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## Overview and Historical Perspectives

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### Abstract

In this overview, we describe the history of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) in two phases. In phase one, between 1977 and 2011, we learned that *E. coli* could produce Shiga toxin and cause both hemorrhagic colitis and the hemolytic-uremic syndrome in humans and that the prototype STEC—*E. coli* O157:H7—adheres to and effaces intestinal epithelial cells by a mechanism similar to that of enteropathogenic *E. coli*. We also recognized that the genes for Stx are typically encoded on a lysogenic phage; that STEC O157:H7 harbors a large pathogenicity island that encodes the elements needed for the characteristic attaching and effacing lesion; and that the most severe cases of human disease are linked to production of Stx type 2a, not Stx type 1a. Phase two began with a large food-borne outbreak of hemorrhagic colitis and hemolytic-uremic syndrome in Germany in 2011. That outbreak was caused by a novel strain consisting of enteroaggregative *E. coli* O104:H4 transduced by a Stx2a-converting phage. From this outbreak we learned that any *E. coli* strain that can adhere tightly to the human bowel (either by a biofilm-like mechanism as in *E. coli* O104:H4 or by an attaching and effacing mechanism as in *E. coli* O157:H7) can cause severe diarrheal and systemic illness when it acquires the capacity to produce Stx2a. This overview provides the basis for the review of current information regarding these fascinating and complex pathogens.

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The scope of topics covered in the forthcoming book *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli* (1) reflects the broad areas of research required for the comprehensive study of Shiga toxin-producing *Escherichia coli* (STEC) infections. Substantial progress has been made in all of these areas since the first edition of this book (2). Although this second edition brings the field up to date in all major areas of research, these pathogens have a long and complicated history, and understanding this history is valuable for a full understanding of this field. The purpose of this chapter is to set the stage for this book by examining the seminal discoveries about STEC biology, epidemiology, and pathogenesis. In this article, we refer to the cytotoxins of *E. coli* O157:H7, *E. coli* O104:H4, and other *E. coli* as Shiga toxins (Stxs; formerly called Shiga-like toxins), hence the nomenclature STEC. However, for reasons described below, a number of investigators

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prefer the term verotoxin (VT). We refer the reader to past discussions of nomenclature (3, 4) for a better understanding of the historical basis for the dichotomy in nomenclature. Additionally, a recently published multi-center study by Scheutz and colleagues (5) provides clear guidance on nomenclature for Stx subtypes. Scheutz reviews that typing scheme in reference 6.

Our understanding of what constitutes a virulent STEC isolate for humans has evolved, as have the organisms themselves. The history of STEC as an emerging pathogen can be divided into two phases. Phase one of STEC chronology is a history of the convergence of two independent laboratory-based research tracks and two independent epidemiology-based areas of investigation. One laboratory-based track focused on Stx, its discovery, characterization, and relationship to *E. coli* cytotoxins, and the other concentrated on enteropathogenic *E. coli* (EPEC) adherence characteristics. These studies led to the realization that a subset of STEC strains, including *E. coli* O157:H7, shares with EPEC both the pathogenic trait of producing attaching and effacing (A/E) intestinal lesions and the genes to provoke that lesion. The epidemiology-based areas include the search for the cause of the well-described but idiopathic hemolyticuremic syndrome (HUS) and the search for the agent responsible for a newly described clinical syndrome called hemorrhagic colitis.

