Shiga Toxin Producing

*Escherichia coli*

Funded by The Beef Checkoff
Shiga Toxin Producing *Escherichia coli*

**Contents**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defining STEC</td>
<td>1</td>
</tr>
<tr>
<td>Virulence Markers</td>
<td>2</td>
</tr>
<tr>
<td>Diagnosis of Shiga Toxin-producing <em>E. coli</em></td>
<td>4</td>
</tr>
<tr>
<td>STEC in Humans</td>
<td>5</td>
</tr>
<tr>
<td>Diseases</td>
<td>5</td>
</tr>
<tr>
<td>STEC in Foods</td>
<td>7</td>
</tr>
<tr>
<td>Beef</td>
<td>7</td>
</tr>
<tr>
<td>Other Meat and Poultry</td>
<td>8</td>
</tr>
<tr>
<td>Fruits, Vegetables and other</td>
<td>8</td>
</tr>
<tr>
<td>STEC in Water and Environment</td>
<td>10</td>
</tr>
<tr>
<td>STEC in Food-producing Animals and Pets</td>
<td>11</td>
</tr>
<tr>
<td>Diseases</td>
<td>13</td>
</tr>
<tr>
<td>Asymptomatic Infections</td>
<td>13</td>
</tr>
<tr>
<td>Outbreaks and Case Reports</td>
<td>13</td>
</tr>
<tr>
<td>USA</td>
<td>13</td>
</tr>
<tr>
<td>Other Countries</td>
<td>14</td>
</tr>
<tr>
<td>Contributions of non-STEC to the Public Health Burden in US</td>
<td>15</td>
</tr>
<tr>
<td>Potential for control of STEC in Foods</td>
<td>18</td>
</tr>
<tr>
<td>Preharvest</td>
<td>18</td>
</tr>
<tr>
<td>Postharvest</td>
<td>19</td>
</tr>
<tr>
<td>Outlook, Implications and Research Needs</td>
<td>19</td>
</tr>
<tr>
<td>References</td>
<td>20</td>
</tr>
</tbody>
</table>

**Defining STEC**

Shiga toxin-producing *Escherichia coli* (STEC) is becoming an ever increasing problem as a foodborne gastrointestinal disease. These bacteria produce a toxin which cannot be distinguished from the toxin produced by *Shigella dysenteriae*, hence the name [1]. *E. coli* 0157:H7 first gained national attention in 1982 when it was isolated from stool samples from victims of a foodborne outbreak caused by hamburgers from a restaurant chain [2]. The level of mortality associated with the outbreak caused a high level of anxiety among the nation’s population and helped push this bacteria into the forefront of food-borne disease surveillance and research. The Centers for Disease Control and Prevention estimated in 1999 that about 73,000 people in the U.S. get sick each year from *E. coli*, with 60 fatalities [3]. Recent outbreaks in a variety of fresh fruits and vegetables have only served to heighten the public’s anxiety.

Infection with STEC is characterized by a short period of abdominal cramps, non-bloody diarrhea and lack of fever. The disease can progress to hemorrhagic colitis with bloody diarrhea and in the worst cases Hemolytic Uremic Syndrome (HUS). Hemolytic Uremic Syndrome is characterized by acute renal failure, thrombocytopenia and
microangiopathic hemolytic anemia. As many as a quarter of patients with STEC can develop HUS and it tends to develop in younger patients [4].

**Virulence Markers**

There is a variety of virulence factors associated with pathogenic STEC. The major virulence factors include Shiga toxins 1 and 2 (stx1, stx2) [5]. There are other virulence factors that are encoded in a region of the chromosomes called the locus of enterocyte effacement (LEE). These factors include enterohemolysin and intimin which help the bacteria adhere to the surface of the intestine. The STEC appear to have gained these virulence factors through horizontal transfer of DNA from other organisms.

There are many different serotypes of STEC, and many different levels of pathogenicity to humans. Most STEC do not cause disease in animals. The animals may act as a reservoir for bacteria that can affect humans. The two different Shiga toxin types, stx1 and stx2, are the most important virulence factors for STEC [6]. A 2001 study identified 2 different stx1 groups and 5 different stx2 groups. Sheep origin stx1 were placed in a separate group than others of that toxin type and the stx2 of deer origin was also found to be separate from the stx2 found in other species. The active sites in all of the toxins were examined and found to be identical to those from STEC of human origin suggesting that animal-origin STEC may be pathogenic to humans.

The pathogenicity of STEC and the appearance of Hemolytic Uremic Syndrome appears more dependent on the type of Shiga toxin (stx) present rather than the amount [7]. About 15% of persons infected with STEC develop HUS. This is particularly true of *E. coli* 0157:H7. There are two main stx types with a variety of subtypes. Orth et al. (2007) found that stx2 and stx2c were associated with the development of HUS while stx2d, stx2e, stx1 and stx1c were not. These strains had no significant differences other than toxin type indicating that it was the difference in toxicity. It is possible for an STEC to produce only one toxin or to be able to code for a combination of toxins [8].

The presence of stx genes may be indicative of potential for human disease transmission even when *E. coli* 0157:H7 is not discovered. In a study looking at 50 dairy farms in Ohio and 50 in Norway, *E. coli* 0157:H7 was not found on any of the Norwegian farms, but was found on 4 of the Ohio farms [9]. A Polymerase Chain Reaction (PCR) run to determine the presence of stx found at least one positive sample on each of the Norwegian farms compared to only 70% of the Ohio farms. The most commonly identified STEC in human infection is 0157:H7 and this study shows its prevalence may be affected by geography, management etc. As more is learned about the role non-0157 STEC play in human disease the widespread dissemination of stx in the environment may become significant.

It is possible to conduct a PCR that can detect multiple virulence factors in a timely manner, enhancing the chance for detection of contamination. A multiplex PCR can
detect the genes for stx1, stx2, intimin and enterohemolysin A in a relatively short time. The most common virulence factors detected were stx1 and enterohemolysin A [10].

