be contaminated with *E. coli* and other bacteria from the environment. Proper pasteurization will kill these

bacteria. Outbreaks of E. coli O157:H7 due to contaminated dairy products are usually associated with unpasteurized milk but there have been some cases of post-pasteurization contamination.

Manure is a valuable fertilizer for crops but manure containing E. coli O157:H7 may be a source of contamination for vegetables or fruits that are not normally cooked before eating. In one study, these bacteria were able to survive for 42 days in manure heaps that were turned and for 90 days in unturned heaps (141) while another study found that E. coli O157:H7 was undetectable after 4 weeks in biowaste compost piles (218). E. coli O157:H7 did not grow or survive in dairy wastewater lagoons (292) but did survive for more than two months in garden soil treated with contaminated manure (183;251).

Field and greenhouse experiments have demonstrated that both E. coli O157:H7-contaminated manure and irrigation water may cause contamination of vegetables. Onions and carrots grown in soils treated with contaminated manure or irrigated with contaminated water had detectable levels of E. coli O157:H7 on their subterranean parts for 2.5 to 5.5 months (183). Lettuce grown in soil amended with contaminated manure did not contain E. coli O157:H7 in leaves (189;190) but spray irrigation of lettuce with contaminated water deposited E. coli O157:H7 on lettuce leaves and these bacteria persisted for up to 30 days (324). Experiments with shredded lettuce, carrots, and cucumbers demonstrated that E. coli O157:H7 could survive and grow on these vegetables even under modified atmospheres used in commercial packaging (2).

Foods can also be contaminated with E. coli O157:H7 by cross-contamination during food preparation and by infected workers who don't practice good hygiene. Several restaurant outbreaks in Oregon and Washington in 1993 were associated with a variety of items from the salad bar but not with steak. All the restaurants obtained their beef from the same source, and it was the practice to trim, macerate, and marinate the beef in the same kitchens used for preparation of fruits and vegetables for the salad bar. It appeared that the beef itself was cooked well enough to destroy E. coli O157:H7 but that some raw beef was the source of contamination for the fresh produce (185).

Contaminated water

Water used for drinking or recreation has been reported as the vehicle of infection for 49 outbreaks: 6 outbreaks associated with water parks and pools, 18 with lakes, springs, canals, and streams, 10 with well water, 12 with "drinking water," and 3 with tap water. Fecal material from ruminant animals, domestic and/or wild, is the probable source of E. coli O157:H7

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in lakes, streams, and wells and for some "drinking water" outbreaks. Drinking water from an unchlorinated source was implicated in a large Missouri outbreak (331). Infected persons are likely the source of bacteria in the pools and water parks and possibly for some other waterborne outbreaks.

RESPONSES TO E. coli 0157:H7 OUTBREAKS

Today, outbreaks of foodborne disease are featured in almost instant and broad media coverage. This causes anxiety among the general population, lawsuits, and requests for action to protect the health and safety of consumers. The Federal Government through the Centers for Disease Control and Prevention (CDC), United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA) is under pressure and scrutiny and this in turn results in new guidelines, directives, and regulations. State and local public health departments may also be pressured to increase surveillance activities and respond more rapidly to outbreaks. In addition, reports of foodborne disease prompt formation of organizations such as STOP (Safe Tables Our Priority) dedicated to the support of survivors and families of outbreak victims and to dissemination of information on methods for preventing foodborne disease.

Surveillance

Animals

There is no regular surveillance program to monitor prevalence of infection with E. coli O157:H7 among ruminant animals in the U.S. Some other countries, including Sweden (8), Denmark (256), and The Netherlands (313), have established national monitoring programs to detect VTEC in herds or cattle at slaughter. Some U.S. surveys indicated that prevalence of E. coli O157:H7 was 13% in feces of one group of summer feedlot cattle (217) and 28% in feedlot cattle presented for slaughter at Midwestern plants (134). A recent review reported that the prevalence of E. coli O157:H7 in beef cattle ranged from 0.2 to 27.8% in published data. In addition, a number of non-O157 VTEC serotypes have been reported from beef cattle (177).

A review of numerous papers on dairy cattle in the U.S., Canada, Europe, Japan, and Brazil indicated that reported prevalence of E. coli O157:H7 varied widely. (Sampling and analytical methods also differed among these studies.) While there were many reports of prevalences of <20%, there were several very high rates of 40-70%. Ranges of prevalence rates in the U.S. for cows, heifers, and calves were,

respectively, 0.2–8.4%, 1.6–3.0%, and 0.4–40% (178). Recent surveys of dairy farms in Minnesota (99) indicated that 4.5–5.2% of cows harbored *E. coli* O157:H7 while 6.5% of dairy cattle in Louisiana tested positive for these bacteria (130). Downer dairy cattle in Wisconsin were found to be three times as likely as healthy cattle to harbor *E. coli* O157:H7 (77).

Many surveys may underestimate the actual carriage of *E. coli* O157:H7 because they are based on analyses of single samples from fecal pats. Rectoanal mucosal swab cultures were found to detect a higher prevalence of infection with *E. coli* O157:H7 (9.5%) than fecal cultures (4.7%) (*157*). A higher prevalence of infection was also observed when fecal pats were sampled in several locations rather than at just one site (*132*).

A survey of fecal samples from livestock at 32 county and state fairs found that some of the animals at 31 fairs were shedding *E. coli* O157:H7. Prevalence among cattle was 11.4% and among sheep and goats was 3.6%. VTEC were also isolated from flies and from environmental samples even after the livestock left the fair (*199*). Nine of 12 county fairs in Minnesota had cattle infected with *E. coli* O157:H7 with 11% of manure samples testing positive (*99*). The relatively high prevalence of *E. coli* O157:H7 in fair animals was surprising because these animals are usually raised individually or in small groups and are washed frequently.

Foods

Although E. coli O157:H7 has been detected in a variety of foods, there is no regulatory surveillance of foods other than ground beef. FSIS began a microbiological testing program for ground beef in October 1994. A subset of plants and retail outlets that grind beef are selected randomly each month for testing. This sampling and testing program is based on data on outbreaks of foodborne illness and on information from the Office of Public Health Science. Testing results are available on the FSIS website (www.fsis.usda.gov/Science/Ground Beef E.Coli Test ing Results/index.asp). Figure 4 shows the number of ground beef samples tested from 2001–2005. Figure 5 shows a dramatic decrease in the number of samples positive for E. coli O157:H7 between 2002 and 2003. This is believed to be a result of a 2002 order by FSIS that beef plants reassess their food safety plans. Most plants made major changes to their operations by installing and validating new technologies to control and test for E. coli O157:H7.

Results from 2006 testing so far indicate that *E. coli* O157:H7 contamination is continuing to decrease with 14 positive samples detected among 7,295 samples tested. However, a further recall of approximately 900 lb. ground beef reported on August

18 demonstrates that there may still be some issues to be addressed at some plants.

Human illness

FoodNet (Foodborne Diseases Active Surveillance Network) is a collaborative active surveillance project to track foodborne illness and involves CDC, USDA, FDA, and ten states in the Emerging Infections Program (CA, CO, CT, GA, MD, MN, NM, NY, OR, and TN). FoodNet began collecting information from five sites in 1996 and has now expanded to monitor about 15% of the U.S. population. In the ten states, public health officials frequently contact directors of over 650 laboratories testing stool samples to find new cases of foodborne disease and HUS and report these to CDC. Goals of FoodNet include: determining the burden of foodborne illness in the U.S., monitoring trends in specific foodborne illness, determining specific foods and settings associated with foodborne illness, and developing and assessing interventions to reduce foodborne illness.

Reports of foodborne illness from clinical laboratories are reported by all state health departments to CDC under The National Notifiable Diseases Surveillance System (NNDSS). However, there is some variation among states in the priority and funding given to investigation of foodborne illness and notifiable illnesses and their aggressiveness in tracking down causes of outbreaks and sporadic cases. Many persons with foodborne illness are not seriously ill and do not seek medical care, and it is likely that only a fraction of cases is reported to CDC by passive surveillance systems.

