FRI FOOD SAFETY REVIEWS

White Paper on Non-O157:H7 Shiga Toxin-Producing *E. coli* from Meat and Non-Meat Sources

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INTRODUCTION

Shiga toxin-producing *E. coli* (STEC) can cause devastating illness, particularly in children, by causing hemolytic uremic syndrome (HUS) leading to kidney failure. Outbreaks of illness caused by STEC have been epidemiologically related to contact with animals and consumption of meat and fresh produce. *E. coli* O157:H7 is the most notorious of the STEC strains causing approximately 73,500 cases in the U.S. each year. CDC estimates that non-O157 STEC are responsible for about 37,000 cases of illness annually but relatively fewer cases of HUS compared to O157:H7. Although many strains of non-O157 STEC appear to be less virulent than *E. coli* O157:H7, a 2008 outbreak of STEC strain O111 in Oklahoma caused illness in at least 314 people, HUS in 17 cases, and one death (*203*). Other non-O157 outbreaks in the U.S. have been traced to contaminated lake water, salad greens, and milk.
Numerous *E. coli* strains are capable of producing one or both Shiga toxins (Stx1 and Stx2), but not all of them are important human pathogens. STEC strains have been divided into 5 seropathotypes (*108;147*):

- **A**, including the O157 strains that are common causes of outbreaks and HUS in most countries;
- **B**, non-O157 strains that cause occasional outbreaks but are fairly common isolates from sporadic cases and HUS (examples: O26:H11, O103:H2, O111:NM, O121:H19, O145:NM);
- **C**, non-O157 strains associated only with sporadic cases;
- **D**, strains associated with diarrhea, not more severe symptoms; and
- **E**, strains not associated with human disease.

Stx2 is the more potent toxin, and those strains producing this toxin are generally associated with more acute illness. Other virulence factors are also important, including intimin, adhesions, enterohemolysin, and those involved in the type III secretion system that participates in the production of characteristic intestinal lesions (*56;59*).

More than 200 virulent non-O157 serotypes have been isolated from outbreaks and sporadic cases of HUS and severe diarrhea in the U.S. and other countries. Serogroups O111, O103, and O26 are among the most frequently detected (*35*). The true incidence of non-O157 STEC infections is probably underestimated because standard stool culture methods routinely used in many clinical laboratories do not detect these bacteria. Recently developed analytical methods for STEC strains detect Stx proteins or genes encoding these proteins (*24;113*). However, the presence of Stx or its genetic determinants in a sample does not necessarily mean that there are viable STEC bacterial cells.

As with *E. coli* O157:H7, non-O157 STEC serotypes are often associated with cattle and other ruminants and surveys have demonstrated their presence in samples from cattle carcasses, retail beef, and raw milk (*53;130;131;133*). Cattle often harbor multiple serotypes, some of which appear to be less of a health risk to humans because they lack one or more important virulence factors. Nevertheless, because microbes can readily exchange genetic information (*137*), the presence of any STEC in food production environments is of concern. A recent review summarized data on the prevalence of STEC in the beef production chain (*227*).

In order to minimize human infections with non-O157 STEC, it is necessary to understand which serotypes are most virulent and all the ways in which people are exposed to these pathogens. A more comprehensive understanding of the epidemiology of infections caused by non-O157 STEC serotypes will lead to improved control methods to prevent illness and reduce economic losses to food producers and processors. This white paper will draw together epidemiological information from the scientific literature and government publications on outbreaks and discuss effectiveness of existing interventions for preventing exposure of humans to pathogenic non-O157 STEC.

EPIDEMIOLOGY OF NON-O157:H7 STEC

Surveillance and Pathogenicity

According to published data, non-O157 STEC were first recognized as a possible cause of sporadic cases of HUS in 1975 in France, where hospital records reported that STEC serotype O103 was present in some patients (*148*). The earliest reported outbreak, caused by serotype O145:H–, occurred in Japan in 1984. No vehicle of infection was determined for this outbreak (*143*). *E. coli* O157:H7 was first identified as a possible human pathogen at about the same time, in a California patient with bloody diarrhea in 1975, and was first associated with a foodborne (ground beef) outbreak of disease in 1982 (*228;266*).

CDC estimates that about a third of STEC infections in the U.S. are caused by non-O157:H7 serotypes. However, this is likely an underestimate because of the challenges in identification of non-O157 strains. Although there are methods for identification of different serotypes, they are not widely available. In addition, many laboratories do not rou-

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tinely screen diarrheal stools for Shiga toxins and may only attempt to isolate pathogens in cases of bloody diarrhea or if there is a suspected outbreak (*268*). It should be noted that there are atypical strains of serogroup O157 designated as O157:H– that can ferment sorbitol and may initially be presumed to be non-O157 strains. Isolates of serotype O157:H– often produce Shiga toxins and have been associated with cattle and with severe illness in children (*146;207*).

Some surveys in the U.S. and elsewhere indicate that non-O157 STEC may cause diarrhea as frequently as *E. coli* O157:H7 even though they are less commonly identified in cases of severe illness, such as HUS. A 2006 review article (*142*) reported results from studies in 17 countries indicating that non-O157 serotypes were responsible for 19–100% of STEC infections from which pathogens were isolated. These studies spanned a 10-year period and examined different patient groups (certain ages or geographical areas), so they do not necessarily reflect a greater prevalence of certain serotypes in different countries.

In more recently published surveys, non-O157 serotypes were reported to be significant causes of STEC infections (% = number of non-O157/total STEC identified):

- 80% in a nationwide survey in the Netherlands (*256*)
- 82% in a laboratory sentinel program in Germany (*267*)
- 74% in a national surveillance program in Denmark (*197*)
- 13% of HUS cases in France (*72*)
- 63% in an enhanced surveillance study in Manitoba (*252*)
- 28% in Ireland in 2008 (*93*)
- 42–61% in Australia during 2004–2006 (*209*)
- 24% (2007) and 35% (2008) in Japan (*7;8*)

Data from summaries of notifiable diseases in the U.S. demonstrate an increasing percentage of cases of STEC infection associated with non-O157:H7 serotypes. In 2002, only 5% of serotyped STEC isolates from human illness were identified as non-O157:H7 strains; in 2005, this had increased to 16% of isolates (*47*). Although relative numbers of virulent non-O157:H7 strains may actually be increasing, more frequent testing for Shiga toxins and different STEC serogroups undoubtedly explains much of the increase in non-O157:H7 isolates. In Idaho, an enhanced surveillance program targeting a "low" STEC incidence area of the state found that with more comprehensive laboratory analysis the reported non-O157 STEC incidence increased from <1 to 11 cases/yr/100,000 population and 56% of serotyped STEC isolates were non-O157 strains (*171*).

During 2003–2005, the most common non-O157:H7 strains identified in the U.S. were O26 (19–25%), O103 (14–18%), O111 (13–17%), O45 (5–13%), O121 (6–7%), and O145 (3.4–7.5%) (*43; 44;45*). Even though a large number of different serogroups are identified in some enhanced surveillance studies of human diarrheal cases, the most common non-O157 STEC strains reported are the six serogroups listed above. Nine to ten serogroups are identified yearly in Wisconsin; the most common serogroups during 2007–2009 were O26 (24–32%), O103 (21–34%), O111 (10–29%), O45 (2–17%), and O121 (2–9%) (*data from J. Archer, Wisconsin Division of Public Health*). No cases of HUS have been associated with non-O157 STEC in Wisconsin in the past 3 years, but some strains of all the common serogroups have caused illness severe enough to require hospitalization of some patients (*J. Archer*). Virulence factors important for human infection are more commonly carried in these strains, resulting in more frequent detection.

