Chapter 9
Enterohemorrhagic *E. coli* (EHEC): Environmental-Vehicle-Human Interface

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Abstract Enterohemorrhagic *Escherichia coli* (EHEC) are a pathogenic subgroup of Shiga toxin-producing *E. coli* (STEC), and have demonstrated ability to cause severe intestinal disease and the hemolytic uremic syndrome (HUS). Cattle are the major reservoir of EHEC, where the bacteria can persist asymptomatically for years. Of particular concern are a small percentage of animals in herds that shed extremely high numbers of EHEC, termed 'supershedders', and are responsible for the majority of EHEC spread and contamination. Another transmission route is through the environment where EHEC can survive for weeks to many months, remaining viable in bovine feces, soil and water. EHEC contamination of meat during slaughter or processing, or contamination of plants via EHEC-containing water or manure are major routes of entry into the food chain. Several hundred outbreaks caused by EHEC O157 as well as non-O157 strains have been identified in industrialized countries worldwide. Current and future research efforts are focused on rapid outbreak identification, development of therapeutics, and implementation of preventative measures.

9.1 Introduction

Most members of the species *E. coli* are part of the physiological flora in the gastrointestinal tracts of humans and animals. In addition to these commensal bacteria, there are pathogenic *E. coli* that cause extraintestinal and intestinal disease. Intestinal pathogenic *E. coli* presently include seven pathogroups: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), adherent invasive *E. coli* (AIEC), diffusely adherent *E. coli* (DAEC) and enterohemorrhagic *E. coli* (EHEC) (Croxen et al. 2013). Each pathotype is associated with unique epidemiology and specific pathological diseases

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that cause significant morbidity and mortality. Zoonotic *E. coli*, of which EHEC are the prototype, pose many challenges to the food industry and public health and are intensively studied in human and veterinary medicine. Ongoing investigations are concerned with both ecology of EHEC in animals and persistence and survival in the environment, and how these factors affect entry into or dissemination along the food chain. Other areas of research are the epidemiology of EHEC infections in humans, diagnostics, pathogenic mechanisms of these bacteria and treatment as there is currently no specific therapy.

EHEC can cause a broad clinical spectrum of disease including watery or bloody diarrhea, and the hemolytic uremic syndrome (HUS), which is an important cause of acute renal failure in children (Tarr et al. 2005). Since the first isolation of an EHEC serotype O157:H7 outbreak strain in the USA in 1982 (Riley et al. 1983), and subsequent identification of involvement of this pathogen in outbreaks of hemorrhagic colitis and HUS (Wells et al. 1983), EHEC has emerged as an important public health concern worldwide. The large EHEC O104:H4 outbreak in Germany in 2011 with 3842 cases, 855 HUS patients and 53 deaths demonstrates the significant impact of an EHEC outbreak on human health (RKI 2011).

9.2 Expression of Shiga Toxins in EHEC

A key characteristic of the EHEC pathotype is the presence of Shiga toxins (Stx). Stx, also known as verocytotoxins (VTs), are members of a large family of cytotoxins that are characterized by a high degree of sequence diversity. The Stx family is divided into two major branches, Stx1 and Stx2, and many toxin subtypes and variants have been described in both branches (Karch et al. 2009; Bergan et al. 2012; Scheutz et al. 2012). Classification of Stx subtypes is used not only for taxonomic purposes, but also serves as an important predictor for the various clinically relevant Stxs found in strains associated with HUS versus other Stx subtypes that are carried by strains causing a milder course of disease (Scheutz et al. 2012). A sequence-based protocol for characterization of the Stx genes has been recently described (Scheutz et al. 2012), and includes three levels of classification: Types, subtypes and variants (see Table 9.1).

1. Types
   The two major branches Stx1 and Stx2 share structure and function but are not cross neutralized with heterologous antibodies. The terms Stx1 and Stx2 should only be used when the subtype is unknown.
2. Subtypes
   Currently the antigenically related members of Stx1 (Stx1a, Stx1c, and Stx1d) and Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g) are distinguished.
3. Variants
   Variants include the subtype-specific prototypic toxins or related toxins within a subtype (that differ by one or more amino acids from the prototype). The

Table 9.1 Types, subtypes and variants of Shiga toxins according to Scheutz et al. (2012)

Types	Subtypes	Variants (examples)
Stx1	Stx1a	Stx1a-O157-EDL933
	Stx1c	Stx1c-O174-DG131-3
	Stx1d	Stx1d-ONT-MHI813
Stx2	Stx2a	Stx2a-O104-G5506
	Stx2b	Stx2b-O111-S-3
	Stx2c	Stx2c-O157-A75
	Stx2d	Stx2d-O91-B2F1
	Stx2e	Stx2e-O26-R107
	Stx2f	Stx2f-O128-T4-97
	Stx2g	Stx2g-O2-S86

variants are designated by toxin subtype, O-antigen group of the host E. coli strain, followed by the strain name or number from which that toxin was described, e.g. Stx1a-O157-EDL933 or Stx2a-O104-G5506 (Scheutz et al. 2012, see Table 9.1). Nucleotide variants within a given Stx subtype are italicized.