Molecular analysis has been used as a tool to determine which of the many non-0157 STECs may cause pathology in humans. Shiga toxin-producing E. coli were examined to determine the distribution of non-LEE effector (nle) genes found in genomic O-islands that encode known virulence factors [11]. Certain O-islands were found to be associated with outbreaks and Hemolytic-Uremic Syndrome (HUS). Strains found to have one complete O-island were not found to be associated with human disease although those with two complete O-islands were significantly associated with human disease. Three or more O-islands were found to cause severe disease. The mere presence of STEC is not sufficient to cause hemolytic-uremic syndrome in humans [12]. It is necessary for the non-0157 STEC bacteria to possess certain virulence factors. The factors are part of a 26-gene pathogenicity island known as O island. This helps to explain why human disease is not common in the face of large numbers of bacteria. These virulence factors alone are not enough to cause disease. The ability of STEC to adhere to intestinal mucosa corresponds to its ability to cause disease [13]. The protein intimin is coded for by the eae gene and allows the bacteria to adhere. This gene was found with much greater frequency in human STEC strains (45%) as compared to bovine (17%) and ovine strains (5%) which possibly explains why human strains are more pathogenic.

Most E. coli strains that cause invasive and non-invasive infections have O antigens as part of their outer membranes. So-called rough strains do not. It was thought that the presence of the O antigen was necessary for infection. Cases of Hemolytic-Uremic Syndrome have been identified that were caused by rough strains of E. coli [14], again raising questions about what is necessary for a strain of E. coli to be pathogenic.

The type of Shiga toxin produced by an STEC has long been thought to have an effect on the virulence of the bacteria. A study in Germany looked for the presence of Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2) in stool samples sent to private laboratories, under certain specific conditions [15]. These conditions were when the patient with diarrhea was 5 years old or younger, the physician asked for an STEC screen or a culture for enteric pathogens, bloody diarrhea was mentioned on the laboratory form or the patient was diagnosed with HUS. A strong relationship was found between patients with gastroenteritis and STEC carrying the stx2 and eae genes. Sixty percent of the STEC isolates carrying both genes were of the serotype 0157:H7.

A large number of infections from non-0157 STEC were detected in Manitoba, Canada. A total of 32 isolates were found representing 10 serogroups and 13 serotypes. The isolates were collected over a 22 month period. Twenty-three isolates were positive for the stx1 gene with five being positive for the stx2 gene. Two isolates contained both genes. Most of the cases were from rural areas and indicate a need for further detection [16].
Diagnosis of Shiga Toxin-producing E. coli.

*Escherichia coli* 0157:H7 ferments lactose quite rapidly and cannot be differentiated from other *E. coli*‘s on a lactose containing media. This strain will not ferment sorbitol, or if it does it is very slow. Colonies of *E. coli* 0157:H7 will appear colorless on this media. Testing these colonies with *E. coli* 0157 antiserum or latex reagents confirms the presence of the bacteria [17]. Not all *E. coli* 0157 have the H7 flagellar antigen, and those few that don’t are usually not pathogenic. Testing for H7 and Shiga toxins 1 and 2 are usually done by reference laboratories because the tests are beyond the capability of most clinical laboratories. Use of MacConkey agar with Sorbitol (SMAC) substituted for the lactose has been a valuable tool in the battle against *E. coli* 0157, but is of questionable efficacy for the detection of non-0157 STECs. The importance of non-0157 STECs may have been underestimated due to the lack of a reliable method of detection [18].

Serotype 0157 is by far the most common *E. coli* to cause human disease. There are over 100 other STECs capable of causing pathology in humans although serotypes O26:H11, O103:H2, O111:NM and O103:H21 are the most common of these [19]. The use of SMAC agar is not useful in the detection of these serotypes. Sheep blood agar is a better choice but it fails to detect about 10% of these serotypes. Polymerase Chain Reaction has been used with great success to detect these serotypes by detecting Shiga toxins 1 and 2 as well as other antigens [20]. There are many different types of PCRs including a real time PCR which gives quick results. A specific PCR was developed to detect *E. coli* serogroups 026 and 0113 by amplifying the wzx and wzy genes. These assays were able to detect bacteria as low as ≤ 10 CFU/ml, showing great promise in the detection of non-0157 STEC [21]. Polymerase Chain Reaction was found to be more sensitive and specific(100%) for identifying shiga toxins as compared to Vero cell cytotoxicity and enterohemolysin activity (91.4%). It is also a quick and easy technique [22].

Enzyme-linked immunosorbent assay (ELISA) is an effective way of detecting *E. coli*. It uses antibodies that are specific for *E. coli*. Enzymes are added that attach to the antibodies causing a color change, indicating presence of the bacteria. ELISA is a quick detection method but it requires specific antigens for the bacteria so it is not very effective in identifying unknown bacteria.

A study was conducted which combined PCR and ELISA to develop a test for *E. coli* 0157:H7 and other STEC in food that was 100 times more sensitive than a PCR by itself. Specific biomarkers were attached to the shiga toxin genes during the PCR amplification process and then detected by the ELISA [23]. The PCR-ELISA test was found to more sensitive and specific than the Vero cell cytotoxicity test when looking for STEC in dairy samples, detecting 14% more positive samples [24]. It is important to use a detection method that will be sensitive enough to detect contamination.

A problem with many detection methods is lack of sensitivity. Many have a limit of 1 ng/ml or more. Immunomagnetic bead time-resolved fluorescence assay (IMB-TRF) detects stx 1 toxin at 50 picograms/milliliter (pg/ml) and stx 2 toxin at 5 pg/ml. This method does not cross react with non–pathogenic microflora present [25].
Determining the prevalence of non-0157 is important in protecting human health. Detection methods used for 0157:H7 may not detect non-0157 STECs. A surveillance program in Michigan that utilized different detection methods found more non-0157 STECs were detected by enzyme immunoassay than by the traditional method of evaluating non-sorbitol-fermenting E. coli assays [26]. A study conducted in Nebraska used three detection methods; cefixime-tellurite sorbitol MacConkey (CT-SMAC) culture, enterohemorrhagic E. coli (EHEC) enzyme immunoassay and stx1,2 polymerase chain reaction (PCR)[27]. The CT-SMAC culture detected the fewest positive samples which is consistent with the previous study. In this study non-0157 serogroups were as prevalent as 0157 serogroups.

As we learn more about STEC in general and non-0157 STEC in particular, we may need to alter some of our detection methods. Novobiocin has been used in STEC enrichment broths to limit the growth of other microflora and allow STEC to grow easier. The antibiotic was added at a concentration of 20 mg l\(^{-1}\) and it has been found that while E. coli 0157:H7 is resistant at this concentration, 31% of the non-0157 STEC strains were inhibited [28]. This may give one explanation as to why non-0157 STECs have not been found as often as 0157:H7. Novobiocin was also shown to slow down the growth of many strains, allowing for the potential of a false negative in cases where the incubation period is short.

