Risk Factors for Shiga Toxin-Producing *Escherichia coli*-Associated Human Diseases

MARTA RIVAS,1 ISABEL CHINEN,1 ELIZABETH MILIWEBSKY,1 and MARCELO MASANA2

1Instituto Nacional de Enfermedades Infecciosas, ANLIS “Dr. C. G. Malbrán,” (1281) Buenos Aires, Argentina; 2Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria, (B1708WAB) Morón, Pcia. de Buenos Aires, Argentina

ABSTRACT We have reviewed the risk factors for the occurrence of Shiga toxin-producing *Escherichia coli* (STEC)-associated human diseases. The analysis of STEC surveillance data and trends shows differences in frequency and severity of the illnesses across countries, whereas the economic and social costs for the affected families, the community, and the health system are better estimated in developed countries. The occurrence of STEC infections is determined by the interaction of the pathogen, the reservoirs, and the biological, cultural, and behavioral aspects of the host. The main risk factors identified in earlier case-control and population-based studies were dietary behaviors and beef consumption. However, in recent years, other risky exposures have also emerged, like the consumption of raw vegetables and sprouts, working or camping in rural areas, visiting farms, and person-to-person transmission. Epidemiological changes have also been determined by the intensification of cattle production, the increase in centralized food production and distribution, and the growth in the volume of international trade of foods. The main lessons learned from recent large outbreaks are knowledge of virulence determinants of new pathogenic strains, recognition of new vehicles of infection, development of new methodologies for detecting STEC in foods and humans, improvement in food regulations and hygiene guidelines, new therapeutic approaches in the treatment of infected patients, establishment of continuous educational programs for food consumers, and enhanced cooperation and teamwork of regional and international networks.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) strains emerged in the late 1970s or early 1980s as highly significant zoonotic threats to public health. In 1982, two outbreaks of severe bloody diarrhea, related to a previously rare serotype of *E. coli*, O157:H7, were reported in the United States (1).

At present, we know that STEC strains are an important cause of morbidity and mortality, with associated loss of life years and diminished health-related quality of life. The clinical manifestations of infection range from symptom-free carriage to nonbloody diarrhea, hemorrhagic colitis (HC), and hemolytic-uremic syndrome (HUS) (2). The linkage between STEC infection and the development of HUS was established by Karmali and colleagues in 1983 to 1985 (3, 4).

HUS is a systemic thrombotic microangiopathy caused by different etiologies and mechanisms, involving acute kidney failure that may result in death or end-stage renal disease (ESRD), a serious chronic condition that reduces life expectancy. Patients with ESRD are initially treated with peritoneal dialysis or hemodialysis and may later be eligible for kidney transplantation (5). The cascade
leading from gastrointestinal infection to renal impairment is complex, the production of Shiga toxin 1, Shiga toxin 2, and/or their subtypes (Stx1a, Stx1c, Stx2a, Stx2b, Stx2c, Stx2dactivatable, and Stx2f) being the primary virulence trait responsible for human disease. However, a mosaic of different virulence traits, comprising several adhesins and other toxins that may play a role in pathogenesis, has also been described (2).

Cattle have been recognized as the main reservoir for STEC for more than 30 years; however, several different surveys have demonstrated that STEC strains occurred in the gastrointestinal tracts of other domestic animals such as sheep, goats, water buffalos, pigs, dogs, and cats (6). Humans usually become infected by eating undercooked beef products, but secondary sources, including leafy green vegetables, apple cider, and dairy products that have been contaminated with manure, are also vehicles for food-borne infection (7). Infections have also been caused by drinking or swimming in contaminated water, person-to-person transmission, or contact with infected animals (6, 8).

STEC isolates that cause human infections belong to a large number of O:H serotypes, and O157:H7 is the most prevalent serotype associated with large outbreaks and sporadic cases of HC and HUS in many countries (9).

In 2003, Karmali et al. (10) proposed classifying STEC serotypes into five seropathotypes (A to E) based on their reported frequencies in human illness and their known associations with outbreaks and severe outcomes, including HC and HUS. Seropathotype A (O157:H7 and O157:NM), considered the most virulent, is associated with the highest incidence in human disease and is often involved in outbreaks. Seropathotype B (O26:H11 and NM; O45:H2 and NM; O103:H2, H11, H25, and NM; O111:H8 and NM; O121:H19 and H7; and O145:NM) is associated with severe human disease, but lower frequency, and is uncommonly involved with outbreaks. Seropathotypes C (O91:H21, O104:H21 and O113:H21, among others) and D have a low incidence in human illness and are rarely associated with outbreaks. Finally, seropathotype E is composed of many serotypes that have not been implicated in human diseases.

**SURVEILLANCE AND DISEASE TRENDS**

Surveillance practices vary considerably among countries, and therefore caution is required when comparing STEC incidence rates among countries.

