Zoonotic Enterohemorrhagic *Escherichia coli*: A One Health Perspective

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Abstract

Escherichia coli O157 and other enterohemorrhagic *E. coli* (EHEC) are food- and waterborne zoonotic pathogens that cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans but little or no discernible disease in their animal reservoirs. Like other zoonotic infections, EHEC are illustrative of the One Health concept as they embody the complex ecology of agricultural animals, wildlife, and the environment in zoonotic transmission of EHEC O157. But compared to the detailed epidemiological and clinical information available for EHEC infection in humans, there is an incomplete understanding of the ecology of EHEC infection in animals and the persistence of EHEC bacteria in the environment. Significant aspects of the microbiology, epidemiology, and host-pathogen interactions of EHEC in animals remain undefined. This review highlights the nature of EHEC infection in humans, provides a One Health perspective on what is known about EHEC in animal and environmental reservoirs, and proposes interventions targeted at pathways of transmission to optimize effective prevention and control measures.

Key Words: animal reservoir; cattle; enterohemorrhagic *E. coli* (EHEC); *Escherichia coli* O157:H7; epidemiology; flies; One Health; zoonosis

Introduction

Escherichia coli O157:H7, the prototype and most virulent enterohemorrhagic *E. coli* (EHEC[1]), was isolated in 1982 from outbreaks of hemorrhagic colitis associated with eating undercooked meat in fast-food restaurants (Riley et al. 1983). EHEC O157 was also isolated from sporadic cases of hemorrhagic colitis (Uyeyama et al. 1982). The recognition of toxin production by EHEC O157 led to the discovery of its causative role in the development of a previously idiopathic condition known as hemolytic uremic syndrome (HUS[1]), a clinical pathological triad consisting of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (Johnson et al. 1983; Karmali et al. 1985; O'Brien et al. 1983).

Although EHEC O157 is the most common serotype isolated from humans in the United States, over 100 other serotypes, characterized collectively as non-O157 EHEC, are recognized by the World Health Organization as zoonotic emerging pathogens (WHO 1998). Non-O157 EHEC have pathogenic and outbreak potential and are associated with diarrhea, hemorrhagic colitis, and HUS in humans (Brooks et al. 2005; Hedican et al. 2009). Genomic comparison of EHEC O157 and three clinically important non-O157 EHEC (O26, O111, and O103) revealed that all share very similar virulence gene sets, providing insight into EHEC parallel evolution (Ogura et al. 2009).

Almost 3 decades after its discovery, EHEC O157 continues to make the headlines as the culprit of major disease outbreaks worldwide. Moreover, recent molecular analyses suggest that certain EHEC O157 strains are apparently more virulent than others (Besser et al. 2007; Kulasekara et al. 2009; Laing et al. 2009; Manning et al. 2008). These findings underscore the need to be vigilant for these pathogens and to apply One Health approaches to minimize the potential for zoonotic transmission and disease outbreaks.

EHEC in Humans

Virulence Factors, Pathogenesis, and Pathophysiology

Shiga toxin (Stx[1])–producing *E. coli* (STEC[1]) strains carry *stx* genes and produce Stx but are not necessarily associated with disease, although some may be capable of causing hemorrhagic enteritis and HUS. EHEC may produce two immunologically distinct toxins, Stx1 or Stx2, alone or in combination. Stx can inhibit protein synthesis (Ogasawara et al. 1988), and O157:H7 strains that produce Stx2 may be associated with an increased risk of systemic complications (Donohue-Rolfe et al. 2000).

stx genes are encoded in bacteriophages and may have different variants based on their genetic sequence (Friedrich et al. 2002). Upon induction, Stx-encoding bacteriophages increase toxin production and play a role in horizontal transfer

