

**Public Health Importance of Non-O157 Shiga Toxin-Producing *Escherichia coli* (non-O157 STEC) in the US Food Supply**

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## **Summary**

Non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC) have emerged as a significant public health issue. Some non-O157 STEC possess the same range of virulence factors as *Escherichia coli* (*E. coli*) O157:H7, including the locus of enterocyte effacement (LEE), production of Shiga toxin, and other plasmid mediated factors, and are capable of causing serious illnesses, or death. Numerous serotypes, including O26, O103, O111 and O145 have been identified as agents of food borne disease. Historically, most *E. coli* O157:H7 STEC outbreaks have been associated with consumption of ground beef. Non-O157 STEC have also been found in ground beef and on cattle hides and feces at levels comparable to those for *E. coli* O157. Bovine feces may be a source of environmental contamination, (e.g., soil or water) which can lead to secondary contamination of produce growing in fields. *E. coli* O157:H7 was implicated in a large outbreak associated with spinach in 2006, and non-O157 STEC have been isolated from produce.

It is difficult to distinguish pathogenic non-O157 STEC strains from non-pathogenic *E. coli* because the former rarely possess any distinguishing phenotypic or biochemical characteristics from the latter. The lack of reliable and validated laboratory methods for testing various food matrices has meant that food is not routinely tested for non-O157 STEC and research is needed to support the development of new and better targeted detection methods. This report describes: the microbiological and molecular characteristics of non-O157 STEC; presents food, animal, environmental, clinical and epidemiologic data; and outlines the laboratory challenges and methodological limitations and capabilities for their detection.

## **Introduction**

*Escherichia coli* (*E. coli*) was first associated with human illness in the early 1940s, when it was linked to infant diarrhea (Bray and Beavan, 1948). Since then, many pathogenic *E. coli* strains have been identified. These are classified on the basis of their virulence properties, mechanisms of pathogenicity, clinical symptoms, and the presence of distinct O and H antigens (Doyle *et al.*, 1997). Groupings include; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), entero-aggregative *E. coli* (EAggEC), and Shiga-toxin producing *E. coli* (STEC)<sup>1</sup>. Of these groupings, STEC organisms have the potential to cause the most severe clinical symptoms.

The first association of STEC with human disease was made in 1982 in the New England region of the United States (Riley *et al.*, 1983). The emergence of this human pathogen, identified as *Escherichia coli* O157:H7 (*E. coli* O157:H7), spurred much interest in the clinical and public health research communities, due to the severity of the ensuing illnesses. The food safety research community became interested in this organism due to its foodborne transmission and apparent ability to survive food-processing procedures that had hitherto assured food safety. In 1994, following a large foodborne outbreak caused by the consumption of under-cooked hamburgers (Centers for Disease Control and Prevention (CDC), 1993), the Food Safety and Inspection Service (FSIS) declared that *E. coli* O157:H7 and *E. coli* O157: non motile (hereafter *E. coli* O157) were to be regarded as an adulterant in raw ground beef, and established a zero-tolerance policy for this pathogen in this food product. As such, FSIS would request a recall if the product had entered commerce. Any raw ground beef found to contain *E. coli* O157 must be disposed of, or sent for further processing involving a lethality step. In that same year FSIS instituted testing of ground beef for the presence of *E. coli* O157.

The current focus of FSIS STEC monitoring remains solely on *E. coli* O157. However, there is growing evidence that some non-O157 STEC are foodborne pathogens. Clinical studies were the first to identify certain non-O157 STEC as causative organisms in illnesses and targeted studies have confirmed the presence of non-O157 STEC in the same reservoirs as *E. coli* O157, with

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<sup>1</sup> The commonly-used terms enterohemorrhagic *E. coli* (EHEC) and verotoxigenic *E. coli* (VTEC) refer to STEC serotypes with the same clinical, pathogenic, and epidemiologic features as *E. coli* O157.

similar survival characteristics. However, the main focus of the food safety research community has remained on *E. coli* O157. While *E. coli* O157 is the STEC most commonly linked to human illness in the United States, other STEC serogroups have also caused cases and outbreaks of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), and in some countries, including Australia, Argentina, Canada, and European Union (EU) nations, non-O157 STEC infections are as prevalent, or more so, than O157 infections. There is increasing evidence to support a focus on the elimination from the US food supply of all pathogenic STEC, rather than just *E. coli* O157.

This report will serve as a comprehensive reference for interested stakeholders by summarizing current scientific literature on the characteristics of non-O157 STEC, the relevant epidemiology, and the laboratory challenges for the detection of these pathogens

## **Section I. Characterization of the Organism**

### **i. Distribution of non-O157 STEC in food and the environment<sup>2</sup>.**

Ruminants, primarily cattle, but also sheep and goats<sup>3</sup>, are the primary source of transmission of STEC to humans (Bettelheim, 2000). In addition to ground beef and unpasteurized milk (Doyle, 1991, Allerburger *et al.*, 2001), highly acidic ready-to-eat foods such as fermented meat (Tilden *et al.*, 1996) and apple cider (Besser *et al.*, 1993) have long been identified as significant sources of STEC foodborne illness (Griffin and Tauxe, 1991; Keene *et al.*, 1997). Other infection routes include manure-contaminated vegetables (Cieslak *et al.*, 1993), person-to-person contact (Reida *et al.*, 1994), animal to person contact (Crump *et al.*, 2002), contaminated water (Keene *et al.*, 1994; Yatsuyanagi *et al.*, 2002) and visiting dairy farms or petting zoos (Zhao *et al.*, 1995; Crump *et al.*, 2002).

There is a relative paucity of studies on the prevalence and distribution of non-O157 STEC in food and in the environment in comparison to the wealth of published research on *E. coli* O157. The non-O157 STEC studies that do exist generally fall into one of two categories; targeted studies that aim to characterize these organisms present in the food supply and the environment, and epidemiological investigations designed to identify causes and routes of infection (Section II). This section will review the former studies, with particular emphasis on meat and poultry products under FSIS jurisdiction.

More STEC outbreaks have been traced to the consumption of ground beef than to any other food (Dean-Nystrom *et al.*, 1997; Hussein and Sakuma, 2005). In the US, 12-19% of ground beef is produced from dairy cattle culled because of health, age, or production reasons (Wilkus, 2007). Studies of US dairy cattle have reported non-O157 STEC prevalence from 0% to 19% (Wachsmuth *et al.*, 1991; Wells *et al.*, 1991; Cray *et al.*, 1996; Thran *et al.*, 2001). Barkocy-Gallagher *et al.*, 2003 reported a prevalence of non-O157 STEC in beef cattle feces at 19.4% and on hides at 56.3%. Pathogen prevalence on hides may reflect several sources of contamination, such as soil, feces from other animals, and the environment (Barkocy-Gallagher *et al.*, 2003). Blanco *et al.*, (2003) studied the dairy farm environment and detected STEC in calf-and cow-feeders, and in both calf-barn surfaces and

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<sup>2</sup> The section focuses on US data only; findings from other countries are addressed elsewhere (Section II, vi).

<sup>3</sup> Data on sheep and goats primarily come from outside the US, and so are outside the focus of this section.

cow-barn surfaces, and proposed that farm environments could remain as sources of STEC for several months.

Barkocy-Gallagher *et al.*, (2003) reported that the prevalence of non-O157 STEC on pre-evisceration carcasses was 58%, dropping to 9% post-processing. Similarly, Arthur *et al.*, (2002) reported that 53.9% of beef carcasses in large processing plants carried at least one type of non-O157 STEC prior to evisceration, but that the prevalence could be reduced to 8.3% with various intervention strategies. A recent retail study by Samadpour *et al.* (2006) reported non-O157 STEC in 2.3% of 1,750 retail raw ground beef samples, compared to *E. coli* O157, found in 1.1% of samples tested. Little information exists on the prevalence of pathogenic non-O157 STEC in FSIS-regulated products other than beef in the US. Doyle and Schoeni (1987) isolated *E. coli* O157 from 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry, and 4 (2.0%) of 205 lamb samples in their survey. Samadpour *et al.* (1994), found non-O157 STEC in 9 (18%) of 51 pork samples, 10 (48%) of 21 lamb samples, 5 (63%) of 8 veal samples, 4 (12%) of 33 chicken samples, 1 (7%) of 15 turkey samples, 6 (10%) of 62 fish samples, and 2 (5%) of 44 shellfish samples tested. Fratamico *et al.*, (2004) determined that 70% of 687 swine fecal samples tested positive for the presence of Shiga toxin, and found that most of the serogroups isolated have been associated with human illness. These authors concluded that swine could be a potential reservoir of STEC strains that cause human illness, but conceded that the extent to which swine play a role in the epidemiology of human infection needs further investigation. In general, pigs, poultry and other non-ruminants are not considered to be a source of STEC and sporadic reports such as these may derive from inadvertent exposure to infected ruminants (Caprioli *et al.*, 2005).

While it might seem reasonable to assume a link between the presence of STEC in food animals and subsequent foodborne illness, not all non-O157 STEC are pathogenic to humans (Section I, ii), and the proportion of non-O157 STEC that can cause disease in humans has not been established. Therefore the implications of prevalence data for these organisms in food must be carefully considered.

