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Emerging Risk evaluation of Enterohaemorrhagic *Escherichia coli* on Public Health

Author
Mesfin Angaw Tesfay
Department of Biology, College of Natural and Computational Science Adigrat University, Ethiopia
PO Box 50, Ethiopia
Email: *mesfinie@gmail.com*

Abstract
Enterohaemorrhagic Escherichia coli (EHEC) include serotypes E. coli O157: H7 and some other serogroups. Current epidemiological studies indicate that strains of enterohemorrhagic Escherichia coli (EHEC) belonging to serotype O157:H7 are most commonly associated with severe human diseases. The ability of EHEC to colonize human and animal intestinal mucosa and to cause disease is associated with a number of virulence factors, including expression of Shiga toxins (Stx) and the capacity to induce attaching/effacing (A/E) lesions. Other virulence factors carried by mobile genetic elements like pathogenicity island (PAI) and plasmids have been recently described. EHEC are zoonotic pathogens. They rarely cause disease in animals, and ruminants are recognized as their main natural reservoir. Cattle are the most important source of human infections with EHEC O157:H7. The organism has also been reported in sheep and goats. The epidemiology of EHEC infections has remarkably changed and an increasing numbers of unusual food vehicles have been associated with human infections. New routes of transmission have emerged, like contact with animals during farm visits and a wide variety of environment-related exposures. As for other zoonotic agents, having animals and raw products that are free from EHEC is not possible in practice. However, their occurrence can be minimised by applying high standards of hygiene in all the steps of the food production chain.
Keywords- *enterohemorrhagic E. coli; E. coli O157; Shiga toxins.*

INTRODUCTION

The epidemiology of microbial food-borne diseases has changed from time to time not only because of a human population increasingly susceptible to diseases and changing life styles but also because of the emergence of newly recognized food-borne [1]. *Escherichia coli* is normal part of the intestinal micro flora of many healthy animals including humans. However certain strains have been associated with gastrointestinal diseases in humans. Shiga toxin-producing *Escherichia coli* (STEC) first recognized as food-borne pathogens in 1982 [2], have recently been identified as major causes of severe illness. Although not all STEC are likely to cause human illness, several serotypes, including *E. coli* O157:H7 and O111 are responsible for many of the food-borne outbreaks of bloody diarrhea and hemolytic uremic syndrome worldwide. These *E. coli* strains have been categorized into pathogenicity groups, based on their virulence properties. One of these groups is characterized by the production of potent cytotoxins that inhibit protein synthesis within eukaryotic cells. These toxins are either termed verocytotoxins (VT), because of their activity on Vero cells, or Shiga toxins (Stx), because of their similarity with the toxin produced by *Shigella dysenteriae* [3]. Therefore, these strains are either termed Stx-producing *E. coli* (STEC) or VT-producing *E. coli* (VTEC). The majority of the cases of disease worldwide are caused by strains of serotype O157:H7, but infections sustained by EHEC strains belonging to serogoups other than

O157, like O26, O111, O103, and O145 have been increasingly reported [3]. These strains are now usually referred to as non–O157 EHEC. This paper will review the recent findings on pathogenesis and virulence factors, trying to seek out what makes a STEC highly pathogenic to humans.

PATHOGENESIS AND VIRULENCE FACTORS

Studies conducted in recent years on virulence and pathogenicity has been to define the combination of virulence factors and the mechanisms that make a STEC strain an EHEC fully pathogenic to humans. The production of Stx appears to be essential but not solely responsible for the pathogenic effects. An increasing number of additional virulence factors have been described, and they are usually carried by mobile genetic elements like plasmids and pathogenicity islands (PAI), large genetic elements carrying virulence genes and inserted in chromosomal loci encoding tRNA [4].