Virulence characteristics vary somewhat among STEC strains but all strains, by definition, produce Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2). Strains producing Stx2 cause more serious illness than those producing only Stx1. Several variants of these proteins have been described and some are more often detected in certain serotypes or certain host animals (*59*). Part of the toxin molecule binds to host cell receptors and facilitates transfer of the toxin into cells. Another toxin subunit has enzymatic activity and interferes with protein synthesis in cells and induces inflammatory responses. In addition to these toxins, virulent STEC often carry the locus of enterocyte effacement (LEE) whose genes code for proteins that form attaching and effacing lesions in the intestines of infected animals. LEE also encodes a type III secretion system to deliver virulence factors to intestinal enterocytes. Many pathogenic STEC also produce enterohemolysin. Several recent reviews discuss these virulence factors in more depth (*21;41;108;139;142*).

Some virulence factors may confer advantages to STEC cells in the environment. Shiga toxins are also toxic to *Tetrahymena*, a common fresh-water protozoan predator of bacteria. STEC may have a survival advantage when these predators are present (*161*). In addition to being an important virulence factor in some STEC infections, the serine protease, espP, also aids in attachment to lettuce leaves and may aid survival in the environment (*154;239*).

Although non-O157 STEC are generally associated with less severe illness than *E. coli* O157:H7,

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this may change because bacteria readily exchange genetic material and are constantly gaining or losing genetic information, such as virulence genes. A genomic comparison of virulent STEC strains of serotypes O157, O26, O103, and O111 revealed that they contained a large number of prophages and transmissible integrative genetic elements containing virulence genes. These strains had distinct evolutionary histories and independently acquired the mobile genetic elements coding for virulence factors (*202*). STEC strains isolated from patients in the early stages of infection may differ from those isolated from fecal samples several days later, indicating that evolution of virulence characteristics occurs even during a matter of days within a host (*179*). Transfer of genes for Shiga toxins through bacteriophage transduction could potentially also occur in foods. However, some experiments examining this process in milk, ground beef, salads, and other foods found that cell numbers would need to be much greater than those normally observed in foods for efficient gene transfer (*137*).

Outbreaks and Sporadic Cases

Reports from public health surveillance studies in many (U.S.) states and from other countries indicate that sporadic cases of non-O157 STEC greatly outnumber outbreak cases (*35;127;163;178; 197;229*). This is also true for *E. coli* O157:H7. According to FoodNet data from 2005, only 23% of 473 confirmed cases of infection with *E. coli* O157:H7 were associated with outbreaks (*48*). Approximately 50 non-O157 STEC cases have been identified annually in Wisconsin during 2007–2009 but these were nearly all sporadic cases (*J. Archer*).

Outbreaks attributed to non-O157:H7 STEC have been reported from the U.S., Europe, Australia, and Japan. Data on 80 outbreaks, from 1984 to 2009, reported in the literature or government websites are presented in Table 1 (*see p. 15*). It is very likely that other outbreaks have occurred but were not recognized because of the difficulties in identifying and characterizing non-O157:H7 STEC serotypes. Information on some other outbreaks may have been published on foreign language websites or in inaccessible journals and were not included here.

In the U.S., the earliest outbreak occurred in Ohio in 1990 among family members. A 2008 outbreak in Oklahoma, caused by serotype O111:NM, affected 341 patrons and workers at a particular restaurant. Despite an extensive investigation by public health authorities, targeting a variety of foods, food handlers and water sources, no specific source of the *E. coli* O111:NM was identified (*203*).

Notable international outbreaks include the 1995 mettwurst outbreak in Australia, caused by serotype O111:NM, which resulted in 23 cases of HUS among 88 persons affected (*38*) and a more recent outbreak in Norway in 2006 caused by a virulent strain of serotype O103:H25, present in a particular kind of mutton sausage. There were 10 cases of HUS and 1 death among the 18 cases that were recognized after an extensive epidemiological investigation (*236*). A Danish outbreak in 2007 due to serotype O26:H11, present in a different type of sausage, was much milder with no cases of HUS or death (*75*). The O103:H25 strain was reported to produce only Shiga toxin 2 whereas the O26:H11 strain produced only Shiga toxin 1.

Another interesting aspect of the Danish outbreak was the epidemiological investigation. Initial interviews with parents of the affected children generated no useful hypotheses. Next, the investigators asked the parents where they shopped in the previous 3 weeks and how much they had spent on food. With this information, they were able to retrieve from the stores' computers exactly what was purchased and they identified one particular organic fermented sausage, bought by several families, as the likely vehicle. The outbreak strain was isolated from the sausage and the sausage was recalled (*75*).

Relative importance of different vehicles of infection for outbreaks is depicted in Figures 1 and 2. In 15 outbreaks (18.8%), no vehicle was identified. Person–person contact was reported to be the cause of about 29% of outbreaks and 20% of cases. Many of these occurred in schools and day-care situations in Japan. Other outbreaks were traced to meat (*9*), dairy products (*8*), water, both drinking water and pool or lake water (*8*), produce (*5*), and other food (*7*). Several small outbreaks (*5*) occurred among visitors to farms and petting zoos. The "other food" category accounted for about 9% of outbreaks but 27% of cases. This was due to two large outbreaks: the 2008 Oklahoma restaurant outbreak, with 341 cases, and a 2004 outbreak associated with unpasteurized cider. Compared to outbreak data gathered for a previous white paper on *E. coli* O157:H7 (*64*), non-O157:H7 STEC strains are much less often associated with meat, water, and produce as outbreak vehicles and much more often attributed to person-to-person contact or unknown vehicles (Table 2). These differences are likely due, in part, to the better analytical methods available for *E. coli* O157:H7. *E. coli* O157:H7 is also more virulent than some non-O157:H7 STEC strains and thus outbreaks are recognized and investigated more rapidly and thoroughly.

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Table 2. Comparison of the relative importance of vehicles associated with outbreaks of non-O157:H7 STEC and *E. coli* O157:H7.

Vehicle	non-O157:H7 STEC	*E. coli* O157:H7(64)
Animal contact	6.2%	9.7%
Water	10.0%	25.6%
Person–person contact	28.8%	6.8%
Dairy	10.0%	12.5%
Meat	11.2%	24.6%
Produce	6.2%	9.2%
Other food	8.8%	5.8%
Unknown	18.8%	5.8%

Figure 1. Outbreaks of non-O157:H7 STEC associated with known vehicles.

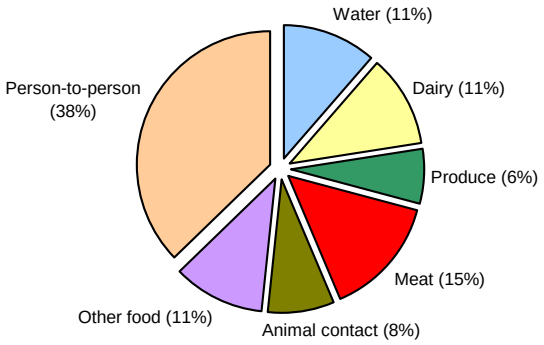
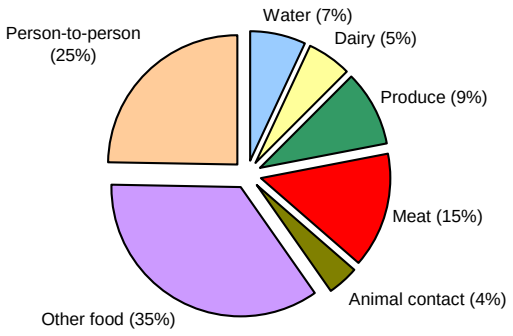


Figure 2. Outbreak cases of non-O157:H7 STEC associated with known vehicles.