The type of broth used in the enrichment medium also seems to pay a role in STEC detection. The two basic media used are Tryptic Soy Broth (TSB) and Escherichia coli (EC) broth. A study looking at the use of Novobiocin, one of the two broths, and different incubation temperatures found that with or without Novobiocin, EC broth was more appropriate for use in detecting non-0157 STEC [29].

The preceding discussion of detection methods show that selecting the proper method is crucial to reaching a definitive diagnosis. The increasing prevalence of foodborne illness and the possible consequences of an incorrect diagnosis point to the need for accurate assessment. In a study that looked at 18 stool samples, 8 samples were found to be positive through growth on SMAC plates [30]. A PCR run on the same samples to detect Shiga toxin resulted in 16 positive samples. The two samples that had not tested positive did so after enrichment. All of the samples were eventually found to be positive, but if the researchers had stopped at any stage during the process they would have had false negatives. Current CDC guidelines recommend a combined protocol utilizing culturing on SMAC plates and PCR analysis for Shiga toxin.

**STEC in Humans**

**Diseases**

A one year study done in France looked at the prevalence and characterization of Shiga toxin-producing E. coli (STEC) isolated from cattle, food, and children. A total of 2,143 samples including 471 fecal samples from healthy cattle, 411 samples from beef, 603 cheese samples and 658 stool samples from hospitalized children were examined by
PCR for shiga toxin-encoding genes. A total of 220 STEC strains were isolated that included 186 from cattle, 18 from beef, 6 from cheese and 10 from children. These strains all came from the same geographical area. The majority of the strains isolated from cattle, beef and cheese were not pathogenic to humans. Although the children sampled in the study were ill, the strains found in them were not found to be responsible for their disease. Strains were detected in this study that had previously been associated with hemolytic-uremic syndrome and they were found to belong to subsets of the STEC types found in the local bovine population [31].

*Escherichia coli* 0157:H7 has been widely cited in scientific and popular media as a cause of bacterial diarrhea. The true incidence of the disease has been hard to determine. Of 30,463 samples taken from 10 hospitals across the country, 118 were positive for 0157:H7. Positives were more likely in samples from northern areas as well as from bloody samples as compared to non-bloody samples [32]. Age was also found to be a factor with the highest number of positives coming from patients from 5 to 9 years of age. It was suggested that patients with bloody diarrhea should always be tested for STEC.

The numbers of foodborne illnesses being diagnosed are on the increase in this country. Some of this increase may be a result of more frequent and better testing. Some is due to an evolution of the bacteria themselves. Bacteria are becoming better able to survive efforts at sanitation. Increasing sophistication in testing has allowed for the detection of non-0157 STEC’s that previously were going undetected. This increased degree of food safety may be a double-edged sword for the food industry, opening it up to a higher level of liability [33].

The relationship between livestock farming and human infection with STEC is well established. The severity of hemolytic-uremic syndrome lends importance to the study of this association [34] . Livestock Density Indicators have been developed which may be used to show an association between human STEC infection and areas of high density livestock agriculture. The strongest association indicators were found to be the ratio of beef cattle to human population and the application of manure to agricultural land.

The incidence of human disease related to non-0157 STEC is uncertain. Beutin et al. (1998) looked at non-0157 STECs that caused clinical disease in humans [35]. They looked at a total of 89 patients, all of whom were infected with a single serotype, except for one patient who had both type 0157:H7 and type 0126:H11. There were also six patients that were also infected with *Salmonella* enterica. They found that clinical disease did not depend on the type of Shiga toxin the serotypes produced. In this study severe disease was associated with the presence of the eae A gene and young age. In two cases family members of patients also developed clinical signs.

Hemolytic Uremic Syndrome (HUS) is sometimes a sequel to severe diarrheal illness. It has been suggested that STEC is the major cause of HUS in developed countries [36]. Because of the time of onset of HUS following the diarrheal disease it is not always possible to determine if an STEC was involved. Eighty-three (HUS) patients from a five
year period were studied to try to determine a cause for their disease. Only seventy of the patient’s stool samples yielded bacterial growth, and 30 grew STEC. Serum samples were taken from 66 of the patients and 53 of them had positive 0157 lipopolysaccharide antibody titers. Sixty of the 83 patients had some evidence of STEC infection. This study indicated that while STEC should be considered the likely cause for post-diarrheal HUS, reaching a definitive determination is not always easy.

**STEC in Foods**

The incidence of STEC contamination in foods is becoming a major health hazard. STEC 0157:H7 is the most well-known contaminant but other STEC serotypes are found as well. Raw milk cheeses were sampled for STEC in southern Iran [37]. Of the 125 samples tested, 6.4% were found to contain stx genes but the bacteria was only isolated from 4% of the samples. Only one of the samples contained the 0157:H7 serotype.

When an unusual number of *E. coli* cases were noticed in Clark County, Washington in 2005 a retrospective study was conducted which identified the source of infection as raw milk from a cow share. This case illustrates some of the difficulties in tracing certain foodborne illnesses because the patients in this outbreak all initially denied drinking raw milk. This case led directly to changes in Washington State law which extended state licensing requirements to cover cow-share programs [38]. The sale of raw milk is legal in California. In 2006 six cases of *E. coli* O157:H7 infection were traced back to a dairy selling raw milk. Two of these case developed HUS. This outbreak resulted in a recall of items from the subject dairy[39].

**Beef**

Ground beef is the most common culprit for foodborne STEC outbreaks. Modern production and distribution can result in a widespread problem. A recent outbreak with 49 cases, in seven states, led to the recall of over 500,000 pounds of ground beef[40]. An outbreak one year prior caused illness in 40 people and resulted in the recall of over 21 million pounds of ground beef[41]. These outbreaks are not only devastating from a health status but also from an economic status.

A study of minced beef samples in Ireland designed to detect *E. coli* 0111 and *E. coli* 026 found an incidence rate of only 0.25% as compared to a previous study that found an incidence rate of 2.8% for *E. coli* 0157 [42].