Incidence of E. coli O157:H7 infections in the U.S. has declined in recent years according to available surveillance data although there was a slight increase in 2005 (Table 3). According to the latest data from FoodNet (2005), incidence of several other foodborne infections has also decreased, including Listeria, Campylobacter, and Salmonella. However, incidence of infections with Vibrio has increased (86). The most dramatic decline in cases, as noted by both FoodNet data and cases included in Notifiable Diseases, was between 2002 and 2003 when there was also a dramatic decrease in positive samples of ground beef detected by FSIS sampling (Figure 6). This trend was also detected in Wisconsin (Figure 7). Enter-Net reports that infections with E. coli O157:H7 decreased by 6% between 2000 and 2004 but infections with non-O157 VTEC serotypes has increased. These serogroups are likely underdiagnosed and underreported in most countries, including the U.S. (140).

In response to the 1992–1993 Jack in the Box outbreak, scientists at CDC subjected bacterial isolates from several western states to DNA fingerprinting by pulsed-field gel electrophoresis (PFGE) thereby aiding in identifying the vehicle of infection and the extent of the outbreak. Prompt recognition of the outbreak probably prevented hundreds of cases of illness. CDC then developed standardized PFGE methods for several foodborne pathogens and created a network of national and state public health and regulatory laboratories that can submit PFGE patterns of bacterial isolates to a central database. In 1998, the inauguration of PulseNet was announced by Vice President Gore, and it has since become instrumental in facilitating early recognition of common source outbreaks (<u>www.cdc.gov/pulsenet/</u>). Database administrators analyze PFGE data submitted by participating laboratories to identify clusters of infection and can electronically alert participants to outbreaks. Epidemiologists throughout the country can also rapidly compare PFGE patterns of local foodborne bacterial pathogens and determine whether there are similar strains causing illness elsewhere (151;160).

Regional and national meetings, such as the First Foodborne Epidemiologist Meeting held in 2004, bring together foodborne epidemiologists from throughout the country. These meetings are being held more frequently and serve to educate public health workers on the latest methods and research and allow participants to share information on state programs. State and federal laboratory personnel present PulseNet data on sporadic and outbreak cases of foodborne illness.

Regulations

Federal regulations for various aspects of food handling and processing are promulgated by FDA and USDA while EPA is in charge of clean water regulations. In addition, many state and local agencies have regulations that impact food, drinking water, swimming beaches and pools, and agricultural fairs and petting zoos. Many of these regulations were established after significant human disease outbreaks and deaths and/or broad media coverage highlighted shortcomings in handling of food, water, or animals. Figure 8 depicts a timeline showing the association between significant outbreaks and new regulations.

Public outrage aroused by the book *The Jungle* lead to the passage and implementation of food inspection laws, including the Federal Meat Inspection Act (FMIA) and the Pure Food and Drug Act for nonmeat products in 1906. The Poultry Products Inspection Act (PPIA) was enacted in 1956. FMIA and PPIA require mandatory inspection of livestock before slaughter and mandatory post-mortem inspection of all carcasses, establish sanitary standards for slaughterhouses and meat processing establishments, and authorize USDA to inspect meat processing and slaughtering operations. Only unadulterated carcasses are approved for further distribution to customers. Meat is considered adulterated "if it bears or contains any poisonous or deleterious substance which may render it injurious to health" or "if it consists in whole or in part of any filthy, putrid, or decomposed substance or is for any other reason unsound, unhealthful, unwholesome, or otherwise unfit for human food"

USDA regulations implementing FMIA and PPIA are found in Title 9 of the Code of Federal Regulations, and FSIS periodically issues Directives that provide instructions for inspectors. In 1967, the Wholesome Meat Act updated FMIA to require inspection of all meat processed and sold within the same state. The Wholesome Poultry Act of 1968 instituted similar requirements for intrastate processing of poultry.

In addition, at least twenty-eight states have their own meat and/or poultry inspection programs covering small and very small establishments. These programs are run cooperatively with FSIS.

<u>Beef</u>

E. coli O157:H7 was first recognized as a foodborne pathogen after a 1982 outbreak affecting 20 people who had consumed undercooked hamburger. During the next 9 years several other outbreaks associated with beef caused illness in 30–70 persons each. Then in 1991 there was a major ground meat-related outbreak in Canada, and in 1992–1993 an outbreak associated with a fast food restaurant affected over 700 people who had eaten undercooked hamburger in the western U.S., with 37 children developing HUS and 4 deaths. This prompted the passage of several regulations by FSIS to improve meat safety (*254*).

- **1994**: Under the authority of the Federal Meat Inspection Act, Michael Taylor of FSIS, in a speech in September, declared *E. coli* O157:H7 to be an adulterant in ground beef. This was challenged in a Texas court but the court decided that USDA had good reason to consider these bacteria as adulterants.
- **1994**: FSIS Directive 7235.1 required the placement of safe handling labels on packages of raw meat and poultry. These labels address storage, cooking, and holding practices to minimize or prevent growth of pathogenic bacteria

(www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/ 7235.1.pdf).

• **1994**: FSIS Notice 50-94 instituted testing of raw ground beef for *E. coli* O157:H7. This notice was later replaced in **1998** by Directive10,010.1.

• **1996**: HACCP (Hazard Analysis and Critical Control Point) systems were mandated as a systematic procedure for determining critical points during processing when meat could be contaminated and instituting appropriate controls to prevent contamination. This rule establishes a testing program for the pathogen, requires slaughter plants to routinely test carcasses for generic *E. coli*, and requires all plants to incorporate an antimicrobial process and have in place sanitation standard operating procedures (SSOPs)

(www.fsis.usda.gov/OPPDE/rdad/FRPubs/93-016F.pdf) (Fed. Register 61(144):38806-38989).

• **1998**: FSIS Directives 6150.1, rev 1 and 6420.1 told inspectors to enforce zero tolerance for visible fecal, ingesta and milk contamination of poultry and livestock carcasses at slaughter. This directive was revised in **2004** to include head, cheek and wesand meats because these may be included in ground beef (www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/

6420.2.pdf).

• **1998**: FSIS Directive10,010.1 revised policy for sample collection and testing to emphasize establishments perceived to be a greater risk. Establishments using validated pathogen reduction interventions on beef carcasses and that had not identified a positive sample within the previous six months would not need to be tested by FSIS

(www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/ 10.010.1.pdf).

- **1998**: Performance standards for lethality and stabilization for cooking of meat were updated (9CFR 318.17).
- **1999**: USDA issued rules allowing irradiation of refrigerated or frozen/uncooked red meat and meat products to destroy pathogenic bacteria including *E. coli* O157:H7 (64 FR 72150) (www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7700.1Rev1.pdf).
- **1999**: Raw ground beef products, including trimmings, would also be considered adulterated if they contain *E. coli* O157:H7 (Fed. Register 64:2803-2805).
- **2002:** FSIS directed producers of ground beef to reassess HACCP plans in light of new epidemiological data on *E. coli* O157:H7, including improvements in analytical tests, data on conditions in feedlot pens as related to shedding of VTEC, and data on contamination of carcasses as related to prevalence of VTEC on hides and in feces. Implementation of critical

controls was required (Fed. Register 67(194):62325).

- 2003: USDA banned all downer cattle from the human food chain. This was intended to prevent possible transmission of BSE but may also have decreased the prevalence of *E. coli* O157:H7 in meat from culled animals since downer dairy cattle have been shown to have a higher prevalence of *E. coli* O157:H7 (77) (www.usda.gov/news/releases/2004/01/0457.ht m).
- 2004: Directive 10.010.1 was revised to require all federally inspected plants that produce raw ground beef products or components be subject to testing for *E. coli* O157:H7. This directive also provided instructions for follow up actions if a sample tested positive and for verifying control of products that are presumptive or proven positive for *E. coli* O157:H7 (www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/

10.010.1.pdf).