Reservoirs of Non-O157:H7 STEC

Understanding the epidemiology of non-O157:H7 STEC serotypes requires a knowledge of where they live and grow in nature (their reservoir) and of how humans come into contact with them. Ruminants have been identified as the major reservoir of *E. coli* O157:H7 and also appear to be a reservoir of non-O157:H7 STEC strains. STEC have been isolated from cattle, sheep, goats, and deer. STEC are occasionally isolated from other wild and domestic animals but it is believed that, in many cases, they are present as transients that the animals acquired from foods or water contaminated by fecal material from ruminants. Nevertheless, some of these transient hosts may be vehicles of infection for humans.

Non-O157:H7 STEC have been detected in numerous species of animals. Two non-O157:H7 STEC outbreaks in Australia were traced to contact with non-ruminants: a 2002 outbreak at a petting zoo with pigs and alpacas infected with STEC serotype O26 (*208*) and a 2007 outbreak at an animal sanctuary likely caused by koalas and/or kangaroos infected with serotype O55:H80 (*110*). Both a child with diarrhea and domestic pigeons in Germany were found to harbor the same STEC serotype, O128:H2 (*246*), and another child in Germany and her cat were found to be excreting identical strains of STEC O145:H– (*36*). A survey of wildlife meat in Germany found a number of non-O157:H7 STEC serotypes present in deer, wild boar, and wild rabbit meats. Some of these STEC were serotypes that have also been detected in cases of human illness (*182*).

Cattle—the primary reservoir

Cattle are probably the most important source of human infections. Of the outbreaks listed in Table 1, 16 appear to be associated directly with cattle. These include two associated with beef, four with "meat," and six with dairy products from cows. In addition there were four outbreaks associated with contact with animals at farms or petting zoos. Other outbreaks associated with contaminated water and fresh produce may be indirectly associated with cattle.

Wide ranges of prevalences of non-O157:H7 STEC in feces from dairy (0.4–74%) and beef (2.1–70.1%) cattle have been reported from various countries. A total of 193 STEC serotypes were reported from dairy and 261 serotypes from beef cattle. About 12–17% of these serotypes have also been isolated from cases of human illness. Many of the apparently non-pathogenic strains appeared to be lacking one or more virulence factors (*131;133*). While it is difficult to compare results of all these studies because of variations in sampling and detection methods, they do indicate that cattle shed a variety of non-O157:H7

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STEC serotypes, some of which are human pathogens.

More recently published studies also demonstrate a large number of serotypes shed by cattle: 26 serotypes detected on organic and conventional dairy farms in Minnesota (*51*), at least 10 detected on beef carcasses in the Pacific northwest (*53*) and 31 serogroups detected in dairy cattle in Japan (*157*). These studies also illustrate an analytical issue that must be considered in analyzing published data. Estimates of prevalence were much higher from PCR or immunoassays detecting Shiga toxins or genes coding for these toxins (23–30%) than estimates obtained from isolation of STEC bacteria (6–12%). It is unclear whether the toxin assays are detecting proteins or DNA from viable or dead bacterial cells.

Several studies have reported the STEC are shed more often by cattle during warm months (*227*). Most isolates of O26 and O111 in Korean cattle were detected during May–October (*140*). Non-O157:H7 STEC were detected more often in Spring and Autumn in Midwestern U.S. beef processing plants (*13*). Seasonality may be related to ambient temperatures, age of cattle, and/or type of feed or pasture consumed at different seasons.

A likely source of infection for cattle is feed or water contaminated with feces of other infected animals. Research has documented the survival of STEC O26 for extended periods in manure: up to three months in manure heaps (*85*) and cow slurry (*86*) and up to a year in manure-amended soil depending on temperature and soil type (*87*). Persistence of these pathogens in an environment contaminated with manure is a concern not only for on-farm transmission and reinfection but also for environments such as county fairs and petting zoos where children may be exposed.

Several on-farm studies have documented the acquisition of STEC by calves. Sera and colostrum of dams and sera of newborn calves were found to contain Stx1-specific antibodies which may help protect newborn animals from infection. Antibody titers decreased rapidly during the first 6 weeks, and by 8 weeks most calves were shedding STEC (*88*). Another study showed that very young calves do not shed STEC (*52*) and calves shed different STEC strains as they age (*238*). About 40% of calf infections were estimated to be acquired from other calves (*170*). Some STEC strains appear to be cleared within a day while other strains persist in calves for several days (*270*). Population dynamics of STEC shedding by beef calves from birth to about two years of age was monitored and modeled to understand changes that occurred over time (*70*). Results from these studies may suggest new approaches for on-farm control of STEC.

Other ruminants

Sheep have been found to carry a great diversity of STEC but *E. coli* O157:H7 is infrequently isolated. Non-O157:H7 STEC have been detected in lambs and/or adult sheep from Australia (*63*), Brazil (*259*), India (*26*), Jordan (*200*), New Zealand (*55*), Norway (*254*), Spain (*30;204;225*), Switzerland (*275;277*), and the U.S. (*135;144*). In all of these surveys, multiple STEC strains were detected but O157:H7, if present, was a minor component of those identified. Several serotypes detected in sheep in different countries were similar, indicating that there may be some serotypes that have adapted to colonizing sheep. STEC serotypes that have been associated with human illness were detected in some studies but the majority of STEC strains present in sheep appeared to be of low virulence because they lacked some important virulence factors (intimin, hemolysin, Stx2).

Data gathered at a commercial lamb processing plant in the U.S. revealed that the prevalences of *E. coli* O157:H7 on pelts, preevisceration carcasses, and postintervention carcasses were 12.8%, 1.6%, and 2.9% respectively. For non-O157:H7 serotypes, prevalences were 86.2%, 78.6%, and 81.6%, respectively. A total of 69 different non-O157:H7 serotypes were identified. About 4% of these serotypes have previously been associated with severe human illness (*144*).

Goats are another reservoir of STEC. Of 13 caprine dairy herds surveyed in Ireland, STEC were isolated from milk filters from 3 farms. Serotypes O157 and O26 were each detected twice (*193*). A longitudinal study of two dairy goat farms in Spain documented chronic shedding of STEC by many adults on both farms but more sporadic shedding by kids. On one farm kids were carrying STEC bacteria within 1 week of age, while on the other farm it was four months before shedding began. Fewer adults were frequent shedders on the latter farm. Serotypes identified were primarily O33, O76, O126, O146, and O166. None of the isolates produced intimin and they appear to be adapted for colonizing the goat intestine (*205*). Non-O157:H7 STEC have also been detected in goats in Jordan (200;249), Bangladesh (*138*), and Vietnam (*128*).

Buffaloes. Nearly one-quarter of the buffalo slaughtered at a facility in Bangladesh contained non-O157:H7 STEC of several serogroups. Most strains produced Stx1 but many did not produce hemolysin or some other virulence factors. *E. coli* O157:H7 was isolated from 14.4% of the buffalo (*138*). A survey of 98 farms in central Vietnam revealed that 70% of

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farms had STEC-positive buffalo. However a minority (5%) of the serotypes identified were commonly associated with human illness (*128*).

Guanaco (*Lama guanicoe*). STEC O26:H11 was isolated from a two-month old guanaco with severe diarrhea in Argentina (*181*).

Deer and elk are present in significant numbers in some environments also used by cattle, sheep and goats, and their droppings may contaminate fresh produce in the field and surface waters. Cultures from fecal pellets from native Idaho ungulates revealed that about 19% were positive for Stx, which is about the same prevalence reported for cattle in that state (*80;96*). There have been numerous reports of *E. coli* O157:H7 in wild deer in the U.S. and other countries (*119*) and a few cases of human illness attributed to deer meat (*152*). Free-ranging wild sheep and deer in Spain were found to shed at least 11 non-O157:H7 STEC serotypes, with O146 being the most commonly detected serogroup (*230*). Several captive ruminants in an Argentine zoo, including alpaca, antelope, deer, eland, sheep, and yak, shed non-O157:H7 STEC (*168*).