The United States Department of Agriculture has recently decided to test for non-0157 STECs. Is this a valid decision or a knee-jerk response to overblown concerns? Sponge samples were collected from beef carcasses at four large processing plants in the United States. The samples were collected over a two month period and were tested for the presence of STECs. Carcasses sampled prior to evisceration had an incidence rate of 54% for non-0157 STEC. The incidence rate was reduced to only 8% for carcasses sampled after antimicrobial interventions such as steam vacuuming, hot water washing, organic
acid washing and steam pasteurization were instituted [43], showing the importance of human intervention.

A survey was conducted in the Pacific Northwest to determine the prevalence of non-0157 STEC in ground beef, beef carcasses and raw milk samples. The samples were tested for Shiga toxin genes and the prevalence rates were 36% for the ground beef, 23% for carcass beef and 21% for raw milk samples. These rates were higher than the rates for isolation of actual bacteria from the samples. The distribution of the toxin types showed stx1 to be predominant in milk, stx2 on carcasses and a mixture of the two in ground beef [44].

A total of 285 samples of ground beef were tested using an stx PCR screen and colony hybridization. Sixteen percent of the samples were found to possess STEC. Testing the isolates for virulence factors rendered a prevalence rate of 95% for the stx1 gene and 65% for the enterohemolysin gene. A total of 18 STEC serotypes were isolated. The most common were serotypes 0174 and 091. This study did not detect the relatively common serotypes 0157, 0111 and 026 [45].

The global marketplace insures that the United States receives the food that it needs, but also leaves us open to possible foodborne infections from outside our borders. The United States imports a large amount of lean, boneless beef trim from Australia, New Zealand and Uruguay to grind into ground beef. The importation of beef products, which are known to be the main source of STEC infection raises the threat of imported foodborne illness. Comparing samples from these countries and from the United States is a logical way of judging risk from the imported samples. Samples from New Zealand had an STEC prevalence of 10% while all of the other samples had a prevalence of 30%. A total of 99 STEC strains were identified, and the serotypes associated with human illness were found had the same prevalence in the domestic samples and the imported samples. There did not seem to be any increased risk from the imported samples [46].

**Other Meat and Poultry**

Sheep are one of the animals known to harbor STEC. The characteristics of STEC isolated from sheep products have not been well-studied. A study looked at a total of 13 STEC strains from sheep dairy products. The strains included eight 0157 and five non-0157 and the study looked at biochemical traits, motility, hemolytic activity, resistance to tellurite-cefixime, maximum growth temperature and antibiotic resistance. The 0157 strains did not show any B-glucuronidase activity and sorbitol, rhamnose and sucrose utilization. In contrast the non-0157 strains did not show any hemolytic activity, while six of the eight 0157 strains did. All of the strains could grow at temperatures up to 45 degrees C. All of the strains tested were found to be resistant to bacitracin, cloxacillin, penicillin and tylosin. The majority of the non-0157 strains were also resistant to gentamicin and streptomycin. These levels of resistance are possibly linked to the use of antibiotics in livestock [47].

**Fruits, Vegetables and other**
The specter of a food contamination problem hangs over anyone who grows fresh fruit and vegetables for the market. While relatively rare, the number of foodborne illnesses involving fresh fruits and vegetables is on the increase and their severity and the coverage they receive in the popular press raise the level of concern to great heights. There has been much debate over the potential benefits of different growing systems such as organic, semi-organic or conventional. A study conducted in Minnesota and Wisconsin looked at this very question, and found that the produce type was more likely to influence *E. coli* contamination rather than farm type. This study, conducted over a two year period did not detect any *E. coli* 0157:H7 but did find other *E. coli*. The most commonly affected produce was cabbage [48].

In recent years fresh fruits and vegetables have been a significant source for human exposure to *E. coli* 0157:H7 [49]. Understanding the manner in which human pathogens come into contact with produce and proliferate once there, will be important in helping to eliminate this hazard. Simple washing of produce will not be enough to reduce the pathogen load. Inorganic material present in wash water has been found to interfere with chlorine-based disinfectants in produce wash water[50]. As sustainable practices regain popularity among produce growers the proper use of manure for fertilizer will need to be addressed. Pathogens may persist in manure for extended periods of time depending on how the manure is handled[51]. The use of manure aged less than 12 months was found to result in a prevalence of *E. coli* 19 times greater than that of farms that used manure aged or composted longer than 12 months. These types of management issues will need to be addressed to prevent human pathogen outbreaks.

In areas with a high concentration of livestock agriculture, a great deal of manure is spread on fields under a variety of conditions. Many factors may affect the persistence of *E. coli* 0157:H7 in the soil. The presence of a cover crop may improve the persistence of *E. coli* in the soil due to its association with the plant roots[52]. The amount of clay in the soil also seems to lengthen 0157 viability as does freezing the sample.

The potential for human outbreaks is real. A study of bagged salad vegetables conducted in London, England in 2001 detected three different *Salmonella* species and one sample of *Listeria Monocytogenes* at a human threat level[53]. Subsequent study of the human population confirmed an outbreak of *Salmonella* consistent with one of the strains present in the salad vegetables. This study is consistent with a study of minimally processed salad vegetables conducted in Brazil in 2007 which found significant concentrations of fecal coliforms in 73% of the samples and *Salmonella* in 3% of the samples[54]. *Listeria* was found in a total of 4 samples. The potential for human contamination from these popular bagged salad greens is very real.

Many things can affect the survivability of *E. coli* 0157:H7 in areas of produce production. One of these is the presence of epiphytic bacteria [55]. Epiphytic bacteria are microorganisms that compete with the pathogenic bacteria for the same nutrients and may interfere with their production by limiting their access to nutrients. The use of competing microorganisms may be a method of pathogen control with great future utility.
A great deal of attention has been paid to the idea that vegetables may be able to take up bacteria from the soil in which they are grown. Johannessseen et al. [56] looked at this question using organic manure inoculated with $10^4$ CFU g$^{-1}$ *E. coli* O157:H7 to fertilize Crisphead lettuce seedlings. The bacteria was found in the soil up to eight weeks after inoculation but was never found in the plants.

This does not mean that vegetables cannot be contaminated in the field. A large outbreak of *E. coli* O157 in Sweden was traced to lettuce that was contaminated as result of irrigation water that received runoff from a cattle operation upstream[57]. In a recent outbreak involving spinach from California, the outbreak strain of STEC was found in feral swine, cattle, surface water, sediment and soil from a ranch one mile from the fields where the spinach was grown. In this case the pathogen may have reached the vegetable fields carried in the intestines of the feral swine[58].