- 2005: Directive 7700.1, FSIS revised and updated instructions regarding irradiation of meat and poultry products in official establishments, including off-site irradiation of product (www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/ 7700.1Rev1.pdf).
- **2006**: Directive 7120.1, Amend 8 provided updated information on safe and suitable ingredients used in the production of meat and poultry products. This includes substances that could be used to destroy bacteria or inhibit their growth.

<u>Juice</u>

Following a multistate outbreak of disease linked to *E. coli* O157:H7 in unpasteurized apple juice in 1996 (*105*), FDA published a final rule (Federal Register 63(130):37029-37056) requiring a warning label on fruit juices that have not been processed to prevent, reduce, or eliminate pathogenic microorganisms. Following another juice-borne outbreak of *Salmonella* in orange juice in 2000, another final rule published by FDA in 2001 required all juice manufacturers to develop and implement a HACCP plan to prevent contamination of juices with dangerous pathogens. Treatments should reduce pathogen levels by 5 logs (Federal Register 66(13):6138-6201).

Fresh produce

FDA has published regulations requiring current good manufacturing practices (GMPs) for food processors under its jurisdiction. However, these regulations do not include "*establishments engaged solely in the harvesting, storage, or distribution of one or more*

raw agricultural commodities. ... FDA will, however, issue special regulations if it is necessary to cover these excluded operations" (21U.S.C. §321(r)). Despite this exclusion, FDA can still regulate fresh produce as "food" subject to the adulteration provisions of the Food, Drug, and Cosmetic Act. A food shall be deemed to be adulterated "if it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health." Therefore, FDA can take enforcement action against an agricultural producer if it determines that food is being produced under insanitary conditions.

In 1998, FDA issued a "Guide to Minimize Microbial Food Safety Hazards for Fruits and Vegetables" that recommended good agricultural practices and GMPs

(www.fda.gov/ohrms/dockets/98fr/97n0451a.pdf).

However, foodborne outbreaks related to fresh produce continued. In a 2005 letter, FDA noted that 8 outbreaks of *E. coli* O157:H7 associated with lettuce and spinach could be traced to Salinas, California. Creeks and rivers in this area test positive for *E. coli* O157:H7 periodically and some areas are highly susceptible to localized flooding. FDA reminded producers that it considers ready-to-eat crops (such as lettuce) to be adulterated if they have been in contact with flood waters due to potential exposure to sewage, animal waste, pathogens, and other contaminants (www.cfsan.fda.gov/~dms/prodltr2.html).

Drinking water

According to EPA regulations, a system that operates at least 60 days per year and serves 25 people or more or has 15 or more service connections is regulated as a public water system under the Safe Drinking Water Act of 1974. If a system is not a public water system as defined by EPA's regulations, it is not regulated under the Safe Drinking Water Act, although it may be regulated by state or local authorities. Revised National Primary Drinking Water regulations are published in the Code of Federal Regulations (40CFR141.1).

Under the Safe Drinking Water Act, EPA requires public water systems to monitor for coliform bacteria. Systems analyze first for total coliforms, because this test is fast. Any time a sample is positive for total coliforms, the same sample must be analyzed for either fecal coliforms or *E. coli*. Both are indicators of contamination with animal waste or human sewage (www.epa.gov/safewater/sdwa/index.html).

Systems serving 25 to 1,000 people typically take one sample per month. Some states reduce this frequency to quarterly for ground water systems if a recent sanitary survey shows that the system is free of sanitary defects. Systems using surface water, rather than ground water, are required to take extra steps to protect against bacterial contamination because surface water sources are more vulnerable to such contamination. At a minimum, all systems using surface waters must disinfect. Disinfection, including chlorination, will kill *E. coli* O157:H7.

Bottled water is regulated by FDA as a food. Standards of quality are listed in the Code of Federal Regulations (21CFR165.110); these include allowable limits for turbidity, color, odor, coliforms, radioactivity and for 70 chemicals. Coliform levels should not exceed 4 cfu/100 mL in any single sample. State and local governments also regulate bottled water.

Swimming pools and beaches

These are regulated by state and local authorities. Pools and water parks are usually tested regularly for fecal contamination that may result from accidents with young children. Lakes and rivers can also be contaminated by human visitors but are perhaps more at risk of contamination during heavy rains when manure may wash into nearby bodies of water. Water at swimming beaches may not be tested as frequently for coliforms but should be tested when there is likely to be a problem with run off.

Fairs and petting zoos

These are regulated by state and county agriculture or public health departments. During the past several years, regulations requiring more hand-washing stations, warning signs, disinfecting of handrails, etc., and cleanliness in animal enclosures have been enacted in a number of states, including North Carolina and Washington, that experienced outbreaks of *E. coli* O157:H7 at county fairs. The CDC has published a compendium of standardized recommendations to prevent disease associated with animals in public settings. The single most important recommendation was proper washing of hands (*57*).

Industry Initiatives: Intervention Strategies

The emergence of *E. coli* O157:H7 as an important foodborne pathogen has prompted industry initiatives to reduce contamination and improve food safety. These included the use of better equipment and testing procedures as well as improved management systems (HACCP). From 1996–2000, the Economic Research Service of USDA estimates that the meat and poultry industry spent about \$180 million per year on improvements in food safety. More than \$7 million has been spent since 1994 on applied research related to control of *E. coli* O157:H7. This research led to commercialization of several processes to reduce pathogens on carcasses during processing: (*a*) vacuuming of carcasses with steam or hot water (*126*); (b) thermal pasteurization in which carcasses are rinsed with water at 180° F; and (c) rinsing with mild organic acids. Steam pasteurization was found to be particularly effective (279). By 1997, the two largest beef packing companies ordered such equipment for all their plants.

Following the large 1992–1993 outbreak in the western U.S. linked to hamburgers, the beef industry created a blue-ribbon task force to develop plans to aggressively address the problem of *E. coli* O157:H7 in cattle and beef. Among the accomplishments of this task force were development and implementation strategies for HACCP programs, safe handling labels on packages of beef, and steam vacuuming technology. The Beef Industry Food Safety Council was formed in 1997 to address the problem of foodborne pathogens in beef. This council facilitates research activities and develops and implements education programs for both consumers and the industry.

Because it is not readily apparent to consumers whether meat or produce is contaminated with E. coli or other bacteria, if a significant outbreak of foodborne disease is associated with a particular company, results can be economically devastating. One of the most well known cases was Hudson Foods which went out of business after its hamburger was implicated in an outbreak and 25 million pounds of product had to be recalled. The industry has instituted a 100% test and hold program for any product that will be ground. Product from positive lots is diverted to cooking using a validated thermal process or else the product is destroyed. It should be noted that this testing, because of sampling limitations, will not detect all lots that contain E. coli O157:H7. However, improvements in cleaning carcasses and testing programs have greatly reduced positive samples of ground beef detected by FSIS in recent years (Figure 5).

Demands by large meat and poultry buyers and many foreign buyers have driven some improvements in food safety practices. After the 1992-1993 outbreak, Jack in the Box canceled all its current contracts with hamburger suppliers and required superior food safety controls including more stringent testing and strict temperature control from its future suppliers. Safer hamburgers were produced using this Bacterial Pathogen Sampling and Testing Program. Many other major meat buyers, including McDonald's, Burger King, Kroger and other fast food and grocery chains, have also mandated their own safety standards in contracts with their suppliers. Ground beef manufacturers are using test and hold procedures to ensure the safety of ground that is shipped out to restaurants and consumers.