Other animals

Swine have been found, in several studies, to be infected with both *E. coli* O157:H7 and non-O157:H7 STEC strains (*22;82;120;141;150;151;199;235;276*), whereas in other surveys only non-O157:H7 STEC were detected (*155;264*). Although virulent STEC strains have been found in a few samples from swine, most researchers conclude that swine are not an important source for human infections because the STEC strains isolated from these animals often lack some important virulence genes and differ from strains usually isolated from cases of human illness (*62;155;264;276*).

Horses do not appear to be an important reservoir for STEC. Only one of 400 fecal samples from horses in Germany tested positive for STEC (serotype O113:H21) and one of 100 horse meat samples tested was positive for STEC (serotype O87:H16) (*218*).

Rabbits, both wild individuals and animals being raised commercially on farms, were reported to harbor non-O157:H7 enterohemorrhagic *E. coli* (*91;164;231*).

Poultry have occasionally tested positive for *E. coli* O157:H7 (*64*), but so far there are no reports of non-O157:H7 STEC in poultry.

A **Cat** and a 2-year-old German girl with bloody diarrhea were found to excrete the same STEC serotype, O145:H–. The cat had no symptoms but was found to excrete this STEC strain for several months and was apparently the source of the child's original infection and/or reinfection (*36*).

Dogs in Brazil were reported to harbor non-O157:H7 STEC that caused diarrhea (*58*).

Shellfish in contaminated waters are known to concentrate some pathogens such as *Cryptosporidium*. *E. coli* is present in human sewage and the possibility exists that pathogenic strains such as STEC could be present in lakes, rivers or coastal waters contaminated by sanitary sewer overflow or runoff from fields containing fecal matter from domestic or wild animals. There have been reports of non-O157:H7 STEC detected in shellfish collected from coastal areas of France (*100*) and India (*160;175*). However, it appears that STEC strains are not a significant contaminant of shellfish.

Transport Hosts

Birds are a potential transport host for STEC because some wild birds harbor these bacteria and might spread them around a farm environment. There are a number of reports of non-O157:H7 STEC in both captive (ornamental, racing) pigeons (*78;102;246*) and in feral pigeons in the city or countryside (*102;156;190*). Stx2f, originally described from pigeon isolates (*234*), has been detected in a number of human diarrheal STEC isolates (*221*). Although a possible case of domestic pigeon-to-human transmission of non-O157:H7 has been reported (*246*), a study in Colorado indicated that wild pigeons may not be a major route of transmission of STEC (*214*).

Other wild birds may also carry non-O157:H7 STEC. A starling on a Danish farm was found to harbor STEC serotype O2:H29 (*198*). STEC serotype O20 was detected in an Oriental turtle dove, and serotype O147 was detected in a barn swallow living near Tokyo Bay (*156*). Stx2 was detected in feces of 30 wild bird species (of 99 species tested) in the UK, but strains were not serotyped (*125*).

Rodents, including mice and rats, are also potential transport hosts for STEC. However, STEC have not been frequently reported from these animals. There is one report of STEC serotype O136:H12 from a rat on a cattle farm in Denmark (*198*). Among animals tested at an Argentine zoo, STEC serotype O146:H28 was detected in a cavy (related to guinea pigs) (*168*).

Flies and beetles, collected on farms with animals shedding *E. coli* O157:H7, have been found to contain detectable levels of these bacteria (*64*). These insects frequent fecal deposits and may transfer these pathogens to foods, feed and water. STEC were recently isolated from flies collected at pig pens and in cattle barns but were not serotyped (*90*).

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Routes of Human Infection

Ruminant fecal material is believed to be the ultimate source of a large percentage of human non-O157 STEC infections. A study in Germany found that there was a positive association between illness caused by a number of non-O157 STEC serotypes and the density of cattle in an area. From data on over 3000 STEC cases, analyses indicated that risk for infection increased by 68% per 100 additional cattle/km^2. Increased risk varied for different serotypes but was greatest for O111 (*81*). A similar association has been documented for *E. coli* O157:H7 (*255*). Sporadic STEC cases have been traced to contact with cattle on farms (*118;196*).

Fecal material may contaminate meat during slaughter, may be washed or blown into lakes or drinking water sources, or may be deposited on fruits and vegetables by use of manure for fertilization or sewage-contaminated water for irrigation. Some animals, such as insects, birds, and rodents, may transport these bacteria from feces to drinking water or foods. In addition, non-O157 STEC may be inadvertently ingested by persons interacting or working with animals. Humans may therefore acquire infections through direct contact with an infected person or animal or their environment or through food, drinking water or surface water containing STEC-contaminated fecal material from an animal or human (*115*).

Direct contact

Numerous outbreaks of enteric zoonotic disease have been associated with animal exhibits at fairs, zoos and other venues. *Cryptosporidium*, *Salmonella* spp., and STEC are the pathogens most commonly identified in these outbreaks (*119;167*). Several non-O157 STEC outbreaks among children who visited farms (*1;245*) or petting zoos (*110;208*) resulted from direct contact with animals and their environment followed by inadequate hand washing. *E. coli* O26:H11 and O111:H– can survive in cattle feces for 10–12 weeks at 15ºC (*89*), and STEC may persist on surfaces at farms and zoos for extended periods (even after animals have stopped shedding) if there is sufficient moisture and temperatures are not excessive (*119*). An outbreak of non-O157 STEC at a Minnesota farm day camp occurred in two consecutive years despite attempts to clean the premises and encourage hand washing (*245*).

Contact with domestic animals has also been a route of STEC infection. A cat and a 2-year-old German girl with bloody diarrhea were both found to excrete O145:H–. Although the cat had no symptoms, it excreted this strain for several months and was apparently the source of the child's original infection and/or reinfection (*36*). Another child with diarrhea and some pigeons harbored the same STEC O128 strain (*246*). Pet rabbits have also been reported to harbor non-O157:H7 enterohemorrhagic *E. coli* (*91*).

Person-to-person spread of non-O157 STEC has been the primary mode of infection in outbreaks in day-cares, schools and senior care facilities (*7;8;35;54*). In many other outbreaks, some cases who consumed contaminated food or water passed the infection directly to friends or others in their family. Although most people apparently stop shedding STEC bacteria within a week or so of recovering from illness, there are some people who continue to shed bacteria for weeks or months afterwards. A study in a German sausage factory over a 21-month period demonstrated that one healthy worker excreted non-O157 STEC intermittently for 7 weeks; another symptomless worker excreted STEC for nearly ten months (*92*).

Contaminated food

Beef, lamb, and mutton can be contaminated during slaughter and processing by exposure to feces or hides containing non-O157 STEC. A 2007 review stated that reported levels of non-O157 STEC in whole cattle carcasses, ground beef, retail beef cuts, and sausage were 1.7–58%, 2.4–30%, 11.4–49.6%, and 17–49.2%, respectively (*129*). Beef trim, which is ground to make hamburger, is believed to be an important source of STEC contamination in ground beef. A survey of boneless beef trim from Australia (220 samples), New Zealand (223 samples), Uruguay (256 samples), and the U.S. (487 samples) revealed that non-O157 STEC were present in 10% of the New Zealand samples and in about 30% of the other samples (*31*). Other surveys have reported the STEC prevalence to be 15% in ground meat in France (*216*), 1.5% in beef in Japan (*113*), 40% in ground lamb in Australia (*14*), 1% in horse meat in Germany (*218*), and 24% in buffalo meat in India (*116*) Procedures for collecting samples and performing analyses differed among these studies, so results are not directly comparable.

Milk from dairy cows, sheep, and goats may be contaminated with *E. coli* and other bacteria from the environment. A review in 2005 summarized numerous surveys that detected STEC in milk and dairy products. Contamination with *E. coli* is generally low, and some of the STEC strains detected in raw milk have not been associated with human disease (*133*). Some non-O157 STEC may be more prevalent in milk than *E. coli* O157:H7. STEC strains have been shown to survive various steps in cheese-making so that raw milk cheeses are potentially a vehicle for STEC infections (*16*).