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[1]Abbreviations used in this article: EHEC, enterohemorrhagic *Escherichia coli*; HUS, hemolytic uremic syndrome; STEC, Shiga toxin–producing *E. coli*; Stx, Shiga toxin

of *stx* genes by infecting other bacteria, as demonstrated in in vivo and in vitro experiments (Acheson et al. 1998; Herold et al. 2004; Wagner et al. 2001). The pathogenesis of EHEC infection has been investigated in mice, rats, New Zealand white rabbits, Dutch belted rabbits, ferrets, dogs, pigs, baboons, and macaques (Brando et al. 2008; Eaton et al. 2008; García et al. 2006, 2008; Gunzer et al. 2002; Pai et al. 1986; Raife et al. 2004; Richardson et al. 1992; Ritchie et al. 2003; Siegler et al. 2003; Sjogren et al. 1994; Suzaki et al. 2002; Wadolkowski et al. 1990; Woods et al. 2002; Zotta et al. 2008).

The current model of pathogenesis indicates that Stx, produced by EHEC during colonization of the intestinal tract, gains entry to the host through epithelial cells and acts on submucosal immune cells that release cytokines; these in turn induce inflammation and increase the expression of the Stx receptor globotriaosylceramide (Gb3) (O'Loughlin and Robins-Browne 2001). Stx then targets the endothelium of organs in which the Gb3 receptor is expressed (e.g., the intestine, kidneys, and brain; Boyd and Lingwood 1989). Because the Gb3 receptor is a glycosphingolipid, variations in the lipid moieties of its structure may influence Stx binding (Kiarash et al. 1994). Stx-mediated endothelial injury activates coagulation, and inhibition of fibrinolysis leads to accumulation of fibrin and thrombosis (Tarr et al. 2005). The combination of Stx and O157 lipopolysaccharide (LPS) induces platelet-leukocyte aggregates and tissue factor release and thus contributes to a prothrombotic state (Stahl et al. 2009). The pathogenic roles of Stx and LPS have been studied in New Zealand white rabbits, mice, and baboons (Barrett et al. 1989; Clayton et al. 2005; Ikeda et al. 2004; Karpman et al. 1997; Keepers et al. 2006; Palermo et al. 2000).

Another important virulence factor of EHEC is an outer membrane protein called intimin, which is encoded by the *eae* gene in the locus of enterocyte effacement (LEE) (Jerse and Kaper 1991; Jerse et al. 1990; Yu and Kaper 1992). During EHEC infection, intimin assists in colonization and induces the characteristic intimate attachment to intestinal epithelial cells and effacement of microvilli (attaching and effacing lesions) by binding to its own receptor (the translocated intimin receptor or Tir), also produced by EHEC and transferred to the host's intestinal epithelial cells by a type 3 secretion system encoded in LEE (Kenny et al. 1997; Paton et al. 1998). Expression of EHEC LEE genes is regulated by quorum sensing and is induced by the host's adrenergic hormones (Sperandio et al. 2003).

Some LEE-negative non-O157 EHEC strains may also produce a novel and highly potent subtilase cytotoxin (SubAB) that, when injected intraperitoneally in mice, results in microvascular thrombosis and necrosis in various organs including the brain, kidneys, and liver (Paton et al. 2004). However, the role of SubAB in human EHEC disease remains to be elucidated. Interestingly, the SubAB receptor is generated by metabolic incorporation of an exogenous glycan derived from food (Byres et al. 2008).

Clinical and Pathologic Manifestations of EHEC Infections in Humans

Human EHEC infection may be asymptomatic or include diarrhea, hemorrhagic colitis, and HUS, a leading cause of acute renal failure in children that is potentially fatal. The clinical progression of *E. coli* O157:H7 infection in children has been well characterized and includes an incubation period of approximately 3 days, followed by diarrhea that may become bloody, and HUS in about 15% of the patients (Tarr et al. 2005).

Approximately 5% of HUS patients do not shed the causative EHEC at the time of microbiological analysis, but do excrete *stx*-negative derivatives of EHEC that lost *stx* during infection (Bielaszewska et al. 2007). The term "incomplete HUS" refers to a clinical presentation in which patients exhibit some but not all of the clinical pathological abnormalities associated with HUS—for example, anemia without azotemia, with or without thrombocytopenia (López et al. 1995; Ray and Liu 2001).