Fresh salad vegetables have also been implicated as a source of foodborne illness. Following the 1998 Guidance from the FDA

(www.fda.gov/ohrms/dockets/98fr/97n0451.pdf) to producers of fresh fruits and vegetables, a majority of the lettuce/leafy green industry adopted the suggested good agricultural practices (GAPs). In April 2006, representatives of trade associations for growers and marketers of fresh produce published a document to aid their members in implementing safeguards during growing, harvesting, value-added operations, distribution, retail, and food service operations: Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain (www.cfsan.fda.gov/~acrobat/lettsup.pdf).

Restaurants have also established their own procedures for ensuring that meat, particularly hamburger, is well-cooked to destroy bacterial pathogens and to prevent cross-contamination during food preparation. The 2005 FDA Model Food Code also was directed toward a more proactive approach through active managerial control in reducing foodborne illness rather than reacting to reported illnesses and outbreaks. This along with the more strenuous recommendation related to no bare hand contact of foods, single use of gloves, and more enhanced reporting of employee health from the employee and restrictions by employers may also have contributed to improved public health as witnessed by the yearly reduction in outbreaks by enteric bacterial pathogens in the U.S. (CDC, personal communication). An important part of this process is education of employees on the importance of cleanliness and proper cooking. Menus contain a warning that the restaurant will not be responsible for illness if patrons request that meat not be fully cooked.

DISCUSSION AND SUMMARY

Epidemiology

Evaluation of current practices

Active surveillance programs, such as FoodNet, identify a greater proportion of cases of *E. coli* O157:H7 than passive programs. It has been estimated that as many as 4–8 times as many symptomatic cases occur for each case identified by active surveillance (56). Sporadic cases of *E. coli* O157:H7, confirmed in laboratories, have remained stable over several years but incidence varies for different states. Many factors may affect whether cases are ascertained:

• It is not always possible to obtain samples of implicated foods after an outbreak is recognized but greater efforts could probably be made to identify pathogens and food vehicles if more resources were put into epidemiological investigations. In Japan, it is the practice to reserve a portion of foods served and store it in the freezer for later analysis, if necessary (*162*).

- Successful investigation of foodborne disease outbreaks requires sufficient resources for collecting and analyzing food and clinical samples. Of 336 foodborne outbreaks reported to FoodNet in 1998–1999, 71% had no identified etiology. Reports on these 237 outbreaks indicated that no stool samples were collected in 156 outbreaks and neither food nor stool samples were analyzed in 130 outbreaks. Causative agents cannot be determined if food and/or clinical samples are not examined.
- Patients may not seek medical attention or doctors may not order analyses of stool samples. For example, the percentage of stool samples with bloody diarrhea that were cultured for *E. coli* O157:H7 was 58% in Georgia and 96% in Connecticut (*56*).
- Not all laboratories routinely culture stool samples for *E. coli* O157:H7 and results are not always reported (*56*). Stool samples from suspected VTEC infections may be cultured for *E. coli* O157:H7 but test negative if a non-O157 serotype is responsible.

Extrapolating from data provided by FoodNet and from data in peer-reviewed articles in the literature can be problematic on two counts. (a) FoodNet sites were not chosen to be representative of the U.S. population as a whole A demographic comparison of the population in the FoodNet sites with the total U.S. population reveals a similar composition by age. However, Hispanics are underrepresented in FoodNet populations as are those in lower socio-economic groups (163). (b) Only a small fraction of outbreaks reported to national surveillance programs are subsequently described in peer-reviewed literature. A review of data on outbreaks of infectious intestinal disease in the UK from 1992 to 2003 pointed out that there were 1763 outbreaks reported to authorities but only 55 were presented in peer-reviewed journals. Of 45 VTEC outbreaks reported to the national surveillance program, only 7 were described in the literature (261). Outbreaks reported in the literature tend to be those for which microbiological and epidemiological evidence is strongest or more out of the ordinary. This may skew perceptions on the frequency of different vehicles of infection as the UK study found that reports in the literature overemphasized the importance of milk/milk products, deserts, and miscellaneous foods and underestimated importance of meat, fish, poultry and eggs.

In addition, if incidence reports are not adjusted for outbreaks, then data may also be misleading. For example, in one day care outbreak in Minnesota, 43 cases were confirmed but most of these were identified because all children and staff at the day care were cultured and many would probably not have been identified if the outbreak hadn't occurred (56).

Recommendations:

- Greater uniformity is needed in statewide investigations and reporting of foodborne illness. Public health systems in some states, such as Minnesota, are very well organized and all specimens are sent to state labs for analysis. Other states with different priorities are less aggressive and rigorous in testing samples and conducting investigations. They should be encouraged to improve funding for public health and to adopt and incorporate better epidemiological procedures and improved laboratory methods.
- Federal grants could be targeted to improving laboratory facilities and training for state epidemiologists, as needed. Greater participation by all states will aid in the rapid identification of multistate outbreaks. (Improving laboratory facilities and expertise at state levels can be considered part of Homeland Security preparations to detect possible outbreaks associated with biological or chemical weapons.)

Interventions—Recent Improvements

Interventions undertaken during recent years by numerous organization concerned with food production and food safety have led to a decline in outbreaks and cases of *E. coli* O157:H7 (Figures 6 and 7). These include:

- Increased emphasis on HACCP and pathogen control by industry and government. This includes improvements at slaughter and processing plants (hide washing, steam vacuuming, hot water and antimicrobial rinses, hold and test programs), at restaurants (proper cooking of hamburger, more care about crosscontamination, notes on menus), and improvements at packing plants and retail stores.
- Increased educational efforts targeted at doctors (1999, American Medical Association Program), workers, and consumers. Numerous web sites and publications maintained by academic institutions, industry trade groups, state and local health departments, and the federal government (e.g. www.fightbac.org/, originally launched in 1996) have made the public more aware of the potential hazard of undercooked hamburger and the

importance of hand washing. Articles in the popular press also reinforce these concepts. Increasingly, agricultural fairs and petting zoos have been posting more signs and providing more hand washing facilities.

- Increased cooperation between federal and state agencies with CDC and FDA sharing more PFGE patterns through PulseNet and federal regulators attending more state and regional meetings on epidemiology and foodborne disease.
- Improved training to make FSIS inspectors more knowledgeable about risk-based inspection.
- Litigation surrounding some high profile outbreaks of foodborne disease. While not primarily intended to be an educational tool, publicity surrounding some outbreaks has undoubtedly alerted consumers to important food safety issues.

Further Research

Much of the research on controlling E. coli O157:H7 has been devoted to ensuring the safety of meat after slaughter. Yet outbreaks of illness associated with meat and fresh produce still occur, indicating that further efforts need to address preslaughter conditions of cattle (78;344;371). Several studies have documented that some individual animals shed very high levels of E. coli O157:H7 while fecal material from other animals in the same herd contains much lower concentrations of these bacteria (223:232:233). Carcass contamination with E. coli O157:H7 is more likely when feces contain higher concentrations of this pathogen (136;267). Research to determine why some cattle are "supershedders" and how to identify them would help reduce transmission in herds and carcass contamination. If super-shedding cattle can be identified, then some interventions noted below could be targeted at those animals.

Although it is impossible to completely eliminate pathogenic bacteria from all cattle, some products and procedures are being tested for efficacy in reducing levels of *E. coli* O157:H7 in cattle. Further research is needed to establish parameters that will make these interventions more consistently effective and commercially useful. These include:

probiotics and competitive exclusion: Probiotic bacteria are harmless or beneficial and compete with pathogens to reduce or prevent their colonization of the gut. Results have not been consistent in "real world" studies but research should be directed at finding more effective combinations of probiotic microbes (*337;365;367*).