#### **ii. Virulence characteristics of STEC.**

Pathogenesis of STEC is a multi-step process (Paton and Paton, 1998), starting with the acid resistance of the strain, which enables the organism to

survive in low-pH foods and in the acid environment of the stomach. The organism must then adhere to and colonize the intestine, invade epithelial cells, and produce toxin. Not all serotypes of STEC are equally pathogenic - there is much evidence of genetic diversity within serotypes, which can affect virulence determinants and, ultimately, pathogenicity (Nataro and Kaper, 1998). Such differences can be manifested, for example, in the infectious dose of the organism (typically 5 - 50 cells (Tilden *et al.*, 1996)), the level and type of toxin produced, the extent of gastrointestinal colonization, the rate of toxin delivery to the endothelial cells and/or the severity of ensuing disease.

Although the set of virulence factors necessary to cause STEC-related disease has not been completely defined, association between the carriage of certain genes and the ability to cause severe disease in humans has been made. Non-O157 STEC typically possess the same range of virulence factors as *E. coli* O157, including the locus of enterocyte effacement (LEE), Shiga toxin production, and other plasmid mediated factors.

**Locus for enterocyte effacement (LEE)** The majority of STEC are capable of colonizing the intestine with a characteristic attaching and effacing (A/E) cytopathology. The A/E lesion is characterized by effacement of microvilli and intimate adherence between the bacteria and the epithelial cell membrane, with accumulation of polymerized actin beneath the adherent bacteria (Nataro and Kaper, 1998). This ability is encoded by a number of genes present on a 'pathogenicity island' referred to as the locus for enterocyte effacement (LEE). The LEE encodes for intimin (an *eaeA* gene product), an outer membrane protein involved in the intimate attachment of bacteria to enterocytes in the gut, and the intimin receptor Tir (encoded by *tir*). Several different intimin types have been identified. STEC most commonly produce intimin  $\gamma$  and  $\epsilon$  (Pelayo *et al.*, 1999).

The LEE also encodes for a type III secretion system that exports LEE effector molecules (including *espA*, *espB* and *espD*) directly into the epithelial cell. Karch *et al.*, (1997) identified a high incidence of *eaeA* positive STEC in HUS patients, particularly children (Beutin *et al.*, 1998; Pradel *et al.*, 2000) suggesting that the presence of this gene is associated with increased virulence in STEC.



The *eaeA* gene is not a universal requirement for virulence (Wieler *et al.*, 1996), and pathogenic strains associated with serious clinical outcomes have been isolated that do not possess this gene (Keskimäki *et al.*, 1997; Pradel *et al.*, 2000; Eklund *et al.*, 2001). Paton *et al.*, 2001 reported the presence of the *saa* gene in an LEE-negative STEC (*E. coli* O113:H21) strain responsible for a HUS outbreak. This gene encodes for an auto-agglutinating adhesion designated Saa (STEC autoagglutinating adhesion). Subsequent investigation by these researchers found homologues of *saa* in several LEE-negative STEC serotypes associated with HUS patients.

**Shiga toxin (Stx)**<sup>4</sup> Konowalchuk *et al.* (1977) were the first to recognize that a toxin produced by some *E. coli* bacteria displayed cytotoxicity against green monkey kidney cells (vero cells). This toxin was initially termed verotoxin, later Shiga toxin (due to its similarity to the toxin produced by *Shigella dysenteriae*), and is now recognized as a primary virulence factor associated with STEC. The vero-cell assay developed by Konowalchuk *et al.* (1977) is still recognized as the 'gold standard' for the confirmation of STEC. Karmali *et al.* (1983) recognized that production of Shiga toxin by *E. coli* O157 was a crucial factor in the pathogenicity of this organism.

STEC serotypes are diverse in their properties, and produce immunologically distinct Shiga toxin (encoded by the Shiga toxin 1 gene (*stx*<sub>1</sub>) and the Shiga toxin 2 gene (*stx*<sub>2</sub>)) (World Health Organization (WHO), 1998; Bower, 1999). Shiga toxins are multimeric cytotoxins consisting of 1 A and 5 B subunits. Cellular binding of Shiga toxin is coordinated through the B subunits, while the A subunit inhibits cellular protein synthesis (Bower, 1999). The cytotoxic effect of Shiga toxin on intestinal epithelial cells causes the characteristic bloody diarrhea associated with STEC infection. The type and/or amount of Shiga toxin produced will determine the capacity of the organism to cause human disease. The *stx*<sub>2</sub> gene can produce a number of variants, termed Shiga toxin 2c, Shiga toxin 2d, Shiga toxin 2e and Shiga toxin 2f, of varying toxicity to humans (Paton and Paton, 1998; Schurman *et al.*, 2000; Bertin *et al.*, 2001). Shiga toxin 2 and Shiga toxin 2c has been cited as 1000 times more cytotoxic than Shiga toxin 1 towards human renal cells and has been more commonly associated with the development of HUS than Shiga toxin 1

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<sup>4</sup> Other bacteria, including *Citrobacter freundii*, *Enterobacter cloacae* and of course *Shigella dysenteriae* can also produce Shiga toxin. However, STEC have emerged as the most significant cause of sporadic human illness associated with this toxin (Acheson and Keusch, 1996).

(Bertin *et al.*, 2001). However, strains producing Shiga toxin 1 only have also been associated with human illness, including HUS (Eklund *et al.*, 2001). As mentioned, not all non-O157 STEC strains that produce Shiga toxin cause HUS. This variability in virulence may have led to an underestimate of the pathogenicity of this diverse set of strains. However, based on data from Europe and Australia, a subset of non-O157 STEC strains are as virulent as *E. coli* O157 (Wickham *et al.*, 2006) with epidemiological evidence of similar incubation periods, symptom onsets, symptom profiles, and comparable proportions of case-patients who develop HUS. Related outbreaks are often indistinguishable from *E. coli* O157:H7 outbreaks (Brooks *et al.*, 2004; Brooks *et al.*, 2005)

**Plasmid-mediated factors** Many STEC possess a highly conserved 97-kb plasmid (pO157), which encodes for several putative virulence factors, including a serine protease (*espP*), a bifunctional catalase peroxidase (*KatP*), enterohemolysin (*ehx*), an immunomodulator (*lif*), and secretion proteins (*etp*) (McNally *et al.*, 2001). Studies have suggested an association between the carriage of the *eaeA* gene and enterohemolysin production (Eklund *et al.*, 2001). The precise role of these genes in the virulence of STEC has not been fully elucidated, though pathogenesis is certainly complex, with many contributory factors (McNally *et al.*, 2001).

In theory, an investigator should be able to pinpoint the cause of illness by identifying the presence of one or more of these virulence factors. However, for practically every virulence factor identified, there is an example of an illness caused by an isolate lacking the gene coding for this trait. Pradel *et al.* (2000) surveyed STEC isolated from cows, children and food (meat and cheese), characterizing the Shiga toxin types of each isolate, and whether or not they contained the *eaeA* gene. They observed a wide diversity of strains and noted that in general the strains isolated from the children were dissimilar to those isolated from the animals in terms of their genetic profile. McNally *et al.* (2001) reported that the comparatively low incidence of human disease attributable to STEC, given its relatively high incidence in cattle, could be attributed to inherent differences between the strains isolated from cattle and from humans. These researchers observed significant differences between human- and bovine-derived strains, and their production of certain LEE-encoded virulence factors, and proposed the possibility of different STEC lineages in cattle and humans. It may be that STEC from bovine sources

exhibit reduced resistance to environmental stresses, and therefore cannot survive food processing and/or digestion.

Because the presence of STEC in food is not a marker for human illness, testing procedures for pathogenic STEC in food must include other screening criteria in addition to Shiga toxin testing. More work is needed to determine the range and scope of distribution of Shiga toxin genes among *E. coli* serotypes, the ease of transfer of these genes among strains in the environment, their distribution in nature, their mode of entry into the food chain, and their potential pathogenicity (Bollinger *et al.*, 2005; Samadpour *et al.*, 2006). The field of STEC virulence and pathogenicity of STEC is an area of much uncertainty, and research is ongoing to identify properties that can be utilized to reliably distinguish pathogenic STEC from non-pathogenic strains.

## **Section II. Public Health Impacts**

### **i. Diseases caused by non-O157 STEC, and their associated morbidity and mortality.**

STEC infection causes symptoms ranging from mild non-bloody diarrhea in healthy adults to more significant health outcomes, sometimes proving fatal, in young, old or immunocompromised individuals. In such susceptible individuals, STEC infection generally causes diarrhea and abdominal cramps, with little or no fever, and resolves itself in 5 to 10 days. However, in some instances, more serious sequelae including hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura can develop.

**Hemorrhagic colitis (HC)** The classic paper entitled "Hemorrhagic colitis associated with a rare *Escherichia coli* serotype" was published by Riley *et al.* in *The New England Journal of Medicine* in 1983 when knowledge of STEC was in its infancy. This paper was the first to propose a link between an STEC (*E. coli* O157) and a significant human disease. Hemorrhagic colitis (HC) is a form of gastroenteritis in which STEC attach to the large intestine and secrete Shiga toxin, leading to bloody diarrhea as a result of damage to the lining of the large intestine. If the toxins are subsequently absorbed into the bloodstream, they can also affect other organs, such as the kidneys. HC can occur in people of all ages but is most common in children and the elderly. Symptoms include the sudden onset of severe abdominal cramps along with watery diarrhea that typically becomes bloody within 24 hours. The diarrhea usually lasts 1 to 8 days. Fever is usually absent or mild but occasionally can exceed 102° F (38.9° C). The prognosis for this disease is good; rarely, death may occur in elderly patients.