In the United States, *E. coli* O157:H7 infection became nationally notifiable in 1995. Since 2000, all STEC infections that cause human illness are notifiable to the Nationally Notifiable Diseases Surveillance System (NNDSS) in the United States. In 2011, the Centers for Disease Control and Prevention (CDC) Emerging Infections Program analyzed the data gathered from the Foodborne Diseases Active Surveillance Network (FoodNet). A total of 521 laboratory-confirmed cases of STEC non-O157 and 463 of STEC O157 infections were identified, with incidence rates of 1.10 and 0.97 per 100,000 persons, respectively. Moreover, FoodNet ascertained 96 HUS cases, including 93 (97%) post-diarrheal HUS cases in 2010. The population under surveillance was 47,505,580, which represents 15.2% of the total U.S. population. According to CDC, illnesses caused by non-O157 STEC serogroups tended to be less severe than those caused by *E. coli* O157:H7 because they required less hospitalization (18% versus 43.4%), the death rate was lower (0.19% versus 0.43%), and HUS developed in fewer patients (1.7% versus 6.3%) (11).

In Canada, STEC infection has been classified as a notifiable disease since 1990. C-EnterNet is a national integrated enteric pathogen surveillance system that collects information on both cases and source of exposure in two sentinel sites, Ontario (since 2005) and British Columbia (since 2010). In 2010, the incidence of illnesses caused by STEC was 2.2 and 2.9 per 100,000 persons in each site, respectively. The national incidence rate was 2.3 per 100,000 persons in 2008 (12).

In 2010, the European Centre for Disease Prevention and Control reported 3,710 confirmed cases of STEC infection, with an incidence of 0.96 per 100,000 persons. The annual Community Summary Report on the European Union gave an incidence of all STEC infections in Austria, Belgium, Finland, and Italy as 1 case per 100,000 or less; and in Germany, the United Kingdom, the Netherlands, Denmark, Sweden, and Ireland, as 1.2, 1.8, 2.9, 3.2, 3.6, and 4.4 cases per 100,000 persons, respectively (13).

In Australia, information on the incidence of STEC infections and HUS is obtained from the Australian NNDSS, and has been mandatory in all jurisdictions since 2000, except Queensland and Western Australia, where the incidence became notifiable in 2001. For the 11-year period from 2000 to 2010, the overall annual rate was 0.4 cases per 100,000 persons, and the annual rate of notification for HUS was 0.07 cases per 100,000 persons, while neighboring New Zealand reported a STEC infection rate of 3.3 cases per 100,000 per year (14).

In Latin America, STEC infections are endemic and contribute to the burden of acute diarrheal syndrome in children less than 5 years of age, being responsible for
2% of total cases of acute diarrhea, and in a few studies correspond to 20 to 30% of bloody diarrhea (8).

Important differences exist in the incidence of STEC infections and HUS in South America. A regional network for surveillance purposes is still nonexistent, and data are restricted to only a few countries. HUS is endemic in some countries of the southern cone region, and reporting is mandatory only in Argentina, Bolivia, Chile, and Paraguay.

In Brazil, STEC infections are important public health issues, at least in some regions, but in general, the incidence is relatively low (15). In Chile, a National Surveillance System was established in 1999, and all clinical laboratories must report and send isolates to the Reference Laboratory. In Uruguay, reports of HUS are not mandatory, and only a few cases are recognized each year (16). In Paraguay, reporting of HUS has been mandatory since 2005, and the estimated annual incidence is 0.6 cases per 100,000 children under 5 years old (8).

In Argentina, STEC-associated illnesses are a serious public health concern. Data on human STEC infections are gathered through different strategies: (i) reporting of clinical HUS cases to the National Health Surveillance System (in Argentina the system is named Sistema Nacional de Vigilancia de la Salud [SNVS]); reports, which have been mandatory since 2000, must be immediate and individualized; (ii) the Sentinel Surveillance System through 25 HUS sentinel units; (iii) the laboratory-based surveillance system through the National Diarrheal and Foodborne Pathogens Network; and (iv) the Molecular Surveillance through the PulseNet of Latin America and Caribbean.

Postdiarrheal HUS is endemic, with the highest rate of pediatric cases globally. Over the last 10 years, approximately 400 HUS cases were reported annually. The incidence ranged from 10 to 17 cases per 100,000 children less than 5 years of age, and lethality was between 1 and 4% (Fig. 1).

Between 2004 and 2010, a total of 1,245 O157 and non-O157 STEC strains, isolated from HUS (597) and bloody (335) and nonbloody (167) diarrhea cases, healthy carriers (74), and unspecified pathologies (72), were confirmed by the laboratory-based surveillance system and the HUS sentinel units. Multiple serotypes were identified, but O157:H7 was the predominant (>70%), and O145:NM (13.6%) was the second most important serotype identified. Among the STEC O157 strains, the \textit{stx}_2a/\textit{stx}_2c/\textit{eae}A/\textit{ehxA} genotype prevailed (>80%). For the non-O157 strains, the genotypes were more diverse, but the full virulent \textit{stx}_2a/\textit{eae}A genotype was prevalent (>60%). In Argentina, outbreaks are identified through the surveillance system of HUS and STEC-associated diseases. The definition of an outbreak used for this analysis is two or more linked cases. Pulsed-field gel electrophoresis (PFGE) and phage typing are used to establish the clonal relatedness of the isolates. In the period 2004 to 2010, a total of 12 outbreaks of bloody diarrhea and HUS cases associated with O157 and non-O157 STEC strains occurred in kindergartens, families, and the community. The outbreak size ranged from 2 to 32 cases, and two patients with HUS died. Person-to-person transmission was the main route identified.

