Risk Profile for Pathogenic Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC)

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Executive Summary

Awareness of non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC) as a food safety concern has increased recently. In October 2009, a Citizen's Petition was filed with the USDA's Food Safety and Inspection Service (FSIS) to declare all enterohemorrhagic (EHEC) Shiga toxin-producing serotypes of *Escherichia coli* (STEC), including non-O157 serotypes, to be adulterants within the meaning of the Federal Meat Inspection Act (FMIA). FSIS considers *Escherichia coli* (*E. coli*) O157:H7 to be an adulterant using the "ordinarily injurious" standard of the Federal Meat Inspection Act. The petition seeks to expand the current definition to include additional Shiga toxin producing *E. coli,* as an interpretive rule.

FSIS undertook the preparation of this risk profile to help clarify the extent of the scientific literature available for evaluating the issues raised by the Citizen's Petition. Risk profiles are defined by Codex Alimentarius as:

> *... a description of a food safety problem and its context that presents in a concise form, the current state of knowledge related to a food safety issue, describes potential microbiological risk management options that have been identified to date, when any, and the food safety policy context that will influence further possible actions... Consideration of the information given in the risk profile may result in a range of initial decisions, such as commissioning a microbiological risk assessment, gathering more information or developing risk knowledge at the level of the risk manager,[or] implementing an immediate and/or temporary decision* (Codex Alimentarius Commission, 2007).

This risk profile provides current scientific information relevant to this issue, i.e. whether certain enterohemorrhagic Shiga toxin producing serotypes of *E. coli*, including non-O157 serotypes, can be considered as adulterants, analogous to *E. coli* O157, within the meaning of the FMIA.

When considering non-O157 STEC, first, the term "enterohemorrhagic Shiga toxin producing serotypes of *E. coli*, including non-O157 serotypes" needed to be more clearly defined. Second, FSIS wanted to determine if this group of organisms was likely to be present in beef produced by establishments under its jurisdiction. Third, FSIS sought to examine the claim that this group of organisms represented an unusual and urgent food safety problem comparable to that posed by *E. coli* O157:H7. This risk profile assesses the scientific and epidemiological literature and other data to address these issues.

(1) What are pathogenic non-O157 STEC, and how can they be distinguished from other STEC?

(2) Are pathogenic non-O157 STEC present in cattle, and beef, including ground beef?

(3) Would traditional and accepted cooking practices for raw ground beef kill pathogenic non-

O157 STEC?

(4) Can small numbers of pathogenic non-O157 STEC cause illness?

(5) Can pathogenic non-O157 STEC cause severe illness including permanent life-threatening damage to major organ systems?

(6) Can pathogenic non-O157 STEC spread from person to person causing illness in settings such as day care facilities?

Note that this Risk Profile does not touch on all of the issues of potential interest in assessing and managing the risks associated with either pathogenic STEC in general or specific strains of non-O157 STEC. For instance, the document focuses on the most severe end of the spectrum of disease associated with non-O157 STEC, but does not address the probability and the likely severity of potential adverse events. Furthermore, the specific virulence profile which makes individual strains of STEC more or less virulent for humans is not fully understood.

Findings:

(1) What are pathogenic non-O157 STEC, and how can they be distinguished from other STEC?
STEC is a group of *E. coli* bacteria that produce Shiga toxin. A subset of STEC causes human illness. We found no consensus in the scientific community about precisely which features, or virulence factors, make an STEC harmful to humans. Therefore, the Risk Profile considers any STEC capable of causing severe human illness to be a pathogenic STEC. The Centers for Disease Control and Prevention (CDC) defines STEC as capable of causing illness of "variable severity characterized by diarrhea (often bloody) and abdominal cramps. Illness may be complicated by hemolytic uremic syndrome (HUS)[1]" (CDC, 2010a).

(2) Are pathogenic non-O157 STEC present in cattle, and beef, including ground beef?
Although the majority of non-O157 STEC infections are attributed to non-beef food sources, surveys indicate that pathogenic non-O157 STEC serogroups may be present in cattle, on beef carcasses, in beef trimmings destined for ground beef production, and in ground beef from federally regulated establishments and retail markets. However, due to lack of baseline data, we cannot make definitive quantitative statements about the national prevalence or the likelihood that pathogenic STEC serogroups may be found in either cattle or ground beef.

(3) Would traditional and accepted cooking practices for raw ground beef kill pathogenic non-O157 STEC?

[1] Although pathogenic STEC infection does not cause thrombotic thrombocytopenic purpura (TTP) (Tarr *et al.*, 2009), HUS may be misdiagnosed as TTP and is included in the CDC definition (CDC, 2010a). Therefore, we include TTP as a severe outcome of STEC infection.

We provide evidence suggesting that traditional and accepted cooking methods would destroy *E. coli* O157:H7 and pathogenic non-O157 STEC at similar rates.

(4) Can small numbers of pathogenic non-O157 STEC cause illness?

This evaluation was based on considerations raised by FSIS in 1994 with regard to *E. coli* O157:H7. We provide evidence suggesting that, for at least some serogroups, a small number of pathogenic non-O157 STEC bacteria can cause illness. This evidence is based on outbreak data that does not provide detailed dose-response information, nor is it specific to outbreaks associated with beef.

(5) Can pathogenic non-O157 STEC cause severe illness including permanent life-threatening damage to major organ systems?

We provide evidence that some pathogenic non-O157 STEC strains can cause permanent, life threatening damage to major organ systems. However, most studies indicate that infection with non-O157 STEC is less likely to result in severe outcomes such as bloody diarrhea and HUS, and that the mortality rate is lower than for *E. coli* O157:H7 infection.

(6) Can pathogenic non-O157 STEC spread from person to person causing illness in settings such as day care facilities?

We provide evidence that it may be possible for some pathogenic non-O157 STEC to be spread from person to person in settings with a high degree of personal contact, however, studies that specifically examine the role of secondary transmission of non-O157 serogroups of interest are not available.

The findings presented in this Risk Profile may be useful to respond to the October 2009 Citizen's Petition and develop an FSIS policy position. However, FSIS' independent peer reviewers raised a number of concerns about the strength of the evidence presented in this Risk Profile for drawing conclusions regarding the actual risk associated with non-O157 (USDA/FSIS, 2011). For instance, the commercially available diagnostic methods for the isolation of non-O157 STEC are currently quite crude, with around a 10% (or less) recovery rate from PCR-positive samples. The FSIS methodology under development will be considerably more sensitive. FSIS will continue to study this issue, and update the Risk Profile as information becomes available.

Background – *E. coli* O157:H7

In September 1994, USDA-FSIS stated that it considered raw ground beef contaminated with *E. coli* O157:H7 to be adulterated within the meaning of the Federal Meat Inspection Act (FMIA[2]), and that the Agency was prepared to use the enforcement provisions of the FMIA to exclude adulterated product from commerce. At the same time, USDA-FSIS indicated that it would begin to sample raw ground beef at federally regulated establishments and in commerce. Two months later, a group of supermarket and meat industry organizations filed suit in the U.S. District Court for the Western District of Texas to stop the Agency's sampling program. The group argued, among other points, that the FSIS sampling program exceeded the Agency's statutory authority, not in the testing of ground beef, but in considering *E. coli* O157:H7 to be an adulterant under the FMIA. The group argued that *E. coli* O157:H7 contaminated ground beef, like raw product contaminated with other pathogens, such as *Salmonella*, is injurious to health only if improperly cooked. The Court determined "that many Americans consider ground beef to be properly cooked rare, medium rare, or medium" [3]. The Court held that *E. coli* O157:H7 is unlike other pathogens because "thorough" cooking and not "proper" cooking is necessary to protect consumers from the pathogen. Thus, ground beef contaminated with *E. coli* O157:H7 is adulterated under the FMIA, and FSIS' sampling program did not exceed the Agency's regulatory authority[3].

During the 1994 proceedings, Mr. Michael R. Taylor, Administrator of FSIS, indicated why ground beef contaminated with *E. coli* O157:H7 should be considered an unusual and urgent food safety problem:

(1) Traditional and accepted cooking practices for raw ground beef (e.g., medium rare or slightly pink hamburger) do not kill *E. coli* O157:H7

(2) *E. coli* O157:H7 requires a small number of bacteria to cause illness

(3) *E. coli* O157:H7 can cause permanent, life-threatening damage to major organ systems especially among children and the elderly

(4) Because of its low infectious dose, *E. coli* O157:H7 can be spread from person to person causing illness, as has been reported in child day care settings[4]

In a 1999 policy issuance, FSIS stated, "…[g]iven the low infectious dose of *E. coli* O157:H7 associated with foodborne disease outbreaks and the very severe consequences of an *E. coli* O157:H7 infection, the Agency believes that the status under the FMIA of beef products

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[2] 21 U.S.C. §601(m)(1))

[3] Texas Food Industry *et al.* v. Mike Espy *et al.*, 870 F. Supp. 143; Dec. 13, 1994

[4] Texas Food Industry Ass'n, *et al.* v. Espy, Civil No.94 CA748IN, Declaration of Michael R. Taylor, Nov. 15, 1994

contaminated with *E. coli* O157:H7 must depend on whether there is adequate assurance that subsequent handling of the product will result in food that is not contaminated when consumed." In the same policy issuance, FSIS indicated that it was concerned with *E. coli* O157:H7 infection associated with consumption of all non-intact beef products, including mechanically tenderized products, due to the potential translocation of contaminants to the interior of the product where it may be protected during cooking[5]. Therefore, non-intact beef and intact cuts of muscle that would be further processed into non-intact product would be considered under the FSIS policy.

The definition of *E. coli* O157:H7 as an "unusual and urgent food safety problem" by FSIS in 1994 was straightforward. This was a single serotype of *E. coli* that had been responsible for illnesses and outbreaks of bloody diarrhea and HUS since 1982. *E. coli* O157:H7 had also been described as "clonal," meaning that the serotype was comprised of closely related genotypes (Whittam *et al.*, 1988). In addition, a large multistate outbreak of *E. coli* O157:H7 illness was linked to the consumption of hamburgers prepared by a restaurant chain in late 1992 and early 1993. In 2001, FSIS completed a quantitative microbial risk assessment for *E. coli* O157:H7 in ground beef (USDA/FSIS, 2001).

In the United States, per 9 CFR 417, all meat and poultry establishments are required to develop and implement a system of preventive controls designed to improve the safety of their products, known as Hazard Analysis Critical Control Points (HACCP). FSIS, through various sampling programs, verifies the effectiveness of establishment's control systems at preventing hazards from entering commerce. The application of HACCP to meat products has been used to control contamination with *E. coli* O157:H7. FSIS required raw beef establishments to reassess their HACCP plans for raw beef products in light of certain scientific data on *E. coli* O157:H7 in 2002[6]. Establishments that determined that the hazard is reasonably likely to occur in the production process were also required to implement critical control points in their HACCP plan. As a result of the reassessment, establishments implemented measures to control contamination of meat products with *E. coli* O157:H7 at pre-harvest, during slaughter, as well as during processing. Such measures include the application of interventions to reduce contamination with *E. coli* O157:H7 as well as establishment sampling. In addition, sampling of a number of raw beef products such as ground beef and components of those products has been utilized by FSIS to verify that establishments' HACCP systems are functioning as intended.