**Hemolytic uremic syndrome (HUS)** The term HUS was coined in the 1950s to describe an acute, often fatal syndrome in children characterized by hemolytic anemia (caused by the destruction of red blood cells), thrombocytopenia (a low platelet count), and severe renal failure. About 5% of HC patients, generally children younger than 5 years and the elderly, go on to develop hemolytic uremic syndrome (HUS) (Banatvala *et al.*, 2001). Some HUS patients develop complications of the nervous system or brain damage.

In children, 90% of HUS cases follow an infectious disease; STEC have been identified as the primary cause (up to 90%) of HUS in temperate climates<sup>5</sup>. Less commonly *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter* have been implicated. The CDC initiated an active surveillance for this condition in 1997, and Dunne *et al.* (2000) reported an annual incidence of 10.6 cases per million for 1997 through 1999 for children under 16. During 2003, a total of 178 cases of HUS were reported from 32 US States; of these, 118 (66%) occurred among children aged <10 years (CDC, 2005<sup>6</sup>). Lynn *et al.*, (2005) reported an increase in pediatric HUS cases in England and Wales from 50 in 1985, to 1087 in 1997. The increased incidence was attributed to improved laboratory techniques, increased reporting, and recognition of the importance of elucidating the causes of diarrheal disease.

HUS is the most common cause of long-term renal failure in children in the US and Britain (Boyce *et al.*, 1995; Chapman, 1995), and has a reported mortality rate of 5-10% in the US (Corrigan and Boineau, 2001).

Approximately 85% of children with classic HUS recover completely with supportive therapy; however, 15-20% of children may develop hypertension 3-5 years after the onset of disease. Adult patients with HUS have a lower mortality rate; however, the renal prognosis is poor in patients who are not treated. Up to 80% of adults with HUS will ultimately require long-term dialysis or renal transplantation.

**Thrombotic thrombocytopenic purpura (TTP)** TTP is a blood disorder characterized by fever, a low platelet count, low red blood cell count (caused by premature breakdown of the cells), compromised kidney function, and neurological abnormalities. Whereas HUS more commonly affects children and the elderly, TTP is more commonly found in non-elderly adults.

Symptoms include fluctuating neurological symptoms, such as bizarre behavior, altered mental status, stroke or headaches, kidney failure, fever, thrombocytopenia (low platelet count) leading to bruising or frank purpura<sup>7</sup>, and microangiopathic hemolytic anemia. The mortality rate associated with TTP approached 100% until the 1980s; today, the survival rate is 80-90% with early diagnosis and treatment with plasma infusion and plasma exchange, however, mortality remains at approximately 95% for untreated

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<sup>5</sup>The remaining 10% of cases are generally associated with an upper respiratory infection.

<sup>6</sup> The patients reported in national notifiable diseases surveillance (CDC, 2005) include only those with antecedent diarrheal illness.

<sup>7</sup> Purpura is the appearance of purple discolorations on the skin caused by bleeding underneath the skin.

cases. Up to one third of patients who survive the initial episode experience a relapse within the following 10 years (Elkins *et al.*, 1996).

## **ii. Recognized sources of infection**

There is limited information on the prevalence of non-O157 STEC in the US food supply. However, it is widely recognized that ruminants, primarily cattle, are the natural reservoirs of STEC (Chapman *et al.*, 1993; Rasmussen *et al.*, 1993; Armstrong *et al.*, 1996). Rangel *et al.* (2005) reviewed CDC *E. coli* O157 outbreak data (1982 through 2002) and reported that ground beef accounted for 75 of the 183 *E. coli* O157 foodborne outbreaks identified. Therefore an understanding of the dissemination and persistence of STEC among cattle is particularly important.

**Ground beef.** Approximately 30% of all cattle are asymptomatic carriers of *E. coli* O157 and other STEC (Elder *et al.*, 2000). *E. coli* O157 and non-O157 STEC alike asymptotically reside in the intestines of cattle, and are regularly shed in feces. Meat can become contaminated during slaughter, and organisms can be mixed into beef when it is ground. Sanitation efforts after slaughter have been shown to reduce the contamination of carcasses with *E. coli* O157 (Elder *et al.*, 2000); however, the ability of these organisms to withstand processing environments, as evidenced by the continuing occurrence of outbreaks linked to the consumption of undercooked ground beef, suggests that additional control measures must be identified and implemented. Methods that focus on reducing STEC populations in food animals before entry to the food chain, and on the elimination of these pathogens by appropriate food processing and handling, will contribute to further reductions in human illnesses.

**Other bovine sources.** Hussein and Sakuma (2005) published a review on the prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. The prevalence of STEC in US dairy cattle ranged from 0.2 to 8.4% (studies performed through the 1990s), with numbers in heifers ranging from 1.6 to 19.0%, and numbers in calves ranging from 0.4 to 40%. Some of the studies reviewed had isolated non-O157 STEC from raw milk,

cheese and milk filters; however, most of the isolated serotypes had never been linked to human illness.

Other known sources of infection, which can often be linked to secondary contamination from bovine sources, include:

**Produce.** The Rangel *et al.* (2005) study found that 38 of 183 *E. coli* O157 foodborne outbreaks identified (1982 through 2002) were attributable to produce, with 26 outbreaks traced to leafy green vegetables. The high-profile “spinach outbreak” in 2006, followed by two lettuce-linked outbreaks, has heightened consumer awareness of the potential for foodborne illness associated with produce. Contamination can arise from seeds, irrigation water, or the use of untreated animal manure harboring the pathogen. Due to the nature of the contamination, the pathogen may become established in the structure of the plant during its development, making it impossible to eliminate by washing (Itoh *et al.*, 1998). Pathogens can exceed  $10^7$  per gram of sprouts produced from inoculated seeds during sprout production, without adversely affecting appearance. Treating seeds and sprouts with chlorinated water or other disinfectants fails to eliminate the pathogens (Taormina *et al.*, 1999), leading the FDA to make the recommendation that raw alfalfa sprouts should be considered potentially contaminated and avoided by persons at high-risk such as the young, elderly and immunocompromised.

**Fermented meats.** Traditionally, fermented meats were not subjected to a heat-lethality process; instead the safety of such products was assured by the low pH achieved during the fermentation process. However the emergence of STEC, which exhibit significant acid-resistance and can thereby survive the fermentation process if present in the raw meat ingredients, meant that traditional fermentation processes were no longer sufficient to assure the safety of such products (Riordan *et al.*, 1998). Today, most fermented meat production processes include a heat-lethality step.

**Unpasteurized milk.** Bacteria present on the cow's udders, hide or on equipment may contaminate raw milk. Raw milk consumption has been

associated with brucellosis, campylobacteriosis, cryptosporidiosis, *E. coli* O157, listeriosis, salmonellosis, staphylococcal enterotoxin poisoning, tuberculosis, and yersiniosis (Potter *et al.*, 1984). The sale of raw milk is prohibited or strictly limited in most US states (Bren, 2004); however, cow-sharing programs and other such initiatives have led to increased availability in some jurisdictions.

**Unpasteurized juice.** Fruit used for juice is typically of poorer quality than that sold as eating-quality fruit. It has been demonstrated that *E. coli* O157 can grow on dropped, damaged or blemished fruit (Dingman, 2000), thereby providing an avenue for pathogen entry (Riordan *et al.*, 2000). In addition, dropped fruit potentially contaminated with manure may be used in juice-making, and though all produce is washed prior to processing, it is very difficult to completely remove pathogens from fruit with traditional washing procedures (Annous *et al.*, 2001). The low pH associated with products such as apple juice, which had traditionally assured their safety, is insufficient to eliminate surviving STEC. A number of outbreaks linked to the consumption of unpasteurized juice (e.g. Besser *et al.*, 1993) led the FDA in 2001 to release Juice HACCP regulations, essentially requiring all juice to be either pasteurized or sold with a warning label.

**Swimming in or drinking sewage-contaminated water.** The very low reported infectious dose of STEC, less than 10 cells in some instances (Tilden *et al.*, 1996), means that manure or fecal contamination of large volumes of water can cause illness in susceptible individuals.

**Person-to-person contact.** The low infectious dose of these pathogens means that bacteria present in the stools of infected persons can be passed from one person to another if hygiene or handwashing habits are inadequate. This is particularly likely among toddlers who are not toilet trained. Family members and playmates of these children are at high risk of becoming infected.



### **iii. Foodborne outbreaks of non-O157 STEC in the United States**

Acheson and Keusch stated in 1996 that 'we cannot let ourselves be complacent in thinking that *E. coli* O157 is the only Shiga toxin producing bacteria that can cause problems'. Evidence from targeted clinical studies suggests that new pathogenic forms of non-O157 STEC continue to emerge, and that testing food for *E. coli* O157 alone is insufficient. Worldwide, the list of non-O157 STEC associated with human illnesses consists of over 100 different serotypes (Eklund *et al.*, 2001). Targeted clinical studies are increasing report evidence of sporadic cases of non-O157 STEC infection in the US. For example, Fey *et al.*, (2000) reported data from clinical studies characterizing STEC isolates from several US locations. Their studies involved testing stools submitted to clinical labs for bacterial culture, and cited numerous sporadic cases of illness associated with non-O157 STEC serotypes. Acheson (2001) reported an incidence of STEC positive stools of 0.75%; 54% *E. coli* O157 serotypes, and 46% non-O157 STEC. Several different non-O157 STEC serotypes were detected, including O26, O103, O121, O111, O145, O165, O69, OX25, O6, OX3, O45, O8, O38, O25, O55, O2 and O1, in addition to a previously undetected outbreak associated with serotype O153 (Fey *et al.*, 2000; Acheson, 2001). These researchers concluded that, overall, the non-O157 STEC, in particular strains O26, O55, O103, O111, and O145, are just as prevalent and clinically significant as *E. coli* O157 in the US.