The Food and Drug Administration developed the Lettuce Safety Initiative as a response to the multiple outbreaks of foodborne illness resulting from cabbage. It was designed to allow consumers to be notified immediately in the event of an outbreak, to assess and improve lettuce safety, perform hazard analysis of lettuce growing and to consider regulatory action to respond to outbreaks[59]. Individual states are pursuing similar actions to safeguard their consumers and their fresh fruit and vegetable industries.

**STEC in Water and Environment**

The increasing incidences of STEC disease outbreaks involving food of non-animal origin are a cause for concern. Infections resulting from sources such as water point to contamination from an outside source. A study in India looked at samples from 188 drinking water sources and found 57 isolates of thermotolerant *E. coli*. Fifteen of the isolates were pathogenic serotypes. All of the pathogenic isolates were determined to be resistant to Bacitracin and various heavy metal ions. Some level of resistant was also demonstrated for Streptomycin, Clotrimazole, Cephaloridine, Polymixin-B and Ampicillin. The ability to transfer antibiotic resistance was found in 9 out of 12 studied. There was also evidence of the transfer of heavy metal resistance [60].

Foodborne illness outbreaks have gained nationwide attention recently. An outbreak of *E. coli* O157:H7 associated with lettuce from Taco John restaurants in Iowa and Minnesota may have been caused by ground water contamination. Investigations of the area in which the lettuce was grown found the outbreak *E. coli* strain in wastewater on two dairy farms adjacent to the fields in which the lettuce was grown. The investigation pointed to several confluence points between the local water districts system and those of the dairy farms. There was also a lack of adequate backflow prevention devices to prevent contamination of the water system with wastewater[61]. This report illustrates how complex this issue can become.

Many parts of the world have a shortage of potable drinking water. Even in the United States we worry about the effect large, confined animal operations may have on the local
aquifer. Monitoring water for possible STEC contamination in areas of high animal density or poor hygiene may shed light on the threat to human health posed by fecal contamination. A total of 188 drinking water samples were collected from a variety of sources in a rural area of India. Fifty-seven isolates of E. coli were found with 45 of them being typable. Half of the serotypes were non-pathogenic and 26.3% were serotypes known to be pathogenic to humans. Many of the water sources from which the samples were taken would not be used for drinking water in the United States but this study does show the potential for contamination.

A large proportion of the world’s population lives near the seashore or an estuary. Determining the ability of E. coli to survive in saltwater is important from a public health standpoint. E. coli 0157:H7 has been found in rivers in and near Tokyo and Osaka, Japan. Osaka has had an outbreak of 0157 [62]. With 0157 in fresh water rivers it is logical to think that it would also be in the bays they drain into. Testing E. coli in water samples containing from 0-8% NaCl it was found that the bacteria could survive a concentration of up to 5%. E. coli survived in natural marine water samples at a pH of 8.1 and an NaCl concentration for up to 15 days. It survived even better in marine water samples that were autoclaved to remove any biological competitors.

Other environmental sources of STEC infection are possible. An outbreak of E. coli O157:H7 in eight children in Evergreen, Colorado had officials stymied until they determined that all eight of the children played soccer on the same field. The soccer field was frequented by elk from the nearby mountains. Elk droppings from the field were found to contain the same strain of E. coli as that infecting the children[63].

**STEC in Food-producing Animals and Pets**

Shiga toxin-producing E. coli is a normal inhabitant of the intestinal tracts of healthy cattle [64]. A study involving fecal samples from 259 healthy cattle found 10.8% to be carrying Shiga toxin-producing E. coli. The serotypes identified were numerous with only 2 animals exhibiting 0157:H7 isolates. Forty percent of the isolates found were from serogroups known to be pathogenic to humans.

To look at the sources of STEC in the environment, fecal samples were taken from 720 healthy animals representing seven different species (cattle, sheep, goats, pigs, chickens, dogs and cats). Shiga toxin-producing producing E. coli were found in all species except for chickens. They were found most frequently in sheep (66.6%), goats (56.1%) and cattle (21.1%). Many serotypes were found, with five occurring in more than one species. Almost 60% of the serotypes found in the study have been found to cause disease in humans [65].

Blanco, et al. collected fecal samples from 32 cattle herds, finding STEC on 84% of the farms. The infection rate on the farms was found to be as high as 63% representing 83 different strains. Almost 45% of the bovine strains found were from subgroups associated with hemorrhagic colitis and hemolytic-uremic syndrome, but only 2% belonged to enterohaemorrhagic E. coli serotypes. The researchers found that only 10% of the
serotypes were positive for the attaching and effacing *E. coli* (eae) gene and suggested that it may be necessary for the strains to be virulent in humans [66].

The density of animals found in an area may have an effect on the risk caused by STEC. In looking at cattle density and STEC it has been found that there is an almost 70% increase in STEC gastroenteritis cases for each 100 cow increase per square kilometer [67]. It was found that serotypes whose prevalence was positively correlated to increased cattle population density accounted for at least 82% of HUS-associated STEC and at least 63% of reported human STEC infection in the geographic area studied.

Cattle are not the only farm animals to serve as reservoirs for STEC. Fecal samples from 222 healthy dairy goats were screened for STEC with 47% of the samples positive. The positive samples were mostly from adult and replacement animals. None of the animals tested showed signs of clinical disease. None of the serotypes found were positive for the eae gene although 16% of the strains were from serotypes associated with clinical disease in humans. The lack of cattle facilities in an area does not necessarily correlate to a low chance of human disease since other animals may serve as a reservoir for the bacteria [68].

In the United States livestock agriculture tends to be predominately cattle, swine and sheep based. This is not necessarily the case in other countries. Water buffalo are important components of farming operations in Southeast Asia. Studying the prevalence and serotypes of the STEC present in these animals can give an indication of how they may be involved in human STEC outbreaks [69]. A year-long study in Vietnam showed 27% of Water buffalo in the study tested positive for STEC. This study also looked at cattle and goats and found 23% and 38.5% respectively to be positive for STEC. A total of 170 different strains were found with varying virulence factors. Only 9 of the STEC found coded for serotypes 026, 091, 0121, 0145 and 0157 which are commonly associated with human disease. This is a relatively low percentage of all the strains found (5%) but does indicate that what we would consider to be non-traditional livestock in the United States are also potential reservoirs of human disease.