Scope of Document

FSIS undertook the preparation of this risk profile to help clarify the extent of the scientific

[5] 64 Fed. Reg. 2,803 (Jan. 19, 1999)

[6] 67 FR 62326

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The following sections attempt to answer these questions using the scientific and epidemiological literature as well as other data available to FSIS.

(1) What are pathogenic non-O157 STEC and how can they be distinguished from other STEC?

There are 300-400 known STEC serotypes, but not all of them have been associated with human illness. STEC can be found in soil, water, and food vehicles, and have been isolated from the gastrointestinal tract of healthy humans. The ability to produce Shiga toxin does not by itself render *E. coli* pathogenic; the presence and expression of additional virulence factor genes are required to cause human illness (Wickham *et al.*, 2006). STEC virulence factors are associated with bacteriophages, plasmids, pathogenicity islands, and O-islands. Researchers have demonstrated that several combinations of virulence factor genes are associated with severe human illness. The genetic characteristics of STEC that are currently most commonly associated with illnesses (including hemorrhagic colitis, HUS, and TTP) can be defined using strains isolated through illness surveillance systems.

There are two major Shiga toxin gene families – *stx* 1 and *stx* 2 – and multiple Shiga toxin genotypes within each family. The *stx* genotype is unrelated to the *in vitro* cytotoxicity or quantity of Shiga toxin produced, but does appear to be associated with the severity of clinical illness (Orth *et al.*, 2007). *Stx*2 and *Stx*2c subtypes are statistically associated with severe symptoms, including bloody diarrhea and HUS (Boerlin *et al.*, 1999, Friedrich *et al.*, 2002, Ethelberg *et al.*, 2004, Zhang *et al.*, 2007, Persson *et al.*, 2007). An enzyme-activated subtype of *Stx*2d has been associated with HUS in STEC strains lacking *eae* (Bielaszewska *et al.*, 2006). Other Shiga toxin variants, such as *stx2d* and *stx2e*, are not as frequently isolated from humans with severe illness (Persson *et al.*, 2007). In a survey of STEC isolates from illnesses in Minnesota from 2000 to 2006, non-O157 STEC were more likely than *E. coli* O157:H7 to have *stx*1 alone, but this finding alone did not explain the apparent differences in the severity of the illnesses (Hedican *et al.*, 2009). *Stx* are located within the DNA of a bacteriophage, which is itself located within the DNA genome of the host bacterium (*E. coli*). *Stx* can be excised from an *E. coli* strain by an unknown mechanism, and could be gained by an *stx*-negative *E. coli* strain if infected by an *stx*-carrying bacteriophage. Excision of *stx*-carrying bacteriophage has been described among STEC isolated during natural infection and during passage in culture (Karch *et al.*, 1995, Feng *et al.*, 2001, Bielaszewska *et al.*, 2007, and Mellman *et al.*, 2008). Bielaszewska demonstrated that loss of *stx*2 in STEC serogroup O26 was associated with the complete excision of the *stx*2-carrying bacteriophage. This occurred in 10-14% of colonies tested (Bielaszewska *et al.*, 2007).

A plasmid, called pO157, which has been detected in 99 to 100% of clinical *E. coli* O157:H7 isolates from humans, carries genes encoding a hemolysin operon (*hly*), a type II secretion system (*etp*C to *etp*O), an extracellular protease (*esp*P), and homologs of large clostridial toxin genes (*tox*A and *tox*B) (Johnson and Nolan, 2009, Burland *et al.*, 1998). Other plasmids containing different combinations and orientations of these and other genes have been detected in non-O157 STEC isolates as well (Brunder *et al.*, 1999). Like bacteriophages, plasmids may be gained or lost from *E.*

coli strains. Other, non plasmid associated virulence factors found in some STEC include a subtilase cytotoxin (*sub*), hemolysin (*hly*), and cytolethal distending toxin (*cdt*) (Paton and Paton, 1998; Beutin *et al.*, 1989; Bielaszewska *et al.* 2004; Paton *et al.* 2004; Khaitan *et al.*, 2007, Wolfson *et al.* 2009).

Pathogenicity islands (PAI) encode groups of virulence genes. The LEE (Locus of Enterocyte Effacement) PAI encodes genes allowing STEC to attach and adhere to enterocytes lining the intestinal tract. This step, called attachment and effacement (A/E), allows STEC to remain in the intestine while enabling Shiga toxin to enter systemic circulation. The LEE PAI encodes virulence genes including adhesin, intimin (*eae*), the intimin receptor (*tir*), and a secretion system (*esp*). As with *stx*, DNA sequence variation has been observed in *eae*. *Eae* subtypes beta, gamma, and theta have been detected in clinical STEC isolates.

Although many pathogenic non-O157 STEC contain the LEE, some do not. The A/E function typically provided by LEE may be replaced or supplemented by fimbrial and non-fimbrial adhesion proteins (*fimA, iha*, *efa1*, *lpfA-O113*) (Galli *et al.*, 2009) or possibly by defects in the lysine decarboxylase pathway (Calderon *et al.,* 2010). The adhesin coded by the *saa* gene has been found in outbreaks of pathogenic non-O157 STEC illness (Paton *et al.*, 2001). O-islands (OI) were first described as DNA segments unique to an *E. coli* O157:H7 strain compared with a non-STEC strain (Perna *et al.*, 2001), and have since been identified in pathogenic non-O157 STEC strains. Many OI have been described. In particular, OI 36, 57, 71, and 122 are more commonly found in STEC strains associated with HUS and outbreaks compared to STEC strains not associated with severe and epidemic disease (Karmali *et al.*, 2003; Wickham *et al.*, 2006, Coombes *et al.*, 2008, Bugarel *et al.*, 2010, Imamovic *et al.*, 2010).

Combinations of virulence genes associated with very severe symptoms (including bloody diarrhea and HUS) are most often found in some non-O157 serogroups: O26, O45, O103, O111, O121, and O145 (Eklund *et al.*, 2002; Brooks *et al.*, 2005; Thompson *et al.*, 2005; Hedican *et al.*, 2009). CDC data indicates that 75-80% of reported and serogrouped non-O157 STEC isolates are from these serogroups (Gould 2009; CDC, National Case Surveillance Data). Table 1 lists serogroups of human non-O157 STEC isolates reported to the CDC between 2003 and 2009. This data shows that among 3,928 STEC isolates whose serogroups were determined, the top six serogroups account for 78% of the isolates. Table 2 provides virulence factors and selected U.S. and international outbreaks associated with the top six STEC serogroups.

In 2003, Karmali and colleagues proposed a seropathotype classification for STEC serotypes based on their reported frequencies (in qualitative terms such as “high,” moderate,” or “rare”) in human illness, and their known associations with outbreaks and severe outcomes including HUS and hemorrhagic colitis (Karmali *et al.*, 2003). There are five seropathotype classifications, A through E. Seropathotype A is associated with the highest incidence in human disease, is commonly involved with outbreaks, and is associated with severe disease. This classification includes STEC serotypes

O157:H7 and O157:NM. Seropathotype B has “moderate” incidence in human disease, is uncommonly involved with outbreaks, and is associated with severe disease. This classification currently includes 13 STEC serotypes O26:H11 and NM; O45:H2 and NM; O103:H2, H11, H25, and NM; O111:H8 and NM; O121:H19 and H7; and O145:NM (Bosilevac and Koohmaraie, 2011). Seropathotypes C and D have “low incidence” in human illness, and are rarely associated with outbreaks. Seropathotype C includes STEC serotypes O91:H21, O104:H21 and O113:H21. Finally, seropathotype E is not associated with human illness, outbreaks or severe illness (Karmali *et al.*, 2003).

There is evidence that strains containing *stx2* and *eae* are associated with severe clinical illness caused by both *E. coli* O157:H7 and non-O157 STEC (Boerlin *et al.*, 1999; Friedrich *et al.*, 2002; Beutin *et al.*, 2004; Ethelberg *et al.*, 2004). Intimin (*eae*) and enterohemolysin (*hly*) have been found in over 90% of STEC illnesses, including HUS, in North America (Jelacic *et al.*, 2003; Brooks *et al.,* 2005). Further, there is evidence of a synergistic effect of *stx*2 and *eae* which together cause severe illness (Boerlin *et al.*, 1999). Over 90% of bloody diarrhea illnesses caused by the top six serogroups of non-O157 STEC referred to the CDC between 1983 and 2002 contained *eae* and/or *hly* (Brooks *et al.*, 2005). Among 21 laboratory-confirmed cases of HUS referred to CDC during this time, 16 of 20 isolates analyzed contain *eae,* and 16 of 20 isolates analyzed contained *hly* (Brooks *et al.*, 2005). Thus, while the precise genetic composition of a “pathogenic STEC” cannot be defined, some genetic characteristics of STEC that are currently most commonly associated with illnesses (including hemorrhagic colitis and HUS) can be identified using strains isolated through illness surveillance systems (provided that the strains are referred to state public health laboratories or referred to CDC for characterization). Table 3 presents selected outbreaks and clusters for which virulence factors were determined and correlates these factors with serogroup and disease severity.

The key issue to addressing the problem of non-O157 STEC in foods is determining how to define those STEC serotypes that pose true health risks. As yet, we cannot definitively determine how "pathogenic" STECs can be distinguished from STECs that do not pose health risks. Pathogenic STECs emerged in the late 1970s or early 1980s as major foodborne pathogens. This emergence was likely the result of several consumer and ecological changes during this time, including an increase in consumption of raw vegetables and undercooked meat, improved adaptation of the bacteria to human hosts, and greater ability of *E. coli* to acquire *stx* genes from free bacteriophages in the environment and in mammalian hosts (Beutin 2006; Zhang *et al.*, 2000). An example of pathogenic STEC emergence is illustrated by a study by Zhang *et al.* (2000). These researchers showed that a subgroup of O26 with a unique virulence profile was detected more frequently in Germany and Czechoslovakia after 1995. Among the 55 strains of O26 studied, *stx2* was never seen prior to 1994; after 1997, *stx2* was present in 97% of strains (Zhang *et al.*, 2000). In this study, these stx2 strains were strongly associated with HUS. Although the reason for this shift is not known, the investigators hypothesized that this change in genotype may have occurred due

to improved adaptation to human hosts, acquisition of genes from bacteriophages, or changes in food consumption.

(2) Are pathogenic non-O157 STEC present in cattle, and beef, including ground beef?

Ruminants, including cattle, are a reservoir of *E. coli* O157:H7 to humans and may be a reservoir for some non-O157 STEC serotypes as well (Bettelheim 2000; Caprioli *et al.*, 2005). *E. coli* O157:H7 and non-O157 STEC alike may reside asymptomatically in the intestines of cattle and may be shed intermittently in feces (Menrath *et al.*, 2010; Pearce *et al.*, 2004). Meat can become contaminated during removal of the hide or the gastrointestinal tract at slaughter, and organisms can be mixed into beef when it is ground (Elder *et al.*, 2000; McEvoy *et al.*, 2003).