Since 1990, 13 outbreaks of non-O157 STEC have been reported in the US (Table 1). *E. coli* O111 was the most frequently reported serogroup, followed by *E. coli* O121. Multiple pathogens were identified in three of the outbreaks. In particular, two non-O157 STEC serogroups were isolated from one outbreak in 2001 (O111 and O51). Of the non-O157 STEC outbreaks reported, none were attributed to FSIS-regulated products although the exposure or vehicle identified in the majority of the outbreaks was generally unconfirmed.

The first reported outbreak of non-O157 STEC in the US occurred in 1994, and was reported by CDC in 1995. In total, 16 of 18 confirmed and

suspected cases developed bloody stools, diarrhea, and abdominal cramps. Three case-patient isolates were identified as *E. coli* O104:H21. The source of the bacteria was thought to be post-pasteurization contamination of milk at a nearby dairy farm.

In June of 1999, 55 of 521 attendees of a cheerleading camp developed gastroenteritis and two were hospitalized after developing HUS (Brooks *et al.*, 2004). The etiologic agent was determined to be *E. coli* O111:H8. This outbreak was clinically indistinguishable from outbreaks caused by *E. coli* O157. The potential vehicle was narrowed down to a particular lunch meal. In August 2006, Utah health officials reported an outbreak involving lettuce from a fast food restaurant (Berger, 2006). Four people became ill, including three who developed kidney failure. The serotype was identified as *E. coli* O121:H19.

It is important to note the limitations of such outbreak investigations. The ability to detect outbreaks depends upon the identification and reporting of diseases in a timely manner. Because diseases are generally underreported, foodborne outbreaks that are recognized and reported only represent a small proportion of all such outbreaks occurring in the US. State laboratories with the ability to serogroup and/or serotype isolates in-house will be better equipped to identify clusters of non-O157 STEC illnesses. However, not all state laboratories have such resources.

#### **iv. USDA-FSIS investigation of non-O157 STEC illnesses**

In 2006, the Foodborne Disease Investigations Branch (FDIB) of the Office of Public Health Science (OPHS) investigated a case-patient ill with *E. coli* O103 infection who had consumed an undercooked ground beef patty one day prior to illness. The state laboratory tested samples from the patient and leftover uncooked ground beef patties and determined them to be indistinguishable by pulsed-field gel electrophoresis (PFGE). However, FSIS was unable to take further action because of possible cross-contamination in the meat

market grinder between product types and because records were not adequate to determine a specific production.

Also in 2006, FDIB investigated a case-patient ill with *E. coli* O157. Leftover ground beef products were collected and tested by the state department of agriculture. The ground beef samples tested positive for Shiga-toxin but were negative for *E. coli* O157, and the ground beef could not be confirmed as the source of the human illness. Nonetheless, product samples were submitted to the CDC for characterization and *E. coli* O6:H34 was subsequently identified.

#### **v. Non-O157 STEC epidemiology in the US**

**National Foodborne Outbreak Surveillance.** *Escherichia coli* O157 has been nationally notifiable since 1994 (Mead and Griffin, 1998). A decade ago, approximately 50% of clinical labs in the US tested bloody stools only for the most common STEC serotype, *E. coli* O157 (Tarr and Neill, 1996). CDC monitors outbreaks of foodborne disease, including outbreaks caused by STEC, and publishes an annual report<sup>8</sup>. Each year, state and territorial epidemiologists report the results of outbreak investigations to CDC. While outbreaks account for a small percentage of the total number of illnesses that occur each year, these reports provide valuable information about sources of foodborne infection and often highlight important prevention opportunities.

Although the true incidence of non-O157 STEC infection in the US population remains unclear, several studies have attempted to quantify the prevalence of these organisms in symptomatic patients. A Nebraska study assessing the prevalence of non-O157 STEC in diarrheal samples positive for STEC identified five different serotypes (Fey *et al.*, 2000). The conclusions of the authors were that non-O157 STEC serotypes were at least as prevalent as serogroup O157 in this small sample.

A study by Klein (2002) examined 1,851 stool samples taken from pediatric patients in an emergency room of a private clinic over a three-year period. The authors found that 2.1% of stool samples contained non-O157 STEC. They found no cases of HUS associated with non-O157 STEC. Although they concluded that *E. coli* O157 was the predominant STEC in this population,

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<sup>8</sup> <http://www.cdc.gov/mmwr/summary.html>

they recommended a combined diagnostic approach using both SMAC for *E. coli* O157 and Enzyme Immunoassay (EIA) for the presence of Shiga toxin since both tests could miss the potentially causative organisms in some cases.

The most extensive assessment to date was published by Brooks *et al.* in 2005, who summarized data from a convenience sample of sporadic cases confirmed by the CDC. The CDC received 940 isolates from a 20-year-period (1983 to 2002). The top six serogroups identified (O26, O45, O103, O111, O121, O145) accounted for 71% of all the isolates while O26, O103, and O111 accounted for more than 50% of isolates. The non-O157 STEC isolates, as a group, were similar to *E. coli* O157 in seasonality, presence in children, and distribution of Shiga toxin genes. However, serogroup O111 was the only serogroup associated with HUS. Interestingly, it is also the most common serotype found in Australia and Germany (Gerber *et al.*, 2002; Elliott *et al.*, 2001).

**Active Surveillance in FoodNet sites.** In 1997 CDC initiated HUS surveillance as part of CDC's Foodborne Diseases Active Surveillance Network (FoodNet). FoodNet is a collaborative project of the CDC, ten Emerging Infections Program sites (CA, CO, CT, GA, MD, MN, NM, NY, OR, and TN), USDA, and FDA. The project consists of active surveillance for foodborne diseases and related epidemiological studies. The core of FoodNet is laboratory-based active surveillance at over 650 clinical laboratories that test stool samples in the ten FoodNet sites. In active surveillance, the laboratories in the catchment areas are contacted regularly by collaborating FoodNet investigators to collect information on all of the laboratory-confirmed cases of diarrheal illness (CDC, 2006b).

In the HUS surveillance study, pediatric nephrologists in catchment areas for sites are regularly surveyed, while adult cases are reported in a passive system, as are cases outside of catchment areas. Thus, HUS caused by non-O157 STEC is more routinely identified (Table 2).

In 2000, the Council for State and Territorial Epidemiologists passed a resolution under which all Shiga toxin-producing *E. coli* were made nationally notifiable under the name Enterohemorrhagic *E. coli* (EHEC); the CDC currently defines Enterohemorrhagic *Escherichia coli* as (a) Enterohemorrhagic *Escherichia coli* O157: H7 or (b) Enterohemorrhagic *Escherichia coli* Shiga toxin positive (not serogrouped) or (c)

Enterohemorrhagic *Escherichia coli* Shiga toxin positive (serogroup non-O157). Information on the actual serotype (other than *E. coli* O157) is not routinely collected, however, and the CDC stated in its summary of notifiable diseases for 2001 (CDC, 2003) that “the number of cases reported for EHEC should be interpreted as an underestimate in a maturing surveillance system”, because few stool specimens are tested in a way that would identify Shiga toxin-producing *E. coli* other than *E. coli* O157. National surveillance for all STEC under this definition began in 2001. Surveillance for laboratory-confirmed cases of *E. coli* O157 has been conducted since FoodNet was established in 1996. In 2000, FoodNet began surveillance for laboratory-confirmed cases of non-O157 STEC infections (Figure 1). From 2000-2006, FoodNet reported 626 laboratory-confirmed cases of non-O157 STEC infection; ranging from 35 in 2002 to 209 cases in 2006 (Figure 2). O-antigen information was available for 488 of the 626 (78%) laboratory-confirmed non-O157 STEC infections. A total of 33 different O antigens were documented; O111 (27%), O103 (21%), and O26 (21%) were the most commonly identified non-O157 serogroups (Table 3). An additional 85 STEC cases with O-antigen undetermined were ascertained during this time period. The majority of cases are identified during the summer months (Figure 3). Information on age was available for 401 (96%) cases; of these, 28% were less than 5 years old (Table 4); 51 (14%) of all cases were hospitalized. Between 2000 and 2005, one death was associated with a non-O157 STEC infection.

**Survey of non-FoodNet sites.** FSIS OPHS epidemiologists examined surveillance data on non-O157 STEC illnesses from 2004 through 2005 was examined to evaluate the public health impact of these organisms, and to determine potential exposures by serogroup, thereby providing additional information on the impact of FSIS-regulated products on illnesses. Foodborne disease epidemiologists in selected states were asked to provide information on the number of *E. coli* O157 and non-O157 STEC case-patients over the study period, any available serotype information on the isolated non-O157 STEC, and any consumption/exposure history. Thirteen sites responded, and eight sites provided exposure histories.

Of all STEC reported 79% were *E. coli* O157 and 18% were non-O157 STEC. Of the non-O157 STEC, the most commonly reported serogroups were O26 (30%), O103 (18%), O111 (12%), O121 (12%), and O45 (13%). The largest

percentages of non-O157 STEC were reported in Virginia (53%), followed by Utah (40%) and Wyoming (32%).

Live animal exposure was cited as a potential vehicle in 55% of cases, including pet dogs and cats, farm animals, birds, reptiles, and even a giraffe. Sixty-three percent of case-patients with STEC serotype O26 reported live animal exposures. Thirty-three percent of non-O157 STEC case-patients reported beef consumption, and 34% reported consumption of other meats. However, evaluation of the food consumption data was difficult: only sparse food history information was available, and individual case-patients often reported multiple food exposures.