- **antibiotics**: These compounds are used to treat illness in animals and to improve feed efficiency and growth. Neomycin treatment has been shown to significantly decrease fecal shedding of *E. coli* O157:H7 in cattle and might be useful under some conditions (78). However, there is a trend to reduce antibiotic use in animals to minimize the potential for inducing drug-resistant pathogenic bacteria. This should be considered in assessing recommendations for future use of antibiotics.
- **bacteriophages:** Phages are viruses that attack bacteria. Some are very specific in regard to the bacteria they destroy. As with probiotics, results in real world studies are not consistent but research should continue (263;315). FDA has recently approved the use of certain phages on foods to reduce or prevent growth of pathogens.
- **vaccines**: Virulence factors secreted by the type III system of *E. coli* O157:H7 were found to be effective components of a vaccine that significantly reduced shedding of these bacteria by cattle in a feedlot setting (284). Further field tests of different vaccine types should determine whether this is a cost effective way to reduce *E. coli* O157:H7 levels in cattle.
- **chlorate**: Chlorate can be metabolized by *E. coli* O157:H7 to form chlorite but as chlorite accumulates the bacteria die. Some experiments indicate this might be a useful way to reduce carriage of *E. coli* O157:H7 shortly before slaughter (*13;321*).
- ruminant diet: In order to improve performance, dairy cattle and finishing beef cattle are often fed high grain diets rather than forage. Some of the starch from grains escapes degradation in the rumen and passes to the colon where it may be fermented by E. coli, including VTEC strains. Several studies have demonstrated that feeding barley grain increases shedding of E. coli O157:H7 (76;114). Other studies have indicated that an abrupt change from finishing grain rations to forage decreases E. coli O157:H7 (78). Longterm feeding of forage rather than grain does not have a dramatic effect on E. coli populations in the gut. However, switching cattle from grain to forage just before slaughter may reduce VTEC populations (372).
- water trough hygiene: *E. coli* O157:H7 can be present and persist in water troughs on farms and in feedlots (*316*;*317*). These bacteria can survive in sediments in troughs for as long as eight months and serve as a continuing source of reinfection for a herd (*216*). More data on the effects of frequency of cleaning troughs and on antimicrobials that might be used for cleaning or

added to water could suggest methods for reducing transmission among cattle on farms and in feedlots.

In addition to reducing levels of *E. coli* O157:H7 in cattle, further efforts are needed to ensure safety of fresh produce. It is very difficult to sanitize raw fruits and vegetables after harvest because pathogens may reside in inaccessible parts of the food. Therefore, strategies for prevention of preharvest contamination from manure or runoff from livestock operations is essential and should be further investigated (*60;61*).

Outbreaks at petting zoos and fairs can be decreased by hand washing but animal owners and caretakers should be aware that E. coli O157:H7 can survive for weeks in feces if conditions are right (58) and can also persist for days on surfaces (356). E. coli O157:H7 can also survive in bedding, such as wood chips and sawdust, and can even grow in bedding contaminated with urine (120). There is some controversy as to whether bedding for animals should be cleaned out daily (with the attendant risk of creating aerosols of the pathogens that may be inhaled or deposited on other surfaces) or whether new clean bedding should be placed on top of the old bedding for several days to cover up and keep the pathogens in place during the fair. Further data on effects of different methods of handling bedding and wastes and on sanitizing surfaces may reduce outbreaks at fairs and petting zoos.

More effective educational efforts may also be useful in minimizing outbreaks at swimming pools, water parks, and beaches.

Disease caused by *E. coli* O157:H7 in humans is still not well understood. Reported data on the use of antibiotics for treatment of infections with *E. coli* O157:H7 is conflicting and should be resolved. As yet there is no good animal model for HUS to test various hypotheses about mechanisms for dispersal of shiga toxins through the body, in particular to the kidney. Mechanisms of action of the toxins, interactions of different virulence factors, and potential for transfer of virulence to other bacteria need to be further delineated.

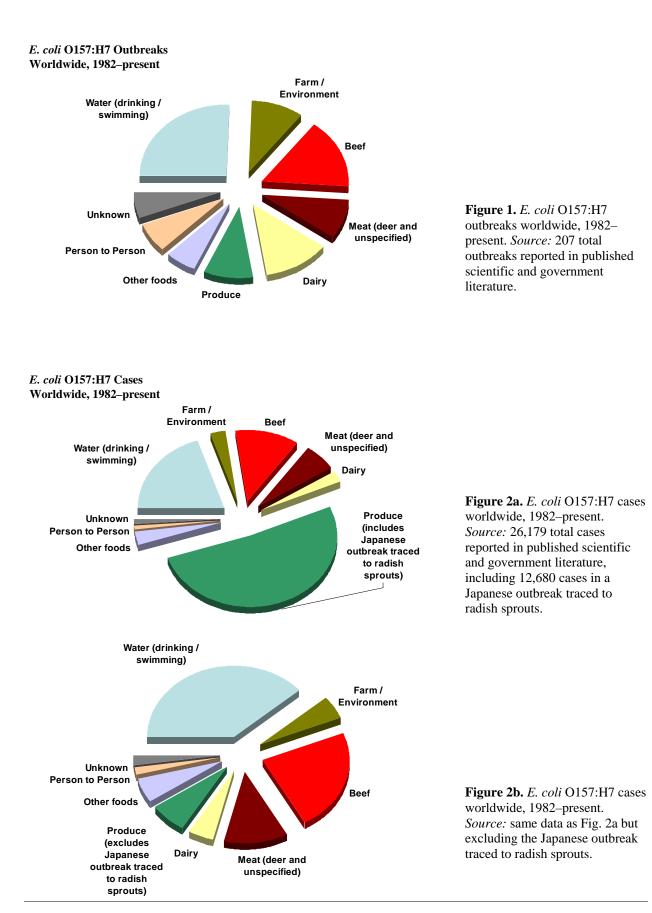
OCTOBER 2006 UPDATE

A large multistate outbreak of *E. coli* O157:H7 associated with packaged fresh spinach occurred between August 19 and September 5, with 203 cases in the U.S. and 1 case in Canada. Cases were first identified in Wisconsin, and this state has the highest number of cases (50) and one confirmed death associated with the outbreak strain. The *E. coli* O157:H7 strain responsible for this outbreak appears to be unusually virulent, with 104 cases requiring hospitalization and 31 cases of HUS (*373;376*).

E. coli O157:H7 has been isolated from open bags of spinach in 10 affected states. This allowed tracing of the spinach to four ranches in the Salinas Valley of California. The outbreak strain of *E. coli* O157:H7 has been isolated from cattle feces and stream water on one ranch (*374*). Samples taken from a wild pig in the area also contained the outbreak strain, and it may be that wild boar trampled fences around the spinach fields and spread the outbreak strain to these fields (*377*).

As noted in a December 2005 FDA letter (<u>http://www.cfsan.fda.gov/~dms/prodltr.html</u>), at least 8 outbreaks of *E. coli* O157:H7 associated with lettuce and spinach in the past ten years have been traced to Salinas, California. This outbreak has resulted in 6 recalls of bagged sinach and salad mixes.

Deposition of *E. coli* O157:H7 on lettuce and spinach leaves may occur from use of contaminated irrigation or washing water or from runoff water flooding the fields. Experiments with shredded lettuce have demonstrated that *E. coli* O157:H7 can survive and grow under modified atmospheres used in commercial packaging (2). Recent data indicate that lettuce plants exposed to soil or water (not sprayed on leaves) containing *E. coli* O157:H7 can take up these bacteria which are then present in internal parts of the plant. This would make it impossible to wash off the bacteria (*375*). Further research is needed to better define routes of contamination of fresh produce at farms and packaging facilities and methods to minimize such contamination.



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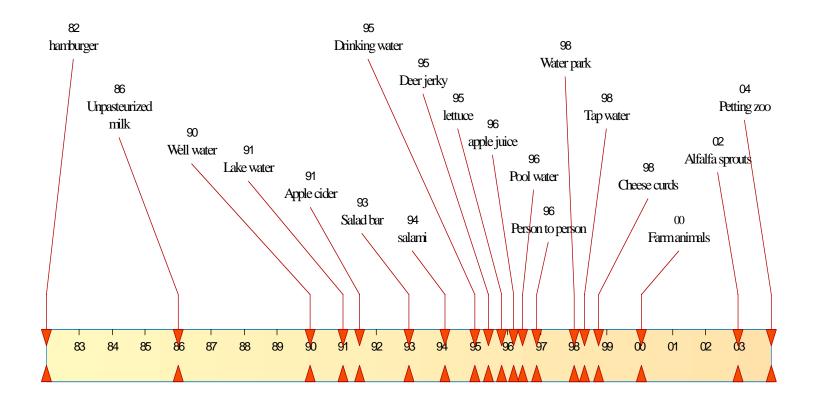


Figure 3. Timeline of appearance of different vehicles for human infection with E. coli O157:H7.