Phase two of the STEC story started with the understanding that the backbone of the *E. coli* strain that produces Stx does not necessarily have to have adherence traits similar to those of EPEC and *E. coli* O157:H7 to cause a large-scale outbreak of human disease. Witness the large 2011 STEC outbreak in Germany in which 3,816 cases were reported (including 54 deaths); 845 of those cases led to HUS (7). The etiologic agent in that outbreak was an enteroaggregative *E. coli* O104:H4 strain that had become transduced with the Stx type 2a (Stx2a)-encoding phage (8). Enteroaggregative *E. coli* (EAEC) strains that do not make Stxs are well-established etiologic agents of diarrhea. The fact that an EAEC isolate can be converted from an exclusively diarrheagenic agent to one that also causes hemorrhagic colitis and HUS in so many patients affirms the preeminent role that Stx plays in these syndromes and illustrates how readily an *E. coli* strain can evolve into a life-threatening pathogen by the acquisition, through horizontal genetic exchange, of the Stx2a-converting phage. Recognizing the essential role Stx plays in the development of severe disease caused by STEC infection, and for chronological reasons, we begin this tale with the discovery and characterization of Stxs.

## HISTORY

### Stxs: History of the Field

In 1898, Kioshi Shiga (9) provided the definitive description of the agent of epidemic bacterial dysentery, *Shigella dysenteriae* type 1 (Shiga's bacillus). Five years following that discovery, Conradi (10) reported that extracts of Shiga's bacillus paralyzed and killed rabbits. Similar findings were published independently by Neisser and Shiga (11). The next nearly 70 years of Stx research led to the clear separation of the endotoxic activity associated with Shiga's bacillus from the activity of the protein Stx; the partial purification of Stx (12); the discovery that high iron concentrations inhibit Stx synthesis (13); the seminal observation by Bridgewater et al. (14) and Howard (15) that Stx appears to target

vascular endothelium in the brain; and the discovery by Vicari et al. that Stx is lethal for certain epithelial cells in culture (16). Although these findings were of interest to toxicologists, none of the results proved a direct role for Stx in the pathogenesis of shigellosis. Only decades later, with the evaluation of data obtained from infection of volunteers (17) and subsequently of monkeys (18) with *S. dysenteriae* type 1, was it clear that production of Stx by the organism exacerbates the severity of the intestinal and systemic lesions in human subjects and increases the intestinal pathology in primate hosts. The ultimate proof of a role for Stx in shigellosis due to Shiga's bacillus was the establishment of a connection between production of this and related toxins with the subsequent development of HUS (see below or references 19 and 20).

In 1972, Keusch and colleagues made the significant finding that Stx alone caused fluid accumulation and enteritis in ligated rabbit intestinal segments (21). This observation revealed that Stx can contribute to the intestinal phase of bacillary dysentery, i.e., bloody diarrhea. That this enterotoxic activity of Stx is a result of the same molecule responsible for its cytotoxic and lethal activities was convincingly demonstrated by the purification of Stx to homogeneity (first by Olsnes and Eiklid [22], followed shortly by reports from O'Brien et al. [23], Brown and colleagues [24], and Donohue-Rolfe and coworkers [25]) and the subsequent testing of that material for all three bioactivities (23).

### **Stxs and Verotoxins Are Different Names for the Same Family of Toxins**

With the availability of purified Stx came the capacity to produce monospecific, cytotoxin-neutralizing rabbit anti-Stx antibodies. O'Brien and colleagues used such sera to ascertain that certain strains of *E. coli* produce a cytotoxin that can be neutralized by anti-Stx (26, 27), an observation that explains the original Shiga-like toxin nomenclature. The preliminary report of that discovery (27) occurred in 1977, the same year that Konowalchuk and colleagues found that certain diarrheagenic *E. coli* strains make a cytotoxin that can kill Vero cells (28), hence the name verotoxin. In 1983, O'Brien and colleagues (29) reported that a Shiga-like toxin was produced by the *E. coli* O157:H7 strain that had caused an outbreak of hemorrhagic colitis in the United States (see below) and that this toxin was the same as the verotoxin shown by Johnson et al. (30) to be produced by *E. coli* O157:H7. Thus, 1983 became the year when the paths of research on Stxs and verotoxins merged. Subsequent genetic studies showed that Stx1 (VT1) differs by none or only a single amino acid from Shiga toxin (31, 32). These studies on the Stx/VT of *E. coli* culminated in a pivotal report published by Karmali et al. (33) in that same year. In that paper Karmali and colleagues proposed that the verotoxin produced by these organisms was linked epidemiologically to the development of HUS (see below).