**FIGURE 1** Number of HUS cases, incidence rates, and percentages of lethality in Argentina, 2002–2011. doi:10.1128/microbiolspec.EHEC-0002-2013.f1
As a part of PulseNet Latin America and Caribbean, national databases were created for O157 and non-O157 E. coli, including strains isolated since 1988 from different sources. Among O157 strains, two patterns, named AREXHX01.011 and AREXHX01.022, are prevalent, representing around 13% of the database (Fig. 2a and 2b). Pattern 011 and other related patterns, with 95% similarity, are part of the hypervirulent clone described in different countries (Fig. 2c). Pattern 011, which has been prevalent in Argentina in the past 10 years, is identical to the most prevalent pattern in Sweden (named SMI-H) and to the most common type, named 047, in human infections in the United States (17). Among the non-O157 strains, the PFGE patterns are more diverse and two patterns, named AREXSX01.0006 (O145) and AREXPX01.0008 (O113), are prevalent.

**COST OF STEC-ASSOCIATED DISEASES**

Severe illnesses with long-term sequelae caused by STEC have a social and economic cost to the community and the health system. However, it is difficult to compare data from cost of illness studies among countries because of differences in definitions of costs, methodologies used, and income distribution.

From FoodNet data (2005–2008), non-O157 STEC strains are estimated to cause 168,698 illnesses each year, and E. coli O157:H7, 96,534 cases in the United States, with more than 3,600 hospitalizations and 30 deaths (18). Frenzen et al. (19) estimated the total annual cost of illness (COI) due to STEC O157 at USD$405 million in 2003, Buzby and Roberts (20) updated this estimate to USD$459 million in 2007, and Scharff et al. (21) suggested that STEC O157 infections cost about USD$990 million in 2009 to U.S. residents.

In the Netherlands, Havelaar et al. (22) described the burden of disease associated with STEC O157 at the population level using the public health indicator “Disability-Adjusted Life Years” (DALYs), and showed that mortality due to HUS, ESRD, and dialysis due to ESRD constitute the main determinants. Tariq et al. (23) evaluated the societal impact of STEC O157 infection using a combination of DALYs and COI, including direct health care costs and indirect non-health care costs. Total annual COI due to STEC O157 infection for the Dutch society was estimated at €9.1 million. The authors concluded that, compared to other foodborne pathogens, STEC O157 infections result in a relatively low burden and low annual costs at the societal level, but the burden and costs per case are high.

In Australia, McPherson et al. (24) estimated the cost of STEC infections at approximately AUD$2.6 million each year.

In Argentina, Caletti et al. (25) evaluated the direct health care costs and indirect non-health care costs of 231 HUS cases attending the Hospital Nacional de Pediatria “Dr. Juan P. Garrahan” in Buenos Aires City, in the period 1987 to 2003. The total annual cost for HUS cases was approximately USD$2 million.

![FIGURE 2](a) Top ten XbaI-PFGE patterns associated with human STEC O157 strains in Argentina. (b) Dendrogram of the top ten XbaI-PFGE patterns. (c) Dendrogram of AREXHX01.0011 pattern and other related patterns. doi:10.1128/microbiolspec.EHEC-0002-2013.f2
RISK FACTORS

Some determinants of the pathogen and its reservoir, the host, and cultural and dietary behaviors have been described as risk factors for acquiring an STEC-associated disease.

Pathogen Factors

Several determinants have been described as risk factors that may play a role in the outcome of an STEC infection, such as the initial bacterial inoculum, the amount and type of Stx produced, and the serotype of the infecting strain; the ability of E. coli to horizontally acquire specific genetic elements known as pathogenicity islands (PAI), and stx genes from free bacteriophages in the environment and in the mammalian hosts; and the improved adaptation of the bacteria to human hosts (26).

Among STEC strains, E. coli O157:H7 has become a significant food-borne pathogen, exhibiting some characteristic features, such as low infectious dose (~100 to 500 organisms) and acid tolerance that certainly favors their transmission to humans by the food chain. In addition to STEC O157, only a restricted number of STEC serotypes (mainly those of seropathotype B) are associated with outbreaks and HUS. Moreover, Stx types differ in their biological activity and association with disease. There is evidence of a linkage of Stx2a (formerly Stx2) with a higher risk of severe human disease. The presence of both stx2a and eae genes in an STEC isolate is considered to be a predictor of HUS (27). However, it has been shown that highly pathogenic strains producing Stx2d activatable are eae negative (28).

Besides Stx and lipopolysaccharide, other putative virulence factors, including adhesins, other toxins and proteases are required to develop disease. Extensive evidence suggests that a major pathogen determinant is the presence of specific PAIs. A number of PAIs, including the locus of enterocyte effacement (LEE), play a major role in enhancing the ability of some serotypes to cause severe human disease. In addition to the proteins encoded in the LEE, the type III secretion system also secretes other effectors encoded outside the LEE. At least three of these non-LEE-encoded effectors have been linked to non-O157 STEC strains that cause HUS (29).