All Stx consist of a single A and five B subunits. The A subunit represents the enzymatically active component. The Stx B pentamer binds to the high and less effective cellular ligand glycosphingolipids (GSLs), globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer), respectively (Müthing et al. 2009). Stx1 and Stx2 share identical binding specificity (Müthing et al. 2009). After binding to the cell surface, the AB_5-Gb3Cer complex is internalized by various endocytic mechanisms and routed from the early endosomes through the *trans*-Golgi-network and the Golgi stacks to the endoplasmic reticulum (Sandvig et al. 2010; Bauwens et al. 2013). Moreover, evidence suggests that Stxs (like other ribosome-inactivating proteins) remove adenine moieties not only from rRNA, but also efficiently depurinate DNA. Stx genes are found within the genomes of temperate bacteriophages, which are mobile elements that can easily integrate at specific sites in the bacterial chromosome. In vitro and in vivo studies have demonstrated that most EHEC can lose the Stx-encoding gene by bacteriophage excision during infection, isolation, or subculture, resulting in *stx*-negative isolates (Mellmann et al. 2009).

9.3 Epidemiology of EHEC in Animals

Several studies have demonstrated that cattle are the main reservoir of human pathogenic EHEC O157:H7, in addition to many pathogenic non-O157 EHEC serotypes (Naylor et al. 2005a). These bacteria have adapted to an oral-fecal cycle in cattle, where EHEC colonization begins with ingestion and subsequent entrance to the rumen and gastrointestinal tract, but they generally do not have a pathogenic effect on adult animals. EHEC has been reported to cause disease in young calves, however, in particular certain non-O157 serogroups (O26, O111, O118) (Naylor et al. 2005a). Prevalence among cattle varies widely, and may be due to several

circumstances including the geographical region, animal age, or the specific farm conditions (Ferens and Hovde 2011). Published prevalence rates vary dramatically, from 0 to 36 % among animals studied in different countries and farm types (Naylor et al. 2005a). Studies have also shown that EHEC prevalence is related to the type of farm (e.g. beef, dairy) and may be influenced by factors such as cattle movement, hygiene management, diet, and husbandry (Menrath et al. 2010; Cobbaut et al. 2009; Ferens and Hovde 2011). While cattle are the major known reservoir of EHEC, other minor reservoirs include sheep, goats, pigs, horses, dogs, poultry, and deer (Naylor et al. 2005a).

The persistence of EHEC O157:H7 in cattle may be due to its ability to colonize a particular niche within the lower gastrointestinal tract (Grauke et al. 2002). Tissue tropism for the colon has been demonstrated by immunofluorescent detection of microcolonies at the lymphoid follicle-dense mucosa at the terminal rectum within 3–5 cm proximal to the rectoanal junction (Grauke et al. 2002; Naylor et al. 2003, 2005b). This rectoanal junction colonization is hypothesized to be responsible for a highlevel of EHEC O157:H7 shedding (10^4 CFU/g of feces) in a minor subset of cattle which are termed ‘supershedders’ and are thought to be responsible for most of the pathogen spread in a farm environment (Menrath et al. 2010). In support of this theory, an association between rectoanal junction colonization and supershedding status has been described (Cobbold et al. 2007; Low 2005). Furthermore, EHEC O157 and non-O157 strains express several fimbrial and afimbrial proteins that likely play a role in ruminant reservoir persistence (Farfan and Torres 2012). In studies that used bovine terminal rectal primary epithelial cells, the H7 flagellum was demonstrated to act as an adhesin to bovine intestinal epithelium, supporting its involvement in the initiating step for colonization of the cattle reservoir (Mahajan 2009). Stx may also play a role in colonization and persistence by blocking the activation of bovine lymphocytes and thus supressing the bovine host’s immune response to the intestinal colonization (Moussay et al. 2006).