Beef and dairy cattle and carcasses

There is no nationwide microbiological baseline data on the prevalence of non-O157 STEC in general or specific serogroups on cattle and cattle carcasses. From a hazard analysis standpoint, however, it is useful to note that a review of the limited studies available over a 25-year period on STEC shedding by beef cattle summarized that 17% of non-O157 STEC serotypes collected from cattle were also isolated from patients with HUS, while 16% of the STEC serotypes were isolated from patients with other illnesses (Hussein 2007). Some of the non-O157 STEC serogroups that are most commonly identified in STEC human illnesses (e.g., O26, O103, and O111) have been isolated from bovine feces (Bettelheim 2007). A USDA Agricultural Research Service (USDA-ARS) study reported that the average prevalence of non-O157 STEC (defined as isolates positive for *stx*) in three Midwestern U.S. feedlots was 19.3% in feces; 57.0% on hides; and 58.2% on pre-evisceration (post-dehiding) beef carcasses (Barkocy-Gallagher *et al.*, 2003). Similar values for pre-evisceration carcasses and post-intervention carcasses were noted in another USDA-ARS study (Arthur *et al.*, 2002). Dairy cows also may be reservoirs of non-O157 STEC (Doyle, 1991; Hussein and Sakuma, 2005), which is relevant because culled dairy cows account for 17% of the ground beef produced in the U.S. (Troutt and Osburn, 1997). A review of studies on STEC shedding by dairy cattle over a 25-year period summarized that 12.4% of STEC serotypes isolated were also isolated from patients with HUS (Hussein and Sakuma, 2005). Studies of U.S. dairy cattle have reported non-O157 STEC fecal prevalence from 0% to 22% (Wells *et al.*, 1991; Cray *et al.*, 1996; Thran *et al.*, 2001; Samadpour *et al.* 2002).

Beef including ground beef

There is no nationwide microbiological baseline data on the prevalence of non-O157 STEC in general or specific serogroups in beef products being prepared for human consumption. From a hazard analysis standpoint, however, it is useful to note that several studies have found non-O157 STEC serogroups in boneless beef trim used for the production of ground beef and in raw ground

beef in the U.S. Judging by the isolation of STEC with serogroups or virulence factors associated with illness, it may be that the prevalence of pathogenic STEC is fairly low. In a review of the limited scientific literature, Hussein found non-O157 STEC in 2.4 to 30.0% of the ground beef samples examined; 48.7% of serotypes isolated from beef products in that study have been associated with HUS or other human illness (Hussein 2007). In a USDA-ARS study of beef trim imported from Australia, New Zealand, and Uruguay, and from the U.S., 99 STECs were isolated among the 1,186 trim samples. A total of 38 non-O157 STEC with serotypes associated with illness were identified in this study, including 13 serotypes associated with HUS (Bosilevac, 2007)[7]. FSIS data indicate that over 1 billion pounds of boneless beef trim is imported to the U.S. on an annual basis, mostly for the production of ground beef.

A 2002 survey of selected sources of retail ground beef packages collected from King County, Washington area grocery chain stores reported 50 (16.9%) of 296 samples were positive for STEC by a colony hybridization method. No additional characterization of serotypes or virulence factors was reported in this study (Samadpour *et al.*, 2002). Another survey of ground beef from the same geographic area reported 61 (3.5%) of 1,750 samples were positive for *stx* and *eae* genes by PCR, which could include *E. coli* O157:H7 as well as non-O157 STEC strains (Samadpour *et al.*, 2006). Another group investigated the prevalence of non-O157 STEC in ground beef available at retail establishments in Washington State. Of 480 ground beef samples, 173 (36%) were positive for *stx*, and 36 (7.5%) were culture-confirmed as non-O157 STEC. Non-O157 STEC isolates from eight (1.7%) samples were positive for *eae* and/or *hly*, or belonged to a serogroup associated with human illness (Cobbold *et al.*, 2008)[8]. In another study conducted by USDA-ARS, *stx1* or *stx2* genes were detected in 1,006 (24.3%) of 4,133 ground beef samples collected by 18 commercial producers. One or more STEC were isolated from 300 (7.3%) of the samples, and STEC that may be considered "a significant food safety threat" (because they carried additional virulence factors) were isolated from 10 (0.2%) samples (Bosilevac and Koohmaraie, 2011). Samadpour (manuscript in preparation) recently examined non-O157 STEC in ground beef collected at selected retail establishments throughout the country. Once the results of the Samadpour study have been subjected to independent peer review, FSIS will incorporate them into this Risk Profile.

These studies indicate that pathogenic STEC can be present in commercially available ground beef and components used to produce ground beef in the U.S. food supply. The absence of data collected under a statistically rigorous sampling design, however, prevents us from drawing conclusions about the national prevalence of pathogenic non-O157 serotypes in either cattle or on

[7] STEC associated with illness and or HUS isolated from boneless beef trim: O168:H+, O113:H21, O113:H4, O15:H27 (3), O163:H19 (4), O165:Hneg, O174:H36 (2), O20:H19 (5), O26:H11, O79:H7, O8:H19 (5), ONT:H+ (2), ONT:H11 (2), ONT:H18, ONT:H19 (2), ONT:H46 (4), Orough:Hneg, ONT:H+

[8] Serogroups associated with human disease isolated from retail ground beef: O8, O18, O35, O46, O175

ground beef.

(3) Would traditional and accepted cooking practices for raw ground beef kill pathogenic non-O157 STEC?

Survival of non-O157 STEC during cooking by consumer

Little data exist to definitively assert whether non-O157 STEC are heat tolerant. However, it is known that *E. coli* O157:H7 can survive during typical or traditional cooking of raw, non-intact beef products and may result in illness (Laine *et al.*, 2005). A study by USDA-ARS (Luchansky *et al*., 2012) examined thermal resistance of STEC-inoculated non-intact beef steaks with strains of *E. coli* O157:H7 and non-O157 STEC (a pooled composite of STEC serogroups O45, O103, O111, O121, and O145). When the steaks were cooked to internal temperatures of 120 to 160 °F, similar reductions of *E. coli* O157:H7 and non-O157 STEC levels were observed for bacteria translocated into steaks cut 1 or 1.5 inches thick. The study demonstrated that cooking steaks from non-intact primals inoculated with high levels of *E. coli* O157:H7 and non-O157 STEC to internal temperatures of 120 to 160°F was insufficient to eliminate the contamination. While the relatively high inoculation levels (3.5 or 6 log microorganisms/g) are unlikely to be representative of typical contamination levels, reductions in non-O157 STEC and *E. coli* O157:H7 log microorganisms/g levels at most of the cooking temperatures and steak thicknesses was comparable. Thus, typical or traditional cooking (defined as an internal temperature of 120 to 160˚F) of non-intact steaks contaminated with non-O157 STEC may result in the consumption of contaminated product after typical or traditional cooking, as has been shown for *E. coli* O157:H7 (Laine *et al.*, 2005). In a study by Duffy *et al.* (2006), decimal reduction values at 55°C (131°F) were determined for strains of *E. coli* O157:H7 and *E. coli* O26 inoculated into minced (ground) beef. A decimal reduction value (D) refers to the time, in minutes, required to reduce the level of inoculated organism by 90%. The D_{55} values for cultures not subjected to a prior heat shock were 10.04 and 8.18 minutes respectively. Additional data on the decimal reduction times for pathogenic non-O157 STEC in ground beef, and the surviving fraction of live cells after typical or traditional cooking are necessary to fully appreciate the effect of consumer handling on the probability of illness.

Acid resistance as a survival characteristic

In general, it is hypothesized that some pathogenic STECs may be more resistant than other foodborne pathogens to deactivation in acidic environments encountered both during fermentation of meat (e.g. dry fermented sausages, pH 4.5-5) (Riordan *et al*., 1998) and during digestion (pH 1.5-2) (Tilden *et al*., 1996). This characteristic may help explain their low infectious dose and why some STEC survive processing techniques that had long been regarded as effective in producing safe food (e.g. fermentation of meat). Studies comparing acid resistance of STEC O157 with non-O157 STEC found no significant differences in acid resistance (Berry *et al*., 2004; Large *et al*., 2005). However we do not have data on specific serogroups associated with severe

disease. As would be expected, there is a range in the survival rates of different STEC serotypes and strains: McKellar and Knight (1999) found that outbreak-associated isolates were more acid tolerant than strains from human or animal sources, suggesting their acid tolerance may well have been a contributing factor to their pathogenicity.

(4) Can small numbers of pathogenic non-O157 STEC cause illness?

Data obtained from outbreak investigations have been used to estimate the minimum level of organisms (expressed in most probable number (MPN) or colony forming units (CFU)) necessary to cause illness. *E. coli* O157:H7 can cause illness at very low levels. Uncooked hamburger patties associated with a large *E. coli* O157:H7 outbreak in 1992-1993 contained 67 ± 5 organisms per patty (range, less than 13.5 to 675 organisms per patty) (Tuttle *et al.*, 1999). An investigation of another *E. coli* O157:H7 outbreak associated with presliced dry fermented salami found very low levels of *E. coli* O157:H7 in the product, and estimated that four case patients consumed between 2 and 45 organisms (Tilden *et al.*, 1996). Limited data are available on dose response for some non-O157 STEC serogroups. Boel *et al*. (manuscript in preparation) recently investigated an outbreak of STEC O26 from fermented beef sausage in Denmark to determine the infectious dose of these microorganisms. Results of this investigation will be included in the risk profile upon publication. From an outbreak of STEC O111 in mettwurst sausage (made from meat from multiple species including both beef and non-ruminant animals) in Australia, investigators extrapolated a dose range of 1 to 10 organisms, given the presence of as few as 1 microorganisms per 10 g of sausage (Paton *et al.*, 1996). Using the concentrations of STEC O145 in contaminated ice cream in an outbreak in Belgium, the estimated infective dose was 400 microorganisms (Buvens *et al.*, 2011).[9] These minimum dose estimates for STEC serogroups O111 and O145 appear to be comparable to minimum dose estimates for *E. coli* O157:H7 based on the more rigorous studies described above (Tilden *et al.*, 1996; Tuttle *et al.*, 1999). Variation in dose response results from complex host-agent interactions and likely depends on factors such as ability of bacteria to survive ingestion, bacterial virulence profiles, and host susceptibility and immunity.

(5) Can pathogenic non-O157 STEC cause severe illness including permanent life-threatening damage to major organ systems?