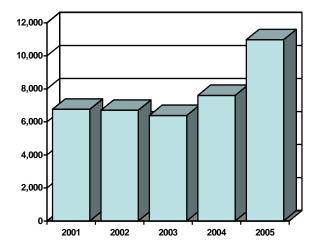


Figure 4. FSIS ground beef samples analyzed by year 2001-2005

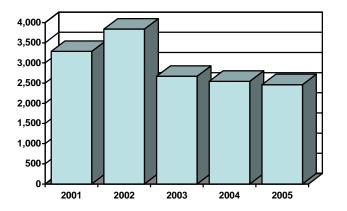


Figure 6. Nationwide E.coli O157:H7 cases by year, 2001–2005

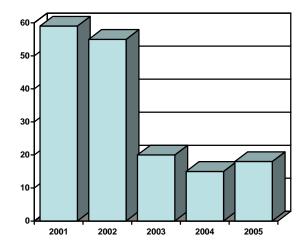


Figure 5. E. coli O157:H7 isolates from FSIS sampled ground beef 2001-2005

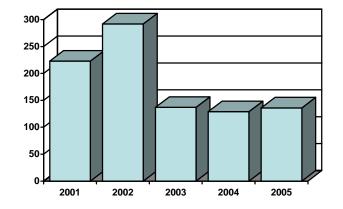


Figure 7. Wisconsin E. coli O157:H7 cases by year, 2001–2005

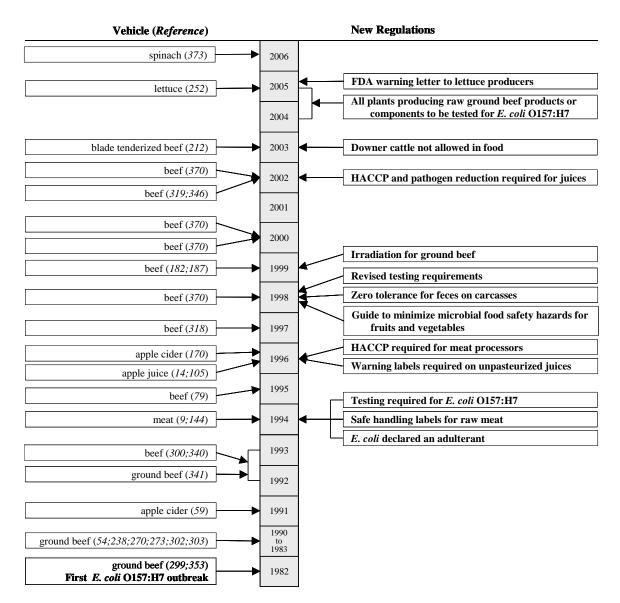


Figure 8. Timeline of important U.S. outbreaks and new regulations to control E. coli O157:H7.

Year	# Cases	Location; Vehicle	Reference
1996	12,680	Sakai, Japan; radish sprouts	(145;241;363)
2000	2,300	Canada; drinking water (Walkerton)	(175)
1999	>1,000	US; well water (New York)	(97)
2000	788	US; raw beef, cross contamination of other foods (Sizzler)	(370)
1992–93	>700	US; hamburger at fast food restaurants (Jack in the Box)	(52;69;119;341)
1995	633	Fife, Scotland; sewage contamination of drinking water	(193)
1991	521	Canada; minced beef and caribou	(268)
1996–67	512	Scotland; meat from one shop	(109)
1996	503	Scotland; lunch foods	(277)
1997	332	UK; restaurant food	(17)
1999	329	US: IL, KY, MO; beef	(182)
1990	243	US: MO; well water	(331)
1999	159	Canada; petting zoo	(283)
2005	157	UK; sliced cooked meat	(305)
1998	157	US: WY; tap water	(266)
1999	143	Canada; salami	(226)
1999	127	US: NY; well water, total of 781 cases, some C. jejuni	(258;260)
2005	120	Sweden; lettuce	(109)
1999	114	UK; milk, pasteurized	(153)
1995–96	110	Sweden; unknown	(368)
2002	109	Canada; salads	(67)
1997	108	US; alfalfa sprouts	(107)
2004	108	US: NC; petting zoo	(116)
2000	102	US: UT; irrigation water used for drinking	(215)
1994	>100	Scotland; pasteurized milk	(342)

Table 1. Large outbreaks of *E. coli* O157:H7 (>100 cases).

Year	Vehicle	Location	Reference
1982	beef, ground (1 st reported outbreak)	US	(299;353)
1985	meat, cooked	Canada	(81;206)
1986	milk cows, unpasteurized	US; Canada	(178;230)
1988	beef, roast	US: WI	(302)
1989	water, well	Canada	(294)
1990	food, restaurant	UK	(229)
1990	person-person transfer	Scotland and Israel	(205;219)
1990	water, drinking	UK	(122)
1990	water, tap	Japan	(5)
1991	yogurt	UK	(248)
1991	water, lake	US: OR	(201)
1991	apple cider	US: MA	(59)
1992–93	cheese, cows', unpasteurized	France	(121)
1992	water, pool	UK: Scotland	(71)
1993	salads	US: OR, WA	(184)
1994	milk, cows pasteurized	UK: Scotland	(301;342)
1994	salami	US: WA, CA	(9)
1994	sandwiches	US: WI	(370)
1994	animals, farm	UK: England	(320)
1995	milk, goats, unpasteurized	Czech Republic	(65)
1995	ham, cooked	UK: England	(359)
1995	lettuce	US and Canada	(3;285)
1995	deer jerky, meat	US: OR	(202;288)
1995	potatoes	UK	(96)
1995–96	sausage	Germany	(12)
1996	apple juice (Odwalla)	US and Canada	(105)
1996	food handler	Australia	(234)
1996	meat, butcher shop	UK: Scotland	(109)
1996	sprouts, radish	Japan	(145;241)
1997	sprouts, alfalfa	US: MI, VA	(70;107)
1997	environment (farm field)	UK	(110)
1997	cakes, cream filled	UK	(262)
1998	cheese curds	US: WI	(131)
1998	cream, unpasteurized	UK	(24)
1998	water park	US: GA	(49)
1999	beach	UK	(31:164)
1999	animals, petting zoo	Canada	(283)
2000	foods, deli	UK	(36)
2000	water, stream	US: CA	(215)
2000	beef, steak	US: MN	(213) (212)
2003	spinach	US: CA	(294)
2003	cheese, goats'	France	(135)

Table 2. E. coli O157:H7: First reports of vehicles/modes of infection.

Notifiable cases (87;88)				rne Outbre oli) (82;83		FoodNet Data (<i>E. coli</i> O157:H7) (84;86)		
Year	<i>E. coli</i> O157:H7	non-O157	Outbreaks	Cases	Deaths	cases/100,000	relative rates	
2006	*2,141							
2005	2,621	908				1.06		
2004	2,544	626				0.9	0.6	
2003	2,671	408				1.06	0.7	
2002	3,840	254				1.68	1.05	
2001	3,287	191				1.60	0.95	
2000	4,528					2.0	1.2	
1999	4,513					2.0	1.25	
1998	3,161					2.4	1.0	
1997	2,555					2.1	1.0	
1996	2,741		11	325	1	3.0	1.0	
1995	2,139		25	393	1			
1994	1,420		25	902	0			
1993			15	1,340	5			
1992			3	19	0			
1991			3	33	0			
1990			2	80	0			
1989			1	3	0			
1988			2	109	0			

Table 3. Data from surveillance summaries of foodborne and notifiable diseases from CDC.