The mid to late 1980s heralded the era of the molecular characterization of the genes encoding the Stx family members (reviewed in reference 34). In the mid-1980s, it was also discovered that Stx1 and Stx2 are usually encoded on lambdoid prophages in *E. coli* (35–39). In contrast, Stx of Shiga's bacillus and Stx2e (edema disease toxin) of animal STEC were later shown to be chromosomally encoded (31, 40). Subsequent genomic analysis revealed that the genome of *E. coli* O157:H7 is riddled with prophage regions that not only encode Stx but also other potential virulence factors (see reference 41). The toxin genes

themselves show considerable variation that can correlate with epidemiological significance, with more than 100 Stx variants so far described (see reference 6).

### Intimate Adherence to Mucosal Epithelium: The Connection between EPEC and EHEC

With the genetics and biology of Stxs fairly well elucidated by the mid to late 1980s, the focus of research on STEC broadened to address the question of how *E. coli* O157:H7 adheres to epithelial cells. The primary finding that initiated a series of discoveries about *E. coli* O157:H7 adherence mechanisms was the observation by Tzipori et al. (42) that *E. coli* O157:H7 causes intestinal A/E lesions in gnotobiotic piglets and that these lesions resemble those produced by Stx-negative EPEC, albeit at different sites in the bowel of the animals. The A/E lesion is characterized by intimate adherence of the bacteria to the enterocyte membrane and effacement of the microvilli. This observation suggested to Levine (43) that these lesions might be a hallmark of *E. coli* O157:H7 and related bacteria. He proposed that the capacity of *E. coli* O157:H7 and related organisms to evoke A/E lesions, together with the production of Stxs by these microbes and the presence of a characteristic large plasmid, was sufficient to define a new category of virulent “enterohemorrhagic” *E. coli* (EHEC). With the identification of pathogenic and genetic markers for this newly recognized group of *E. coli* came the realization that the *E. coli* O26:H11 serotype, which had long been considered a classic EPEC serotype, should be reclassified as an EHEC. This reclassification was supported by the findings that strains of the O26:H11 sero-type also produced Stx and possessed the large plasmid found in O157:H7 (44). Thus, the O26:H11 serotype is one example of an STEC serotype that had been associated with diarrheal disease (reviewed in reference 45) long before the discovery of *E. coli* O157:H7.

The pathognomonic A/E histopathology of EPEC, *E. coli* O157:H7, and a few additional serotypes of STEC was subsequently shown by Knutton et al. (46) to correspond *in vitro* to a lesion characterized by bacterial microcolonies intimately adherent to the surface of tissue culture cells, with accumulation of cytoskeletal actin under the bacteria. The actin accumulation was visualized in that study by a fluorescent actin stain (FAS) test in which fluoresceinated phalloidin was used as a probe. The FAS-positive phenotype of EPEC bound to HEp-2 laryngeal epithelial cells was used by Jerse et al. (47) to screen EPEC for genes required for this intimate adherence. These investigators identified the gene *eae* (for *E. coli* attaching and effacing), which was also present in O157:H7. The *eae* gene product, appropriately named intimin, was subsequently shown to be required for EPEC to cause A/E lesions in gnotobiotic pigs (48). Shortly after the discovery of EPEC *eae*, the homologous gene was cloned and sequenced from two strains of *E. coli* O157:H7 (49, 50). The intimin of *E. coli* O157:H7 was also shown to be necessary but not sufficient to induce A/E lesions *in vitro* and *in vivo* (51, 52).

