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Emerging Public Health Challenges of Shiga Toxin–Producing *Escherichia coli* Related to Changes in the Pathogen, the Population, and the Environment

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Emerging public health challenges of Shiga toxin (*stx*)–producing *Escherichia coli* (STEC) include the occurrence of more frequent or severe disease and risk factors shifts associated with changes, often interconnected, in the pathogen, the population, and the environment. In 3 outbreaks with heightened severity attributed to enhanced pathogen virulence, including the acquisition of an *stx2* phage in 1 outbreak, population and environmental factors likely contributed significantly to disease outcomes. Evolving population risk factors that are associated with more severe disease include consumption of fresh produce, contact with STEC-contaminated environments, demographics, socioeconomic status, and immunity. Risks of increasing STEC environmental pollution are related to continued intensification of agriculture and super-shedder cattle. Mitigation strategies include surveillance and research on emerging STEC, development of effective communications and public education strategies, and improved policies and interventions to mitigate risks, including those related to the contamination of produce and the environment, using a "One Health" approach.

Keywords. STEC disease severity; emerging STEC; population risk factors; environmental risks; One Health.

Shiga toxin (*stx*)–producing *Escherichia coli* (STEC) [1, 2] cause diarrhea, often epidemic, that may be complicated by hemorrhagic colitis [3] and hemolytic uremic syndrome (HUS) [4, 5], the leading cause of acute renal failure in children [1]. Of the 2 major types of the phage-encoded *stxs*, *stx1* and *stx2*, HUS is associated more commonly with strains that produce *stx2* alone or in combination with *stx1* rather than those that produce *stx1* alone [6, 7]. Most cases of STEC infection are acquired by consuming food of bovine origin; however, other foodstuffs, water, environmental contact, and person-to-person transmission are also important sources [8]. Most human STEC infections are associated with serotype O157:H7, but awareness of the public health importance of non-O157 serotypes is growing [9–11].

In the last 2 decades the frequency of STEC O157:H7 infections, especially in North America, has decreased [12, 13] due largely to improvements in meat safety [14]. Despite this, some worrying public health challenges are emerging, including the occurrence of more frequent or severe HUS associated with particular STEC serotypes or subtypes [15–17] and with novel "hybrid" strains that have acquired *stx2* phage by horizontal gene transfer with [18]; and changing risk factors for disease. These challenges are driven mostly by changes in the pathogen, the population, and the environment.

Here, I review how changes in the pathogen, the population, and the environment contribute, often in an interdependent manner, to emerging challenges and consider how these can be mitigated.

CHANGES IN THE PATHOGEN

The risk of developing HUS following STEC infection depends on several host factors, including demography [19], immunity [20], lifestyle [8], and use of antimicrobial and antimotility agents [21]; and on pathogen factors, such as serotype [1, 2], toxin type [7], other virulence factors such as the locus of enterocyte effacement (LEE) [2], and exposure dose [22].

Examples of situations where pathogen factors have been linked to higher-than-expected severity or frequency of HUS include STEC O157:H7 clade 8 produce-associated outbreaks [16]; an STEC O157:H7 strain Xuzhou21 outbreak in China [17]; *stx2*-producing STEC O26:H11/H- [15]; and novel "hybrid" *stx2*-producing serotype O104:H4 [18] and O80:H2 disease [23].

STEC O157:H7 Clade 8 Strains Linked to Fresh Produce

In 2006, multistate outbreaks of STEC O157:H7 infection occurred in the United States associated with the consumption of fresh produce [24–26], in which the frequencies of HUS and/or hospitalization were significantly higher than expected [27].

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A subsequent analysis confirmed that outbreaks attributed to foods generally consumed raw, potentially containing viable pathogenic organisms [24], including leafy vegetables, fruits, unpasteurized dairy, and sprouts, caused higher morbidity than those attributed to foods generally consumed cooked (beef, poultry, and other meats) [8].

Manning et al [16] postulated that the increased morbidity in produce-associated outbreaks was related to enhanced virulence in the pathogen. They genotyped more than 500 STEC O157 clinical isolates and identified 9 distinct clades of which the produce-associated outbreak clade 8 was specifically associated with more severe disease. Further research [28] showed that clade 8 strains had higher adherence to epithelial cells and a higher expression of several virulence genes, including *stx2*, compared to other clades. The high expression levels of *stx2* correlated with more severe pathology in *in-vivo* mouse and rabbit models of infection [29]. While these findings make an association between clade 8 strains and severe disease conceivable, the consumption of uncooked fresh produce, that was contaminated with the pathogen [24], likely also contributed to disease severity.