*includes all serotypes of VTEC E. coli as of October 1, 2006.

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Appendix 1

Chronological List of Outbreaks of E. coli O157:H7

Date	Location	Cases	Deaths	HUS	Vehicle	Ref #
1982 (a)	US: OR, MI	20			beef, ground	(299;353)
1982 (b)	Canada	31	1	0	beef, ground	(294)
1984	US: NE	34	4	1	beef, ground (nursing home)	(303)
985	Canada (nursing home)	73	17	12	meat;person-person	(81;206)
986 (a)	US: WA	40	2	1	beef, ground	(270)
986 (b)	US: WI	2	0	2	milk, unpasteurized	(230)
986 (c)	Canada	74		1	milk, unpasteurized	(178)
988 (a)	US: MN	32	0	0	meat patties	(54)
988 (b)	US: WI	61	0	0	beef, roast	(302)
988 (c)	US: UT	51	4	8	beef, ground	(273)
.989	Canada	7	1		water, well (nursing home)	(294)
990 (a)	Japan	14	2	14	water, tap	(5)
990 (b)	US: MO	243	4	2	water, well	(331)
990 (c)	UK: Scotland	11	4		person-person;geriatric hospital	(205)
990 (d)	UK	16		4	restaurant food	(229)
990 (e)	UK	4	0	4	water, drinking	(122)
990 (f)	Israel	4	0	0	person-person	(219)
990 (g)	US: ND	70	0	2	beef, roast	(238)
991 (a)	Canada	521	2	22	beef, ground?;person-person	(268)
991 (a) 991 (b)	US: MA	18	0	0	apple cider	(59)
991 (c)	UK	16	0	5	yogurt	(248)
991 (d)	US: OR	21	0	3	water, lake	(248)
.991 (u) .992 (a)	Germany	45	1	3	person-person	(201)
.992 (a) .992 (b)	Scotland	6	1	1	water, pool	(71)
992 (b) 992-3 (c)	US: WA, ID, CA, NV (Jack in Box)	>700	3	37	beef, ground	(71) (52;69;100;119;314;341
992-3 (f)	France	4	0	4	cheese, cows' unpasteurized	(121)
992-3 (1)	US: OR	14	0		milk, unpasteurized	(200)
992-4 993 (a)	UK: Wales	8	0	1	beef, ground	(360)
993 (b)	US: OR, WA,	93	0	0	salad bar ?	(184)
993 (c)	Netherlands	11	0	4	water, lake	(111)
.993 (d)	US: CA	11	0		beef, ground	(340)
993 (u) 993 (e)	UK	6	1	3	water, paddling pool	(171)
.993 (f)	US: CT	20	0	0	beef, ground	(300)
	Germany	6	0	1		(224)
993 (g)		20	0		person-person	
994 (a)	US: VA	20	0	1	beef, ground	(144)
994 (b)	US: WA, CA		-	2	salami	(9)
994 (c)	Italy	15	1	15	poultry ?	(338)
994 (d)	UK: Scotland	>100	1	9	milk, pasteurized	(342)
994 (e)	US: NY	12	0	2	water, lake	(4)
994 (f)	UK: Scotland	22			beef, ground	(118;278)
994 (g)	UK: Scotland	71	2	10	milk, pasteurized	(301)
994 (h)	UK: Scotland	26	0	1	beef, ground	(117)
994 (i)	US: CT	21			supermarket, food?	(47)
994 (j)	UK: England	7		4	animals, farm	(320)
994 (k)	US: WI	26	0	0	sandwiches	(370)
994 (l)	UK: Scotland	22		1	cheese	(178)
995 (a)	US: IL	12		3	water, lake	(348)
995 (b)	US: Mont.	92		1	lettuce, leaf	(3)
995 (c)	UK: Wales	31		2	person-person	(6)
995 (d)	US: GA, TN	10		1	beef, ground	(79)
995 (e)	US: IL	12			water, lake	(250)
995 (f)	US: OR	11	0	0	deer jerky	(202)
995 (g)	UK	8		1	potatoes, person-person	(96)
995 (h)	Canada	21	0	0	lettuce	(285)
995 (j)	UK: Scotland	633	0	2	water, tap	(193)
995 (k)	UK	14	0	2	meats, precooked	(327)
	UK	26	0	2	sandwiches, takeaway	(237)
995 (1)	UK					

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1995 (n)	US: WI	27	0	0	water	(370)
995 (o)	UK: England	11	0	4	ham, cooked	(359)
1995 (p)	US: MN	33			water, drinking	(220)
995-6 (a)	Sweden	110	0	29	unknown	(368)
995-6 (b)	Germany	28	3	28	sausage	(12)
996 (a)	US and Canada (Odwalla)	70	1	14	apple juice	(14;105)
996 (b)	US: CT, IL	61		3	lettuce, mesclun	(169)
996 (c)	US: GA	18		1	water, pool	(143)
996 (d)	Japan, Sakai	12,680	12	121	sprouts, radish (schools)	(145;241;350;363)
996 (e)	US: CT	14	0	4	apple cider	(170)
996 (f)	Japan	47	1	3	sprouts,radish (factory)	(351)
996 (g)	Scotland (Wishaw)	503	20	34	meats, cooked	(128;277;278)
996 (h)	US: CO	24	0	1	person-person	(357)
996 (i)	Australia	6	0	0	food handler	(234)
996 (j)	US: FL	2			water, well	(220)
996 (k)	UK	3	0	0	milk, unpasteurized	(15)
996 (l)	UK	12	0	1	milk, raw and "pasteurized"	(104)
996-7	Scotland	512	17		meat from a shop	(109)
997	Europe	15	0	3	water, well	(275)
997 (a)	US: CO (Hudson)	15			beef, ground	(318)
997 (c)	US: MI, VA	108	0	4	sprouts, alfalfa	(70;107)
997 (d)	UK: England	5		2	animals, farm	(287)
997 (e)	UK	8	0	1	environment (dairy farm field)	(21;110)
997 (f)	UK	45	0	0	cakes, cream-filled	(262)
997 (g)	Finland	14			water, lake	(272)
997 (h)	UK	332	0	1	restaurant food	(17)
997 (i)	UK	2	0	0	cheese, cows' unpasteurized	(23)
997 (j)	UK	3	0	2	animals, farm	(92;242)
997 (k)	Europe (travel overseas)	10	0	3	water, drinking ?	(18)
997 (l)	UK: Scotland	6			unknown (nursing home)	(19)
997 (m)	UK: Scotland	10			meat, cooked, butcher shop	(20)
997 (n)	UK: Scotland	5		1	unknown:farm animals?	(16)
<u>997 (o)</u>	UK: Scotland	34			unknown; hospital	(22)
<u>997 (p)</u>	US: WA	4			water, well	(49)
997 (q)	US: MO	8	0		water, lake	(49)
998 (a)	US: WI	69	0	1	cheese curds	(131; 370)
998 (b)	US: WY	157		4	water, tap	(266)
.998 (c)	US: CA, NV	8	0		sprouts, alfalfa	(244)
.998 (d)	Canada	39	0	2	salami	(358)
998 (e)	Canada	11	0	0	apple cider	(334)
998 (f)	UK (Dorset)	10	0	0	water, drinking	(26)
998 (g)	UK	7	0	0	cream, unpasteurized	(24)
998 (h)	-	1	0	1	cheese, cows' unpasteurized	(25)
998 (i)	US: WI	47	0	3	fruit salad	(370)
998 (j)	US: WI	13	0	0	beef, ground	(370)
998 (k)	US: GA	26			water park	(49)
998 (l)	US: MN	5			water, lake	(49)
998 (m)	US: IL	3	2	1.4	water, well	(49)
999 (a)	US: NY (Wash. Co. fair)	127	2	14	water, well; 781cases <i>E. coli</i> and/or	(258;260)
999 (b)	UK	114		3	<i>C. jejuni</i> milk, pasteurized	(153)
999 (b) 999 (c)	Canada	114	0	6	salami	(226)
999 (c) 999 (d)	US: CA, NV, AZ	143	0	3	beef, tacos	(187)
999 (d) 999 (e)	US: CA, NV, AZ US: CT	13	0	3	deer meat	(288)
999 (e) 999 (f)	US: UI US: WA	37	0	3	water, lake	(74;306)
999 (1) 999 (g)	Scotland	6	0	3	water, drinking	(221)
999 (g) 999 (h)	UK: Wales	24		3	animals, farm	(274)
999 (ll) 999 (i)	Canada	7	0	0	beef, ground	(225)
999 (1) 999 (j)	US: NY	>1,000	2	11	water, well	(97)
999 (j) 999 (k)	UK	>1,000	0	0	cheese, cows' unpasteurized	(97)
999 (k) 999 (l)	UK	9	0	3	milk?; person-person	(30)
	UK	27	0	0	unknown	(28)
999 (m)	UK	5	0			(28)
.999 (n) .999 (o)	UK	14		2	unknown-visit to Turkey beach	(31;164)
			1	3		
.999 (p)	US: CA	7	0	0	water, lake	(137)