An additional twist to the similarities in pathogenic mechanisms between EPEC and EHEC was the provocative finding of McDaniel et al. (53) that *eae* lies within a pathogenicity island of approximately 35 kb and that this island encodes genes for attachment, FAS reactivity, a type III secretion apparatus, and secreted proteins (53). The island was named locus of enterocyte effacement (LEE) (53). In reference 54, Stevens and Frankel review the

LEE pathogenicity island, intimin, the type III secretion system, and other associated virulence factors of EHEC.

The discovery and characterization of LEE prompted the realization that the term EHEC represents a subset of STEC since not all STEC strains contain LEE and the large EHEC plasmid (see reference 6). Although the majority of STEC strains associated with human disease possess LEE and the large plasmid, some strains lacking these factors, most notably O104:H4, have been implicated in human disease, thereby leading to the use of the more general term STEC rather than EHEC.

### Hemolytic-Uremic Syndrome

Hemolytic-uremic syndrome (HUS), first described in 1955 by Gasser et al. (55) in Switzerland, is defined by a triad of clinical features that include acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. HUS is a leading cause of acute renal failure in children, and in some studies it is the most common cause of renal failure in this age group. A variety of agents, including drugs, chemicals, toxins, and various microbes, had been proposed as the cause of HUS; indeed, before 1983, most nephrologists thought that HUS was a multifactorial disease that could result from a number of initiating events (56). Because HUS occasionally occurred in outbreaks, an infectious cause was sought. The strongest documented linkage between HUS and a microorganism was the association with *S. dysenteriae* type 1, but numerous microorganisms, including *Salmonella typhi*, *Campylobacter jejuni*, *Yersinia pseudotuberculosis*, *Streptococcus pneumoniae*, rickettsia-like organisms, coxsackievirus, echovirus, and Epstein-Barr virus, were proposed as the causative agent (reviewed in reference 56). Several studies noted that many, if not the majority, of HUS cases were preceded by diarrhea. Interestingly, a survey of HUS in South Africa led Kibel and Barnard in 1968 (57) to speculate that HUS is caused by an enteropathogenic strain of *E. coli* that had acquired a bacteriophage. These authors also raised concerns that treatment with antibiotics might lead to excessive bacterial destruction and enhanced absorption of toxin, with an adverse clinical outcome (see reference 58). Additional information on the history of HUS and various proposed pathogenic mechanisms can be found in several reviews (19, 20, 56, 59, 60).

The key event in the linkage of HUS and STEC was the 1983 report in *The Lancet* by Karmali et al. (33) that sporadic cases of HUS were linked to the presence of verotoxin, which O'Brien et al. (29) reported was equivalent to Stx [see above], and/or *E. coli* that produced Stx in patients' stools. The toxigenic *E. coli* strains characterized by Karmali and colleagues belonged to different serogroups, thus ruling out a single strain as the cause of this disease, and serum collected from several patients contained rising titers of neutralizing antibody activity against verotoxin. This initial report was confirmed by a prospective case-control study that linked cases of HUS with isolation from stool of STEC belonging to at least six O serogroups (O26, O111, O113, O121, O145, and O157) (61). The 1985 article in *Journal of Infectious Diseases* describing the case-control study was reprinted by that journal in 2004 along with a commentary (62) describing it as one of the landmark papers published in that journal over the first centennial of its history. The connection between Stx (VT) production by an *E. coli* strain and the development of HUS after infection with that

organism was most recently substantiated by the 2011 O104:H4 outbreak, showing that diarrheagenic enteroaggregative *E. coli* could cause HUS after acquiring the capacity to produce Stx2 (see below).

### Hemorrhagic Colitis

In 1982, two outbreaks of a severe bloody diarrheal syndrome in Oregon and Michigan were linked to the consumption of hamburgers from a specific restaurant chain (63). This syndrome, called hemorrhagic colitis, was characterized by severe abdominal cramps, grossly bloody stools, little or no fever, and evidence of colonic mucosal edema, erosion, or hemorrhage (64). *E. coli* strains of a previously rare serotype, O157:H7, were isolated from the stools of about half the cases but from none of the healthy controls. Strains of this serotype were subsequently shown to produce Stx (see above). Numerous studies have since confirmed that O157:H7 is an important cause of hemorrhagic colitis, nonbloody diarrhea, and HUS in the United States, Canada, the United Kingdom, and Japan, as reviewed in reference 1.