Shiga Toxins (Stx)

Stx are considered to be the major virulence factor of EHEC and comprise a family of structurally related cytotoxins with similar biological activity. The two main groups consist of Shiga toxin1 (Stx1), which is nearly identical to the toxin of *S. dysenteriae* type 1, and Shiga toxin2 (Stx2), which shares less than 60% amino acid sequence with Stx1 [4]. The genetic information for the production of Stx1 and Stx2 is located in the genome of lambdoid prophages integrated in the STEC chromosome [4]. Whereas Stx1 shows only little sequence variations [5], several variants of Stx2 with altered antigenic or biological characteristics have been described. Such toxins have been termed Stx2c, Stx2d, Stx2e and Stx2f [6]. Epidemiological studies have revealed that Stx2 is more associated with severe human disease than Stx1 [7]. Among the Stx2 variants, Stx2 and Stx2c have been frequently found in strains isolated from patients with HUS, while strains producing Stx2d are usually isolated from cases of uncomplicated diarrhoea [8], Other variants are produced by strains of animal origin and are rarely observed in human isolates.

The Pathogenesis Process

EHEC are highly infectious to human beings. The infection is usually acquired by ingestion of contaminated food or water or by person-to-person spread through close contact. Outbreaks or incidents of illness are believed to result from a very low infective dose, e.g. < 100 cells, but people may carry EHEC as part of their transient gut microflora without disease [9]. When these organisms do cause illness, very serious clinical manifestations can occur, including haemorrhagic colitis and HUS [10]. This latter condition usually occurs in children under five years of age and is the major cause of acute renal failure in children [9]. The pathogen and host factors that contribute to the clinical manifestations of EHEC infection are the subject of considerable ongoing investigations and the pathogenesis process is still not fully understood. Stx production is a prerequisite for EHEC mediated diseases. The toxin is able to pass through the intestinal epithelium to reach its target on endothelial cells lining small blood vessels that supply the gut, kidney and other viscera [11]. In addition to exacerbating the intestinal damage associated with infection, Stx are responsible for life-threatening post-diarrhoeal complications due to their action on glomerular and brain microvascular endothelial cells and the activation of prothrombotic and proinflammatory cascades that lead to the development of HUS and central nervous system complications [11].

Besides Stx production, colonisation of the host intestinal mucosa is another key determinant of virulence. In conclusion, not all the strains of STEC are able to cause haemorrhagic colitis or HUS, and those that do carry virulence determinants in addition to Stx. However, it is still not clear why only the subset of STEC defined EHEC have narrow host specificity and is human pathogens.

ANIMAL RESERVOIRS AND ECOLOGY

A reservoir host is "an organism in which a parasite that is pathogenic for some other species lives and multiplies without damaging its host." The reservoir of EHEC O157 generally includes ruminant animals, particularly cattle, since they periodically or seasonally ubiquitously shed EHEC O157 at prevalence ranging from single digits to near 100%, yet suffer no apparent illness from colonization and shedding. But there may be other important reservoirs of EHEC O157. As we discuss below, colonization of cattle is transient and varies strongly by season, yet specific strain types may stably exist on single farms over at least several years, raising the question of the possible existence of other, more stable reservoirs.

Prevalence and Shedding of EHEC O157 and Non-O157 in Domestic Ruminants

Detected fecal prevalence of EHEC O157 in cattle ranges widely, depending on the age group, the season, and the isolation technology [11]. One study evaluating previously published reports in beef cattle found that prevalence was 0.3–19.7% in feedlots and 0.7–27.3% on pasture, whereas the prevalence of non-O157 was 4.6–55.9% and 4.7–44.8%, respectively [11]. Another study evaluating published reports on fecal testing of dairy cattle also showed wide ranges of prevalence rates for O157 (0.2–48.8%) and non-O157 (0.4–74%) [12]. Specific strain types of EHEC O157 can exist stably on a particular farm for up to several years ([13], [14], [15]. Research has not determined whether persistence of these strain types is due to rare long-term carriage by ruminants, to persistence in environmental reservoirs, or to the existence of other, as yet unidentified animal reservoirs that are more persistently infected than ruminants. Most studies in in many regions of the world have seen a strong seasonal pattern of shedding, with prevalence peaking in summer and early autumn [16].