As the population of STEC O157 strains increased in frequency and spread geographically, it has genetically diversified. Isolates of STEC O157 from clinical and bovine sources have been shown to be genotypically diverse by different methods, including PFGE, octomer-based genome scanning, and multilocus variable number of tandem repeats analysis. Studies of prophage and prophage remnants in STEC O157 strains have indicated that genotypic diversity is largely attributable to bacteriophage-related insertions, deletions, and duplications of variable sizes of DNA fragments (30).

After the description of the hypervirulent clade of O157 associated with the raw spinach outbreak in the United States in 2006 (30), several studies were performed in the northern hemisphere countries to assess the frequency of this clade in human disease and cattle. However, no data were available from the southern hemisphere countries.

As notable differences were observed in the prevalence and severity of human diseases caused by O157 isolates in Argentina (high) and Australia (low), Mellor et al. (31) compared human and bovine O157 isolates from both countries. The locus-specific polymorphism analysis genotyping revealed that lineage I/II (LI/II) E. coli O157 isolates were the most prevalent in Argentina (88%) and Australia (88%). Argentinean LI/II isolates were shown to belong to clades 4 (30%) and 8 (65%) while Australian LI/II isolates were identified as clades 6 (15%), 7 (80%), and 8 (2%). In Argentina, clade 8 isolates dominated in both cattle (50%) and humans (80%); meanwhile, in Australia clade 7 dominated in both cattle (70%) and humans (90%).

Host Factors

Several host factors influence the risk of acquiring STEC infection, including age, immunity, health status, the use of antibiotics and antimotility agents, stress, and genetic factors.

The highest age-specific frequency of HUS is in infants and young children. It declines with increasing age and increases again in the elderly, probably due to changes in the immunity status. Gastric acidity is an important initial host barrier to ingested pathogens. Its protective role against E. coli O157:H7 infection has been suggested because individuals with low gastric acidity are at a significantly higher risk for HUS developing than those with normal physiological gastric function.

The possible role of stress as a risk factor for severe disease, and host genetic factors that may influence host-pathogen interactions, including the innate immune response to infection and the nature of the toxin-cell interaction, have been described. The genes that regulate the gut colonization by E. coli O157:H7 may be modulated by hormone-like soluble factors produced by other bacteria in a process known as quorum sensing. The quorum-sensing pathway could be activated by host stress hormones such as epinephrine and norepinephrine (32).
Behavior and Cultural Factors
Since the emergence of STEC, case-control and population-based studies, varying in sizes and rigor, have been conducted to examine risk factors in associated infections.

In the 1990s, studies to evaluate risk factors were focused on sporadic cases of E. coli O157 infection. The consumption of undercooked hamburgers and meat, eating in restaurants or fast-food establishments, living or working on or visiting a cattle farm, drinking untreated surface water, swimming in contaminated water, contact with animal feces, and consumption of raw milk were the main risk factors identified.

In a literature review of several articles published in the past decade, it was observed that several studies were conducted to learn more about the risk factors of both non-O157 and STEC O157 infections. The main findings of some of these studies are summarized in Table 1.

Cattle Management Factors
The role of cattle as a source of human infection has been extensively studied and reviewed, mostly in reference to STEC O157 (42). Prevalence of STEC O157 in cattle feces and hides is highly variable (43), dependent on region, farm and cattle type, age, and season, among other factors. The degree of herd infection is uneven, with herds usually being colonized by a low number of predominant strains (44). In Argentina, a similar pattern was found at the abattoirs, where STEC O157 was detected in approximately 15% of arriving lots, with an average prevalence in feces of 4.1% (45).

In the animal production environment, the prevalence of other STEC serogroups, mainly those named “big six” (O26, O45, O103, O111, O121, O145), is less well known. Recent studies have shown that only a small fraction of strains from those O groups isolated from cattle carry Stx genes (46, 47).

Risk of infection could be increased in spring and summer, as most reports state that during warmer months there is a higher prevalence of STEC in cattle (48). The risk of colonization and shedding through the use of different cattle diets is a complex issue, thoroughly revised lately (49). Attention has been given to finishing diets with an increased ratio of grains that could enhance STEC O157 shedding. Inclusion of orange peel (50) or a dietary shift to forage has been proposed to decrease STEC O157 shedding. However, much less is known about the effect of diets on the ecology of other STEC serotypes.

The emergence of super-shedding bovines is a relevant risk for STEC O157 contamination of the beef supply chain. Super-shedders have been linked to the diversity of STEC O157 prevalence in cattle populations (51) and to the increasing spread of hide contamination in feedlot cattle (52). These studies and others (53) have shown that by controlling super-shedding bovines there would be a high impact on preventing STEC O157 infection in humans.

STEC strains are able to survive some months in the environment, feces slurries, and cattle manure (54–56). A recent and systematic review (57) concluded that there is a complex relationship among animal reservoirs, pathogens, and the environment leading to the contamination of fresh produce from the environment. Manure and fecal contamination of irrigation water were the most important media for STEC presence in fresh produce at preharvest. The importance of minimizing these sources of contamination has been highlighted by STEC outbreaks with fresh produce such as spinach (58).