During human disease outbreaks livestock operations often find fingers being pointed at them as the cause of the outbreak. One group of animals that is rarely looked at are those animals housed in zoological parks. A study conducted in a zoo in Argentina collected fecal samples from 112 animals from the order Cetartiodactyla comprising 17 different species (guanaco, Pere David’s Deer etc.) and 15 rodents found in the enclosures with them. Ten species of Cetartiodactyla and one species of Rodentia were determined to be new carriers of STEC. A total of 38.5% of the animals tested were found to be positive for STEC. Seven different serotypes were found including serotypes 012:H25 and 013:H6 which were new. Serotype 0146:H28 accounted for 24% of the positives is a serotype frequently found in human infections [70].

Other animals that are not typically kept as pets or used for food may play a role in the dissemination of STEC. The wide geographic areas covered by birds could make them a serious potential threat if they are harboring hazardous bacteria. A total of 50 fecal
samples from seagulls were examined in Japan to determine if they contained STEC. One sample was found to contain two different STEC serotypes. They were 0136:H16 and 0153:H-. The 0136 serotype was found to produce stx2 toxin and the 0153 serotype was found to produce stx1 toxin. Neither serotype possessed hlyA or eaeA genes. The stx toxins were found to be very similar to the human stx toxins and pose the possibility that birds may be an important carrier of human disease [71].

Diseases

Asymptomatic Infections

The importance of Shiga Toxin-producing E. coli as a human pathogen is well-established. Cattle are a major reservoir for these bacteria, but they do not display clinical illness. The prevalence of E. coli 0157:H7 does not seem to vary depending on whether the cattle are fed grain or grass-fed[72]. A study conducted in Kansas did find a correlation between the feeding of dried distiller’s grains and the fecal shedding of E. coli 0157:H7 [73]. The concentration of E. coli 0157 was higher in the fecal samples of calves fed the dried distillers grains as well as in the samples taken from the calves upon necropsy.

There are many different subtypes of E. coli and a wide variety of different virulence factors. The presence of E. coli in livestock reservoirs does not necessarily prove a connection between these bacteria and the bacteria causing human disease. A study in Denmark genotypes E. coli strains from cattle herds and from cattle at slaughter plants and characterized them by the their virulence factors. The strains of bacteria isolated from cattle were then compared to human strains taken at the same time. The strains found in the cattle had all of the typical virulence factors, but had significant differences from the human strains indicating that cattle might not be the most important source of human infection in Denmark[74].

Escherichia coli does not usually cause symptomatic disease in cattle, but does in humans. Clinical disease does not always result from human infection with E. coli. Eighty percent of human urinary tract infections are caused by E. coli, but it is possible to have asymptomatic bacteruria with E. coli. The lack of clinical symptoms seems to be related to whether the strains have functional fimbriae. Some of the bacteria that cause the asymptomatic infections have been shown to compete with the uropathogenic strains of E. coli and suppress them, making them a potential tool for use against these pathogenic strains[75].

Outbreaks and Case Reports

USA

Outbreaks of E. coli in various leafy vegetables have received a great deal of press coverage in recent years but STEC can be found in many products. Pressing, bottling and selling apple cider is a fall tradition in many parts of the country. This is a tradition that
has been changed by the increasing incidence of *E. coli* contamination. In October 1996 eight cases of *E. coli* 0157:H7 infection were traced back to unpasteurized apple cider. Some of the apples used in the cider were “drop” apples which had been picked up off the ground[76].

A six year old girl who presented with a history of eight hours of abdominal and left-flank pain, vomiting and dysuria was found to be infected with *E. coli* 0103:H3. Initially diagnosed with pyelonephritis this child progressed to HUS which required 20 days of hospitalization including eleven days of hemodialysis. It was determined that this case had arisen from a urinary tract infection caused by the bacteria[77].

Research suggests that STEC are the main cause of post-diarrheal HUS in developed countries. A prospective study was done to determine the frequency of STEC involvement in HUS. A total of 83 patients were looked at and of the 70 whose stool samples grew bacteria, 30 (43%) yielded STEC. Twenty-five of those were *E. coli* O157 and five were non-O157. Sixty-six of the patients had serum samples examined and 53 (80%) had positive O157 antibody titers. Of the 83 total patients 60 (72%) had some evidence of STEC infection suggesting that STEC infection should be considered as a probable cause in all cases of post-diarrheal HUS[36].

**Other Countries**

Outbreaks of STEC are not limited to the United States. In the year 2006 there were three concurrent unrelated outbreaks in the United Kingdom. The first outbreak was linked to a Scottish daycare center with 13 confirmed cases and another 10 possible cases. Seven of the confirmed cases were asymptomatic carriers and four of them developed Hemolytic-Uremic Syndrome. A second outbreak in Scotland occurred within days of the first but was not linked to it. There were a total of three cases in this outbreak, linked to a local butcher shop. A third outbreak occurred at the same time in England involving four patients, one of whom died from HUS [78]. While the numbers of affected individuals were not huge in these outbreaks, the fact that three unrelated outbreaks were occurring at the same time give an idea of the scope of the problem worldwide.

*Escherichia coli* O157:H7 detection is relatively easy compared to testing for many other serotypes. Many laboratories do not have the ability to test for non-O157 STEC. An outbreak of HUS in Australia yielded both O157:H- and O111:H- because the reference laboratory used had the capability of finding both types. Further testing indicated that the cause of the outbreak was actually the O111:H- type. A laboratory with less capability might have only found the O157 serotype and ascribed the outbreak to it. It is possible that some of the outbreaks for which O157 gets credit are actually caused by serotypes for which the laboratory cannot test [79].

An outbreak of *E. coli* occurred among school aged children in south Wales in 2008 resulting from contaminated meat served at schools[80]. There were a total of 158 cases spread over 42 schools, with 33 needing hospitalization. This outbreak led to the
recommendation of stricter guidelines for additional guidance from the UK food standards agency to businesses handling raw and cooked meats.