The abrupt appearance of *E. coli* O157:H7 in 1982 raised questions as to whether this organism had recently emerged as a pathogen or had always been present and had simply been unrecognized. To address this issue, investigators at national laboratories in the United States, Canada, and the United Kingdom reviewed their records and *E. coli* collections and found archived *E. coli* O157:H7 strains recovered before 1982 from the stool of one patient in the United States, one patient in the United Kingdom, and six patients in Canada, some of whom had bloody diarrhea (reviewed in reference 59). The clinical syndrome of hemorrhagic colitis is so distinctive that outbreaks are unlikely to have been overlooked, although occasional cases of a hemorrhagic colitis-like syndrome of unknown etiology were reported in the 1960s and 1970s (reviewed in references 56 and 59). Thus, the available evidence indicates that the incidence of infections with O157:H7 and other STEC strains increased in the 1980s and 1990s. However, this conclusion is confounded by the increase in the number of laboratories seeking this pathogen (56) and by the 25 to 75% of patients with O157:H7 infection who present with nonbloody diarrhea (56, 65), a clinical manifestation that may go unrecognized as one of the manifestations of O157:H7 disease. Studies by Whittam and colleagues using multilocus enzyme electrophoresis demonstrated the stepwise evolution of STEC O157:H7 from an O55:H7 ancestor (66, 67), a Stx-negative serotype of EPEC that had previously been only associated with nonbloody diarrhea.

The comprehensive study of the Oregon and Michigan O157:H7 outbreaks of hemorrhagic colitis (in which no cases of HUS were noted) was published in the March 24, 1983, issue of *New England Journal of Medicine* (68). Karmali's study linking verotoxin-producing *E. coli* strains of different serogroups to HUS was published in the March 19, 1983, issue of *Lancet* (33), and 1 week later, in the March 26, 1983, issue of *Lancet*, O'Brien and colleagues (29) reported that a Shiga-like toxin was produced by the *E. coli* O157:H7 strains from the Oregon and Michigan outbreaks of hemorrhagic colitis and that this toxin was the same as the verotoxin previously shown to be produced by *E. coli* O157:H7 (30). Thus, March 1983 was a momentous month in which numerous laboratory, clinical, and epidemiological

studies on HUS, bloody diarrhea, verotoxins, Shiga toxin, and *E. coli* came together to establish the field of STEC infections.

#### O104:H4

In May 2011, a large outbreak of gastroenteritis and HUS began in Germany, one of the largest outbreaks of STEC yet reported. In 3 months, 3,816 cases (including 54 deaths) were reported, of which 845 (22%) were HUS (7). The etiologic agent was identified as Stx-producing *E. coli* O104:H4, and sprouts were identified as the outbreak vehicle (69). Sprouts had previously been identified as the vehicle in STEC outbreaks, most notably the 1996 outbreak in Sakai City, Japan, where 12,680 cases were reported (70). The most striking clinical and epidemiological finding in the 2011 outbreak was the very high number of HUS cases (22% of all cases), with 88% of the HUS cases occurring in adults rather than in children. In contrast, the incidence of HUS in the 1996 Japan outbreak was 1% and all cases were in children. The dramatic features of the 2011 outbreak suggested that the causative agent might be a novel pathogenic variant of STEC.