It is important to reiterate that the data presented here are incomplete, and do not provide a reliable estimate of the total burden of non-O157 STEC in the US. Several factors influence surveillance data, including laboratory practices, and these are known to vary across sites from which these data were collected.

#### **vi. Non-O157 STEC illnesses in other countries**

Worldwide, the list of non-O157 STEC associated with human illnesses consists of over 100 different serotypes (Eklund *et al.*, 2001). While *E. coli* O157 is the principal STEC strain isolated from implicated food and clinical isolates in the US, non-O157 STEC predominate in other countries including Australia, Brazil, Canada, Germany and the UK, among others (Beutin *et al.*, 1998; Dekoninck *et al.*, 1998; Keskimäki *et al.*, 1998; Pradel *et al.*, 2000; Baffone *et al.*, 2001; Guth *et al.*, 2003) (Table 5). Many studies have been performed throughout the world to assess the prevalence of *E. coli* O157 and other STEC in retail foods, in order to determine the public health risk posed by these organisms. Limited comparisons can be made, however, due to the variety of sampling and testing methods, and study designs used.

### **Section III. Methods**

#### **i. Difficulties in distinguishing non-O157 STEC from non-pathogenic *E. coli*.**

Samadpour *et al.*, (1994) were among the first to speculate that the apparent predominance of *E. coli* O157:H7 among STEC in the US was probably a result of its relative ease of isolation, and stated that the lower perceived incidence of non-O157 STEC in comparison to *E. coli* O157:H7 was likely a consequence of the detection and isolation methods used, which selectively excluded most non-O157 STEC. As already stated, the detection of toxin or toxin genes, the single 'universal' virulence factor among STEC, is not sufficient to formulate a definite association with illness as some strains may produce toxin but lack other necessary virulence factors (as seems to be the case in many non-bovine ruminant strains). Mounting data on virulence factors associated with STEC has led to the development of targeted molecular techniques for their detection while concurrently demonstrating the genetic heterogeneity of these organisms. The ongoing development of new technologies has provided an expanded capability for testing isolates, using toxin and/or molecular based technologies, which do not focus on phenotypic characteristics.

The difficulty in distinguishing pathogenic non-O157 STEC strains from non-pathogenic commensal *E. coli* obscures the true clinical significance of non-O157 STEC in the US. The perceived lack of a problem with non-O157 STEC has resulted in little commercial or research interest in developing reliable 'routine' detection methods for these organisms, thereby contributing to the fact that foods are generally not routinely tested for non-O157 STEC, and the magnitude of the problems associated with non-O157 STEC remains largely unrecognized. However, data from targeted epidemiological investigations, clinical studies, lab diagnoses in individual cases, and the increased incidence of HUS demonstrate that the increasing significance of non-O157 STEC becomes apparent when diagnostic methods that can detect these serotypes are employed (Keskimäki *et al.*, 1998). This has resulted in an increasing awareness among the scientific community of the proliferation of non-O157 STEC in the environment and in cases of human gastrointestinal illness.

## **ii. Considerations when choosing an analytical method for non-O157 STEC.**

Goldwater and Bettelheim (2000) stated that “the current heavy reliance on very specific tests based on either the use of specific nucleic acid sequences or monoclonal antibodies will become a severe disadvantage in detecting ...newly emerging pathogens.” It was precisely such selective procedures, in a more low-tech format, i.e. the development of the highly selective SMAC medium in 1986 by March and Ratnam, which contributed to the delay in recognition of the prevalence and importance of non-O157 STEC. Finally, and perhaps most significantly for most laboratories, it may be necessary to use a specific analytical method, or one deemed equivalent to some specified standard, e.g. an AOAC approved method.

In order to establish routine testing of food for non-O157 STEC, regulatory Agencies would be faced with the task of choosing the most appropriate method for non-O157 STEC detection for their purposes, mindful of the above considerations, with the exception that there are as yet no existing standards to meet. Indeed, the choices such agencies might make in regards to this pathogen would likely influence future research into this organism.

## **iii. Testing for *E. coli* O157 and non-O157 STEC.**

The difficulties inherent in the isolation and identification of non-O157 STEC are well recognized. When reporting on the 1995 mettwurst STEC outbreak in Australia, which was ultimately linked to *E. coli* O111, Goldwater and Bettelheim (1996) stated that when investigating the cause of this outbreak, “if (their) laboratory had to rely on conventional microbiologic culture procedures, including sorbitol-MacConkey agar, strains of serogroup O157 would have been identified from three patients, as well as from the epidemiologically incriminated mettwurst. The laboratory would not have found the O111 strains because they all fermented sorbitol readily and would have been discarded as normal flora as would the other enterohemorrhagic *E. coli* serotypes”. Some years later Bettelheim (2003) stated that the relatively easy identification of non-O157 STEC, in the same manner as *E. coli* O157 “may never be achievable”. As already mentioned, the problem is the lack of a single distinctive phenotypic characteristic common to all pathogenic non-O157 STEC that can be reliably harnessed to some selective medium, biochemical test, or other procedure. Existing technologies for the



detection of *E. coli* O157 could potentially be adapted by manufacturers of diagnostics products for the development of non-O157 STEC detection methods, if they perceived a commercial market for these products. An unscientific poll of exhibitors at the 2007 International Association for Food Protection (IAFP) Annual Meeting revealed that while the manufacturers are aware of the existence of non-O157 STEC and the associated problems, as yet there is little impetus for them to develop commercial kits for the detection of these organisms, because there is no market for them: food producers, even those with large *E. coli* O157 testing programs, generally do not test their products for these organisms. Without a drive to push development of non-O157 STEC methods, the introduction of regulatory testing requirements might result in the adoption of sub-optimal technology by the industry, in the initial rush to introduce testing programs.

Commercial testing systems for *E. coli* O157 and non-O157 STEC detection and identification are presented here. *E. coli* O157 test methods are detailed because it is quite likely that non-O157 STEC-specific tests will be developed as an extension of an *E. coli* O157 test.

#### **iv. Method Components.**

The basic routine for pathogen detection can be broken down into three stages: enrichment, screening and confirmation.

**Enrichment.** When choosing an enrichment medium appropriate for the isolation of a target organism in a substrate such as raw ground beef (or any material commonly host to heterogeneous microflora), it is important to strike the right balance between providing sufficient nutrients for the target organism to be resuscitated (if necessary) and grow, while restricting the growth of non-target organisms. An ideal medium will have high productivity without compromising selectivity, and will allow the target organism to proliferate while suppressing background flora. For non-O157 STEC (including serotypes O26 and O111) this has been achieved by enrichment in media that have lower nutrient levels (e.g. buffered peptone water (BPW)) than other, richer media (e.g. tryptone soy broth (TSB)), and incubating at 41-42°C rather than at 37°C (Catarama *et al.*, 2003; Drysdale *et al.*, 2004). Addition of vancomycin (8mg/l) has been reported as optimal for non-O157 STEC recovery (Drysdale *et al.*, 2004). Many researchers have found that using mEC+N (i.e. *E. coli* broth with 1.12 g/l bile salts, and 20mg novobiocin/l), the enrichment medium for *E. coli* O157 detection used by

FSIS, results in significantly reduced recovery of non-O157 STEC (e.g. Drysdale *et al.*, 2004). Many of the *E. coli* O157 screening methods listed below have attendant suggested enrichment broths: it is likely that development of screening methods specific for non-O157 STEC would include the development of optimal enrichment broths.

**Screening.** Few commercially available screening kits have been developed for non-O157 STEC, for reasons already discussed. Those on the market include Denka Seiken's EHEC Immunomagnetic Separation (IMS) kits for *E. coli* O157, *E. coli* O26 and *E. coli* O111; and Dynal Biotech's Dynabeads® for *E. coli* serotypes O26, O103, O111, O145 and O157, which also use IMS. Another commercial test for non-O157 STEC is the Oxoid Dryspot™ *E. coli* Seroscreen, a single-screen latex agglutination system that can detect serotypes O26, O91, O103, O111, O128, O145 and O157. Screening tools for *E. coli* O157 are presented below:

- PCR-based assays

PCR assays can be completed in as few as 2 to 4 hours post-enrichment, acting as an effective, rapid and very specific screening tool. Conventional PCR assays require amplification of one specific target gene in a thermocycler, separation of PCR products by gel electrophoresis, followed by visualization and analysis of the resultant electrophoretic patterns. The Marshfield Clinic *E. coli* O157 Test Method<sup>9</sup>, which can detect the *eae* gene present in *E. coli* O157 and other EHEC strains with a confirmed positive or negative result in less than 12 h, is an example of such an assay.

Multiplex PCR assays increase the specificity of the test by allowing multiple genes to be targeted at once. A carefully designed multiplex PCR can be used as a screen to test composite samples as it allows rapid detection of any STEC carrying any individual or combination of the target genes (Sharma *et al.*, 1999). Fratamico *et al.* developed the first multiplex PCR for *E. coli* O157 in 1995, and since then gene clusters specific to many clinically significant non-O157 STEC serotypes including *E. coli* O26 (D'Souza *et al.*, 2002), *E. coli* O103 (Fratamico *et al.*, 2005), *E. coli* O111 (Wang *et al.*, 1998), *E. coli* O121 (Fratamico *et al.*, 2003), *E. coli* O145 (Perelle *et al.*, 2003), among others (Paton and Paton, 1998; Sharma *et al.*, 1999;

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<sup>9</sup> AOAC Performance Tested Method 070502

Fratamico *et al.*, 2000; DebRoy *et al.*, 2004; Perelle *et al.*, 2004) have been identified and incorporated into multiplex PCRs.