Most later surveys also report a relatively low prevalence of non-O157 STEC in raw milk but there

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were some positive samples indicating the need for proper treatment of milk (*173;174*). STEC strains were isolated from 11% of bulk milk tank samples, 4% of cheese curds, and 5% of cheese in Spain (*226*) and in 21% of raw milk samples in France (*216*). In a Swiss study, non-O157 STEC were isolated from 16 of 744 raw milk hard and semi-hard cheeses (*248*). Analyses of 40 STEC strains isolated from raw milk and cheese in France found that most of the strains lacked some virulence factors found in isolates from human disease (*220*). Proper pasteurization kills *E. coli*; so outbreaks of STEC due to contaminated dairy products are usually associated with unpasteurized milk (*2;4;61*) but there has been an outbreak due to post-pasteurization contamination (*189*).

Field and greenhouse experiments have demonstrated that both *E. coli* O157:H7-contaminated manure and irrigation water may cause contamination of vegetables and this is probably true for non-O157 STEC as well. Manure is a valuable fertilizer for crops but manure containing STEC may be a source of contamination for vegetables or fruits that are not normally cooked before eating. In one study, non-O157 STEC were able to survive for 42 days in manure heaps that were turned and for 90 days in unturned heaps (*85*). STEC O26 survived for at least 90 days in cow slurry (an effluent comprised of feces, urine, water, spilt feed, and bedding) (*86*). Serotype O26 was also detectable in manure-amended loam soil for more than 9 months at 4ºC and for more than 6 months at 20ºC (*87*).

Foods can also be contaminated with STEC by cross-contamination during food preparation and by infected workers who don't practice good hygiene. There have been several outbreaks attributed to restaurant food. Cross-contamination in food preparation areas or infected food handlers might have contributed to these outbreaks. An outbreak in a prison was traced to a food handler (*46*).

Contaminated water

Water used for drinking or recreation has been reported as the vehicle of infection for 7 outbreaks. One outbreak in 1988 in Czechoslovakia was associated with tap water. Several outbreaks occurred among children playing or swimming in pool or lake water. Other infected children may have been the source of bacteria for these cases. Other outbreaks were traced to water consumed at summer camps. Fecal material from domestic and/or wild ruminant animals may have contaminated lakes, rivers, and some "drinking water."

Surveys of some surface waters have detected *stx* genes in beach and stream water in a park in Pennsylvania (243), and in river water in Michigan and Indiana (*67*) and in India (*223*). The significance of these findings is unclear because the presence of *stx* genes was not correlated with numbers of viable bacteria present. Some strains of the non-O157 STEC serotypes O26 and O111 have been reported to survive in untreated well water for over 56 days at 10ºC. Cells die off more quickly at 22ºC but do persist in significant numbers for four weeks (*265*).

INTERVENTIONS FOR CONTROL OF NON-O157:H7 STEC

Research on prevention of STEC contamination of foods and water and strategies to kill or severely limit growth of any STEC that might be present in foods has concentrated primarily on *E. coli* O157:H7. In the following sections, data will be presented for non-O157 STEC when available and summarized for *E. coli* O157:H7 or other *E. coli*. Susceptibility of non-O157 STEC to various intervention techniques is probably similar to that of other *E. coli* although there are known differences among strains in acid tolerance and sensitivity to some other agents. Some recent reviews discussing intervention techniques have been published (*119;134;224;257*).

Effects of processing technologies on *E. coli* and other bacteria in meat were recently reviewed (*10*). Among the procedures discussed were irradiation, high hydrostatic pressure, natural antimicrobials, active packaging, and thermal treatments. Comparisons between *E. coli* O157:H7 and non-O157 STEC were not discussed, but conditions generally effective against *E. coli* were described.

Pre-Harvest Interventions

Dietary Interventions

Feed. Results of experiments published about ten years ago indicated that different diets fed to cattle may affect concentrations of *E. coli* O157:H7 shed in feces. Feeding of high grain rations to feedlot cattle to increase feed efficiency causes some starch from the grains to escape fermentation in the rumen and pass to the hindgut where it is fermented by other bacteria. This can change the pH of the rumen and hindgut thereby affecting survival of some bacteria, such as STEC strains. A recent review paper discussed various experiments conducted since that time to determine whether dietary interventions could reduce numbers of STEC bacteria excreted by cattle (*37*). Subsequent experiments have shown that dietary differences do affect *E. coli* populations in cattle but the effects varied in magnitude and impact. Some studies suggest that the tannins and phenolic acids in forage may be the important components affecting shedding of STEC while other experiments, in which

barley and distillers grains were fed to cattle, demonstrated an increase in shedding of *E. coli* O157:H7. Dietary experiments that tested for genes coding STEC virulence factors in cattle feces (which would measure effects on all STEC not just *E. coli* O157:H7) suggested that a diet with more roughage may reduce concentrations of STEC bacteria that cause human disease. However, the cattle in this study were excreting low levels of STEC, and further experiments are needed to determine the significance of these results (*95*).

Probiotics, commensal bacteria fed to animals to reduce numbers of pathogens, have been suggested as a strategy to prevent growth of STEC in young ruminants. In one experiment with calves, a three-strain mixture of non-pathogenic *E. coli* was fed to calves three days after challenge with one of three STEC serotypes. The probiotic *E. coli* mixture decreased fecal shedding of O157:H7 and O111:NM but did not affect shedding of O26:H11. At necropsy, all of the calves challenged with O26:H11 and four of twelve calves challenged with the other STEC still harbored viable STEC (*253*). *E. coli* strains used as probiotics may produce colicins that kill STEC strains (*232*). Other experiments have tested the effects of lactic acid bacteria on STEC survival. Some inhibition of STEC has been observed in tests in vitro (*98;126*). Some variability in practical results of tests with probiotics may be due to different management practices, including the effects of subtherapeutic levels of antibiotics fed to livestock to enhance growth (*119*).

Bacteriophages are viruses that can kill bacteria and have been proposed as potential control agents for STEC. In experiments with an artificial rumen system, phage D22, specific for *E. coli* O157:H7, eliminated these bacteria from the fermentor within four hours. However, in experiments with lambs, inoculated first with *E. coli* O157:H7 and then two days later with the phage, there was no decrease in numbers of *E. coli* O157:H7 shed. The phage did not persist in these animals (*11*). To avoid the problem of inactivation of phage in an animal's digestive tract, another experiment tested the effects of two phages (one of which could also kill some non-O157 STEC strains); the phages and *E. coli* O157:H7 were applied directly to the rectoanal junction of steers. Phage therapy reduced the average number of *E. coli* O157:H7 detected in feces but did not eliminate these bacteria from most of the animals (*240*). Although bacteriophages have not yet emerged as a practical preharvest solution to STEC shedding by ruminants, some research continues to find other phages that might be more effective (*262*).

Chlorate is metabolized by some bacteria, including *E. coli*, to a toxic compound, chlorite. Feeding chlorate to cattle in feed and water prior to slaughter can significantly reduce concentrations of *E. coli* O157:H7 and other *E. coli* in feces at slaughter. This is a potentially useful strategy for reducing contamination of meat during processing at slaughter facilities (*6*). Chlorate in drinking water is also effective in reducing *E. coli* populations (*119*).

Drinking water may be contaminated by fecal material and is known to be a source of infection for cattle (*241*). Four chemical treatments of drinking water using lactic acid, acidic calcium sulfate and one of the following: benzoate, caprylic acid, butyric acid, chlorine dioxide resulted in >3 log reduction in numbers of *E. coli* O157:H7, O26:H11, and O111:NM in contaminated trough water. However, cattle consumed much less water when these chemicals were present, so they should not be used continuously (*274*).