Investigators quickly established that the O104:H4 strain lacked the LEE pathogenicity island present in O157:H7 and other common EHEC strains. Instead, the strain possessed an unusual combination of virulence factors that were typical of EAEC in addition to Stx (71). EAEC strains that do not make Stxs are well-established etiologic agents of nonbloody diarrhea that can be either acute or persistent in duration. Disease is seen in both children and adults, in travelers, and in people infected with human immunodeficiency virus in both the developed and developing world (reviewed in reference 72). The term “enteroaggregative” is derived from the “stacked-brick” appearance of EAEC on intestinal epithelial cells, in which large numbers of bacteria closely adhere to enterocytes in a biofilm. The pathogenesis of EAEC is poorly understood, but a variety of virulence factors have been described, including aggregative adherence fimbria (AAF) that mediate intestinal adherence and induce inflammation, several serine protease auto-transporters of *Enterobacteriaceae* (SPATEs) implicated in mucosal damage and colonization, and several other putative adhesins and toxins (reviewed in reference 73).

Analysis of the genome sequence of the O104:H4 strain from the Germany outbreak revealed that it closely resembled other EAEC strains, but that it had become transduced with the Stx2a-encoding phage (8). The genome sequence also revealed the presence of an unusual combination of SPATEs and several antibiotic-resistance factors. Other *E. coli* O104:H4 strains unrelated to the Germany outbreak did not possess Stx. Further discussion of the pathogenesis of O104:H4 is presented in reference 74.

Although more than 100 different serotypes of *E. coli* have been shown to produce Stx, the majority of such STEC strains are not considered to be pathogens. Several serotypes such as O26:H11, O111:NM, and O121:H19 contain the LEE and other pathogenicity islands found in O157:H7 and are clearly pathogens. A few other STEC serotypes, such as O91:H21 and O113:H21, lack the LEE but have additional virulence factors and have been epidemiologically implicated as pathogens (75). The different STEC serotypes and their epidemiological significance are reviewed in reference 6. In the case of the German Shiga toxin-producing enteroaggregative *E. coli* O104:H4, the addition of the Stx2a phage to a

pathotype of *E. coli* that was already capable of avidly adhering to and damaging the intestinal epithelium produced a novel pathogen with profound clinical and epidemiological consequences.

## THE PRESENT

The current themes and directions of STEC research span an astonishing range of topics. Multiple disciplines encompass epidemiology, animal ecology, food safety, clinical microbiology, gastroenterology, nephrology, infectious disease, toxicology, bacterial pathogenesis, cell biology, and immunology. Topics in this area of research range from farm management of livestock and manure to clinical management of end-stage renal disease. *Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli* (1), edited and written by internationally recognized experts in this area, reflects this breadth of topics.

The first section of the volume describes the microbiology of STEC. In chapter 2 (6), Flemming Scheutz describes the taxonomy of STEC and Stx toxins and relates this information to the public health significance of the different serotypes and toxin subtypes. The discussion of Stx toxin continues in chapter 3 where Angela Melton-Celsa reviews the structure and function of these toxins (76). Sadiq and colleagues take a genomic perspective in chapter 4 (41) to review the history of typing and genetic analysis from distinguishing STEC strains using pregenomic methodologies to the current technology, where the genome sequences of multiple strains can be determined in a single day.

The pathogenesis of STEC infections is covered in section two. In chapter 5 (19), Obata and Obrig discuss the role of Stx toxins in pathogenesis, with a particular emphasis on effects in the renal system. In chapter 6, Stevens and Frankel review the LEE pathogenicity island and virulence factors encoded therein, as well as other virulence factors encoded outside the LEE (54). Colonization of the intestinal tract is an essential first step in STEC pathogenesis, and a variety of potential adherence factors have been described, as reviewed by McWilliams and Torres in chapter 7 (77). Unfortunately, there is no single animal model that reproduces all aspects of STEC disease, but Ritchie reviews the various models available and their advantages and disadvantages in chapter 8 (78). The range of environments where STEC can be found—from the farm environment to the human intestine—requires numerous regulatory genetic elements to optimize expression of virulence factors and survival factors. Mellies and Lorenzen (79) describe the complex regulation of STEC virulence in chapter 9.