Mapping studies have given epidemiological evidence of the significance of environmental contamination for STEC human infection in rural areas (59–61). In Argentina, Tanaro et al. (62) have shown a degree of contamination of surface waters in rural areas of a cattle-producing region of Entre Rios Province.

Armstrong et al. (63) suggested, as a probable cause for the emergence of STEC O157, the changes in modern livestock and food processing industries, characterized by the concentration, homogenization, and increased scale of operations. Within the abattoir, the hide-removal operation is a critical point for carcass contamination (53). In addition, lairage areas have been identified as key for STEC dissemination as most isolates from carcasses were traceable to the lairage environment rather than to the original feedlot (64).

Studies conducted in Argentina showed that 11 STEC strains (ten O157:H7 and one O178:H19) isolated in abattoirs had similar phage type-XbaI-PFGE pattern-stx genotype profile as those responsible for 19 HUS cases in the same period (45, 63). For STEC O157 it was possible to link 12% of reported human infections to the bovine reservoir (66). In the same study, it was estimated that, at slaughter, ca. 38,000 bovines would carry STEC O157 in their feces per each clinical case of HUS and bloody and nonbloody diarrhea cases reported.

Risk factors identified in cattle production and at the abattoir can be integrated as components of risk assessment models that allow estimates of the risk of STEC infection for the population from consumption of a specific meat product (67). They are also useful tools to evaluate the outcome of mitigation strategies in cattle production and the meat industry (67, 68). Risk assessment studies have also been conducted to estimate the risk of STEC in ground beef hamburgers in Argentina (69).
NEW SCENARIO AND LEARNED LESSONS FROM OUTBREAKS

The epidemiological profile of food-borne diseases has changed dramatically in recent decades. Some of the contributing factors for the emergence of outbreaks with different epidemiological characteristics and for the widespread epidemics are (i) the development of new food processing technologies and foods, (ii) the more centralized and rapid food distribution systems, (iii) the changes in consumer preferences and behaviors, and (iv) the considerable increase in the volume of food products traded internationally. Additionally, the enormous increase in global travel allows individuals to be infected in one country and to become ill on their return to their country of origin (70).

This epidemiological change was also influenced by the genetic variation and “relentless evolution” of the O157 pathogen population (71). Mellmann et al. (72) remarked that bacterial evolution is an ongoing process that undoubtedly will lead to the emergence of other successful pathogenic clones of E. coli in the future.

Since the emergence of STEC as a food-borne pathogen in 1982 (1), large outbreaks of gastrointestinal disease, involving numerous persons and associated with different sources and vehicles of transmission, have been described worldwide.

Each outbreak showed different aspects of complexity, from the detection of the source and the vehicles of transmission to the need for the development of control strategies to avoid the occurrence of new cases. These large outbreaks were of great concern and challenge for the public health system, compelling the food regulatory and health agencies and the food industry to establish improved guidelines to control and prevent new incidents.

Large outbreaks have been commonly notified in industrialized countries like the United States (73), Japan (74), Germany (75), Canada (76), among others. In these countries, the advanced epidemiological system has contributed to a better investigation and understanding of these events. Furthermore, small indoor outbreaks were described worldwide, mainly in families and child-care centers with a lower frequency (77, 78).

In this context, it is interesting to point out the lessons learned about risk factors, treatment, diagnosis, and advances in health and food regulations from some emblematic outbreaks (Table 2).

The large outbreak of E. coli O157:H7 infection that occurred in four western states of the United States in February 1993 was the first event with a great population and media impact. For the first time, people were massively informed about good practices for food handling regarding this pathogen. The outbreak investigation allowed us to know about the low infectious dose of this organism, its capacity to survive for a long time (more than 2 months) in frozen storage, and the temperature and time combinations (68°C, 15 sec) that ensure a safe hamburger cooking condition. These findings were used as strong arguments to enforce a zero tolerance policy for this microorganism in processed food and for the need for a pronounced decrease in the contamination of raw ground beef (73, 79, 80).

At present, the Sakai outbreak of E. coli O157 infection in July 1996 was the largest outbreak ever experienced, because of the number of people affected (74). As described by Fukushima et al. (81), lunch foods contaminated with E. coli O157 and supplied to elementary schools by a centralized distribution system were the cause of this massive outbreak. It is interesting to note the clinical experiences in the treatment received by the affected children. Almost all patients were treated with antibiotics, fosfomycin and lactobacilli, from the early days of the illness. An evaluation of the outcomes revealed that these treatments were more than satisfactory compared to previous reports. However, the favorable outcome could not be only attributed to the effectiveness of antibiotics. Other factors, like age and racial differences, might also be responsible.

The spinach outbreak in the United States in July 2006 highlighted the importance of fresh produce as a vehicle in STEC infections and also the role of international trade because the product was exported to Canada and Mexico. During the epidemiological survey, most of the patients (95%) reported consuming uncooked fresh spinach during the 10 days before the onset of the illness (58). Women (71%) were the most affected, probably reflecting that women are more likely to consume fresh vegetables. Moreover, the outbreak was related to the practice of consuming prewashed, bagged leafy greens, as the general public assumed food handling in farms and in processing facilities to be safe. The sequencing of the genome of the TW14359 strain, responsible of this outbreak, helped identify the genetic factors that enhanced the ability of this strain to cause such a high number of HUS cases (82).