Vaccines

Some infected calves develop an immune response to STEC, and vaccines targeting some important STEC proteins may be useful in preventing the establishment of STEC in calves. A recent review article mentioned 7 vaccines that have been described in the literature. There is not much information available on most of them because of proprietary considerations. Some have been tested in cattle but others have apparently been tested only in pigs or laboratory animals. Some vaccines, if demonstrated to be effective in cattle, may offer protection against non-O157 strains if they induce antibodies to a common virulence factor (*257*). Two recent articles described some new vaccines but they have not been tested in cattle as yet (*105;169*). Large-scale testing of a vaccine to reduce carriage of *E. coli* O157:H7 in cattle has begun in Colorado. A New York Times article on December 4, 2009 described the program and the obstacles faced by vaccine producers in getting approval for use of this product.

Processing Interventions

Many interventions that aid in control of *E. coli* O157:H7 are likely to be effective for non-O157 STEC also. Effectiveness of interventions for decontaminating meat was recently reviewed (*10*). Use of hot water and lactic acid washes and steam to clean carcasses effectively reduces contamination with *E. coli* O157:H7 (*32;158*). Intervention techniques to remove *E. coli* O157:H7 from the surface of beef carcasses were found to be similarly effective against O26:H11 and O111:H8 (*57*).

Stress tolerance to heat, salt and acid has been observed in many STEC strains and should be considered when devising interventions in food processing. Some non-O157 STEC are more susceptible or

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resistant to stresses so that effectiveness of procedures needs to be tested with more than one serotype. For example, *E. coli* O157:H7 was found to be more resistant to acid in a model stomach system than some non-O157 STEC serotypes (*18*). However, other tests in different media found *E. coli* O157:H7 to be more sensitive to acid than other non-O157 STEC (*20;162;186*). Acid-resistant non-O157 STEC survive longer in fermented raw sausage than non-acid resistant strains (*188*). Both sodium lactate and sodium benzoate inhibit non-O157 STEC but the extent of inhibition is temperature dependent (*117;166*).

STEC serotypes do not grow at refrigeration temperatures but can remain viable in food for extended periods in the cold (*211*), and *E. coli* O157:H7 can survive at least for several days in meat and yogurt when frozen at –18ºC (*104*). Thermal treatments can destroy STEC, but one study reported that STEC O26 was less heat sensitive than *E. coli* O157:H7 in minced beef heated at 55ºC (*66*).

Effectiveness of sanitizers and disinfectants has been tested against STEC O111 and O26 (*159;242; 258*). Several STEC serotypes tested were more resistant to desiccation on paper discs than *Shigella* and non-pathogenic *E. coli*. STEC also survived for months in chocolate (*17;121*).

Zoo and Farm Environmental Interventions

CDC published in 2009 an updated version of recommended measures to prevent disease associated with animals in public settings. Million of human–animal interactions occur annually in a variety of settings. Hand washing is the most important preventive step for reducing disease transmission. In addition, CDC recommends prohibition of food in animal areas, education of visitors about disease risk and prevention, proper care and management of animals, and transition areas between animal and non-animal locations. The updated guidelines also discuss risks associated with baby poultry, reptiles, rodents, and aquatic animals (*49*).

Composting of manure from animals shedding STEC can eliminate STEC when temperatures and the presence of other bacteria are optimized (*97*).

ANALYTICAL METHODS FOR DETECTING NON-O157:H7 STEC

Introduction

E. coli O157:H7 can usually be readily identified in the laboratory because of its inability to ferment sorbitol or cleave the fluorogenic substrate 4-methylumbelliferyl-B-d-glucuronide within 24 hours, which distinguishes it from other *E. coli* and most of the other bacteria in its environment. Nearly all *E. coli* O157:H7 produce Shiga-like toxins or harbor genes (*stx*) encoding the toxins so a culture-positive result is assumed to be positive for STEC. It should be noted that there are atypical strains of serogroup O157, designated as O157:H–, which can ferment sorbitol and may initially be presumed to be non-O157 strains. Isolates of serotype O157:H– often produce Shiga toxins and have been associated with cattle and with severe illness in children (*146;207*).

Detection and identification of non-O157:H7 STEC serotypes in a timely fashion are more difficult. These strains do ferment sorbitol so they are not detectable on sorbitol MacConkey agar plates. Even though six non-O157 serogroups (O26, O45, O103, O111, O121, O145) cause most of the reported cases of non-O157 infection, over 150 STEC serotypes have been associated with illness. In addition, not all strains of these serogroups produce stx. Therefore, CDC recommended in a recent report that laboratories simultaneously (1) test samples for the toxins with enzyme immunoassays or for *stx* with PCR methods and also (2) isolate and grow the bacteria in pure culture (*99*). If Shiga toxin is detected, then cultures will be immediately available for serotyping and molecular characterization.

Over 100 reports in scientific journals describe analytical methods for detection of non-O157 STEC. Immunoassays, PCR methods, and molecular analytical methods, developed in the past five years, are highlighted in the discussions below. Some useful descriptions of culture and enrichment methods are also included. Several comparisons of the accuracy of different methods and evaluations of commercially available detection methods will be discussed in the final section along with future research needs.

Enrichment and Culture

Isolation of non-O157:H7 serotypes from animals and foods containing large numbers of a variety of other bacteria is challenging because of the genetic and biochemical diversity of these STEC and their similarity to some non-pathogenic bacteria. In a 2006 review article, enrichment/culture protocols, described in 132 papers published since 1997, were discussed (*261*). Many researchers used media containing bile salts to inhibit non-Enterobacteriaceae and antibiotics, such as novobiocin, which inhibit primarily Gram-positive bacteria. Incubation times and temperatures varied. However, because of variations in experimental procedures, no definitive conclusions could be drawn about the relative effective-

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ness of the protocols for enrichment from different environmental samples.

Other antibiotics (cefixime, vancomycin) and selective agents (tellurite) have been used to inhibit non-STEC bacteria (*132*) but there are reports that some non-O157:H7 STEC are sensitive to these agents (*15;206*). Other experiments demonstrated that growth of some non-O157:H7 STEC is inhibited by novobiocin in media and use of this antibiotic was not recommended (*260*). Universal preenrichment broth (which does not contain antibiotics), incubated at 42ºC, was reported to be more effective for detection of STEC O26 and O157 on beef, poultry, and radish sprouts than modified *Escherichia coli* broth with novobiocin (*145*).

An acid enrichment procedure was recently reported to substantially decrease background flora in fecal specimens and enhance recovery of STEC strains (*124*). In a comparison of three enrichment protocols, a USDA procedure, an FDA procedure and acid enrichment, *stx*2 gene was detected in more samples of swine waste treated with acid than samples enriched by the other methods. All strains were non-O157:H7 STEC. The acid enrichment media was found to support the growth of all of 31 STEC strains tested while FDA media supported growth of 23 strains and USDA medium supported growth of 5 strains (*101*).

Identification of STEC Serotypes

Culture methods

There are no completely reliable culture methods for identifying non-O157 STEC strains, although research indicates that some specific nutritional requirements or capabilities are associated with certain STEC serotypes. O26 strains that produce Shiga toxins are often unable to ferment rhamnose and can usually (but not always) be distinguished from non-toxigenic strains on rhamnose MacConkey agar (*77*). A procedure using a consecutive series of differential and confirmation media was developed to distinguish 4 STEC serotypes (O26, O103, O111, O145). Samples of artificially contaminated food and fecal samples were first enriched in media containing novobiocin, vancomycin, rifampicin, bile salts and tellurite. On the first differential media containing sorbose and sucrose, the non-O157 STEC strains produced different colored colonies. These were then plated to media containing D-arabinose, D-raffinose, L-rhamnose, or dulcitol for confirmation. Isolation efficiency of all serotypes from different sources was 100%, 82.3%, 88.5%, 65.9%, 64.3%, and 13.6% for STEC in raw milk, cheese made from pasteurized milk, cheese made from raw milk, ground beef, fermented meat, and cattle feces, respectively (*219*).