Evidence from population surveillance systems

All STECs (initially called EHECs) became nationally notifiable in 2000. Investigators in several U.S. states have shown that when complete testing of specimens isolated from ill patients is

[9] Accurate dietary history was not obtained, but the authors estimated an average consumption amount of 200 g.

performed, non-O157 STECs are isolated as frequently as or more frequently than *E. coli* O157:H7 (Fey *et al.*, 2000; Jelacic *et al.*, 2003; CDC, 2007; Lathrop *et al.*, 2009; Hedican *et al.*, 2009; Manning *et al.*, 2007). CDC analyzed FoodNet data from 2005-2008 and estimated that in the U.S. there may be nearly twice as many non-O157 STEC illnesses as *E. coli* O157 illnesses (Scallan *et al.*, 2011). Specifically, CDC estimates that there may be 168,698 (range 17,163 to 428,522) illnesses due to non-O157 STEC and 96,534 (range 26,982-227,891) illnesses due to *E. coli* O157:H7 (Scallan *et al.*, 2011). CDC estimates that 82% of non-O157 STEC illnesses, or 112,752 (range 11,467-287,321) illnesses, are domestically acquired from food; 68% of O157:H7 illnesses, or 63,153 (range 17,587-149,631) illnesses, are domestically acquired from food (Scallan *et al.*, 2011). Specific foodborne vehicles were not determined by CDC in this analysis. Data on U.S. non-O157 STEC illnesses and outbreaks (from all causes) are summarized in Tables 4 and 5. Seasonal trends in the incidence of human infection with both *E. coli* O157:H7 and non-O157 STEC are comparable, with the highest rates in the U.S. occurring in the summer months (Luna *et al.*, 2010; Brooks *et al.*, 2005; Slutsker *et al.*, 1997).

There are several different channels through which surveillance data are gathered. CDC's FoodNet is an active surveillance system and tracks enteric illness from several participating states and counties. FoodNet has been collecting information on non-O157 STECs since 2000. The National Notifiable Diseases Surveillance System (NNDSS) is a joint effort of CDC and the Council for State and Territorial Epidemiologists. CDC's Enteric Diseases Epidemiology and Laboratory Branches compile illness data and reports on enteric bacterial illnesses including non-O157 STEC. Its National *E. coli* Reference Laboratory provides serotyping and molecular characterization of virulence factors for non-O157 STECs submitted by state public health laboratories. Since 2003, CDC's Enteric Diseases Epidemiology Branch (EDEB) has published an annual surveillance report, incorporating laboratory data from the Enteric Diseases Laboratory Branch (EDLB), as well as NNDSS and Public Health Laboratory Information System (PHLIS) data (PHLIS is a state public health laboratory isolation-based reporting system). CDC collects reports of foodborne outbreaks due to enteric bacterial, viral, parasitic, and chemical agents. State, local, and territorial public health agencies report these outbreaks through the National Outbreak Reporting System (NORS). The NORS surveillance team conducts analyses of these data to improve understanding of the human health impact of foodborne outbreaks and the pathogens, foods, settings, and contributing factors involved in these outbreaks.

Analysis of FoodNet data reveals that the number of laboratory-confirmed reported cases of non-O157 STEC illness increased 284% (range 169% to 450% increase) from 2000 to 2009 (Henao, personal communication) (Table 6). These increases may be partially attributed to improved identification and reporting (CDC, 2008) and thus would not necessarily represent a true measure of changes in incidence of non-O157 STEC (Henao, personal communication). Data from 2000 through 2007 show that as more clinical laboratories tested for non-O157, more isolates were

found (Gould 2009). Most clinical laboratories do not routinely test for STEC all human fecal specimens submitted for culture (Hoefer *et al*. 2011); CDC estimates that for every case of non-O157 STEC illness diagnosed, there may be 106.8 illnesses that are not diagnosed (Scallan *et al.*, 2011). This potential level of underdiagnosis is over four times higher than *E. coli* O157:H7; for every case of *E. coli* O157:H7 diagnosed, there are 26.1 cases that are not (Scallan *et al.*, 2011). CDC strongly encourages laboratories to test for non-O157 STEC in its recently published updated recommendations for clinical laboratory diagnosis (CDC, 2009).

Although the true incidence of non-O157 STEC infection in the U.S. population remains unclear, researchers have attempted to quantify the presence of these organisms in symptomatic patients. Studies of STEC serotypes from human feces by Acheson (1998) and Fey *et al.* (2000) suggest that, overall, non-O157 STEC, in particular serogroups O26, O45, O103, O111, and O145, may be as common and clinically significant as *E. coli* O157:H7 in the U.S. These findings are consistent with U.S. surveillance data. For the first time, CDC FoodNet data showed in 2010 that non-O157 STEC reported illnesses and incidence surpassed those of *E. coli* O157:H7. In 2010, FoodNet reported 451 cases of non-O157 STEC; the estimated incidence rate was 1.00 per 100,000 population in the surveillance area for adults, and 5.00 non-O157 STEC infections per 100,000 population for children under 5 years of age (CDC, 2011). For comparison, 2010 FoodNet data indicate that the incidence rate for *E. coli* O157:H7 was 0.90 per 100,000, and 3.3 *E. coli* O157:H7 infections per 100,000 population for children under 5 years of age. From 2000-2010, FoodNet reported 1,842 cases of non-O157 STEC infection. In addition, FoodNet data from 2006-2010 indicates that over 200 non-O157 STEC confirmed illnesses were reported annually from the FoodNet catchment area (Table 5). As laboratory-confirmed illnesses from *E. coli* O157:H7 have generally remained the same since 2001, confirmed illnesses from non-O157 STEC have increased during this period (Figure 1). This increase is likely due to increasing use of Shiga toxin detection assay as more laboratories have increased their capability to detect these pathogens. A survey evaluating testing practices of clinical laboratories within FoodNet catchment sites found that in 2007, most labs followed testing recommendations for *E. coli* O157 but not for non-O157 STECs (Hoefer *et al*., 2011). Between 2003 and 2007, more laboratories reported testing fecal samples using a method that would detect non-O157 STECs, but this remained low, at 11% (Hoefer *et al*., 2011). Recommendations for diagnosis of STEC infections by clinical laboratories were recently updated (CDC, 2009), which should lead to improved detection of non-O157 STEC illnesses and outbreaks.

CDC's EDEB is responsible for surveillance of bacterial enteric pathogens. EDEB encourages state health laboratories to forward suspected non-O157 STEC isolates to CDC's National *Escherichia coli* Reference Laboratory, where confirmatory testing for Shiga toxin genes and serotyping are offered. Since 2003, EDEB has published an annual surveillance report, incorporating CDC's own laboratory data, and other available data. Surveillance reports from PHLIS and the CDC Reference Laboratory indicate a rising number of reported cases and/or isolates every year.

Evidence from outbreak investigations

Through compilation of non-O157 STEC outbreak data from Foodborne Disease Outbreak Surveillance System (FDOSS)[10], from published literature, and from additional queries of health departments, FSIS and CDC have identified 41 confirmed non-O157 STEC outbreaks from 1990-2010, resulting in over 1,500 illnesses (through November 4, 2010). It is important to note that not all of these outbreaks involved were caused by only non-O157 STEC; in seven of these outbreaks, other enteric pathogens were also detected. Among those caused by non-O157 STEC, the most common serogroups involved in these outbreaks have been O26, O111, and O121, but in total 12 serogroups have been isolated among these outbreaks (Table 4). The largest domestic non-O157 STEC outbreak occurred in Oklahoma in August 2008: 341 illnesses, 72 hospitalizations, 26 HUS cases, and 1 death were attributed to the outbreak. STEC serogroup O111 was determined to be responsible for the outbreak. The source of the outbreak was a single restaurant, but a food or environmental source was never determined (Oklahoma State Department of Health, 2009).

Evidence from outbreaks involving beef products

The majority of reported non-O157 outbreaks have been attributed to non-beef food sources. FSIS is aware of outbreak investigations in which beef products are considered to be suspect vehicles or definitively linked to infection. In 2007 an outbreak of STEC O111 occurred among 23 people attending a wedding reception in North Dakota. Guests consumed a variety of foods, including ground beef meatballs, chicken, beans, salads, bread, and dessert. STEC was not detected in the foods that were tested. The ground beef meatballs themselves were not available for testing, and no additional, specific information about the ground beef was available. There is no direct evidence that contaminated ground beef resulted in the outbreak. The North Dakota Department of Health, which performed its own investigation, considers ground beef a suspect vehicle in this outbreak (North Dakota Department of Public Health, 2007).

In 2006, FSIS investigated a case-patient with STEC O103 infection who had consumed an undercooked ground beef patty 1 day prior to illness onset. The state laboratory tested samples from the patient and from leftover uncooked ground beef patties, and determined them to be indistinguishable by pulsed-field gel electrophoresis (PFGE). In 2009, FSIS investigated a non-O157 STEC illness where the case-patient consumed ground beef and became ill. The state laboratory tested and confirmed that the case-patient's STEC PFGE pattern was indistinguishable from that of STEC isolated from the ground beef, but CDC was not able to determine the serotype. In both of these cases, non-O157 STEC illness was traced back to ground beef. FSIS was unable to take further action in either case because records were inadequate to trace contamination back to an establishment. Although no regulatory action could be taken, these two cases illustrate that pathogenic non-O157 STEC has been found in FSIS-regulated product.

[11] Available at www.cdc.gov/outbreaknet/surveillance_data.html

In August 2010, FSIS became aware of an outbreak of *E. coli* O26 involving three case-patients (in Maine and New York). All of the case-patients reported exposure to ground beef prior to illness onset. FSIS established that the suspect product originated from a Pennsylvania establishment. The suspect product was traced from two separate retail stores, one in New York and the other in Maine. Further investigation also established that product from this Pennsylvania establishment was available for sale at stores listed by the third case patient. New York State Department of Health collected leftover ground beef from the case-patient's home for testing. Product tested positive for Shiga toxin 1 and STEC O26 with a PFGE pattern indistinguishable from the outbreak strain (isolates were uploaded to PulseNet on September 2, 2010). On August 28, 2010, the Pennsylvania establishment recalled ~8,500 pounds of ground beef that may be contaminated with *E. coli* O26, marking the first definitive non-O157 STEC outbreak associated with beef in the U.S., and the first time that FSIS requested a recall of non-O157 STEC contaminated beef in the U.S.

Worldwide, eight confirmed outbreaks of non-O157 STEC illness due to consumption of ruminant meat have occurred in Australia, Denmark, France, Germany, Italy, Norway, and the U.S. (Caprioli, *et al.*, 1994; CDC, 1995; Henning *et al.*, 1998; Werber *et al.*, 2002; Espié *et al.*, 2006; Schimmer *et al.*, 2008; Ethelberg *et al.*, 2009; King *et al.*, 2010). Six of these outbreaks involved beef products. These eight outbreaks resulted in 228 confirmed cases, including 45 cases of HUS and 3 deaths (Table 7). In five of these outbreaks, the pathogen caused HUS as well as other severe illness. In all of these outbreaks, the Shiga toxin gene or product was detected. Intimin (*eae*) was detected in all of these outbreaks (except one) when researchers screened for it.

Non-O157 STEC illnesses in other countries

In some countries, including Australia and some European nations, non-O157 STEC infections are at least as common as, or more common than *E. coli* O157:H7 infections (Blanco *et al*., 2004; Elliott *et al*., 2001; Nielsen *et al.*, 2006, Vally *et al*., 2012). Analysis of risk factors for sporadic STEC infection among Argentinean children identified eating beef outside the home and eating undercooked beef among risk factors for developing STEC illness (Rivas *et al.*, 2008). Risk factors specific to developing illness from non-O157 STEC were drinking from a bottle left at room temperature, drinking formula (a factor not identified in the full group), eating a piece of beef outside the home, contact with a child <5 years of age with diarrhea, wearing diapers, living in overcrowded conditions, and teething on undercooked beef. Although teething on undercooked beef is assumed to be an uncommon practice in the U.S., it indicates that exposure to very young children of undercooked beef can present risk of non-O157 STEC infection.