Commercially available kits that use PCR for the detection of *E. coli* O157 in raw ground beef include Applied Biosystems' TaqMan® *Escherichia coli* O157:H7 Detection Kit, BioControl's Genetic Detection System<sup>10</sup> O157 Assay and Shiga-toxin gene assay, the Dupont BAX system<sup>11</sup>, (currently used by FSIS to screen for *E. coli* O157), and Warnex Diagnostics' Genevision™ Rapid Pathogen Detection *Escherichia coli* O157 and *E. coli* O157:H7 kits.<sup>12</sup>

- Shiga toxin tests

The vero cell assay has been regarded as the "gold standard" for Shiga toxin detection since the discovery of this toxin family by Konowalchuk *et al.* in 1977. However, the constraints inherent in tissue culture, as well as the associated prolonged turnaround time for results and lack of specificity with this procedure, mean that this method is not routinely used for the screening of STEC (Rahn *et al.*, 1996), though it can be used for confirmation purposes.

Commercial kits for Shiga toxin detection include the Antex Biologica VeroTest, the Diffchamb Transia Plate Verotoxin kit, the Merck Duopath Verotoxin test<sup>13</sup>, Meridian Diagnostics' Premier™ EHEC, Oxoid's VTEC-RPLA (Reversed Passive Latex Agglutination) for VT1 and VT2, and r-Biopharm's RidaScreen Verotoxin test. Most tests require an isolated colony, however, thereby significantly increasing the time required for a result.

- Immunoassay based methods

There is a wide variety of immunoassay-based methods available, each typically involving a pre-enrichment (from <8h to 24+h) of the test sample followed by specific detection of the cellular antigen in either a lateral flow device or by immunomagnetic capture (10-45 min post-enrichment). Commercially available lateral flow systems include Strategic Diagnostic's RapidChek® system<sup>14</sup>, Diffchamb's Transia™ Card *E. coli* O157:H7<sup>15</sup> (both of which are listed as *E. coli* O157 screening tools in Chapter 5 of the FSIS

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<sup>10</sup> AOAC Official Methods AOAC 2005.04 and AOAC 2005.05 respectively

<sup>11</sup> AOAC Performance Tested Method Nos. 010401 and 050501.

<sup>12</sup> AOAC Performance Tested Methods 010408 and 010409 respectively.

<sup>13</sup> AOAC Performance Tested Method 020402

<sup>14</sup> AOAC Performance Tested Method 030301

<sup>15</sup> AOAC Performance Tested Method 010402

Microbiology Laboratory Guidebook), BioControl's VIP for EHEC<sup>16</sup>, the Centrus International Envisio system, DuPont's™ Lateral Flow System™<sup>17</sup>, Meridian Diagnostics ImmunoCard STAT!<sup>®</sup> *E. coli* O157 Plus, Merck's Singlepath<sup>®</sup> *E. coli* O157<sup>18</sup>, and the Neogen Reveal<sup>®</sup> system<sup>19</sup> (formerly used by FSIS to screen for *E. coli* O157).

Some non-lateral flow immunoassay-based tests include BioControl Systems' Assurance EHEC EIA<sup>20</sup>, the bioMérieux VIDAS<sup>®</sup> *E. coli* O157 (ECO) test with O157:H7 ID Agar<sup>21</sup>, Diffchamb's Transia Plate *E. coli* O157<sup>22</sup>, IGEN International's PATHIGEN *E. coli* O157 test<sup>23</sup>, the Matrix Bioscience PATHATRIX *E. coli* O157 test system<sup>24</sup>, Neogen's GeneQuence™ *E. coli* O157, and TECRA's *E. coli* O157 VIA<sup>25</sup>.

Several immuno-latex agglutination kits for *E. coli* O157 are available, including the Denka Seiken EHEC O157 kit already mentioned, Microgen Bioproducts Ltd. Microscreen *E. coli* O157, Pro-Lab Diagnostics' Prolex™ *E. coli* O157 with *E. coli* H7 Flagellar Antigen Latex Reagent test, Remel's RIM *E. coli* O157:H7 Latex Test, and the Wellcolex *E. coli* O157 and O157:H7 tests.

- Other Methods

Neogen's ISO-GRID™ for enumeration of total *E. coli* and *E. coli* O157:H7<sup>26</sup> utilizes hydrophobic grid membrane filter technology to detect and quantify target organisms. The BioControl Assurance GDS™ *E. coli* O157:H7 assay<sup>27</sup> and Shiga Toxin gene assay<sup>28</sup> combine technologies by incorporating immunomagnetic separation and highly specific primers in their system for the detection of *E. coli* O157 and the Shiga toxin gene respectively.

**Confirmation.** A positive test result for *E. coli* O157 by any of the above screening tests is generally confirmed by a PCR for the H7 antigen in industry testing, where time is of the essence. Confirmation of the presence of Shiga

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<sup>16</sup> AOAC Official Method 996.09.

<sup>17</sup> AOAC Performance Tested Method 010401

<sup>18</sup> AOAC Performance Tested Method 010407)

<sup>19</sup> AOAC Official Method 2000.13 (8h enrichment) and AOAC Official Method 2000.14 (20h enrichment)

<sup>20</sup> AOAC Official Method 996.10

<sup>21</sup> AOAC Performance Tested Method 010502

<sup>22</sup> AOAC Performance Tested Method 040401

<sup>23</sup> AOAC Performance Tested Method 010301

<sup>24</sup> AOAC Performance Tested Method 030202

<sup>25</sup> AOAC Performance Tested Method 001101

<sup>26</sup> AOAC Official Method 997.11

<sup>27</sup> AOAC Official Method 2005.04

<sup>28</sup> AOAC Official Method 2005.05

toxin and additional cultural methods are required for FSIS test samples. Initially, STEC culture media development was based on the phenotypic characteristics of *E. coli* O157. In 1986, March and Ratnam developed sorbitol MacConkey agar (SMAC) as a selective agar for the detection of *E. coli* O157, based on the inability of most *E. coli* O157 to ferment sorbitol, and although sorbitol-fermenting *E. coli* O157 have subsequently been identified that do ferment sorbitol (Karch *et al.*, 1997; Bielaszewska *et al.*, 1998) SMAC remains the selective medium of choice for identifying this organism. Potassium tellurite and cefixime have been added to SMAC to decrease the numbers of background flora (Zadik *et al.*, 1993). In addition, a number of selective agar supplements have been designed based on the inability of *E. coli* O157 to produce  $\beta$ -Glucuronidase (Doyle and Schoeni, 1984), though a small number of *E. coli* O157 have subsequently been identified as  $\beta$ -Glucuronidase-positive (Keskimäki *et al.*, 1998). Other selective media for *E. coli* O157 include Rainbow Agar™ (Bettelheim, 1998a), CHROMagar™ (Bettelheim, 1998b), and bioMérieux' O157:H7 ID; all chromogenic media that rely on reactions that distinguish target colonies from background flora by color changes. Unfortunately, most of these media will not permit distinction of non-O157 STEC from background flora, and, as yet there is not one medium for non-O157 STEC that is as widespread and as validated as SMAC agar is for *E. coli* O157. However, media have been developed to exploit the enterohemolytic characteristic of STEC (Sugiyama *et al.*, 2001). Such media are composed of blood agar with various additives, e.g. BVCC (blood agar with vancomycin cefixime cefsulodin) and WMBA (washed sheep blood agar with mitomycin C). The addition of rhamnose to MacConkey agar (RMAC) results in a medium selective for *E. coli* O26, with target colonies growing a distinctive brown color (Hiramatsu *et al.*, 2002). Research is ongoing in this field to increase the specificity of selective media for non-O157 STEC.

#### **Section IV. Conclusion**

Examination of FoodNet (2001 – 2006) and national surveillance data (2004-2005) shows a continued increase in the number of reported non O157 STEC infections. Of the five most commonly reported serogroups from both FoodNet sites and non-FoodNet sites, four are shared between both groups (although ranking varies): O26, O45, O103, and O111. The fifth most common serogroup for FoodNet sites is O145, for non-FoodNet sites it is O121. This is consistent with an analysis of non-O157 STEC isolates submitted to CDC, which documented these six as the most commonly isolated serogroups (Brooks *et al.*, 2005). The number of reported cases in FoodNet sites increased 256% from 2000 to 2005, while national surveillance data have documented a 193% increase from 2001 to 2005. Additionally, national surveillance data have shown a 1935% increase in EHEC that has not been serogrouped over the same time-period, while *E. coli* O157 decreased 20%. This demonstrates that although the incidence of *E. coli* O157:H7 infection decreased over this time period, the incidence of infection with non-O157 STEC serotypes did not follow the same trend.

The Healthy People 2010 objective for the incidence of *E. coli* O157:H7 infection was set as 1.00 infections per 100,000 population; no national health objective has been set for non-O157 STEC infections. The preliminary overall 2006 incidence of non-O157 STEC infection in FoodNet sites was 0.46 per 100,000 population, ranging from 0.12 per 100,000 in Tennessee to 1.19 per 100,000 in New Mexico. Several non-FoodNet sites had 2005 non-O157 STEC incidence rates above 1.00 per 100,000; for example, Idaho with 1.26 per 100,000 and Utah with 1.21 per 100,000, reflecting incidences similar to those seen in FoodNet sites in recent years, and those for STEC O157 infection. Furthermore, Virginia reported more cases of non-O157 STEC than *E. coli* O157:H7. This suggests not only regional differences in incidence, but a higher burden of illness than was previously thought.