Immunoassays

E. coli serotypes can be identified by immunoassays that target the O and H antigens on cell surfaces. Antisera for the most common non-O157 serotypes (O26, O45, O103, O111, O121, O145) are available commercially and can be used to identify many STEC isolates (*99*). Antibodies may be coated on immunomagnetic-separation beads and are potentially useful for detecting cattle shedding a large number of STEC of certain serotypes, but these methods are not as reliable if STEC numbers are low (*109*). Immunomagnetic beads have also been used to detect O26 and O111 in ground beef (*201*).

PCR for serotype specific genes

PCR (polymerase chain reaction) methods targeting DNA variants specific to different serotypes have been developed recently. These are often combined with PCR assays detecting genes coding for Shiga toxins or other virulence markers. Some assays target a gene associated with the O antigen such as the *wzx* gene in serotype O26 (*172*) and in serotype O103 (*215*), while others target genes associated with both the O and H antigens as in methods described for O111:H8 (*69*) and O26:H11 (*68*). More recent multiplex methods detect specific genes present in O-antigen gene clusters of four or five different O groups (*23;83;84;187;217*). These procedures can identify serotypes isolated from foods and fecal samples.

Shiga Toxin Detection

Immunoassays for toxins

Six commercial immunoassays have been approved by FDA for the diagnosis of STEC infections (*99*):

- Biostar OIA SHIGATOX, an optical immunoassay which does not distinguish between stx1 and stx2, can detect toxin in broths and fecal samples (*251*). (This will be withdrawn from market in 2009.)
- Duopath Verotoxin test detects and differentiates Shiga toxins 1 and 2 in less than one hour in cultures of isolated cells (*212*).
- Immunocard STAT!EHEC also differentiates Shiga toxins 1 and 2 in less than an hour in enrichment broths and cultures of isolated cells (*99*).
- Premier EHEC does not distinguish between Stx1 and Stx2 and takes several hours to perform. It can detect toxins in stool samples and enrichment broths. Stx concentrations in fecal samples are typically very low, however, and detection is better in enrichment broths (*153*).
- ProSpecT Shiga Toxin *E. coli* Microplate Assay does not differentiate Stx1 and Stx2 and takes several hours to perform. It can detect toxins in stool samples as well as enrichment broths.

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Results are generally better in testing broth cultures (*94*).

- VTEC Screen "Seiken" does differentiate Stx1 and Stx2 and takes several hours to perform. It detects toxins in cultures of isolated cells (*42*).

An evaluation of the Ridascreen Verotoxin Immunoassay published in 2007 noted that it could detect all known variants of Stx1 and Stx2 in routine screening of bacterial isolates (*24*). A more recent evaluation of this assay and the Premier EHEC and ProSpecT assays found that none of the tests could detect some Stx2 variants. The ProSpecT assay was about ten-fold less sensitive than the other two assays. Premier EHEC assay may be useful in screening cattle (*271*).

Nanoparticle assay for toxin proteins

Stx bind to cell surface receptors containing terminal Gal-α1,4-Gal disaccharides. Glycopolydiacetylene nanoparticles, with Gal-α1,4-Gal disaccharides on their surfaces, were found to change color from purple to brown when bound to stx. These nanoparticles could distinguish between *E. coli* strains that did and did not produce Stx within 10 min (*194*).

PCR for toxin genes

PCR assays for *stx* genes are generally designed for testing isolated cells from media or bacteria growing in enrichment broths rather than bacterial DNA extracted directly from foods or fecal specimens. PCR procedures have been developed and evaluated for identification of STEC from human stool samples (*103*), cattle feces (*237*), and foods (*9;84*). Some multiplex PCR assays are designed to screen for different types of diarrheagenic *E. coli* targeting virulence genes found in enterohemorrhagic, enteroinvasive, enteropathogenic, enterotoxigenic, and enteroaggregative strains (*107*). An evaluation of the GeneDisc assay, a multiplex assay targeting genes for *stx*, intimin, and DNA sequences characteristic of O26, O103, O111, and O157, found that it was very sensitive and capable of detecting 2 to 3 STEC colonies in a lawn of 50,000 bacteria (*25*). A highly sensitive immuno-PCR utilizing an immunoassay with antibody capture and DNA amplification detected as little as 10 pg purified *stx*2/ml (compared to 1 ng detected by a commercial immunoassay (*273*).

LAMP (loop mediated isothermal amplification) assay for toxin genes

This method for nucleic acid amplification differs from PCR in that 4 or 6 primers are used to amplify the target gene at a single temperature step. Amplification products can be detected by turbidity because a by-product of the reactions, magnesium pyrophosphate, is insoluble. Turbidity caused by this precipitate correlates with the amount of DNA synthesized (*112*). DNA was extracted from an enrichment from ground beef and tested with a LAMP assay targeting *stx*. Several STEC serotypes were detected (*111*).

Comparison of different stx detection methods

STEC strains have been detected in the past by determining their cytotoxic effects on Vero (monkey) cells in culture. PCR assays for *stx* give results that are more than 90% in concordance with the Vero cell assay (*272*).

Subtyping Methods

Particularly in suspected outbreak situations, it is important to specifically identify the causative pathogen in order to trace pathways of contamination and determine the extent of outbreaks. There is great genetic diversity within STEC due to insertions and deletions in certain parts of the chromosome and genetic information carried by bacteriophages (*202*). PFGE (pulsed field gel electrophoresis) is a widely used technique that analyses patterns of chromosome fragments generated by restriction enzymes that cause breaks at certain DNA sequences. Such analyses aided in detection of foods associated with an outbreak caused by STEC O103:H25 in fermented sausage in Norway in 2006 (*236*) and in tracing the contamination of ice cream associated with a Belgian outbreak (*60*). Other molecular analyses of multiple genetic loci have provided information on important virulence mechanisms and evolutionary relationships among various STEC strains (*56;180;202;244;250*).

FUTURE RESEARCH NEEDS

More recently published studies also demonstrate that a large number of *E. coli* serotypes may be present in animals: at least 10 detected on beef carcasses in the Pacific northwest (*53*) and 31 serogroups detected in dairy cattle in Japan (*157*). Estimates of prevalence are much higher from PCR or immunoassays detecting Shiga toxins or genes coding for these toxins (23–30%) than estimates obtained from isolation of STEC bacteria (6–12%) (*157*). Many of these STEC strains may not be virulent but it would be useful to know how readily these strains can exchange genetic information and acquire virulence factors. Although multiplex PCR methods are available for confirmation of virulence genes in isolates, initial screening methods that segregate the diverse populations of *E. coli*, that are commonly encountered in clinical, environmental, and food samples, would facilitate detection and identification of seropathotypes. A combination of effective selective and differential plating media and molecular typing methods are needed to make accurate and comparable prevalence

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determinations and environmentally track specific serotypes or strains.

Many studies have documented the effects of various intervention techniques on *E. coli* O157:H7. Some interventions should also be tested on pathogenic non-O157 serotypes. The need for these validations arises from the variability of growth and survival properties within a given species such as *E. coli*. With multiple serotypes comprising the non-O157 STEC, there is greater diversity and a need to ascertain if certain serotypes, seropathotypes, or strains have growth or survival properties that differ significantly from *E. coli* O157:H7. Because of variations in resistance to environmental stresses, it is possible that the lethality of interventions and processes used to control *E. coli* O157:H7 will need to be evaluated with a select set of non-O157 STEC.