A 2 year-old girl in Japan who developed watery diarrhea which then progressed to bloody diarrhea and eventually HUS was found to be positive for stx-2 positive STEC 0121. Stool samples from her seven family members yielded two with stx-2 positive STEC 0121 and two with stx-1 positive STEC 091, while three were negative. Further research determined that the source of infection was drinking water that had been contaminated by the family’s cattle which were housed near the well. The water samples were intermittently positive for stx-2 positive STEC 0121, but never showed the presence of stx-1 positive STEC 091. The cattle were tested and two were found to be positive for the exact same strain of stx-2 positive STEC 0121 as found in the family members and water samples as well as stx-1 positive STEC 0111. None of the cattle were found to positive for the stx-1 positive STEC 091 [81].

The importance of non-0157 STECs as emerging human pathogens is a topic of much research. In one two-month period in Australia a total of 23 cases of Hemolytic-Uremic Syndrome were reported in children under the age of 16. The median age of the patients in this study were four years of age, and most were male. Sixteen required kidney dialysis. Escherichia coli 0111, non-motile (NM) was isolated from 16 of the patients, while other E. coli strains were detected in three patients. Ten sausage samples were removed from the homes of nine patients and eight were positive for stx1 and stx2. Four of the samples were positive for E. coli 0111 NM[82]. Studies, such as this, point to the need for further research in non-0157 STECs.

A study in France [83] isolated six STEC strains from stool samples of patients with Hemolytic-Uremic Syndrome. Each of the isolates was of a different serotype. None of the serotypes isolated was 0157:H7. All of the serotypes were positive for Shiga toxin type 2 and two were positive for enterohemolysin-encoding genes. None of the isolates were positive for the eae gene. This study points to the difficulty in determining the reason why some STEC are virulent in humans and others are not.

**Contributions of non-STEC to the Public Health Burden in US**

Non-0157 STEC may cause clinical disease in human beings. The tendency in this country has been to test for E. coli O157:H7 and testing for the other serotypes does not necessarily occur on a routine basis. Table 1. shows the recognized foodborne outbreaks due to non-O157 E. coli that occurred in the United States from 1994-2006.
The United States Department of Agriculture has recently decided to test for six non-O157 STECs in addition to the E. coli O157:H7 they are already doing [84]. The testing is for study purposes and will not result in a mandatory food recall if they are detected which leads to disturbing questions. If STECs are detected in a food and the USDA does not issue a recall, is the producer liable for any illness caused by the STEC? Is the USDA opening up a virtual Pandora’s Box of liability issues by taking this step? For years public health has been focused on detecting and controlling E. coli O157 H7 to the exclusion of other Shiga toxin producing E. coli’s. The most prevalent Shiga toxin producing E. coli in this and other countries is E. coli O157:H7. Other STECs are present and this paper will try to assess the need for increased surveillance for them.

A survey for STEC in raw milk and beef conducted in the Northwestern United States found prevalence rates for the detection of Shiga toxin gene (stx) in retail beef, beef carcasses and raw milk of 36%, 23% and 21% respectively. STEC serotypes known to cause human disease identified in this study included not only O157 but also O175, O35, O8, O46, O128, O108, O160, O116 and a previously unidentified serotype. This study suggested that non-O157 prevalence rates were similar to those of O157 but their detection and isolation vary considerably, indicating that we may be getting a large number of false positives. The authors suggested the need for standardized tests to detect non-O157 STEC [44].

A growing concern among the public health community is that non-O157 STEC’s are being under-diagnosed. Two rare sorbitol-fermenting STECs were isolated from stool samples from two related patients with clinical diarrhea [85]. One carried the stx2 gene and the other carried the stx1 and eae genes. The sample carrying the stx2 gene caused bloody diarrhea, leaving the patient hospitalized while the other sample caused non-bloody diarrhea that did not result in hospitalization.

### Serogroup of non-O157 STEC isolates from humans sent to CDC, 2003-2005

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<thead>
<tr>
<th>Serogroup</th>
<th>Number</th>
<th>Percent</th>
<th>Serogroup</th>
<th>Number</th>
<th>Percent</th>
<th>Serogroup</th>
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From CDC etc.
E. coli O157:H7 is the most prevalent STEC detected in this country but that doesn’t mean that other serotypes capable of causing human disease are not being found. Table 2. shows the various non-O157 human isolates that were sent to the CDC from 2003-2005 indicating that the number of different isolates found in the United States is large.

More than 100 different Serotypes of STEC have been found to cause human disease of varying intensity. E. coli O157:H7 is the serotype which has garnered the most attention as it has appeared which the greatest frequency, and causes severe disease. Research suggests that improved testing for other serotypes would show some of them are more prevalent than previously thought and some of them are capable of causing disease as severe as that caused by the O157 serotype.

Figure 1. illustrates the increase in detection of non-O157 human isolates at the CDC from 1983-2002. The graph illustrates that significant increases correlate well with the introduction of a commercial Shiga toxin enzyme linked immunoassay and with non-O157 serotypes becoming reportable [86]. As testing for non-O157 STEC improves and becomes more common we may see non-O157 isolates reach numbers similar to those of the O157 serotype.

Laboratories are more likely to test for E. coli O157:H7 than they are for any other STEC. This is partly due to the fact that it has been historically the most prevalent but also because many laboratories are not able to test for other STECs. This inability to test for other STECS may lead to a false impression that they are not important pathogens. An outbreak of diarrhea with two cases of HUS occurred in 1999 in Texas, and showed signs indistinguishable from E. coli O157:H7. In this particular case a total of 55 persons were ill but O157 was never identified. Eleven stool samples were done and two were positive for E. coli O111:H8 [87]. Laboratories need to look for other STEC serotypes.
Potential for control of STEC in Foods

Preharvest

The mechanisms for pre-harvest control of STEC can be split into two categories. The first deals with changing existing management strategies and the other is developing a new procedure[88]. The first method deals with taking steps such as improving cleanliness, to decrease bacterial load. The second method has not shown a great deal of promise to date but may be areas of future research.

The dissemination of STEC into the environment and its ability to survive is an important factor in pre-harvest contamination. Non-0157 STEC was inoculated into four manure heaps that were managed in two different ways. Two of the heaps were turned regularly and two were not turned. The heaps were monitored for temperature, pH, moisture content and oxido-reduction potential. The moisture level at the surface of the heaps decreased from 76.5% to 42% for the turned heaps and the temperature reached 65°C at the center of all of the heaps. The *E. coli* survived for up to 42 days in the turned heaps as compared to 90 days for those that were not turned showing the ability of *E. coli* to survive long-term under differing conditions. Proper hygiene is required to minimize contamination potential [89].