The incidence, epidemiology, and ecology of STEC are reviewed in the third section. In chapter 10, Terajima et al. review the incidence and epidemiology of STEC in Japan, the site of the largest STEC outbreak reported (80). Animals, particularly cattle, serve as the reservoir of STEC infections, and Persad and LeJeune review this critical reservoir in chapter 11 (81). Transmission to humans most often involves consumption of contaminated food items. Initial outbreaks of STEC disease involved improperly cooked hamburgers, an issue that was relatively easy to address by increasing cooking temperatures. However, as reviewed by Feng in chapter 12 (82), most outbreaks in recent years involved produce that is consumed raw. Caprioli et al. (83) review in chapter 13 the epidemiology and other public



health aspects of STEC infection, with a particular emphasis on Europe. Methods for detecting STEC from nonhuman sources and strain typing are reviewed by Beutin and Fach in chapter 14 (84).

Clinical, pathological, and pathophysiological aspects of human disease are reviewed in chapters 15 through 17. Tarr and coauthors review in chapter 15 (58) the clinical features of STEC infections in humans, including outcomes and prognosis, and provide insights from both gastroenterological and nephrological perspectives. The inflammatory response to STEC infection and the virulence factors these pathogens have evolved to thwart this response are discussed by Pearson and Hartland in chapter 16 (85). Unfortunately, no ideal therapy is available for STEC infections, and the use of antimicrobials is contraindicated, at least for typical EHEC infections, although investigators of the German O104:H4 outbreak reported the benefit of azithromycin treatment to reduce fecal shedding of the organism. This issue, along with novel therapeutic interventions under study, is reviewed by Melton-Celsa and O'Brien in chapter 17 (86).

Host determinants of disease and host responses encompass factors ranging from cultural and dietary practices to host genetics and immune status to intestinal microbiota, all of which can play important roles in STEC infection and outcome. Risk factors for STEC infections are discussed by Rivas et al. in chapter 18 (87). The host response and other aspects of STEC pathogenesis are reviewed by Karpman and Stahl in chapter 19 (20). With the recognition that regulation of EHEC virulence factors can be influenced by commensal intestinal bacteria, the interplay between the microbiota and EHEC can be important, as reviewed by Pifer and Sperandio in chapter 20 (88).

Prevention and control strategies to reduce or eliminate the risk of STEC infections are particularly important in the control of STEC infections. Preharvest and peri- and postharvest food safety factors are reviewed by Besser and colleagues in chapter 21 and by Moxley and Acuff in chapter 22 (89, 90). A veterinary public health approach to managing pathogenic STEC in the agri-food chain is discussed by Duffy and McCabe in chapter 23 (91). Vaccines have been critical in reducing the disease burden in humans for many infectious diseases, but for STEC infections, vaccines to reduce carriage in the bovine reservoir to reduce transmission to humans may hold more promise than direct immunization of humans. In chapter 24, Szu and Ahmed present data showing that parenteral O157 lipopolysaccharide conjugate vaccines are safe and immunogenic in children and adults (92). However, there are multiple potential problems in vaccinating humans against STEC disease, including finding a population with a high enough incidence in which to determine vaccine efficacy and identifying an appropriate target population to vaccinate once vaccine efficacy is established (reviewed in reference 93). Success in vaccinating cattle to reduce fecal shedding of STEC has been achieved, and David Smith reviews these studies in chapter 25 (94).

The emergence of STEC-EAEC O104:H4 in 2011 was a landmark development in the history of STEC infections. The virulence factors that combined to produce this highly virulent strain are discussed by Navarro-Garcia in chapter 26 (74). Such a development makes one wonder what the future holds for the STEC field, and Vanessa Sperandio offers

in the final chapter (95) some speculations on current questions and future directions for investigating this fascinating and ever-changing pathogen.

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