Worldwide, 59 recorded outbreaks involving non-O157 STECs have occurred from 1984-2009 (Kaspar *et al.*, 2009). A very large recent outbreak in Germany involves an unusual serotype, STEC O104:H4. Investigations have suggested that raw sprouts consumption and secondary transmission are likely responsible for this outbreak.

Non-O157 STEC illness severity relative to *E. coli* O157:H7

Surveillance data shows that illnesses caused by non-O157 STEC serogroups in the U.S. tend to be less severe than those caused by *E. coli* O157:H7 (CDC, 2007; Gould, 2009). CDC estimates that 46.2% of *E. coli* O157:H7 patients develop illness severe enough to require hospitalization, compared with 12.8% of all lab confirmed non-O157 STEC patients (Scallan *et al.*, 2011). CDC estimates that the death rate of non-O157 STEC infections is 0.3%, while for *E. coli* O157:H7 it is 0.5% (Scallan *et al.*, 2011). FoodNet data from 2000-2008 shows that 6.3% of *E. coli* O157:H7 patients developed HUS, while 1.7% of non-O157 STEC patients did (Gould, 2009). In developed countries, post-diarrheal HUS is most commonly caused by infection with Shiga-toxin producing *E. coli* (Bantavala *et al.*, 2001; Tarr *et al.*, 1990). Among HUS cases in the U.S. caused by STEC, it is estimated that 60-80% are caused by *E. coli* O157:H7 (Bantavala *et al.*, 2001; Tarr *et al.*, 1990; Siegler *et al.*, 1994).

Although in general, *E. coli* O157:H7 causes severe illness more frequently than non-O157 STEC, pathogenic non O157 STEC have been shown to cause the same range of symptoms as *E. coli* O157:H7, ranging from mild non-bloody diarrhea to more significant health outcomes, including HUS and death, especially in young, elderly or immunocompromised individuals (Brooks *et al*., 2005; Johnson *et al*., 2006). On a regional level, differences have been observed that indicate non-O157 STECs can cause severe illness as frequently as or more frequently than *E. coli* O157:H7. For example, data from two surveillance sites in Minnesota showed that O157 correlated with more severe illness than non-O157 STEC. *E. coli* O157 cases were more likely than non-O157 cases to involve bloody diarrhea (78% vs. 54%), hospitalization (34% vs. 8%), and HUS (7% vs. 0%) (Hedican *et al.*, 2009). Among patients with non-O157 STEC infections, serogroups O26, O45, O103, O111, O121, and O145 were the most common cause of hospitalizations (CDC, 2010b).

Studies performed in other countries indicate that *E. coli* O157:H7 infection is the most common cause of severe illness. However, non-O157 STEC infection has also been reported as an important cause of illness, sometimes with severe outcomes such as bloody diarrhea and HUS. For example, in a retrospective study performed in Hungary, 63% of patients infected with *E. coli* O157:H7 presented with bloody diarrhea or hemorrhagic colitis, compared to 46% of those infected with non-O157. In this study, two patients with STEC O26:H11 infection developed HUS (Mag *et al.*, 2010). In a study in Germany, 66.4% of HUS cases were caused by *E. coli* O157:H7 or *E. coli* O157:H-; STEC O26 was second, causing 13% of HUS illnesses (Friedrich *et al.*, 2002). Among less severe cases (diarrhea without HUS), *E. coli* O157:H7 was isolated from 35.5% of cases while non-O157 STECs were isolated from 64.5% of illnesses. Similarly, a prospective study in Austria and Germany found that among HUS patients, 15.4% were infected with STEC O26:H11, making it the second most frequently detected serotype behind O157, which was detected in 53.3% of cases (Zimmerhackl *et al.*, 2010). In a prospective case-control study in Argentina, *E. coli* O157:H7 was isolated in 60% of cases of HUS, with non-O157 STEC isolated in the remainder (Rivas *et al.*, 2008).

In other nations, studies have shown non-O157 STECs to cause severe illness such as HUS as or more frequently than O157. For example, another German study showed that non-O157 STEC infections developed into bloody diarrhea and HUS at similar rates as O157: 39.2% of bloody diarrhea and 47.6% of HUS cases were caused by non-O157 STECs (Beutin *et al.*, 2004). In Denmark, a study showed that 47.6% of patients with HUS and 55.2% of patients with bloody diarrhea were infected with non-O157 STEC (Ethelberg *et al.*, 2004). In a prospective national study in Switzerland, STEC was isolated in 60% of all HUS cases; of these, 25% were *E. coli* O157:H7 with 75% being non-O157 STEC (Schiffferli *et al.* 2010). Older surveillance data from Australia showed that STEC O111 caused the majority of HUS; *E. coli* O157:H7 was not isolated from any HUS cases (Elliott *et al.*, 2001). A more recent Australian study cited O157, O111, O26, O113, O55 and O86 as those STEC most commonly detected in the OzFoodNet database between 2000 and 2009, with O157 by far the most commonly reported STEC (Vally *et al*., 2012). Studies have shown that HUS was correlated with specific virulence factors that were variably present in both O157 and non-O157 STECs (Beutin *et al.*, 2004; Ethelberg *et al.*, 2004; Gerber *et al.*, 2002).

In the U.S., certain non-O157 STECs have caused severe illness at the same rates and with the same severity as *E. coli* O157:H7. For example, an outbreak of STEC O111:H8 in Texas in 1999 was determined to be "clinically indistinguishable from outbreaks due to *E. coli* O157:H7," in that both pathogens had a similar incubation period and symptom profile (Brooks *et al.*, 2004). Especially significant was that the same proportion of patients with non-O157 STEC infections in this outbreak developed HUS as those ill from *E. coli* O157:H7 in outbreaks reported in 1998 and 1999 (Brooks *et al.*, 2004). This also occurred in another outbreak of a non-O157 STEC (also STEC O111), in Oklahoma in 2008. In addition to patients developing HUS at the same rate as *E. coli* O157:H7, "compared with O157-related HUS, HUS caused by STEC O111 [in this outbreak] had a similar proportion of patients with bloody stools and a similar or higher rate of acute complication" (Piercefield *et al.*, 2010).

(6) Can pathogenic non-O157 STEC spread from person to person causing illness in settings such as day care facilities?

STEC may spread from person to person (referred to as secondary transmission) during outbreaks, especially in nursing homes and day care settings, where there is close contact between persons with immature or compromised immune systems and/or underdeveloped personal hygiene skills (Paton and Paton, 1998, Rangel *et al.*, 2005, Kaspar *et al.*, 2009, Snedeker *et al.*, 2009). Both symptomatic and asymptomatic individuals present a risk for transmission of infection due to fecal shedding and subsequent contamination of food or fomites. An analysis of 90 *E. coli* O157:H7 outbreaks occurring in Great Britain, Ireland, Scandinavia, Canada, the United States and Japan found that an average of 19% of the outbreak cases resulted from secondary transmission. In outbreaks occurring in nurseries, or where the median age of cases was below 6 years, the

percentage of secondary transmission has been observed to be even higher (Snedeker *et al.*, 2009). Shedding of STEC in feces is an important determinant of secondary transmission. STEC has been detected in feces shed by symptomatic and asymptomatic patients, even for extended periods (Swerdlow *et al.*, 1997). Prolonged and intermittent shedding of serotypes O26:H11 (31 and 37 days observed over two events), and O145:NM (19 days) was observed among children in day care centers in Argentina (Miliwebsky *et al.*, 2007). The Japanese Ministry of Health, Labour and Welfare (MHLW) conducts extensive investigation of all foodborne outbreaks of EHEC illness, including testing of the surrounding population. Higher rates of asymptomatic infection (up to or exceeding 50%) occur in adults 30-59 years of age than in younger and older age groups (Infectious Disease Surveillance Center, 2010). In Argentina, the Ministry of Health considers each HUS case to be part of an outbreak and collects fecal samples from household and institutional (e.g. daycare, school) contacts of each case patient, including both symptomatic and asymptomatic contacts (Rivas, personal communication). There have been many reports of *E. coli* O157:H7 outbreaks in the U.S. that reportedly involved secondary transmission (Reida *et al.*, 1994, Rangel *et al.*, 2005, Snedeker *et al.*, 2009). Among 14 EHEC outbreaks with 10 or more case-patients investigated by the Japanese MHLW, the settings for nine were in nursery schools, and secondary transmission was suspected in seven of these outbreaks (Infectious Disease Surveillance Center, 2010). O121:H19, O26:HNT (2 outbreaks), O26:H11, O157:H7 (2 outbreaks), and O145:H- were involved (Infectious Disease Surveillance Center, 2010). Japan has also reported two outbreaks of STEC O103 in a nursery and in a welfare facility for the aged (Muraoka *et al*., 2007; Infectious Disease Surveillance Center, 2008). Several U.S. outbreaks of non-O157 STEC illness have reportedly involved secondary transmission (Table 4). These include 9 outbreaks associated with day care centers involving STEC serogroups O26 (3 outbreaks), O111 (4 outbreaks[11]), O121 (1 outbreak), and O145 (1 outbreaks). An STEC O111 outbreak in Texas associated with a cheerleading camp found that the illness, once introduced by food and water at the first meal of the camp, may have been spread through consumption of ice from open barrels contaminated by direct contact with campers (Brooks *et al.*, 2004). An STEC O45 outbreak in a New York correctional facility was attributed to an ill food worker (CDC, 2006). Observations from other countries, especially Japan, where EHEC outbreaks are extensively investigated, support that non-O157 STEC, like *E. coli* O157:H7, are capable of asymptomatic infection as well as secondary transmission.