Efforts by the research community to identify factors associated with the presence and persistence of non-O157 STEC will facilitate development of targeted, successful mitigation strategies, as has been the case for *E. coli* O157:H7. However, this will likely not be an easy task: Schurman *et al.* (2000) stated that there is 'no one area in the food chain where stringent intervention will eliminate STEC as a concern. Highly controlled slaughter is crucial to the production of safe food, but not to the exclusion of other critical

control points in the food chain, beginning on the farm and ending on the consumers table.'

FSIS is unique among the world's public health agencies in the way it routinely tests raw ground beef and raw ground beef components for *E. coli* O157, and in its ability to request a recall of any product found to be contaminated with this organism. This has led to significant changes in the raw ground beef production industry since *E. coli* O157 was declared an adulterant in 1994. Manufacturers, in their desire to produce a safe product, have implemented many targeted food safety interventions that have been proven to significantly reduce the numbers of ground beef samples testing positive for *E. coli* O157 (Naugle *et al.*, 2005). Non-O157 STEC pose just as great a risk to public health as *E. coli* O157. However, the introduction of a new regulatory requirement to combat these organisms will not be undertaken lightly. Even with the overwhelming evidence of the inherent dangers of non-O157 STEC, and the availability of options presented for their detection and identification in food, the cost and other practicalities of the introduction of control measures must be carefully considered.

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**Table 1. Outbreaks of Shiga toxin-producing *E. coli* non-O157 infections in the United States, 1990-2006 \***

Year	Serogroup	State	Number of Ill Persons	Serologically Confirmed	Suspected Exposure or Vehicle	HUS Reported	Exposure or Vehicle Confirmed
1990	O111 <sup>†</sup>	Ohio	5	Yes	Unknown	Yes	No
1994	O104 <sup>†</sup>	Montana	18	Yes	Milk	No	No
1998	O121 <sup>‡</sup>	Montana	40	Unknown	Unknown	Unknown	Unknown
1999	O111 <sup>†</sup>	Texas	56	Yes	Salad Bar	Yes	No
	O121 <sup>†</sup>	Connecticut	11	Yes	Lake Water	Yes	No
2000	O103 <sup>†</sup>	Washington	18	Yes	Punch	Yes	No
	O111 <sup>§</sup>	Minnesota	59 <sup>□</sup>	Yes	Calves	No	No
2001	O26 <sup>†</sup>	Minnesota	4	No	Lake Water	No	No
	O111 <sup>§</sup> O51 <sup>¶</sup>	Minnesota	25 <sup>¶</sup>	Yes	Calves	No	Yes, Calves
	O111 <sup>†</sup>	South Dakota	3	No	Daycare	No	No
2004	O111 <sup>‡</sup>	New York North Carolina <sup>**</sup>	212 <sup>††</sup>	Yes	Unpasteurized Apple Cider	No <sup>**</sup>	Yes, Environmental Sample
2005	O45 <sup>‡‡</sup>	New York	52 <sup>§§</sup>	Yes	Ill Food Worker	No	No
2006	O121 <sup>□□</sup>	Utah	4	Yes	Lettuce	Yes	No

Table adapted from Brooks *et al*, 2005.

\*Provisional data from January 1, 2006 to June 30, 2006

<sup>†</sup> Brooks, JT, *et al*. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. *J Infect Dis*. 2005; 192:1426.

<sup>‡</sup>CDC outbreak surveillance data

<sup>§</sup>Minnesota Department of Health Disease Control Newsletter, Volume 33, Number 2 (pages 13-20), March/April 2005

<sup>□</sup> Smith KE, *et al*. Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatr Infect Dis J*. 2004; 23:1098-104; two case-patients tested positive for O111 as part of a camp outbreak, *Cryptosporidium parvum*, *Campylobacter jejuni*, and *Salmonella* Typhimurium were also identified among other confirmed case-patients

<sup>¶</sup> Smith KE, *et al*. Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. *Pediatr Infect Dis J*. 2004; 23:1098-104; 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, *E. coli* O157:H7 and *Cryptosporidium parvum* were also identified among other confirmed case-patients

<sup>\*\*</sup> CDC Electronic Foodborne Outbreak Reporting System (eFORS)

<sup>††</sup> *Escherichia coli* O111 and *Cryptosporidium parvum* were identified



<sup>##</sup> CDC. Importance of Culture Confirmation of Shiga Toxin-producing *Escherichia coli* Infection as Illustrated by Outbreaks of Gastroenteritis --- New York and North Carolina, 2005. MMWR Weekly. 2006; 55:1042-1045

<sup>##</sup>Sixteen samples were stx1 positive by PCR; three samples sent to the CDC were positive for O45:NM

□□Weber-Morgan Health Department August 7, 2006 *E. coli* News Release

**Table 2. Non-O157 STEC infections by serogroups, 2000-2005<sup>29</sup>**

Serogroup	Year						2000-2005	
	2000	2001	2002	2003	2004	2005	n	%
	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>		
0	0	3	0	0	0	0	3	0.8
8	1	0	0	0	0	0	1	0.3
22	0	0	0	0	2	1	3	0.8
26	5	17	9	7	12	31	81	21.2
33	0	0	0	1	0	0	1	0.3
45	3	8	3	4	6	7	31	8.1
51	0	1	0	0	0	1	2	0.5
76	0	0	0	1	1	2	4	1.0
84	0	0	0	1	0	0	1	0.3
87	0	0	0	0	0	1	1	0.3
88	1	1	0	0	1	0	3	0.8
91	1	1	1	0	0	3	6	1.6
103	6	13	6	9	15	30	79	20.7
110	0	0	0	0	1	0	1	0.3
111	10	11	9	13	41	20	104	27.2
113	0	0	0	1	0	0	1	0.3
117	0	0	0	0	0	1	1	0.3
118	1	1	0	2	1	1	6	1.6
119	1	0	0	0	0	0	1	0.3
121	2	0	2	3	0	8	15	3.9
126	0	0	0	0	1	0	1	0.3
128	0	1	0	0	0	0	1	0.3
132	0	0	0	0	1	0	1	0.3
145	3	2	2	3	5	3	18	4.7
146	0	0	2	0	0	1	3	0.8
153	0	0	1	0	1	1	3	0.8
159	1	0	0	0	0	0	1	0.3
163	1	0	0	0	0	0	1	0.3
165	0	1	0	2	0	0	3	0.8
174	0	1	0	0	2	0	3	0.8
177	0	0	0	0	0	1	1	0.3
181	0	0	0	0	0	1	1	0.3
<b>Total serogroups</b>	<b>36</b>	<b>61</b>	<b>35</b>	<b>47</b>	<b>90</b>	<b>113</b>	<b>382</b>	<b>100.0</b>
<i>Missing serogroups</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>20</i>	<i>15</i>	<i>35</i>	

<sup>29</sup> Source: CDC, 2006. Personal communication.

**Table 3. Number of laboratory-confirmed non-O157 STEC infections ascertained in FoodNet, by age group, 2000-2005<sup>30</sup>**

<b>Age Categories</b>	<b>Non-O157 STEC</b>	
	<b><i>n</i></b>	<b>%</b>
<5	112	27.9
5-9	50	12.5
10-19	104	25.9
20-29	41	10.2
30-39	25	6.2
40-49	18	4.5
50-59	16	4.0
60-69	15	3.7
70+	20	5.0
<b>Total responses</b>	<b>401</b>	<b>100.0</b>
<i>Missing responses</i>	<i>16</i>	

<sup>30</sup> Source: CDC, 2006. Personal communication.

**Table 4. Shiga-toxin *E. coli* O157 and non-O157 in 13 non-FoodNet states, United States, 2004-July 2006<sup>\*31</sup>**