To more completely define the epidemiology of non-O157 STEC, additional information on animal, environmental, and asymptomatic human hosts is needed. A more complete understanding of localization within hosts as well as the growth, persistence and dissemination in the environment would be beneficial. As with *E. coli* O157:H7, identifying where the pathogen is located in and on the animal can lead to more effective harvesting and processing strategies to reduce contamination of raw food products. Identification of preferred environmental niches can lead to possible on-farm interventions and reduction of pathogen prevalence. Lastly, the incidence of person-to-person transmission by non-O157 STEC requires further investigation to fully understand the role humans may play in the dissemination of this diverse group of pathogens.

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http://fri.wisc.edu/docs/pdf/FRI_Brief_NonO157STEC_4_10.pdf
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Table 1. Reported outbreaks caused by non-O157 STEC.

Date	Strain	Location	Cases	HUS	Deaths	Vehicle	Reference(s)	Other details
1984	O145:H–	Japan	100		0	Unknown	(*143*)	school
1986	O111:H–	Japan	22	1	1	Unknown	(*12*)	orphanage
1988	O111:H2	Australia	2	2	0	Unknown	(*12*)	family
1988	O26:H11	Czech Republic	5	5	1	Water, tap	(*27*)	
1990	O111:NM	US: Ohio	5	1	0	Unknown	(*12*)	family
1991	O111:H–	Japan	234			Unknown	(*12*)	primary school
1992	O111:B4	France	26	10	0	Person–person	(*33*;*39*)	school
1992	O111	Italy	9	9	1	Unknown	(*4*;*40*)	
1992–93	O119:B14	France	4	4	1	Cheese, unpasteurized	(*4*;*61*)	bovine and caprine milk
1994	O103	France	4	4	0	Cheese, raw caprine milk	(*4*)	
1994	O104:H21	US: Montana	18	0	0	Milk, pasteurized	(*79*;*189*)	post-pasteurization contamination
1995	O111:H–	Spain	13			Water, drinking	(*29*)	summer camp
1995	O111:NM	Australia	88	23	1	Sausage	(*38*;*71*;*213*)	mettwurst
1995–96	O157:H–	Germany	28	28	3	Sausage	(*5*)	
1996	O118:H2	Japan	131	0	0	Salads	(*114*)	junior high school
1997	O26:H11	Japan	27	0	0	Watermelon; sprouts; spinach	(*122*)	nursery
1999	O26	Ireland	10	0	0	Unknown	(*28*)	child care facility
1999	O26:H11	Germany	3	3	0	Unknown	(*184*)	3 children, small town
1999	O111:H8	US: Texas	58	2	0	Unknown: salad and/or ice?	(*19*;*34*)	teenage campers
1999	O121:H19	US: Connecticut	11	3	0	Water, lake	(*177*)	swimming in lake
2000	O26:H11	Germany	11	0	0	Beef	(*269*)	day care centers
2000	O103	US: Washington	18	+		Punch	(*35*)	
2000	O111	US: Utah	102			Water, drinking	(*165*)	also O157:H7 & *C. jejuni*
2000–01	O51:H11, O111	US: Minnesota	7	0	0	Calves, contact	(*245*)	farm day camp, 2 years; other enterics
2001	O26:H11	Japan	11	0	0	Water, river	(*123*)	water used for washing
2001	O26	US: Minnesota	4	0	0	Water, lake	(*35*)	
2001	O26:H–	Austria	2	2	0	Milk, cow's, raw	*(2)*	
2001	O111	US: South Dakota	3	0	0	Person–person	(*35*)	day care centers
2001	O111	US: Minnesota	2	2	1	Unknown	(*183*)	
2002	O148	France	10	2	0	Mutton, lightly roasted	(*73*)	wedding reception
2002	O26	Australia	5			Animal contact: pigs; alpacas	(*208*)	petting zoo
2002	O157:H–	Germany	38	38	4	Unknown	(*3*)	
2003	O111	Australia	13	0	0	Person–person	(*54*)	old folks home
2004	O86:H27	Australia	4	2	0	Cattle; person–person	(*191*)	1st case from cattle farm
2004	O111:NM	Japan	107	0	0	Meat	(*149*)	students traveling to Korea
2004	O121:H19, O121:H7	Japan	63	0	0	Animal exposure: cattle	(*1*)	children, farm visit
2004	O111	US: New York	213			Cider, unpasteurized	(*263*)	also *Cryptosporidium*
2005	O26	Italy	6	6	1	Milk products; buffalo	(*173*)	
2005	O26 & O80	France	16	16		Cheese from unpasteurized cow's milk	*(72)*	
2005	O26:H11	Japan	12	0	0	Person–person	(*136*)	kindergarten, also norovirus
2005	O45	US: New York	52	0	0	Food handler	(*46*)	prison
2006	O26	US: Massachusetts	5		0	Strawberries; blueberries	(*50*)	
2006	O26	Japan	16	0	0	Food, restaurant	(*185*)	served at one restaurant

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Date	Strain	Location	Cases	HUS	Deaths	Vehicle	Reference(s)	Other details
2006	O26:H11	Japan	33	0	0	Person–person	(*247*)	nursery school
2006	O80:H– & O145	Germany	59	1		Milk, unpasteurized	(*65*)	camp
2006	O103:H2	Japan	12	0	0	Person–person	(*192*)	nursery school
2006	O103:H25	Norway	18	10	1	Mutton; sausage	(*106*;*233*;*236*)	
2006	O121:H19	US: Utah	69	4		Lettuce	(*176*)	restaurant
2007	O26 & O145	Belgium	13	5	0	Ice cream	(*60*)	contamination by food handlers ?
2007	O26	US: Iowa	3			Water, pool	(*176*)	
2007	O26:H–	Japan	29			Person–person	(*7*)	nursery school
2007	O26:H11	Denmark	20	0	0	Sausage	(*74*;*75*)	
2007	O26:H11	Japan	19			Person–person	(*7*)	nursery school
2007	O26:H11	Japan	19			Person–person	(*7*)	nursery school
2007	O26:H11	Japan	31			Person–person	(*7*)	nursery school
2007	O55:H80	Australia	3	1		Animal contact	(*110*)	kangaroos and koalas at an animal sanctuary; also infected with Salmonella Virchow
2007	O103:H11	Japan	20			Unknown	(*7*)	old folks home
2007	O111:H–	Japan	8			Person–person	(*7*)	kindergarten
2007	O111:H–	Japan	28			Unknown	(*7*)	nursery school
2007	O111:H–	Japan	22			Person–person	(*7*)	nursery school
2007	O111	US: Maine	8			Food	(*203*)	
2007	O111	US: North Dakota	6			Person–person	(*203*)	
2007	O111:NM	US: North Dakota	23	0	0	Beef, ground	(*195*)	wedding reception
2008	O111:NM	US: Oklahoma	341	25	1	Food, restaurant	(*203*)	traced to 1 restaurant
2008	O26:H11	Japan	91			Food, high school	(*8*)	high school
2008	O26:H11	Japan	27			Person–person	(*8*)	nursery school
2008	O26:H11	Japan	52			Unknown	(*8*)	school excursion
2008	O26:H11	Japan	8			Person–person	(*8*)	nursery school
2008	O26:H11	Japan	84			Person–person	(*8*)	kindergarten
2008	O26:H11	Japan	10			Person–person	(*8*)	nursery school
2008	O26:H11	Japan	11			Person–person	(*8*)	nursery school
2008	O111:H–	Japan	67			Unknown	(*8*)	hospital
2008	O111:H–	Japan	13			Unknown	(*8*)	
2008	O26:H–	Japan	32			Person–person	(*8*)	nursery school
2008	O26:H–	Japan	14			Person–person	(*8*)	nursery school
2008	O145:H–	Japan	13			Person–person	(*8*)	nursery school
2008	O111:H–	Japan	61			Person–person	(*8*)	nursery school
2008	O115:H NM	Japan	2			Chicken or egg	(*76*)	
2008	Unknown, not O157	Australia	7			Water, drinking	(*210*)	camp
2009	O121	Japan	31			Person–person	(*8*)	nursery school

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