Manning et al. (30) published a comparison among outbreaks with different severity of the reported illness. The 1993 outbreak in western North America and the large 1996 outbreak in Japan had low rates of hospitalization and HUS, whereas the 2006 North American spinach outbreak had high rates of both hospitalization (50%) and HUS (10%). Single nucleotide polymorphism genotyping revealed the genetic variability...
<table>
<thead>
<tr>
<th>Year of study</th>
<th>Country</th>
<th>Study design and size</th>
<th>Risk factors identified</th>
<th>Conclusions</th>
<th>Advice to change behaviors and reduce health risks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000–2001</td>
<td>France</td>
<td>Matched case-control study to evaluate risk factors for sporadic HUS cases in children. 105 cases: 196 controls.</td>
<td>Eating undercooked ground beef. Contact with a person with diarrhea. Drinking well water during the summer period.</td>
<td>Adequate cooking of ground beef may reduce the incidence of STEC infection. Assiduous attention to hygienic measures to prevent the spread of STEC within families and child-care facilities has the potential to further reduce HUS episodes in childhood.</td>
<td>Proper cooking of ground beef (National Public Health Campaign, 2006). Revision of safety control measures at all levels of the ground beef food chain. Specific education program for professionals involved in the meat industry.</td>
<td>33</td>
</tr>
<tr>
<td>2001–2002</td>
<td>Argentina</td>
<td>Matched case-control study to evaluate risk factors for sporadic STEC infections in children enrolled in two sites, Mendoza (urban and semirural area) and Buenos Aires (urban area). 150 cases: 299 controls.</td>
<td>Eating undercooked beef at any place. Contact with a young child with diarrhea. Attending a daycare center or kindergarten. Living in or visiting a place with farm animals. Contact with farm animals and cattle manure. Having nonparental income.</td>
<td>Meat-related dietary habits and animal exposures were linked to illness. Person-to-person spread was an important mode of transmission. Some risk factors were specific by location. Eating a wider variety of fruits and vegetables and washing hands after handling raw beef, especially with soap and water, were protective factors.</td>
<td>Apply effective safe practices at all stages of the food chain (industry, government, and consumers). Ensuring that beef is well cooked at home and outside home. Establish an educational program to avoid risks of dietary habits and behaviors, and recommendations to protect people through hand washing.</td>
<td>34</td>
</tr>
<tr>
<td>2001–2003</td>
<td>Germany</td>
<td>Matched case-control study to evaluate risk factors for sporadic STEC infections in different age groups. 202 cases: 202 controls.</td>
<td>Contact with small ruminants and consuming raw milk (children &lt;3 years). Playing in a sandbox (children &lt;3 years and aged 3–9 years). Swimming in nonpublic swimming pools (children aged 3–9 years). Consuming lamb meat and raw fermented spreadable sausages (cases ≥10 years or older).</td>
<td>Risk factors were age specific. In children, food-borne transmission played a lesser role in both acquiring STEC infection and developing HUS. Consumption of lamb meat and raw spreadable sausages was identified as risk factor for the first time.</td>
<td>Modify the upper pH limit (5.6) of raw spreadable sausage because STEC survives under acidic conditions (pH 4.0).</td>
<td>35</td>
</tr>
<tr>
<td>1997–2006</td>
<td>Finland</td>
<td>Population-based study applying a statistical model to distinguish between risk factors for occurrence and incidence of STEC diseases. 131 cases.</td>
<td>Increased occurrence and incidence: Proportion of beef cattle to human population. Proportion of population with higher education related to consumption habits of undercooked meat. Increased incidence: Proportion of fresh water. Number of cultivated farms. Proportion of low-income households with children.</td>
<td>Socioeconomic factors, like low-level income and education, were important for acquiring STEC infections. Ecological factors such as the relation between population and density of beef cattle were important for the incidence of the disease.</td>
<td>Applying good hygiene in animal and slaughter process and food retail. Consumer education. Proper food handling. Up-to-date national legislation and regulation.</td>
<td>36</td>
</tr>
</tbody>
</table>
### Risk Factors for Shiga Toxin-Producing E. coli-Associated Human Diseases