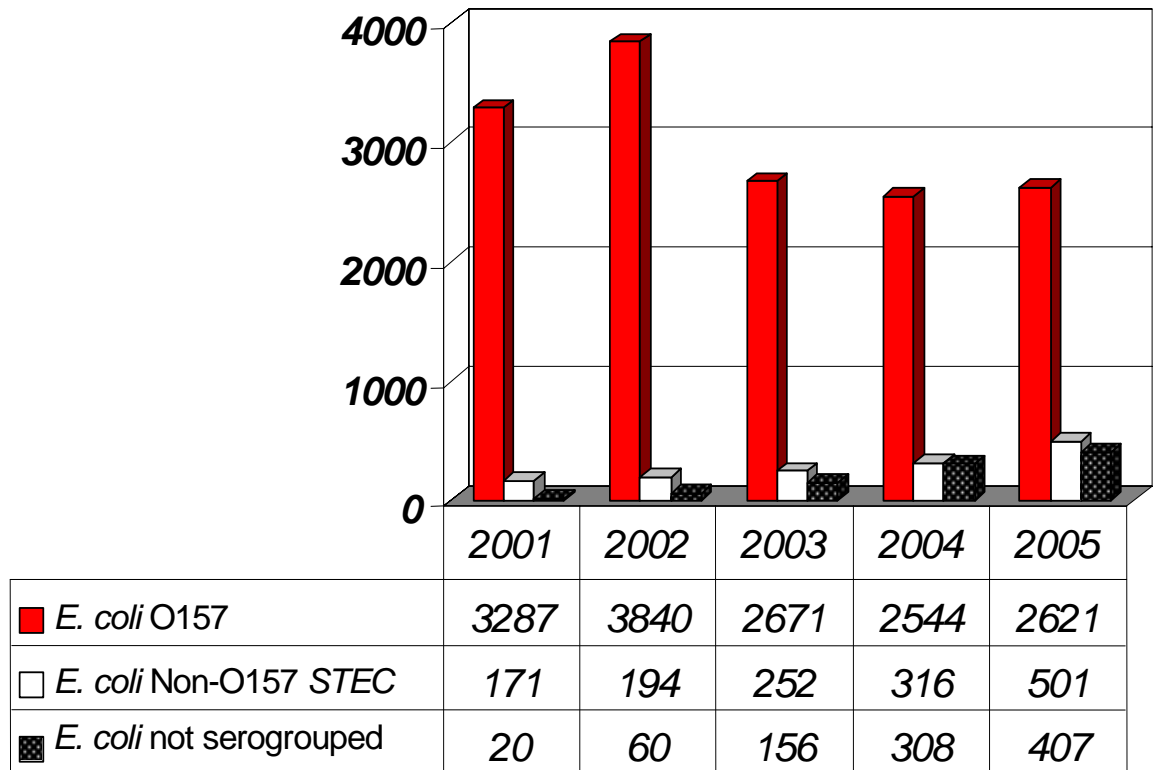
	CO	ID	FL	MI	MO	NC	OK	PA	SD	UT	VA	WA	WY		
<b><i>E. coli</i></b>															
<b>O157</b>	145	129	153	196	200	135	70	150	67	99	113	361	28	1846	78.5%
<b>non-O157</b>															
<b>STEC</b>	19	39	14	19	42	20	6	51	2	58	130	6	13	419	17.8%
<b>O4</b>	1													1	0.4%
<b>O8</b>								1						1	0.4%
<b>O11</b>	2													2	0.7%
<b>O19</b>					1									1	0.4%
<b>O26</b>	10	15		1	8	2	1	8		26	9	3		83	30.4%
<b>O28</b>		1												1	0.4%
<b>O33</b>					1									1	0.4%
<b>O45</b>				5		7	1	16		1	5			35	12.8%
<b>O49</b>				1										1	0.4%
<b>O51</b>							1							1	0.4%
<b>O63</b>					1									1	0.4%
<b>O77</b>								1						1	0.4%
<b>O84</b>								1						1	0.4%
<b>O91</b>					2					1	1			4	1.5%
<b>O103</b>	2	3		2	9	6		13		4	10		1	50	18.3%
<b>O111</b>	2	7		2	2	2		4	1	9	1			30	11.7%
<b>O112</b>	1													1	0.4%
<b>O116</b>								1						1	0.4%
<b>O118</b>								1						1	0.4%
<b>O121</b>		10				2		1	1	15		1	2	32	11.7%
<b>O128</b>		1												1	0.4%
<b>O156</b>	1													1	0.4%
<b>O145</b>		1		2	2	1		2		1	1		1	11	4.0%
<b>O158</b>											1			1	0.4%
<b>O165</b>		1			2			2						5	1.8%
<b>O174</b>												1		1	0.4%
<b>O177</b>											1	1		2	0.7%
<b>O179</b>					1									1	0.4%
<b>O181</b>					1									1	0.4%
<b>missing</b>	0	0	14	6	12	0	3	0	0	1	101	0	9	146	
<b>STEC</b>															
<b>O undet</b>	2	6	53	2	0	0	2	8	5	1	3	0	4	86	3.7%

<sup>31</sup> Source: FSIS Human Health Sciences Division (2006) Internal report.

**Table 5. Prevalence of STEC in retail foods in non-US countries**

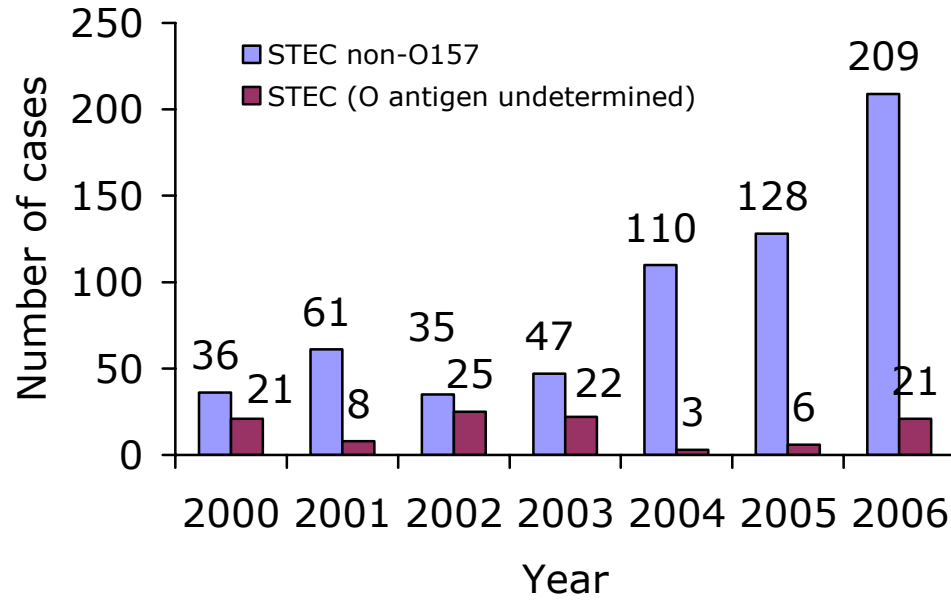
<b>Country</b>	<b>Organism</b>	<b>Reported prevalence</b>	<b>Reference</b>
Argentina	<i>E. coli</i> O157:H7	4.8% of fresh sausages; 3.8% of raw ground beef; 3.3% of dry sausages	Chinen <i>et al.</i> , 2001
Belgium	All STEC	4.6% of raw meat samples (beef, mutton and venison)	Pierard <i>et al.</i> , 1997
Botswana	<i>E. coli</i> O157:H7	5.2% of meat cubes; 3.8% of raw ground beef; 2.3% of fresh sausages	Magwira <i>et al.</i> , 2005
England	<i>E. coli</i> O157:H7	2.9% of lamb products; 1.1% of beef products	Chapman <i>et al.</i> , 2000
France	All STEC	11% of beef; 10% of cheese	Pradel <i>et al.</i> , 2000
France	<i>E. coli</i> O157:H7	0.1% of raw ground beef	Vernozy-Rozand <i>et al.</i> , 2002
India	non-O157 STEC	2/60 fish samples; 3/48 clam samples	Sanath Kumar <i>et al.</i> , 2001
Italy	<i>E. coli</i> O157:H7	0.4% of raw ground beef	Conedera <i>et al.</i> , 2004
New Zealand	All STEC	17% of lamb; 12% of beef; 4% of pork; 0% of chicken	Brooks <i>et al.</i> , 2001
Sweden	<i>E. coli</i> O157:H7 All STEC	0.06-0.5% of raw ground beef 4% of raw ground beef	Lindqvist <i>et al.</i> , 1998

**Figure 1. Cases of enterohemorrhagic *E. coli* reported in the United States, 2001-2005<sup>32</sup>**



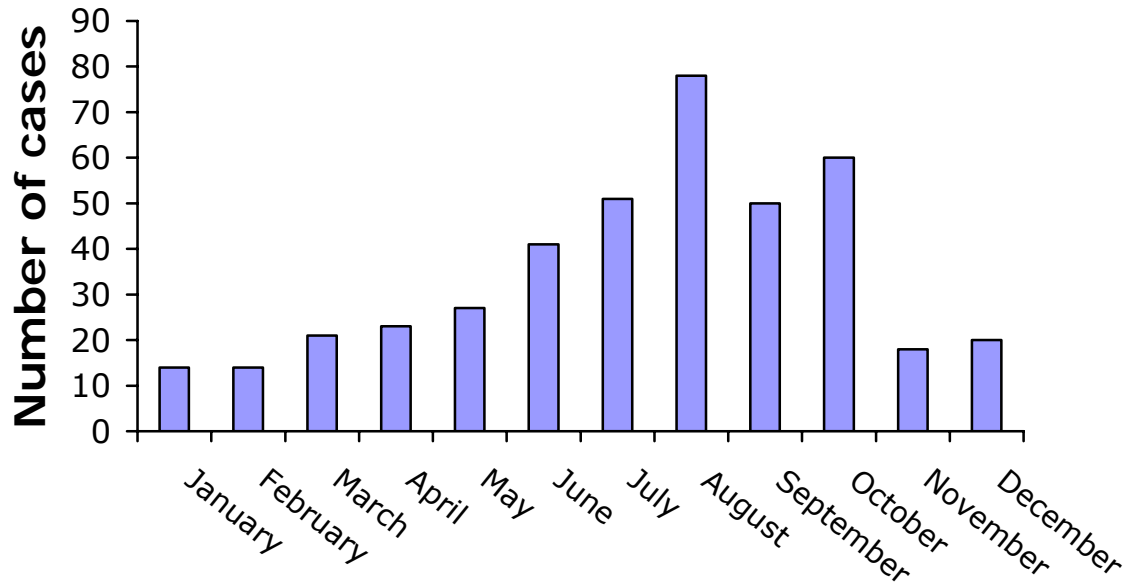
<sup>32</sup> Source: CDC (2006) Summary of Notifiable Diseases, 2001-2005. *MMWR*

**Figure 2. Laboratory-confirmed non-O157 STEC and O-antigen undetermined infections ascertained in FoodNet, 2000-2006<sup>33</sup>**



<sup>33</sup> 2006 - preliminary data

Figure 3. Laboratory-confirmed non-O157 STEC infections ascertained in FoodNet by month, 2000-2005<sup>34</sup>



<sup>34</sup> Source: CDC, 2006. Personal communication.