Another strong pattern is relatively higher-prevalence shedding in sub adult cattle, aged 2 months (weaning) to 2 years (first calving), compared to either younger or older animals [17]. This age group typically includes most feedlot cattle that are slaughtered for high-quality beef.

The biological basis for either seasonal or age-related peak shedding by cattle is unknown. Hypotheses include seasonal exposures of cattle to EHEC O157 due to the pathogen's environmental replication to infectious doses; seasonal variation in day length affecting hormone levels, with effects on the intestinal environment; and seasonal presence of increased numbers of young, high shedders [17].

OTHER RUMINANT SPECIES

STEC, including EHEC O157 and other serogroups associated with human infections like O91, O128 and O146, have been frequently isolated from the intestinal content of sheep [18]. EHEC O157 has also been found in both meat and milk and sheep are now considered as an important reservoir for human infection. EHEC O157 has also been isolated from goats [19], and goat milk has been associated with an outbreak. Small ruminant flocks may also have a relevant role in spreading STEC contamination in the environment [20]. The water buffalo is another potential source for STEC infections. A recent survey conducted in southern Italy (unpublished results) showed that buffalo dairy herds were frequently colonised by EHEC O157; yet the organism was not found in a study conducted on mozzarella cheese prepared with unpasteurised buffalo milk [21]. STEC can be found in wild ruminants, and the possible role of these animals as reservoirs for domestic ruminants sharing the same environment has been suggested. EHEC O157 has been repeatedly isolated from deer and the consumption of deer venison has been associated with human infections [21]; these episodes also underline the risk of products derived from private slaughtering.

NON RUMINANT MAMMALS

Investigations of the prevalence of EHEC O157 in nonruminants on cattle farms are typically part of larger epidemiologic studies focusing on cattle or

food sources. Evaluation of the data from these investigations should account for their use of various diagnostic techniques for isolation and/or detection of EHEC. One study involved the isolation of EHEC O157 from horses (1.1%), dogs (3.1%), pooled bird feces (0.5%), pooled flies (3.3%). but not from rodents (N = 300) or other wildlife species (N = 34) sampled on dairy farms (Hancock et al. 1998a). Another report identified horses and dogs, based on isolation of EHEC O157 with identical genotypes, as potential reservoirs of human O157:H7 infections [15]. In this study, an O157:H7 strain (phage type 4) was isolated from the stool of a 1-year old child with bloody diarrhea after he visited a small farm with goats, a pony, a heifer and a calf, and two dogs. Twelve days after the boy's illness a similar O157 strain (phage type 4) was isolated from the pony's feces and subsequently from the dog's feces. Other investigations have provided evidence that dogs with diarrhea can excrete STEC [21] and have reported the detection of STEC strains including O157 and non-O157 in 16.6%, 14.6%, 3.2%, and 7.1% of isolates from cows, calves, farm dogs, and humans, respectively, in dairy farms in Trinidad [22].

EMERGING MODES OF TRANSMISSION

EHEC infections may be sporadic, in small clusters, or manifest as larger outbreaks. Transmission is via the fecal-oral route and frequently occurs through ingestion of contaminated food or water; direct contact with infected animals, humans, or objects; or, rarely, inhalation [20]. Outbreaks of EHEC infection may result from contamination originating in restaurants, home kitchens, farms, petting zoos, nursing homes, day care centers, recreational pools/lakes, and schools . Irrigation water can also contaminate Produce [21]. EHEC O157 survival and replication in a soil protozoan (*Acanthamoeba polyphaga*) suggests a potential environmental reservoir for transmission [8]. The infective dose in humans has been estimated at 4 to 24 organisms, similar to that of *Shigella* spp. [16]. Infected individuals are highly contagious and may be considered a public health hazard [10]. Approximately 20% of the *E. coli* O157:H7 cases diagnosed during an outbreak are the result of secondary transmission; rates of such transmission are particularly high in outbreaks that affect children with a median age of less than 6 years and those in nurseries [14].