9.4 EHEC in the Environment

EHEC can survive in bovine feces long-term, making this a likely vehicle for transmission to cattle, food and the environment. Survival in feces can range from 1 to 18 weeks depending on the temperature (5, 15 and 25 °C were tested) (Fukushima et al.1999). Entry of EHEC to the environment may occur through direct deposit of feces onto land or through drainage runoff of fecal material in soil, especially after heavy rainfalls (Thurston-Enriquez et al. 2005). Moreover, under experimental conditions, EHEC can survive for more than 1 year in various manure-amended soils at different temperatures (Fremaux et al. 2008). Long-term survival of EHEC in lake water (13 weeks) and in cold river water has also been demonstrated (Wang and Doyle 1998; Maule 2000). This extended persistence in the environment likely plays a significant role in the colonization of cattle and subsequent human infection (Fremaux et al. 2008).

EHEC O157:H7 is also able to colonize various types of plants and fruits. For example, EHEC O157:H7 has been shown to form bacterial aggregates on apples (Janes et al. 2005) as well as on the surface of lettuce leaves (Seo and Frank 1999; Auty et al. 2005). Furthermore, studies have found EHEC in the internal inner tissues of plants, including radishes, carrots and lettuce (Itoh et al. 1998; Solomon et al. 2002). These subsurface localizations may be protective to the bacteria as they are inaccessible to other competitive bacteria as well as surface treatments and washing.

9.5 EHEC Infections in Humans

After ingestion of EHEC, a 3–12 day incubation period is typically followed by development of watery diarrhea accompanied with abdominal cramping and pain. Most patients will subsequently suffer from bloody diarrhea. About 1 week after the initial onset of diarrhea, HUS develops in a variable proportion of cases, depending on the serotype of the causative EHEC strain and the Stx subtype (Tarr et al. 2005). HUS patients present with widespread thrombotic microvascular lesions in the kidneys, the gastrointestinal tract, and other organs (Richardson et al. 1988). Since EHEC infections are rarely bacteremic, i.e. bacteria do not penetrate the circulatory system and are not found in patient blood cultures (Bielaszewska and Karch 2005), it is hypothesized that HUS results from vascular endothelial injury by circulating Stx. According to the generally accepted model of HUS pathogenesis, Stx is released by EHEC in the intestine, absorbed across the gut epithelium into the circulation (Hurley et al. 2001; Müthing et al. 2009), and transported to small vessel endothelial cells.

HUS is the most common cause of acute renal failure in children. The mortality rate can be up to 3% (Karch et al. 2005). While 70% of EHEC-infected patients were fully recovered within 5 years after diagnosis, the remaining 30% still experienced persistent hypertension (9%), neurological symptoms (4%), decreased glomerular filtration rate (7%), and/or proteinuria (18%) (Rosales et al. 2012). There is currently no effective causative therapy, and antibiotic treatment appears to be ineffective if not harmful (Wong et al. 2000; Davis et al. 2013). In contrast to cattle, EHEC O157:H7 colonizes humans only for a limited time of about 4 weeks (Fig. 9.1; Karch et al. 1995). Moreover, whereas in cattle many different EHEC O157:H7 PFGE subtypes can co-exist in a single animal (Jacob et al. 2011), human patients are infected mostly by a distinct O157:H7 PFGE subtype.

EHEC O157:H7 is the most prevalent EHEC serotype identified as a cause of sporadic HUS cases (Tarr et al. 2005; Karch et al. 2005). Still, non-O157:H7 EHEC (especially O26:H11, O103:H2, O111:H8, O145:H28/H25 and sorbitol-fermenting (SF) O157:H$^-$) represent a significant portion of EHEC infections leading to HUS complications (Karch et al. 2005; Mellmann et al. 2008; Bielaszewska et al. 2013).