[11] In one of these outbreak, there was co-infection with *Cryptosporidium*

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Appendix: Tables and Figures

Table 1: Non-O157 STEC isolates characterized at the National *Escherichia coli* Reference Laboratory, by serogroups

Serogroup	Number of isolates reported, 2003-2009	Percentage of total isolates serogrouped
26	918	23.2%
103	806	20.4%
111	643	16.3%
45	290	7.3%
121	248	6.3%
145	179	4.5%
69	71	1.8%
118	71	1.8%
91	60	1.5%
76	52	1.3%
165	45	1.1%
228	28	0.7%
174	27	0.7%
123	23	0.6%
177	22	0.6%
153	21	0.5%
28	20	0.5%
84	19	0.5%
128	19	0.5%
146	18	0.5%
113	17	0.4%
119	15	0.4%
8	14	0.4%
55	14	0.4%
172	12	0.3%
130	10	0.3%
156	10	0.3%
178	10	0.3%
63	9	0.2%
7	8	0.2%
2	7	0.2%
14	7	0.2%
22	7	0.2%
88	7	0.2%
126	7	0.2%
9	6	0.2%
110	6	0.2%
112	6	0.2%

117	6	0.2%
175	6	0.2%
179	6	0.2%
6	5	0.1%
43	5	0.1%
71	5	0.1%
141	5	0.1%
181	5	0.1%
1	4	0.1%
33	4	0.1%
50	4	0.1%
80	4	0.1%
98	4	0.1%
116	4	0.1%
132	4	0.1%
166	4	0.1%
51	3	0.1%
60	3	0.1%
73	3	0.1%
79	3	0.1%
82	3	0.1%
86	3	0.1%
109	3	0.1%
125	3	0.1%
162	3	0.1%
163	3	0.1%
168	3	0.1%
5	2	0.1%
11	2	0.1%
18	2	0.1%
20	2	0.1%
21	2	0.1%
25	2	0.1%
38	2	0.1%
42	2	0.1%
49	2	0.1%
74	2	0.1%
75	2	0.1%
77	2	0.1%
85	2	0.1%
100	2	0.1%
104	2	0.1%
124	2	0.1%
136	2	0.1%
137	2	0.1%
143	2	0.1%
149	2	0.1%
158	2	0.1%
160	2	0.1%
3	1	0.0%
4	1	0.0%
12	1	0.0%
19	1	0.0%
24	1	0.0%
27	1	0.0%
52	1	0.0%

53	1	0.0%
61	1	0.0%
70	1	0.0%
87	1	0.0%
96	1	0.0%
101	1	0.0%
105	1	0.0%
115	1	0.0%
131	1	0.0%
134	1	0.0%
135	1	0.0%
140	1	0.0%
150	1	0.0%
151	1	0.0%
152	1	0.0%
154	1	0.0%
180	1	0.0%

Table adapted and data from CDC Bacterial Foodborne and Diarrheal Disease National Case Surveillance Annual Reports, 2003-2006

* Excludes any isolates for which serogroup could not be determined (including isolates in unknown, undetermined, and rough categories)

Table 2: Comparison of characteristics of serogroups of pathogenic STEC

Serogroup	H-types; common virulence factors	Severe outcomes reported	Major outbreaks (vehicles) and nations where commonly isolated from patients	Infective dose	Thermal resistance
O157	H7; H-; *stx1, stx2, eae, hly, katP, espP, etpD, efa1, genes associated with LEE and O-island 122 (efa1-lifA)*[28-34]	HUS, Hemorrhagic colitis, acute renal failure,[34-36]	US (lettuce; spinach; ground beef; cheese)[37-40]; also commonly isolated in Japan, U.K., and other European nations[41-42]	10 cells[43]; 31-35 cells[44]; <700 cells[45]	D-value: 11.13 to 139.2 minutes[46]
O26	H11 is most common, H-; *stx1, stx2, eae, hly, irp2, espP, katP, etpD, efa1,hly*[1,2,3], genes associated with O-islands 71 and 122[4]	HUS[2,5,6,7], Hemorrhagic Colitis[5,6,8]	Denmark (beef sausage); Austria (raw milk); many outbreaks have occurred in daycare (person-to-person transmission). Many outbreaks have no vehicle found. Commonly isolated in U.S., New Zealand, Germany, Japan, Australia, Czech Republic, Scotland, Brazil, Canada, Belgium, U.K., Spain, Denmark, Finland, Argentina, Chile, and Italy [2,7,8,9]	Data to be published[10]	Similar thermal tolerance at 55 C in minced beef compared with *E. coli* O157:H7[11]
O45	H2, non-motile; *stx1, stx2*, eae, hly*, genes associated with O-islands 71 and 122[4,6]	Hemorrhagic Colitis[6,12]	New York (ill food worker); Commonly isolated in U.S.[9]	No data available	Similar inactivation of pooled isolates (O45, O103, O111, O121, O145) in inoculated non-intact properly cooked steaks compared with *E. coli* O157:H7[13]

O103	H2, H-, H25; *stx1*, *stx2**, *eae, hly*. A common O103:H2 virulence profile is [*stx1, eae, hly*, and genes associated with O-islands 71 and 122][4,16]	HUS[14,15], Hemorrhagic Colitis[6,14]	Norway (mutton sausage; stx2 only); Washington state (water-based punch); Commonly isolated in U.S., Canada, Germany, Italy, France, Denmark, Finland, Argentina[9]	No data available	Similar inactivation of pooled isolates (O45, O103, O111, O121, O145) in inoculated non-intact properly cooked steaks compared with *E. coli* O157:H7[13]
O111	H8, H10, H-; *stx1, stx2, eae*, E-*hly* [6,17] A common O111:H8 virulence profile is [*stx1/stx2, eae, hly*, and genes associated with O-islands 71 and 122[4]	HUS[2,6,18-20], Hemorrhagic Colitis[2,6,18-20]. Among HUS patients: seizures, pneumonia, cerebrovascular events, persistent psychiatric problems, severe colitis resulting in destruction of colon requiring surgical removal and colostomy[21]	Oklahoma (buffet); Texas (camp); Italy (ground beef), Australia (mettwurst sausage made from beef and non-ruminant meat). Commonly isolated in U.S., Canada, Germany, Italy, Czech Republic, Belgium, Denmark, Australia, Chile, Japan[9]	1-10 cells[22]	Similar inactivation of pooled isolates (O45, O103, O111, O121, O145) in inoculated non-intact properly cooked steaks compared with *E. coli* O157:H7[13]
O121	H19; *stx1*, *stx2*, *eae, hly,* and genes associated with O-islands 71 and 122[4,6,16]	HUS [6,23,24], Hemorrhagic Colitis[6,23]	Utah (lettuce);Connecticut (lake water); Commonly isolated in U.S., Denmark, Sweden, Argentina[9]	No data available	Similar inactivation of pooled isolates (O45, O103, O111, O121, O145) in inoculated non-intact properly cooked steaks compared with *E. coli* O157:H7[13]
O145	H8, H16, H25, H28, H-[15,25]; A common O145:H28 virulence profile is [*stx1/stx2, eae,*	HUS [6,25-27], Hemorrhagic Colitis[6,25-27]	Multiple U.S. states (lettuce); Belgium (ice cream); commonly isolated in U.S., Canada, Germany, U.K., Spain, Italy,	400 cells [3]	Similar inactivation of pooled isolates (O45, O103,

	hly, and genes associated with O-islands 71 and 122.[4] Other genes associated with HUS-causing strains include *espP*, *katP*, and *etpD*[3]		Denmark, Finland, Argentina, Japan[9]		O111, O121, O145) in inoculated non-intact properly cooked steaks compared with *E. coli* O157:H7[13]
O1[47]	H7;H-; *stx1*, *stx2*, *eae*, *hly*	HUS		No data available	No data available
O8[47]	H19; *stx2*, *hly*	HUS		No data available	No data available
O46[47]	H31; *stx2*	HUS		No data available	No data available
O73[16]	H18; stx2	Hemorrhagic colitis		No data available	No data available
O91[47]	H21, H-; *stx1*, *stx2*,*hly*	HUS		No data available	No data available
O98[47]	H-; *stx1, eae, hly*	HUS		No data available	No data available
O113[47]	H21; *stx2, hly*	HUS		No data available	No data available
O118[47]	H16; *stx 1, eae, hly*	HUS		No data available	No data available

O128[47]	H2; *stx1, stx2, eae, hly*	HUS		No data available	No data available
O165[16]	H25; stx2	Hemorrhagic colitis		No data available	No data available
O172[47]	H-; *stx2*, *eae*, *hly*	HUS		No data available	No data available
O174[47]	H-; *stx2*	HUS		No data available	No data available
O177[16]	NM; stx1, stx2	Hemorrhagic colitis		No data available	No data available
O181[16]	H49; stx2	Hemorrhagic colitis		No data available	No data available

* this serotype is uncommon in the U.S.

1. Bielaszewska, Zhang, *et al.*, 2007
2. Murinda SE and Oliver SP, 2006. Presence of some other virulence factors depends on whether the strain is positive for stx1, stx2, or both.
3. Buvens *et al.*, 2011 (see also supplemental table mentioned in this study).
4. Bugarel *et al.*, 2010. In this study of pathogenic non-O157 STECs which had caused illness, the O103:H25 strain isolated from the Norway sausage outbreak had stx2 not stx1, and all these virulence factors plus nleF and nleA.
5. Allerberger *et al.*, 2003
6. Brooks *et al.*, 2005
7. Lorusso *et al.*, 2009
8. Ethelberg *et al.*, 2009
9. Johnson *et al.*, 2006
10. Boel *et al.*, 2009
11. Duffy *et al.*, 2006
12. CDC, 2006a
13. Luchansky *et al*,, 2012.

14. Schimmer *et al.*, 2008
15. CDC, OutbreakNet Foodborne Outbreak Online Database; Luna *et al.*, 2010
16. Jelacic *et al*., 2003
17. Bielaszewska, Köck, *et al.,* 2007
18. Piercefield *et al.*, 2010
19. Brooks *et al.*, 2004
20. CDC, 1995
21. Henning *et al.*, 1998
22. Paton *et al.*, 1996
23. Weber-Morgan Health District (Utah), 2007
24. McCarthy *et al.*,2001
25. De Schrijver *et al.*, 2008
26. Fratamico *et al.*, 2009
27. CDC, 2010a
28. Imamovic *et al*., 2010
29. Pennington 2010.
30. Jaeger and Acheson 2000.
31. Caprioli *et al.*, 2005
32. Posse´ *et al.*, 2007
33. Eklund *et al.*, 2006
34. Kappeli *et al.*, 2010
35. Rangel *et al.*, 2005
36. Blanco *et al.*, 2004
37. Sodha *et al.* 2010
38. Rangel *et al.*, 2005
39. CDC, 2006b
40. CDC, 2010a
41. Kaper *et al.*, 2004
42. Lim *et al.*, 2010
43. Tilden *et al.*, 1996
44. Teunis *et al.*, 2004
45. Tuttle *et al.*, 1999
46. Duffy *et al.*, 2006; inactivated in minced meat at 55°C.
47. Blanco *et al.*, 2004. All information in these rows was from this reference

Table 3: Serogroups and associated virulence factors for selected outbreaks and clusters

Serogroup	Outbreak/Cluster	Illnesses	Bloody diarrhea (no. of cases)	HUS (no. of cases)	Deaths	Virulence factors				
						stx1	stx2	eae	hly	other
O26	(C): Germany (1999)[1]	3	3	3	0		*stx2*	*eaeβ*	*hly*	*etp*
	(O): Austria (2001)[2]	2	2	1	0		*stx2*	*eae*	*hly*	
	(O): Denmark (2007)[3]	20	1	0	0	*stx1*		*eae*		
	(O): Germany (2000)[4]	6	0	0	0	*stx1*		*eae*	*hly*	*katP*
	(O): New York and Maine (2010)[5]	3	0	0	0	*stx1*		*eaeA*	*hly*	*nleA*
O45	(O): New York (2005)[6]	53	17	0	0	*stx1*				
	(O): North Carolina (2006)[7]	11	0	0	0	*stx1*		*eae*	*hly*	
O103	(O): Norway (2006)[8]	17	14	10	1		*stx2*	*eae*		
	(O): Japan (2006)[9]	12	0	0	0	*stx1*				
O111	(O): OK (2008)[10]	341	140	26	1	*stx1*	*stx2*			
	(O): Australia (1995)[11]	161	30	23	1	*stx1*	*stx2*	*eae*	*hly*	
	(O): TX (1999)[12]	56	20	2	0	*stx1*	*stx2*	*eae*	*hly*	
	(C): Ohio (1990)[13]	5		5		*stx1*	*stx2*			
O121	(O): Utah (2006)[14]	42	8	1	0	N/A				
	(O): Connecticut (1999)[15]	11	3	1	0	N/A				
	(O): Japan[16]	63	2	0	0		*stx2*			
O145	(O): Argentina (2005)[17]									
	(C): Argentina (2006)[18]	4	3	3	0		*stx2*	*eae*	*hly*	