CONTROL STRATEGIES

As for other zoonotic agents, having animals and raw products that are free from STEC is not possible in practice. However, their occurrence can be minimised by applying high standards of hygiene in all the steps of the food production chain.

At the farm level, classical eradication strategies based on the elimination of positive animals are not feasible, due to the high Enterohaemorrhagic *E. coli* prevalence of colonisation, its transient nature, and the technical difficulties in detecting low levels of the organism in animal faeces. Many approaches have been attempted to reduce the intestinal colonisation in cattle. These include interventions on the diet of the animals, the administration of probiotics as competitive micro flora and the use of bacteriophages active on EHEC O157 [23].

These approaches have produced inconclusive and sometimes conflicting results. Moreover the feed regimens and the treatments adopted in experimental trials are often difficult to apply to farming practices. Recently, experimental vaccines aiming at reducing the shedding of EHEC O157 in cattle were developed. Subcutaneous administrations of type III secreted proteins, are able to decrease shedding of EHEC O157 by cattle [20]. Transgenic tobacco plant cells that express the host cell-binding domain of EHEC O157 intimin have also been tested successfully in a mouse model [23]. Although transgenic plants are not likely to be used for cattle vaccination, at least in Europe, this latter result suggests that an intimin-based vaccination strategy could be successful. However, as for

other infectious diseases, good hygiene and management practices remain at the present the best way to reduce the spread and persistence of EHEC O157 on the farm. As discussed in the above paragraphs, these may include cleaning the water troughs where EHEC O157 can survive and even grow up [22] reducing faecal contamination and humidification of feed, and a correct preparation of silage. Other factors that could favour colonisation and shedding of EHEC O157 like sudden modifications in the diet and the stress derived from movement or overcrowding should be reduced.

Since environmental contamination may have an important role in the transmission of the infection to humans, the handling of the animal dejections represents an important issue. STEC can survive in bovine faeces for a considerable time, therefore manure and slurries should be properly composted to ensure sterilisation or at least the reduction of the microbial load [5]. As far as the transmission through the direct contact with animals is concerned, both farmers and people visiting farms should apply hygiene practices. In particular, farms receiving school visits must ensure that adults always control children, facilities for hand washing are easily available, and areas for food consumption are clearly separated from those where the animals are kept. At the abattoir level, no specific procedures for STEC elimination can be applied. However, good hygiene and manufacturing practices as well as implementation of HACCP will contribute to reducing faecal contamination of carcasses.

The general principles of food hygiene will also be effective in preventing EHEC infections at the processing and retail levels of the food chain. In particular, cross contamination between raw and ready to eat products must be avoided, bearing in mind that several large outbreaks (Upton P., and Coia J.E., 1994) have originated from gross failures in this basic point. Microbiological testing of meat lots consumed by persons who have become ill suggests that the infectious dose for EHEC O157 might be very low (*Phillips, Navabpour, Hicks, et al* 2016). This represents a strong argument for enforcing zero tolerance for this organism in processed food and for markedly decreasing contamination of raw ground beef.

CONCLUSION

Despite the great efforts, the studies on the virulence and the evolution of EHEC have only unraveled part of these complicated phenomena. Understanding the factors that govern the development of severe disease in human beings, and the colonisation of the animal hosts would provide the insights for more effective intervention on both these aspects. Moreover, defining the combination of virulence genes and the mechanisms that make a STEC strain fully pathogenic will be pivotal to improve the efficacy of both the diagnostics of human infections and the surveillance of animal reservoirs and the assessment of public health risks. Human infections result from diverse exposures including contaminated foods of animal (especially bovine) origin, direct contact with shedding or contaminated animals, direct contact with environmental (water) contaminants, and ingestion of other foods (especially produce) contaminated with EHEC O157. In particular, the isolation of EHEC from a growing spectrum of animal species, which can either act as true natural hosts or merely as occasional vectors, suggests that investigations on episodes of human disease with a potential link to a rural environment should be conducted with an open mind and that previously non described animal reservoirs, or food, or environmental vehicles should be considered and tested.

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