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1999 (q)	Canada	159			animals, petting zoo	(283)
1999 (r)	Sweden	11	0	0	lettuce	(352)
1999 (s)	US: TX	22	0	0	water, drinking	(215)
1999 (t)	US: IL, KY, MO	329			beef	(182)
2000 (a)	US: WA	5	0		animals, farm	(147)
2000 (b)	Canada (Walkerton)	2,300	7	27	water, drinking	(175)
2000 (c)	UK: Scotland	2,500	/	21	environment (agr. showground)	(173)
2000 (d)	UK	6	0	1	milk, unpasteurized (2 outbrk)	(35)
2000 (u) 2000 (e)	UK	9	0	1	foods, deli	(36)
2000 (e) 2000 (f)	UK	15	0	3	unknown	(36)
2000 (I) 2000 (g)	UK	7	0	5	person-person	(33)
2000 (g) 2000 (h)	UK	45	0	0	foods? (prison)	(34)
	UK: Scotland	18	0	0	animals, farm ?; camp	
2000 (i)		-	1	4		(32)
2000 (j)	US: WI (Sizzler)	788	1	4	beef, unknown foods	(370)
2000 (k)	US: OH	48	0	2	water, drinking	(294)
2000 (1)	Netherlands		0	1	animals, petting zoo	(166)
2000 (m)	US: PA	51	0	8	animals, farm	(113)
2000 (n)	US: WI	15	0	3	unknown food	(370)
2000 (o)	US: WI	9	0	1	beef, ground	(370)
2000 (p)	US: ID	4			water, canal	(215)
2000 (q)	US: CA	5			water, stream	(215)
2000 (r)	US: UT	102			water, irrigation; also C. jejuni	(215)
2000 (s)	US: ID	15			water, spring	(215)
2000 (t)	US: CT	11			water, lake	(215)
2001 (a)	US: OH	23		1	environment (fair building)	(345)
2001 (b)	Canada	5	0	2	milk, goat, unpasteurized	(239)
2001 (c)	Canada	4	0	0	water, lake	(75)
2001 (d)	Austria	2	0	1	milk, unpasteurized	(11)
2001 (e)	UK	20		1	person-person	(38)
2001 (f)	UK: Scotland	15			water private well	(37)
2001 (g)	China	39	32	28	water, animals?(91%>60yrs)	(366)
2001 (h)	UK	30	0	2	meat (butcher shop)	(290)
2001 (i)	US: MN	4	0	0	animals, farm	(322)
2001 (j)	US: WI	55		1	animals, fair	(370)
2001 (k)	US: WI	34	0	1	environment (stock pavilion)	(370)
2001 (1)	US: MN	20			water, lake	(364)
2001 (m)	US: SC	45			water, lake	(364)
2001 (n)	US: ME	9			water, wading pool	(364)
2002 (a)	US: CO, etc. (ConAgra)	28		5	beef, ground	(319;346)
2002 (c)	Canada	13	0	2	cheese, cows' unpasteurized	(172)
2002 (d)	Canada	17	0	2	person-person;daycare	(148)
2002 (e)	Canada	109	2	0	salads	(67)
2002 (f)	UK, France	10	0	0	salad, cucumber	(127)
2002 (g)	UK: Wales	16	0	0	water, drinking	(41)
2002 (h)	UK	16			person-person	(40)
2002 (i)	UK	15			water, drinking	(39)
2002 (j)	US: WI	54	0	0	beef, ground	(370)
2002 (k)	US: WA	76		~	lettuce, romaine	Ecoliblog
2002 (1)	US: KY	2			water, well	(66)
2002 (n)	US: OR	60		12	environment, animal dust (fair)	(57)
2002 (m) 2003 (a)	US: MN, CO	20			sprouts, alfalfa	(138)
2003 (b)	US	12		1	beef, steak, blade tenderized	(212)
2003 (c)	Canada	45	0	0	animals, petting zoo	(115)
2003 (d)	Canada	8	0	3	person-person	(1)
2003 (d) 2003 (e)	Australia	3	0	0	foods?	(106)
2003 (f)	US: CA	32	2	3	spinach (nursing home)	(294)
2003 (1) 2003?	Slovakia	9	2	3	milk, unpasteurized	(222)
2003-4	Denmark	25	0	0	milk, pasteurized	(188)
2003-4 2004 (a)	Japan: Okinawa	6	0	0	beef, ground	(207)
2004 (a) 2004 (b)	France	3	U	2	cheese, goats'	(135)
2004 (b) 2004 (c)	Canada	34	0	2	beef, ground	(330)
	Canada	13			water park	(152)
2004 (d)	UK: Scotland	5	0	1 2	*	(152)
2004 (e) 2004 (f)	Japan: Mie	23	U		water, drinking	
	Japan: Ivne	23		1	person-person	(329)
2004 (I) 2004 (g)	UK: England	7	0	0	water, stream	(180)

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FRI BRIEFINGS: E. coli O157:H7 Human Illness from Food and Non-food Sources

2004 (h)	US: NC	108		15	animals, petting zoo	(116)
2005 (a)	France	13		13	beef, ground	(142)
2005 (b)	UK: Wales	157	1	2	meat, cooked	(305)
2005 (c)	UK: Scotland; Turkey	15	0	0	unknown	(323)
2005 (d)	Sweden	120	0	7	lettuce	(333)
2005 (e)	Ireland	18	0	2	water, drinking; person-person	(228)
2005 (f)	UK	79	0		beef	(44)
2005 (g)	Japan: Kumamoto	11	0	0	beef, offal	(231)
2005 (h)	US: FL	73	0	12	animals, petting zoo	(116)
2005 (i)	US: AZ	2			animals, petting zoo	(116)
2005 (j)	US: WA	18	0	2	milk, unpasteurized	(51)
2005 (k)	Netherlands	32	0	0	beef, steak tartare	(125)
2005 (1)	US: MN, WI, OR	25	1		lettuce, packaged (Dole)	(252)
2006 (a)	UK: Scotland (Fife)	23	0	4	person-person? (nursery)	(43)
2006 (b)	UK: Scotland	3	0	0	meat (butcher shop,Lanarkshire)	(43)
2006 (c)	UK: England	4	1	4	unknown (nursery)	(43)
2006 (d)	UK: England	25			meat, cooked, butcher shop	(45)
2006 (e)	US: TN	10		3	person-person (daycare)	(282)
2006 (f)	US: MN	17	2	1	ground beef	(173)
2006 (g)	US: 26 states; Canada	204	3	31	spinach	(373;376)