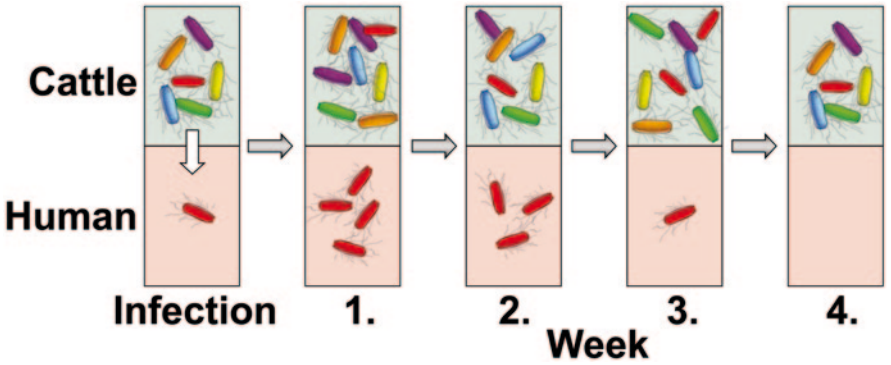

Fig. 9.1 Schematic illustration of EHEC O157:H7 infection in cattle and humans. In contrast to cattle, EHEC O157:H7 colonizes humans only for a limited time of about 4 weeks. Moreover, whereas in cattle many different EHEC O157:H7 PFGE subtypes can co-exist in a single animal, human patients are infected mostly by a distinct O157:H7 PFGE subtype. Different EHEC O157:H7 PFGE subtypes are indicated by different colors

Though EHEC strains are often considered as a pathogroup, there may be important differences between serotypes.

SF EHEC O157:H$^-$ represent a significant serotype in Europe which has not yet been detected in North America. These strains are characterized by a specific combination of their phenotypic and virulence characteristics that differentiates them from classical non-SF EHEC O157:H7 (Karch and Bielaszewska 2001). This combination includes the ability to ferment sorbitol overnight and to produce β-D-glucuronidase. A gene cluster termed *sfp*, which encodes fimbriae and mediates mannose-resistant hemagglutination, has been identified on the large plasmid of SF STEC O157:H$^-$ (Brunder et al. 2001). Notably, Sfp-encoding genes are absent in EHEC O157:H7.

The minimum infectious dose of EHEC in humans is extremely low, with approximately 10–50 bacteria needed for colonization (Teunis et al. 2004). In meat implicated as an outbreak source in the USA in 1993 there were less than 700 EHEC O157:H7 bacterial cells per hamburger patty prior to cooking (Tuttle et al. 1999). Moreover, a high degree of tolerance to acid and drying enables EHEC to survive in food items, the consumption of which had been previously considered safe with respect to the ability to cause foodborne illness (e.g., apple cider, semi-dry fermented sausage). Three principal routes of transmission of EHEC infection have been identified: (1) contaminated food and contaminated water used for drinking or swimming, (2) person-to-person transmission, and (3) animal contact, for example in petting zoos housing domesticated sheep, goats and other small animals or (occupational) farm exposure (Crump et al. 2002; Karch et al. 2005).

Table 9.2 Example of outbreaks caused by EHEC O157:H7

Year	Country	Cases/HUS/deaths[a]	Source	Reference
1982	USA	47/0/0	Hamburger[b]	Riley et al. 1983
1992–1993	USA	501/45/3	Hamburger[b]	Bell et al. 1994
1996	Scotland	345/34/16	Meat[b]	Dundas et al. 2001
1996	Japan	>6000/n.a./2	Radish sprouts	Watanabe et al. 1996
2000	Canada	~2300/28/7	Drinking water[b]	Hrudey et al. 2003
2005	Sweden	135/11/0	Lettuce	Söderström et al. 2008
2006	USA	199/31/3	Spinach[b]	CDC 2006
2006	USA	77/7/0	Iceberg lettuce	Sodha et al. 2011
2011	USA	15/4/2	Strawberries[b]	Laidler et al. 2013

n.a. Not available

[a] number of persons involved in the outbreak/number of HUS cases/number of deaths

[b] Strain isolated from the source

9.6 EHEC Outbreaks

EHEC is the cause of hundreds of outbreaks worldwide (Griffin et al. 1988; Michino et al. 1999; Karch et al. 1999). Examples of large outbreaks, including clinical impact and source, caused by EHEC O157:H7 and non-O157 are described in Tables 9.2 and 9.3, respectively. Consumption of raw or undercooked food items of bovine origin, particularly ground beef (hamburger), are common modes of EHEC O157:H7 transmission (Table 9.2). Moreover, contaminated radish sprouts, lettuce, spinach, strawberries, and contaminated water have been implicated in transmitting EHEC O157:H7 (Table 9.2).

One of the largest outbreaks to date occurred in Japan, in Sakai City, in 1996 (Watanabe et al. 1996; Michino et al. 1999), where thousands were affected, mostly school children. White radish sprouts served during school lunches were the most probable vehicle of the infection. In the winter 1992–1993, the largest outbreak of EHEC O157:H7 infection in the United States affected 501 persons in four western states including Washington, Idaho, Nevada and California (Bell et al. 1994) where 45 persons, mostly children, developed HUS and three children died. Hamburgers from a single fast-food restaurant chain were identified as the vehicle of the infection (Bell et al. 1994). The largest outbreak caused by contaminated drinking water occurred in Canada in 2000. Approximately 2300 people became seriously ill and seven died from exposure to drinking water contaminated with EHEC O157:H7. In Europe, a large EHEC O157:H7 outbreak occurred in Central Scotland in 1996; 345 people contracted an infection after consuming meat from a single butcher's shop, and 16 died (Dundas et al. 2001).