STEC O157:H7 Strain Xuzhou21 Outbreak in China

In an extraordinary outbreak of STEC O157:H7 infection in China [17], there were 195 individuals with HUS, of whom 90% died. Although the exact frequency of HUS in this outbreak is not known, the severity was exceptionally high. Most patients (85.6%) were aged >50 years and female (62.1%). The induced level of *stx-2* was significantly higher in Xuzhou21 than in the Sakai and EDL 933 reference strains, and Xuzhou21 induced significantly higher levels of either interleukin-6 or interleukin-8 in peripheral blood mononuclear cells than did the reference strains; these features potentially contributed to disease severity. However, population and environmental factors also likely contributed to disease outcome given that most affected individuals were elderly, lived in poor social conditions in close proximity to infected animals, and were heavily exposed to a fecally contaminated environment.

Stx2-producing STEC O26:H11/H-

Non-O157 STEC has been less frequently associated with HUS than STEC O157:H7 [21]. In the 1980s and 1990s most non-O157 strains associated with HUS, including those of serotype O26:H11/H-, produced *stx1* alone [30]. Toward the end of the 1990s, a genotype shift in serotype O26:H11/H- was observed from strains that contained *stx1* toward those that contained *stx2* alone. Bialaszewska et al [15] investigated the correlation between *stx* genotypes and disease phenotype in more than 200 O26 human isolates from 7 European countries between 1996 and 2012. They found that strains with the *stx2* genotype with or without *stx1* were more frequently associated with HUS than were the *stx1*-only strains. In the United States, *stx2*-producing non-O157 STEC strains are more commonly associated with HUS than are *stx1*-producing strains [11].

Novel "Hybrid" *stx2*-Producing Serotype O104:H4 and O80:H2 Disease

In 2011 a massive, unexpected outbreak of severe disease linked to the consumption of raw fenugreek sprouts [31] occurred in central Europe. It was caused by a novel strain of enteroaggregative *E. coli* (EAEC) serotype O104:H4 that had acquired *stx2* phage by horizontal gene transfer [18]. In Germany 3816 cases were identified, mostly adult women, including 845 (22%) persons with HUS, of whom 36 (4.2%, mostly elderly women) died [18]. The coupling of *stx2* with EAEC, which already has a well-honed virulence and adherence machinery [2], including the ability to translocate *stx2* from the gut, proved to be a "deadly combination" [32].

The O104:H4 strain is not the first highly pathogenic hybrid strain to emerge. All enterohemorrhagic *E. coli* (EHEC) strains, such as those of serotype O157:H7 and O26:H11/H-, are hybrid strains that evolved through the acquisition of *stx* genes by atypical enteropathogenic *E. coli* (EPEC) [33, 34], which already had established virulence attributes including LEE [2]. This suggests that, "out of the blue," emergence of new highly pathogenic STEC strains will be the result of the acquisition of *stx2* phage by a pathogen with established virulence potential, as illustrated by the *stx2*-producing hybrid strain of serotype O80:H2 [23].

Hybrid *stx2*-positive O80:H2 strains were first recognized in 2005 in France where, in 2014, they were the second leading cause of pediatric HUS after STEC O157:H7. Of 54 patients identified between 2005 and 2014, about 90% had HUS, and 2 cases developed invasive disease. The strains exhibited varying combinations of *stx2a*, *stx2c*, and *stx2d* genes.

The hybrid O80:H2 strains had virulence characteristics of both STEC and extra intestinal pathogenic *E. coli* (ExPEC). They contained a rare intimin gene, *eae* zeta, and a plasmid with characteristics of ExPEC plasmid pS88 that is associated with bacteremia and neonatal meningitis, which may explain the bacteremia that occurred in 2 patients.

The Role of *stx* Phages in the Emergence of Novel Pathogenic STEC

It is noteworthy that the 2 novel hybrid *stx2*-positive pathogens of serotype O104:H4 and O80:H2, respectively, acquired *stx2* phages, rather than *stx1* phages, by horizontal transfer. This is not surprising because *stx2* phages far outnumber *stx1* phages in human and animal fecal wastes in the environment [35], a phenomenon related to the different biology and lifestyles of the 2 phages in the bacterial host [35–37]. *stx1* tends to be transcribed while the phage is stably integrated in its host genome; *stx2* is typically transcribed when the phage is induced, for instance, by stressors in the human or animal gut (eg, antibiotics) and enters the lytic cycle, replicating to large numbers. The progeny *stx2* phages are dispersed in the environment where they can infect other bacteria depending on their host range [38, 39].

CHANGES IN THE POPULATION

Several evolving trends are associated with an increased population risk of STEC infection and HUS, including changing lifestyles, demographics, socioeconomic status (SES), and immunity.

Evolving Lifestyle Changes

Two lifestyle trends that are associated with an increased risk of STEC-related morbidity are the consumption of produce, especially leafy greens [8], and environmental contact [6, 8, 9].