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Study Type</th>
<th>Case Details</th>
<th>Risk Factors</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003–2007</td>
<td>Australia</td>
<td>Case-control study to evaluate risk factors in sporadic O157 and non-O157 STEC infections.</td>
<td>213 cases; 304 controls.</td>
<td>STEC O157: Consumption of hamburgers. Eating at restaurants. Having occupational exposure to red meat. Non-O157 STEC (O111, O26, O103, O113, O172): Occupational exposure to animals. Consumption of sliced processed chicken meat and sliced corned beef. Eating out at a catered event. Camping in a bush. Risk factors were serogroup specific. Hamburgers and ground beef have not been implicated in outbreaks of STEC and have not been previously considered a source of infection.</td>
<td>Design educational programs for people who live or work with animals or raw meat, as well as for those who enjoy camping. Recommend that consumers and food handlers cook hamburgers thoroughly.</td>
</tr>
<tr>
<td>2000–2009</td>
<td>United States</td>
<td>Population-based study to compare risk factors in patients infected with O157 and non-O157 STEC.</td>
<td>392 patients.</td>
<td>O157 and O103 STEC: Eating pink hamburger. Handling raw ground beef. Non-O157 STEC (O111, O103, O26): International travel in the 7 days prior to symptoms. Non-O157 STEC (O111, O103, O26, O45): Consumption of untreated surface water.</td>
<td>Some STEC serogroups such as O103 seem to have an epidemiological and exposure profile similar to O157, and they likely occupy a similar ecologic niche. Other serogroups are quite different (O45) and may not be able to be managed through identical measures to control O157. Continue population-level monitoring of the epidemiology of STEC to determine longer term trends and opportunities for control.</td>
</tr>
<tr>
<td>2002–2009</td>
<td>Argentina</td>
<td>Case-control study to identify risks factors for sporadic STEC infections in children aged up to 6 from the Central Eastern area.</td>
<td>63 cases; 374 controls.</td>
<td>Eating food prepared outside home.</td>
<td>Protective effects of a diet that includes vegetables. Plan strategies for local prevention to diminish the incidence of HUS in the region under study.</td>
</tr>
<tr>
<td>2007</td>
<td>Argentina</td>
<td>Epidemiological survey to evaluate risk factors for STEC infections in different socioeconomic groups.</td>
<td>883 students aged 10–12 years from elementary public schools of an urban area of Buenos Aires Province.</td>
<td>Eating commercially prepared precooked or homemade hamburgers. Exposure to water of swimming pools. No hand wash after going to the toilet or before eating food.</td>
<td>Differences in the frequency of hamburger consumption were observed among children from different socioeconomic strata. Children from high and medium strata attending private swimming pools and children from low stratum attending public swimming-pools were at risk. Improve educational programs to enhance personal hygiene, adequate meat handling and cooking techniques, and maintenance of safe recreational water.</td>
</tr>
<tr>
<td>2003–2012</td>
<td>Argentina</td>
<td>Epidemiological survey to evaluate risk factors for sporadic HUS cases and STEC infections in urban and semirural area of Rio Negro Province.</td>
<td>42 cases.</td>
<td>Eating undercooked ground beef, sausages, barbecue, and unpasteurized milk. Contact with farm animals. Poor hygiene in food and father with rural activities, and poor hygiene of work clothes.</td>
<td>Food, contact with rural workers, and environment were risk factors for children. Improve the surveillance system, considering the particular conditions of the region. Establish a health education program sustained over time.</td>
</tr>
</tbody>
</table>
### TABLE 2
Features of major food-borne outbreaks associated with Shiga toxin-producing *E. coli*

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>No. of cases</th>
<th>No. of hosp.</th>
<th>No. of HUS</th>
<th>No. of deaths</th>
<th>Associated pathogen</th>
<th>Impact</th>
<th>Vehicle</th>
<th>Risk factors</th>
<th>Learned lessons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan, Sakai City, 1996</td>
<td>12,680&lt;sup&gt;b&lt;/sup&gt;</td>
<td>398&lt;sup&gt;c&lt;/sup&gt;</td>
<td>121&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>STEC O157:H7 (&lt;i&gt;stx&lt;/i&gt;&lt;sub&gt;1a&lt;/sub&gt;/&lt;i&gt;stx&lt;/i&gt;&lt;sub&gt;2a&lt;/sub&gt;)</td>
<td>Massive and widespread outbreak. Children in elementary schools in different districts affected.</td>
<td>Radish sprouts, among others.</td>
<td>Centralized distribution of school lunch food. Raw vegetables.</td>
<td></td>
</tr>
<tr>
<td>United States, 26 states, 2006</td>
<td>205&lt;sup&gt;d&lt;/sup&gt;</td>
<td>95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>STEC O157:H7 (&lt;i&gt;stx&lt;/i&gt;&lt;sub&gt;2a&lt;/sub&gt;)</td>
<td>Large outbreak with high number of hospitalizations and HUS cases.</td>
<td>Fresh spinach.</td>
<td>Consumption of prewashed, bagged fresh produce. Emergence of hypervirulent <em>E. coli</em> O157 strain. International trade.</td>
<td></td>
</tr>
<tr>
<td>Germany and other 13 European countries, United States, and Canada, 2011</td>
<td>3,842&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td>855&lt;sup&gt;e&lt;/sup&gt;</td>
<td>53&lt;sup&gt;e&lt;/sup&gt;</td>
<td>STEC O104:H4 (&lt;i&gt;stx&lt;/i&gt;&lt;sub&gt;2a&lt;/sub&gt;)</td>
<td>Large outbreak with: High proportion of adults affected, mainly women. Unusually high number of HUS in adults. Patients with severe neurological symptoms followed by death.</td>
<td>Fenugreek sprouts/seeds.</td>
<td>Eating salads. Emergence of hypervirulent hybrid EAEC-STEC O104 strain, with enhanced colonization and long-term shedding.</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from reference<sup>79</sup>.<br><sup>b</sup>Data from reference<sup>81</sup>.<br><sup>c</sup>Data from reference<sup>74</sup>.<br><sup>d</sup>Data from reference<sup>58</sup>.<br><sup>e</sup>Data from reference<sup>84</sup>.<br>ND, no data.
among pathogenic strains associated with clinical infection. Their results support the hypothesis that the clade 8 lineage has recently acquired novel factors that contribute to the enhanced virulence.