	(O): Belgium (2007)[19]	12	7	5	0		*stx2*	*eae*	*hly*	*etpD*
	(O): Michigan, Ohio, New York, Tennessee, Pennsylvania (2010)[20]	33	28	3	0		*stx2*	eae		

1. Misselwitz *et al.*, 2003. Cluster of hemolytic-uremic syndrome caused by Shiga toxin-producing *Escherichia coli* O26:H11.
2. Allerberger *et al.*, 2003.
3. Ethelberg *et al.*, 2009
4. Werber *et al.*, 2002.
5. Ongoing FSIS investigation (with New York State Department of Health and Maine Department of Agriculture, Food and Rural Resources). Results are from NY case. Full test results not yet available from ME lab.
6. CDC, 2006. It is not known whether virulence factors other than stx were tested.
7. Luna *et al.*, 2010. Confirmed that stx1, eae, and hly were found.
8. Schimmer *et al.*, 2008. All HUS cases but one were preceded by bloody diarrhea. Only stx1/stx2 and eae were tested.
9. Muraoka *et al.*, 2007. It was not known whether virulence factors other than stx were tested.
10. Piercefield *et al.*, 2010 and Oklahoma State Department of Health, 2009. 10/10 specimens from HUS patients were positive for both stx1 and stx2; 17/18 specimens patients without HUS were positive for both stx1 and stx2.
11. CDC, 1995 and Elliot *et al.*, 2001. The 30 bloody diarrhea cases did not go on to develop HUS. All HUS cases were post-diarrheal. 20 cases were positive for both stx1 and stx2; one case was positive for only stx2.
12. Brooks *et al.*, 2004 and Brooks *et al.*, 2005. 11 samples were available for culture for non-O157 STEC; two were positive for the listed virulence factors. One was from an HUS patient; one was from a patient with bloody stool (not diarrhea). Both HUS cases were preceded by bloody diarrhea.
13. Banatvala *et al.*, 1996. No other virulence factors were tested.
14. Weber-Morgan Health District (Utah), 2007 and G. Kinney, 2010 (personal communication). 227 people were interviewed in this investigation; 8 people reported bloody diarrhea.
15. McCarthy *et al.*, 2001. An ELISA test was done that detected both stx1 and stx2 but did not differentiate.
16. Akiba *et al.*, 2005. One patient was co-infected with STEC O157.
17. Miliwebsky *et al.*, 2007.
18. Gómez *et al.*, 2010.
19. Buvens *et al.*, 2011 and De Schrijver *et al.*, 2008. One HUS patient was co-infected with O26 carrying *stx1, eae, hly, espP, katP*
20. CDC, 2010a and Gerner-Smidt (personal communication). An outbreak timeline indicated that 28 people reported bloody diarrhea.

Table 4: Outbreaks of non-O157 Shiga toxin-producing *E. coli* infections in the United States, 1990-2010*

Year	Serogroup	State	Number of ill persons	HUS reported?	Hospitalizations	Other enteric pathogens detected	Known or suspected exposure/vehicle
1990	O111[1]	Ohio	5	1/5	1/5		Private home/Family cluster[2]
1994	O104[1]	Montana	18	0/18	4/18		Pasteurized Milk
1998	O121[3]	Montana	8[4]	unknown	unknown		Camp[2]
1999	O111[5]	Texas	55	2/55	2/54		Salad Bar, Ice from barrel
1999	O121[1]	Connecticut	11	3/11	3/11		Lake Water
1999	O145[6]	Minnesota	2	0/1	0/1		Daycare
2000	O103[1]	Washington	18	2/18	2/16		Water-based punch
2000	O111[7]	Minnesota	61[8]	0/59	1/59	*C. parvum,* *C. jejuni,* *S.* Typhimurium	Animal contact (calves)
2000	O111[9]	Utah	126	0/126	0/126	*C. jejuni,* *E. coli* O157:H7 *Shigella* sp.	Irrigation Water
2001	O26[1]	Minnesota	4	0/4	0/4		Lake Water
2001	O111,[7] O-rough[10]	Minnesota	31[8]	0/25	3/25	*C. jejuni,* *E. coli* O157:H7	Animal Contact (Calves)
2001	O111[2]	Minnesota	3	3/3	3/3 (1 death)		Family cluster (animal exposure reported for one patient)
Year	**Serogroup**	**State**	**Number of ill persons**	**HUS reported?**	**Hospitalizations**	**Other enteric pathogens**	**Known or suspected exposure/vehicle**

						detected	
2001	O111[1]	South Dakota	3	0/3	1/3		Daycare
2004	O111[12]	New York	213	0/212	15/212	*C. parvum*	Unpasteurized Apple Cider
2005	O45[13]	New York	52	0/52	3/52		Ill Food Worker(s)
2005	O145[14]	Oregon	60	0/60	0/59	*C. jejuni,* *E. coli* O157:H7	Drinking water
2005	***O26***[10]	Nevada	4	unknown	unknown		Daycare[2]
2006	O121[15]	Utah	42	3/42	3/42		Lettuce
2006	O26[16]	Massachusetts	5	0/4	1/4		Berries
2006	O121[10]	Nebraska	5	4/5	5/5		Daycare
2006	***O45***[10]	North Carolina	11	0/11	0/11		Animal contact (goats)
2006	O165[2]	Oregon	3	0/3	1/3		Correctional facility
2007	O111[17]	North Dakota	23	0/23	0/23		Private home (ground beef)
2007	O111[3]	Maine	8	0/8	0/8	*Cryptosporidium* spp.	Daycare[2]
2007	O121,O26, O84[3]	Colorado	135	0/135	10/135		Correctional facility outbreak; Ill food workers (Pasteurized American cheese, margarine)[2,3]
Year	**Serogroup**	**State**	**Number of**	**HUS**		**Other enteric**	**Known or suspected**

			ill persons	reported?	Hospitalizations	pathogens detected	exposure/vehicle
2007	***O45***[10]	New Hampshire	5	0/5	0/5		Animal Contact
2007	***O111***[10]	North Dakota	6	0/6	1/6		Elementary school[2]
2007	O26[2]	Iowa	2	0/2	unknown		Daycare
2008	O111[18]	Oklahoma	344[19]	26/341	72/341 (1 death)		Restaurant
2008	***O111***[10]	Minnesota	3	0/3	0/3		Daycare[2]
2008	O111[2]	Nebraska	34	0/ 34	2/34		Catered event (BBQ pork)
2008	O141[2]	Maryland	191	0/191	4/191	Norovirus	Hotel outbreak; ill food worker(s)
2009	O26[2]	Wisconsin	8	0/8	0/8		Family picnic
2009	O111[2]	South Dakota	13	pending	pending		Daycare
2009	O26[2]	California	3	not reported	0/3		Daycare
2009	O121[2]	Washington	3	pending	pending		Raw milk
2010	O26[2]	Washington	6	pending	pending		Raw milk
2010	O145[20]	Michigan, Ohio, New York, Tennessee, Pennsylvania	33	3/30	12/30		Lettuce
2010	O111[21]	Colorado	11				
2010	O26[22]	Maine, New York	3	pending	pending		Ground beef
2010	Pending[2]	Tennessee	3	1/3	1/3		Daycare

Table adapted from Brooks *et al*, 2005. Table compiled in collaboration with CDC.

Hospitalizations and deaths reported by CDC (R. Luna, personal communication)

* Reported as of November 4, 2010. This table is subject to change as information is reported to CDC.

1. Brooks *et al.,* 2005
2. Luna, 2010 (personal communication). In the the 2008 outbreak in MD, STEC O141 was isolated from two patients.
3. CDC OutbreakNet Foodborne Outbreak Online Database
4. 40 cases were reported to the CDC NORS database; CDC clarified with Minnesota officials that there were 8 cases.
5. Brooks *et al*., 2004
6. Minnesota Department of Health, 2000
7. Minnesota Department of Health, 2005
8. Smith *et al.,* 2004. Two case-patients tested positive for O111 as part of a camp outbreak. The 2000 outbreak included 2 secondary cases; the 2001 outbreak included 6 secondary cases.
9. CDC, 2002. CDC clarified with Utah officials that there were 126 cases. STEC O111 was isolated from 3 patients.
10. Luna *et al.*, 2010
11. Smith *et al.,* 200*4.* Two case-patients tested positive for O111 and two tested positive for O rough:H11, which was concluded to be indistinguishable from O51:H11, which was isolated from calves as part of a camp outbreak.
12. Vojdani *et al.*, 2008.
13. CDC, 2006. Sixteen samples were stx1 positive by PCR and negative for O157-specific DNA; three samples sent to the CDC were positive for O45:NM.
14. Oregon Department of Human Services, 2006. Among patients tested, *E. coli* O157 was isolated from 9 patients; *E.coli* O145, 2 patients; *Campylobacter sp*, 3 patients; O157 & *Campylobacter,* 3 patients.
15. Weber-Morgan Health District (Utah), 2007
16. Massachusetts Department of Public Health, 2008
17. North Dakota Department of Health, 2007.
18. Piercefield *et al.*, 2010.
19. Oklahoma State Department of Health, 2009. Includes 3 secondary cases.
20. CDC, 2010b. Includes confirmed and probable case-patients.
21. This is a recent outbreak; information is preliminary.
22. Ongoing FSIS investigation (with New York State Department of Health and Maine Department of Agriculture, Food and Rural Resources). Results are from NY case. Full test results not yet available from ME lab.

Table 5: Surveillance of Shiga toxin-producing *E. coli* non-O157 infections in the United States, 2001-2010

Year	Number of cases or isolates reported by data source				
	FoodNet[1]		NNDSS: Nationally Notifiable Diseases Surveillance System, Summary of Notifiable Diseases (cases)[2]	PHLIS: Public Health Laboratory Information System (isolates)[3]	CDC's National *E. coli* Reference Laboratory (isolates)[3]
	cases	incidence			
2000	57	0.19/100,000			
2001	61	0.18/100,000	171[4]		Not published
2002	35	0.09/100,000	194[4]		Not published
2003	47	0.11/100,000	252[4]	166	239[6]
2004	110	0.25/100,000	308[4]	139	248[7]
2005	128	0.28/100,000	501[4]	224	348[8]
2006	212	0.47/100,000	N/A[5]	423	554[9]
2007	272	0.59/100,000	N/A[5]		Not yet published
2008	205	0.45/100,000	N/A[5]		Not yet published
2009	264	0.57/100,000	N/A[5]		Not yet published
2010	451	1.0/100,000	Not yet published		Not yet published

This table is meant to show that the number of detected and reported non-O157 STEC cases has increased. Due to variability in these surveillance systems, the numbers cannot be compared to each other or used to determine national incidence, except for FoodNet. FoodNet is an active surveillance system in 10 states and represents approximately 15% of the U.S. population. The Nationally Notifiable Diseases Surveillance System is not a single surveillance or reporting system; NNDSS compiles an annual summary of data reported to CDC from various sources. PHLIS is a nationwide passive reporting system and relies on voluntary reporting of cases by state public health laboratories. CDC's

National *E. coli* Reference Laboratory receives isolates submitted by state public health laboratories and performs confirmatory testing.