Consumption of Fresh Raw Produce

The consumption of leafy green vegetables was the second most common source, after beef, of foodborne outbreaks in the United States (2003–2012).

The consumption of fresh produce globally has increased greatly over the past 2 decades for many reasons including people's desire to lead healthier lives [40]. At the same time, foodborne outbreaks from contaminated fresh produce, sometimes with devastating effect [18], are being recognized worldwide [40]. Microbial contamination, sometimes at high levels, can occur during any step of production, including irrigation with contaminated environmental water, to handling in the home [40]. Contamination of crops with polluted irrigation or runoff water has been linked to serious outbreaks of infection associated with fresh produce [41, 42].

Environmental Exposures

Contact with recreational water, especially lakes [8], and the agricultural environment [6, 8, 9, 43, 44], including rural residence, contact with animals and animal wastes, and visiting farms and petting zoos, is a growing concern not only as a risk factor for STEC infection but also, as a result of high exposure doses [45], for HUS, especially in children [46].

Demographics, Socioeconomic Status, and Immunity

Age and Immunity

Symptomatic STEC infection and HUS occur most often in young children and tend to decrease with increasing age and then rise again in old age [12, 19], with significant mortality in the elderly [19]. The age incidence of HUS correlates inversely with the age-related frequency of antibodies to *stx1* and *stx2* [20], suggesting that these antibodies are protective against HUS.

Gender

Female predominance in both STEC infection and HUS or hospitalizations has been observed in STEC O157 outbreaks in the United States [8] and the United Kingdom [6]. This predominance was also observed in a large population-based study of demographic risk factors for HUS following STEC O157 infection [19]. The US study [8] noted that the proportion of female patients was higher in outbreaks attributed to produce than in those associated with other foods and suggested that the predominance of females in produce-related infection was linked to gender-specific food preferences, as was also suggested in the STEC O104:H4 sprout-associated outbreak [18]. However, an explanation for the female predominance in either STEC infection or HUS was not found in the UK study [6], suggesting that further research may be necessary to identify other possible causes for this, including host factors [6, 20, 47].

Socioeconomic Status and Immunity

High SES has been linked to the development of HUS (but not necessarily STEC infection) in the United States [48, 49] and in women in the STEC O104:H4 sprout-associated outbreak (Dr. D. Werber, written personal communication, May 2016). In the 1960s in South Africa, lower environmental exposure and, supposedly, lower immunity to a presumed etiological agent was thought to be a risk factor for HUS in persons of high SES compared to those of low SES with higher environmental exposures, and, presumably, with immunity to the hypothetical etiological agent [50].

While serum *stx* antibodies are thought to protect against the development of HUS [20], antibodies to bacterial colonization factors have been correlated with immunity to pathogen colonization.

EHEC share the LEE with the closely related EPEC, which is the most common cause of infantile diarrhea in many low- and middle-income countries (LMICs) and in emerging economies such as Brazil, Mexico, and South Africa [2]. The LEE-encoded adhesin intimin elicits an immune response that confers cross-immunity between EHEC and EPEC. This is thought to explain the low incidence of EHEC infections and HUS in Brazil [51] and, by extension, possibly in other LMICs.

In the 1940s and 1950s EPEC was a major cause of outbreaks of severe infantile gastroenteritis in hospitals and nurseries in Europe and the United States [2, 52], but EPEC outbreaks declined in the early 1970s for reasons that are not fully understood [52]. It has been speculated (The Late Dr. Peter C. Fleming, Oral Personal Communication, 1988) that the gradual decline of population immunity to EPEC in the 1960s and 1970s may have influenced the sudden emergence of EHEC infection in North America in the 1980s. Similarly, a decline in population immunity to EPEC infection in LMICs as standards of living rise, may result in increased risks of EHEC infection in these countries.

CHANGES IN THE ENVIRONMENT

STEC is widespread in agricultural and open environments and in domestic and wild animals. Ruminants, especially cattle, are the major reservoir for human STEC infection, and STEC can persist for long periods in the agricultural environment, including in soil [53]. Contact with the agricultural environment is a well-known risk factor for human STEC infection [44], and STEC-contaminated fecal wastes can be washed into water sources following heavy rainfall [8].

Environmental contamination by STEC poses a significant public health challenge because systematic efforts to reduce fecal shedding of pathogenic STEC by livestock at the preharvest level have not been successfully implemented [1]. Furthermore, environmental contamination may continue to increase because of growth in intensive agriculture, especially in emerging economies, and the presence of "super-shedder" cattle, as discussed below. Also, circumstances that bring people and animals (wild and/or domesticated) together, including changes in land use for agriculture through deforestation [54], provide opportunities for horizontal gene transfer of *stx2* phages found in human and animal wastes [35], thus enhancing risks of new STEC pathogens emerging.