Table 9.3 describes several examples of large outbreaks caused by non-O157 EHEC strains. These include a wide range of serotypes, with the largest non-O157 outbreak occurring in Germany in 2011 associated with the contamination of fenugreek sprouts by EHEC O104:H4 (RKI 2011; Karch et al. 2012).

Table 9.3 Examples of outbreaks caused by non-O157 EHEC

Year	Serotype	Country	Cases/HUS/Deaths[a]	Source	Reference
1994	O104:H21	USA	18/0/0	Past. cow milk	CDC 1994
1995	O111:H$^-$	Australia	n.a./20/1	Sausage[b,c]	Paton et al. 1996
1999	O111:H8	USA	55/2/0	Salad bar	Brooks et al. 2004
2001	O26:H11	Germany	11/0/0	Beef	Werber et al. 2002
2004	O111:H$^-$	USA	27/0/0	Apple cider	Schaffzin et al. 2012
2006	O103:H25	Norway	17/10/1	Mutton sausage[b]	Schimmer et al. 2008
2007	O26:H11	Denmark	20/0/0	Beef sausage[b]	Ethelberg et al. 2009
2007	O145 + O26	Belgium	12/5/0	Ice cream[b]	De Schrijver et al. 2008
2009	O145:H28	Norway	16/0/0	Sheep	Wahl et al. 2011
2010	O145:H$^-$	USA	33/3/0	Romanian lettuce[b]	CDC 2010
2011	O104:H4	Germany	3842/855/53	Fenugreek sprouts	RKI 2011

n.a. not available

[a] number of persons involved in the outbreak/number of HUS cases/number of deaths

[b] Strain isolated from the source

[c] "Mettwurst", German sausage made from raw minced pork

9.7 Future Strategies and Unresolved Issues

Advances in rapid alert systems for the early detection of EHEC outbreaks have created greater awareness for both the public as well as the clinical community. Moreover, an increasing number of clinical microbiological laboratories routinely screen for EHEC by detection of Stx genes and/or toxin production. Diagnosed cases are now legally required to be reported in nearly every country. New high resolution techniques including next generation sequencing (NGS) are becoming more accessible and widely used, which enable the rapid identification of outbreaks at the earliest stages (Mellmann et al. 2011). In the future, databases and nationwide reporting systems could be in place to facilitate outbreak prevention and public health. The value of such strain linkage analysis is obvious. Common sources of infection can be identified accurately and rapidly. This is especially important considering the emerging epidemiology of foodborne infections. In particular, foodborne outbreaks nowadays less frequently follow the "church picnic" model, in which small isolated clusters of illness can easily be identified with case interviews. Instead, current outbreaks now more frequently result from the dissemination of vehicles that are contaminated by relatively low levels of pathogens. Such outbreaks can occur across state lines and international borders.

Another area where considerable efforts are being expended to bring improvement are the farming practices and environmental factors that affect infection of animals with EHEC. EHEC transmits readily between ruminants in the farm setting and wild animals can represent important vectors. For many years, the cattle industry and researchers have focused on improving the safety of meat products after slaughter. Postslaughter antimicrobial treatments of carcasses and HACCP policies in slaughter plants have been shown to significantly reduce meat contamination (Elder et al. 2000).

Due to the widespread distribution of EHEC O157 and non-O157 in farm cattle, its control will require intervention at the individual farm level. Recently, two vaccines against EHEC O157:H7 that are designed for use in cattle have been developed. While use of these vaccines could reduce the risk of EHEC in cattle by 50 %, which translates to approximately 85 % reduction in human cases, these vaccines have not yet been widely accepted by farmers due to several factors including burden of responsibility and economic factors (Matthews et al. 2013). An alternative route for the control of EHEC in cattle may be the feeding of probiotic bacteria, which can compete and interfere with pathogenic strains by producing metabolites that are inhibitory to EHEC. Still, more research is needed to develop viable strategies targeting the different levels (cattle, food, person-to-person spread, etc.) to control EHEC.

Further research is also needed to address effective therapies for humans after EHEC infection. Ongoing investigations are focused on topics such as toxin binders and Stx neutralizing immunoglobulin preparations.

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