The massive outbreak of bloody diarrhea and HUS that occurred in 2011 in Germany and other 13 European countries, the United States, and Canada was a challenge for clinicians and microbiologists given its atypical presentation. The seriousness of the illness and the fatalities, coupled with the lack of a definitive source of the causative agent, created a highly negative impact on the population and gained the front page of newspapers around the world (83).

This unprecedented outbreak affected mainly adults (90%), predominantly women, and resulted in an unusually high number of HUS cases (n=855). The augmented adherence of the strain to the intestinal epithelium facilitating systemic absorption of Stx could explain the high progression to HUS. The outbreak provided important new insight into novel antibiotic strategies in the treatment of HUS in adults and for decolonization of long-term STEC carriers (84).

The characterization of the O104:H4 outbreak strain revealed an unusual combination of pathogenic features typical of enteroaggregative \textit{E. coli} combined with the capacity to produce Stx. Additionally, isolates displayed an extended-spectrum β-lactamase phenotype, carrying plasmid-borne blaCTX-M-15 and blaTEM-I genes (85). DNA sequencing data rapidly revealed that outbreak strain was a new hybrid of two types of pathogenic \textit{E. coli}. Up to date, nine O104:H4 isolates have been sequenced (https://github.com/ehec-outbreak-crowdsourced/BGI-data-analysis/wiki; http://www.bgisequence.com/eu/index.php?clid=194). Different methodologies for detection and characterization of the O104:H4 strain in clinical and food samples were developed in a short time (85, 86). Possible mitigation options for safe consumption of raw vegetables were advised after a fast-track assessment of the consumer exposure to STEC through this type of food (87).

A restaurant cohort study and the trace-back and trace-forward data analysis of the Task Force EHEC contributed to the identification of fenugreek seeds as sources of transmission. This study also contributed to confirm the implications of international trade in foodborne outbreaks (88).

One of the most important lessons from the O104 outbreak is the successful cooperation among health and food networks and agencies. This type of work could in a short time produce valuable epidemiological and microbiological information, essential for developing public health measures to improve the management of future outbreak situations (75). This outbreak emphasized the importance of common alert and surveillance systems for the early detection of international outbreaks and for a better assessment of their spread.

**CONCLUSIONS**

Differences in the frequency and the severity of STEC-associated human diseases are observed from country to country. The more reliable information is provided by developed countries as better enteric pathogen surveillance systems are in place. Because of the severity and the long-term sequelae of STEC-associated illnesses, they have a high social and economic cost for both the affected families and the health system. In Argentina, because of the number of HUS cases reported each year, those social and economic costs are particularly significant.

The risks for acquiring an STEC infection are associated with several determinants of the pathogen and its reservoir and with biological and cultural factors of the host. The best knowledge about risk factors was obtained from case-control and population-based studies. Main risk factors identified in earlier studies were dietary behaviors related to beef consumption, but at present they include a wider range of foods, such as fresh produce or sprouts. Other risky behaviors identified have been connected to environmental sources, as living in, working in, or visiting rural areas; swimming and camping in recreational areas; and being in contact with farm animals. Risk factors for STEC infection have also been identified in cattle management and at the abattoir, such as the effect of finishing diets, the existence of super-shedders in a herd, the cross-contamination in lairage areas, and the hide-removal step at the abattoir, among the most important ones. Another important risk factor is person-to-person transmission, especially for young children. In Argentina, beef is a traditional component of diet, with an average consumption of ca. 62.5 kg per capita per year. This high rate of consumption, and particularly some meat-related dietary habits, could be risk factors for STEC illness in our population.

A new risk scenario has emerged in the last decades due to the bacterial evolution that gave rise to the emergence of hypervirulent O157 clones with a worldwide distribution, and other STEC strains with unusual combinations of pathogenic features, such as the O104:H4 strain. The epidemiological changes were also influenced by the increase in centralized food production and distribution systems and the growth in the volume of international trade of food ingredients.
The learned lessons from large and emblematic outbreaks could be summarized as (i) the advances in the knowledge of virulence determinants of new pathogenic strains; (ii) the recognition of new vehicles of infection; (iii) the development of new methodologies for STEC detection in foods and humans; (iv) the improvement of food regulations and hygiene guidelines; (v) the new therapeutic approaches in the treatment of STEC-infected patients, especially HUS in adults; (vi) the establishment of continuous educational programs for food consumers; and (vii) the enhanced cooperation and teamwork of regional and international networks.

ACKNOWLEDGMENT
We declare no conflicts of interest with regard to the manuscript.

REFERENCES
Risk Factors for Shiga Toxin-Producing E. coli-Associated Human Diseases


32. Clarke MB, Sperandio V. 2003. Events at the host-microbial interface of the gastrointestinal tract III. Cell-to-cell signalling among microbial flora, host, and pathogens: there is a whole lot of talking going on. Am J Physiol Gastrointest Liver Physiol 288:G1105–G1109.