The high population growth projection, including a burgeoning middle class, in LMICs and emerging economies in the next 50 years will greatly expand demand for protein. This demand will, in turn, lead to continued expansion of animal production and, consequently, increased contamination of the environment by STEC [55, 56].

Some cattle, known as super-shedders, excrete very high concentrations of organisms, resulting in increasing levels of STEC contamination in the agricultural environment [45] This may increase the risks of HUS in populations subjected to such high-exposure doses. Modeling studies [45, 57] indicate that 80% of the transmission of STEC O157:H7 occurs from 20% of super-shedder animals and suggest that interventions (eg, vaccination, probiotics, and bacteriophages) directed at such animals may have a disproportionately large effect in reducing environmental contamination. However, identification of super-shedders is difficult, and the factors responsible for super-shedding are unknown. These may include specific characteristics of the STEC pathogen, the environment, and the animal host, including the composition of the animal fecal microbiome, which itself is influenced by multiple factors including diet [58].

Antimicrobials continue to be used in many countries for growth promotion in intensive animal production and can lead to the induction and environmental dissemination of *stx2* phages [59], which may infect other bacteria and lead, potentially, to the emergence of new *stx2*-positive pathogens. The projected growth in intensive agriculture in emerging economies will lead to a substantial increase in antimicrobial use in animals [60], despite gradual restrictions of this in Europe [61].

CONCLUSIONS AND MITIGATION STRATEGY

Emerging public health challenges of STEC include the occurrence of more frequent or serious HUS and risk factor shifts associated with changes in the pathogen, the population, and the environment, often in an interconnected manner. For example, in STEC O157:H7 clade 8 and Xuzhou21 outbreaks [16, 17], the clade 8 and Xuzhou21 strains expressed high levels of certain virulence factors, including *stx2*, which makes an association with more severe disease conceivable [29]. In the *stx2*-positive EAEC O104:H4 outbreak [18], the acquisition of an *stx2*-encoding phage was critical for the serious disease outcome. However, population and/or environmental factors also contributed to severe outcomes in all 3 outbreaks through a dietary preference for raw produce in 2 (clade 8 and *stx2*+ve O104:H4), old age (Xuzhou21 and *stx2*+ve O104:H4), and exposure to a heavily contaminated environment (Xuzhou21). In sporadic cases of STEC O26:H11/H-, more frequent HUS was associated with a genotype shift from *stx1* to *stx2*, while the ability of serotype O104:H4 and O80:H2 strains to cause HUS was enabled by the acquisition of *stx2* phages, which are more commonly present in the environment than *stx1* phages [35].

Although consumption of contaminated beef remains a major source of STEC O157:H7 infection [8], a decline in beef-associated infection is lending greater prominence to fresh produce consumption [8] and contact with fecally contaminated agricultural environments as causes of severe STEC O157:H7 disease [6, 8, 9]. Increases in environmental pollution by STEC will exacerbate the risks of produce contamination through irrigation with polluted water [40]. Consumption of fresh produce is expected to continue to grow as part of people's desire to lead healthier lives [40], and environments may become increasingly contaminated because of the continued growth in intensive agriculture [55, 56] and the presence of super-shedder animals [45, 58]. Additionally, environmental contamination by *stx2* phages from human and animal fecal wastes [35] may lead to the emergence of new *stx2*-positive pathogens via horizontal acquisition of *stx2* phages.

The demographic transition that is under way in high-income nations, with lower birth rates and increased longevity [62], will lead to a growing pool of seniors susceptible to HUS [20]. Improvement in SES may lead to increased susceptibility to HUS [49] in high-income countries, whereas continued social development in LMICs is likely to lead to EPEC infection becoming replaced with EHEC infection [51] with its attendant risk of HUS.

Mitigation Strategies

Given the complex interplay of human, animal, and environmental factors that contribute to emerging public health challenges of STEC, a mitigation strategy that incorporates a multidisciplinary "One Health" approach [53, 55] should include the following: surveillance of STEC infection (by all pathogenic serotypes) and HUS and adequate characterization of pathogens to monitor emergence of novel virulent STEC strains; research to identify changes in risk factors for STEC infection (by all pathogenic serotypes) and HUS and development of interventions to mitigate them; effective communication programs to alert the public of the risk of STEC infections and educational programs to aid in their mitigation; ongoing improvements in national and international food safety policies for the safe production and distribution of fresh produce and their implementation; reduction in the use of antimicrobials for

animal growth promotion; and research to identify super-shedder animals and to better understand the determinants of super-shedding so that suitable preharvest interventions can be implemented to reduce environmental contamination by STEC, ensuring that suitable incentives are developed for farmers and industry to do this.

Notes

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