1. CDC FoodNet Reports (2000-2007) and Preliminary Data (2008, 2009, 2010)
2. CDC Summary of Notifiable Diseases (2001-2007)
3. CDC Bacterial Foodborne and Diarrheal Disease National Case Surveillance Annual Reports (2003-2006)
4. Additional isolates that were not serogrouped: 2005, 407; 2004, 316; 2003, 156; 2002, 60; 2001, 20
5. Source did not differentiate between *E. coli* O157:H7 and non-O157 STEC
6. 239 isolates were forwarded by 32 states to the CDC's National *Escherichia coli* Reference Laboratory and Epidemic Investigation and Surveillance Laboratory at CDC. There were 32 different O groups, with predominant groups being O26 (25%), O111 (17%), O103 (14%), O145 (8%), O121 (6%), and O45 (5%). These six O groups made up 75% of all isolates
7. 248 isolates were forwarded by 30 states to the CDC's National *Escherichia coli* Reference Laboratory and Epidemic Investigation and Surveillance Laboratory at CDC. There were 30 different O groups, with predominant groups being O26 (19%) , O103 (18%), O45 (13%), O111 (13%), O145 (6%), and O121 (7%). These six O groups made up 76% of all isolates.
8. 348 isolates were forwarded by 39 states to the CDC's National *Escherichia coli* Reference Laboratory and Epidemic Investigation and Surveillance Laboratory at CDC. There were 29 different O groups, with predominant groups being O26 (24%), O103 (17%), O111 (13%), O45 (8%), O121 (7 %), and O145 (3.4%). These six O groups made up 72.4% of all isolates.
9. 554 isolates were forwarded by 42 states to the CDC's National *Escherichia coli* Reference Laboratory and Epidemic Investigation and Surveillance Laboratory at CDC. There were 50 O groups, with predominant groups being O26 (22%), O103 (17%), O111 (14%), O121 (5%), O45 (5%), and O145 (4%). These six O groups made up 67% of all isolates.

Table 6: Change in laboratory-confirmed infections with STEC O157 and non-O157 STEC compared with 2001-2003 and 2006-2008, United States

Pathogen	Percent change in 2009 (95% confidence interval)	
	Compared with 2006-2008	**Compared with 2001-2003**
STEC O157	24% decrease (36% decrease to 10% decrease)	27% decrease (39% decrease to 13% decrease)
STEC non-O157	6% increase (22% decrease to 45% increase)	284% increase (169% increase to 450% increase)

Data source and graph: CDC FoodNet and O. Henao, CDC

Table 7: Outbreaks due to non-O157 STEC associated with red meat products worldwide

Year	Country	Cases	Deaths	Illness types	HUS?	Non-O157 STEC involved	Virulence factors detected[1]	Evidence of food source?
1992	Italy	9[2]	1	HUS (all), bloody and non-bloody diarrhea	Yes	O111:NM	Vero cytotoxin via assay	No; ground beef epidemiologically linked[2]
1995	Australia	161[3]	1	Ranging from: mild to severe diarrhea only (105), bloody diarrhea (30), post-diarrheal HUS (23), TTP (3). Among HUS patients: seizures, pneumonia, cerebrovascular events, persistent psychiatric problems, severe colitis resulting in destruction of colon requiring surgical removal and colostomy[4]	Yes	O111:H$^-$	*stx1*, *stx2, eae, hly*	Yes; uncooked, semi-dry fermented beef sausage (made from beef and meat from non ruminants) (mettwurst)
2000	Germany	6[5]	None	Non-bloody diarrhea	No	O26:H11	*stx1*, *eae*, *hly*, *katP*	No; Seemerolle cut of beef epidemiologically linked[5]
2002	France	10[6]	None	Post-diarrheal HUS (2), hemorrhagic colitis (7), non-bloody diarrhea	Yes	O148:H8	*stx2* (*eae* and *hly* negative)	No; mutton epidemiologically linked[6]
Year	**Country**	**Cases**	**Deaths**	**Illness types**	**HUS?**	**Non-O157 STEC involved**	**Virulence factors detected**[1]	**Evidence of food source?**

2006	Norway	17[7]	1	Post-diarrheal HUS (10), bloody diarrhea (14), non-bloody diarrhea (2), asymptomatic (1)	Yes	O103:H25	*stx2, eae, hly;* pathogenicity island OI122 genes *ent/espL2, nleB, nleE;* pathogenicity island OI77 genes *nleF, nleH1-2, nleA*[8]	Yes, uncooked dry cured mutton sausage
2007	Denmark	20[9]	None	One case of bloody diarrhea; remainder mild gastrointestinal illness	No	O26:H11	*stx1*, *eae*	Yes; fermented beef sausage
2009	France	2[10]	0	Post-diarrheal HUS (1), bloody diarrhea (1), non-bloody diarrhea (1)	Yes	O123:H-	*stx2, eae, hly*	Yes; ground beef burger
2010	U.S.	3[11]	0	Non-bloody diarrhea (3), abdominal cramps (2), vomiting (1)	No	O26	*stx1, eae, hly, nleA*	Yes; ground beef

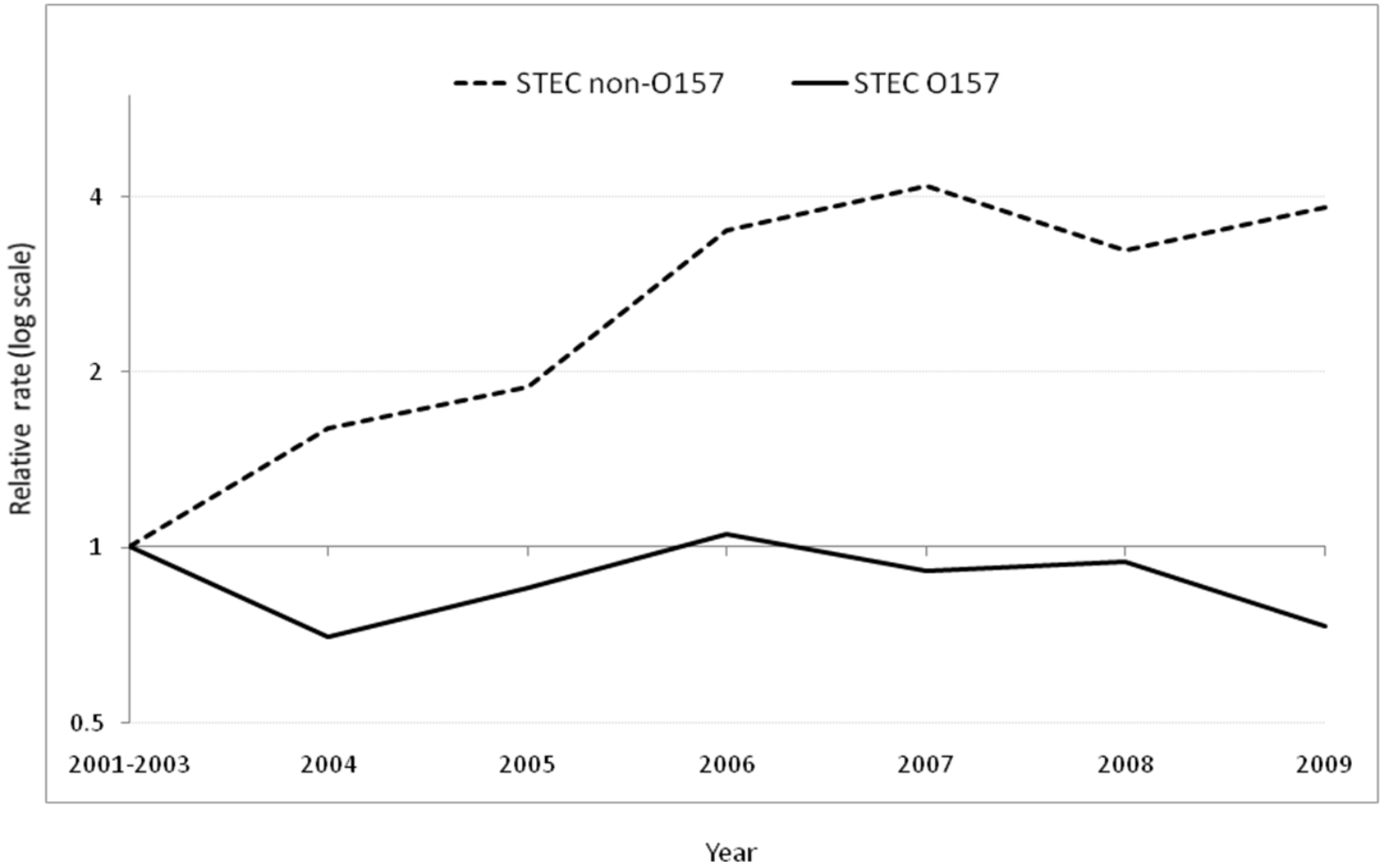
Table adapted from Bollinger, 2004

1. Other than *stx2* and those virulence factors identified, no other virulence factors were sought by investigators, unless otherwise noted.
2. Caprioli *et al.*, 1994. All case patients had HUS and were children; the outbreak was detected via hospitals participating in an HUS surveillance program. A questionnaire sent to area hospitals to identify possible additional patients did not uncover any. Parents of seven patients were interviewed; six patients had consumed ground beef purchased from six different retailers. Two retailers reported obtaining the meat from the same distributor; traceback investigations could not be completed.
3. CDC, 1995 and Elliot *et al.*, 2001
4. Henning *et al.*, 1998
5. Werber *et al.*, 2002. Purchase of Seemerolle cut of beef was statistically associated with supplying kitchens of case institutions; however, traceback investigations could not be completed.
6. Espié *et al.*, 2006. Microbiological investigations showed that the three STEC isolates from the mutton were indistinguishable from the isolate from one of

the HUS patients (*stx2c* toxin type, P2 PFGE pattern, M2 ribotype and O148 serogroup). Epidemiological, microbiological and environmental investigations together implicated mutton.

7. Schimmer *et al.*, 2008
8. Bugarel *et al.*, 2010
9. Ethelberg *et al.*, 2009. Some patients also tested positive for *Campylobacter*, *Yersinia*, Norovirus, and *stx*-negative/ *eae*-positive *E. coli*
10. King *et al.*, 2010. Both patients were siblings living in the same household.
11. Ongoing FSIS investigation (with New York State Department of Health and Maine Department of Health and Human Services).

Figure 1: Relative rates of laboratory-confirmed infections with STEC O157 and non-O157 STEC compared with 2001-2003, by year, United States



Data source and graph: CDC FoodNet and O. Henao, CDC

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