Understanding the shedding patterns of *E. coli* from feedlot cattle is one step in learning to reduce contamination. Testing of fecal and environmental samples from a feedlot yielded peaks in shedding activity. The lowest level of shedding was on day 13. The incidence of stx samples was also found to be dependent on the day tested with days 0, 2 and 62 being significantly higher and days 27, 34, 55 and 69 being significantly lower. A total of 37 different STEC were isolated and assigned to virulence groups based on the presence of stx1, stx2, eae A and ehx A. A total of 8 virulence groups were defined [90]. The potential to minimize STEC contamination by management practices is real. Hygiene control should be practiced by not allowing feed and water to be contaminated, minimizing hide contamination preslaughter and separating animals based on risk level (calves versus cattle).

The amount and duration of fecal shedding of STEC can also vary by serotype [91]. It was found that 0157 shedding persisted from less than 1 week to as many as 10 weeks while 026 went from less than 1 week to less than 3 weeks. A higher prevalence of 0157 shedding was found in calves aged 5-6 months while 026 was found predominately in calf aged 1-2 months and 0157 had a higher incidence in the summer as compared to spring for 026. This study also detected a difference between breeds with the beef type Japanese Black cattle having the greatest excretion of 0157 and Holstein cattle having a higher excretion of 026. The fact that 0157 was shed for longer periods and in higher numbers than 026 may indicate that it causes more human disease because it has more chance to cause human disease. The differences in incidence rate of the two serotypes, between the two cattle breeds, is at the very least interesting.
Most interventions to minimize meat contamination with STEC have focused on the slaughterhouse. It is possible to make an impact at the feedlot level and possibly decrease the pathogen load at the slaughterhouse [88]. Two avenues for intervention include the modification of existing management strategies and the development and implementation of novel targeted intervention technologies. The second avenue would probably be too difficult and expensive to be practical. One management strategy that is useful is feeding direct-fed microbials to block or compete with the STEC. The most effective is the Lactobacillus-based strain NP51. Bacterin vaccines have also shown some promise as a means to decrease the shedding of STEC. Using Sodium Chlorate as a metabolic poison that the E. coli picks up and metabolizes to the highly toxic chlorate form has also been effective. The use of antimicrobials to decrease shedding is intuitive and has been shown to be effective. Neomycin sulfate fed for two days greatly decreased E. coli shedding in market ready cattle. Using antimicrobials in this manner is a cause for debate and could lead to microbial resistance. While none of these methods may be the silver bullet that helps to eliminate STEC contamination of meat, they do show promise for decrease the pathogen load entering the slaughterhouse.

Postharvest

Samples taken from beef carcasses collected in a two month period in the summer of 1999 from four processing plants in the United States yielded large numbers of non-O157 STEC. Fifty-four percent of carcasses sampled prior to evisceration were found to be positive for non-O157 STEC, totaling 361 isolates representing 41 different O serogroups. The prevalence on the carcasses after antimicrobial interventions was only 8%, showing that it is possible to have a large positive impact in the beef industry post-harvest[43].

A study of 1216 retail ground beef samples to look for non-O157 STEC resulted in twenty-three positives (1.9%). Serotypes found included O26, O103, O113, O121 and O145. Most of these serotypes were positive for the stx and eae virulence factors. The level of positive results indicates a need for increased testing for non-O157 STEC[92].

The act of harvesting and processing can cause tissue damage in vegetables. Romaine lettuce which had sustained damage due to harvest was inoculated with E. coli O157:H7 and within 4 hours of inoculation the bacteria multiplied by a factor of four. This was double the increase found in lettuce that was undamaged. Minimizing the damage to produce during harvesting and processing may help to minimize contamination with bacteria[93].

Outlook, Implications and Research Needs

With increased awareness, the diagnosis of non-O157 STEC infections will likely continue to rise worldwide. There are several interrelated critical research needs that have implications for the understanding the significance of these infections.
1. The detection of non-O157 STEC remains problematic. Until such time that sensitive methods allow for the isolation of organisms and subsequent molecular characterization, it will be difficult to fully understand their ecology and epidemiology in cattle, the environment, the food production environment, in foods and in people. Studies conducted on STEC strains that are more easily recovered may not fully represent the behavior of all STEC in animals, foods, the environment and people.

2. There is a need to better understand the relationships between molecular subtyping and disease causing potential. Clearly all STEC do not pose the same level of public health risk. Bacterial genetics of disease-causing and non-disease causing organisms may also impact their survival and transmission.

3. The source and reservoirs of non-O157 STEC pathogenic to humans has yet to be fully defined. Unlike, O157, non-O157 that encode the constellation of virulence factors typically associated with severe disease in humans have not been identified. Identifying the primary source(s) of infections for human is imperative if progress towards prevention is to be expected.

Despite these limitations, regulatory officials (USDA) have proposed to test ground beef for non-O157 STEC. It is likely that these results will be made publically available. We know that STEC are common commensal flora present in the gastrointestinal tract of many animals, including healthy cattle. Available data would indicate that the majority of non-O157 STEC strains recovered from US cattle pose minimal food safety and public health risk. Nevertheless, the biggest challenges facing the beef industry is to communicate the (in)significance of non-O157 in beef products while at the same time not being viewed as completely dismissing the concern that these organisms occasionally contaminate the food supply.

Until such time that some of the research needs listed above (especially Need #2), are addressed, testing for non-O157 STEC in beef products, in addition to O157 testing will likely not significantly contribute to increased safety of the food supply. Given our current level of understanding, beyond those used for *E. coli* O157, there are no additional preventive control measures recommended for the control of non-O157 STEC in either beef or live cattle.

Control measures instituted for *E. coli* O157 should, in theory, be equally effective against non-O157 STEC as well. Responding to these challenges will require a concerted effort by multiple players in the food production and public health arenas. Attribution of the source non-O157 STEC infections is a prerequisite for disease prevention. This requires involvement of physicians, epidemiologists and laboratories. Answers to other questions will involve longer-term research on the part of scientists. Those projects which build on collaboration with producers and processors are especially important as they can put “what is possible to occur” in the research lab into perspective as to what is “probable to occur”.
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