Studies assessing the long-term renal prognosis of patients with HUS have found microalbuminuria and mild decreases in glomerular filtration rate 5 years after HUS recovery; however, the clinical relevance of these findings has not been determined (Garg et al. 2008).

During infection and HUS, severe colonic pathology may manifest with ischemic changes and pseudomembrane formation resembling *Clostridium difficile* colitis (Kendrick et al. 2007; Richardson et al. 1988). Pathological renal effects in HUS include vascular lesions characteristic of thrombotic microangiopathy (TMA), which typically leads to thrombotic occlusion of small renal arteries and arterioles, while endothelial damage in the glomeruli causes formation of microthrombi in the glomerular capillaries (Benz and Amann 2009). Central nervous system involvement can be a major complication of HUS and may manifest clinically as seizures, coma, and/or dysregulated breathing (Theobald et al. 2001).

Recent Epidemiologic Trends

A recent study involving 2000–2006 data from the Foodborne Diseases Active Surveillance Network reported that death occurred in 0.6% of all patients with O157:H7 infection and in 4.6% of those with HUS and that the highest proportion of HUS cases (15.3%) occurred among children less than 5 years old (Gould et al. 2009b). Patients aged 60 years or older had the highest rate of death due to O157:H7 infection—33% in patients with HUS and only 1.9% in those without HUS (Gould et al. 2009b).

In 2006 there were a total of 1,270 foodborne disease outbreaks in the United States that resulted in 27,634 cases and 11 deaths, 10 of which were attributed to bacterial etiologies and 6 to O157:H7 (Ayers et al. 2009). During 2007 there were 4,847 reported cases of STEC infection in humans and 292 cases of postdiarrheal HUS; most of the latter were associated with O157:H7 infection and occurred among children aged 1 to 4 years (Hall-Baker et al. 2009).

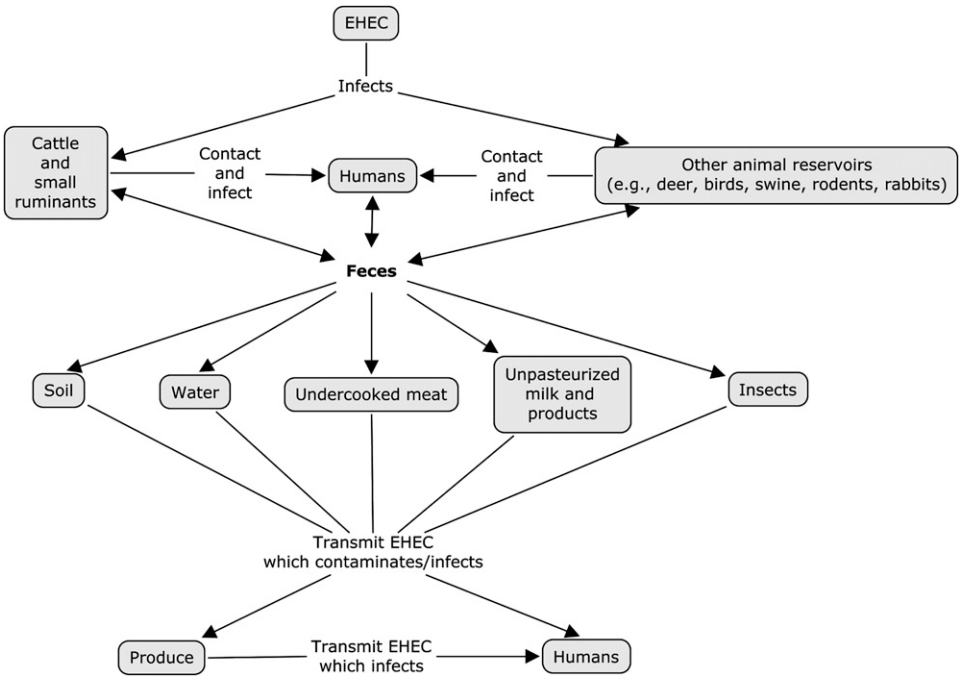

Figure 1 Concept map illustrating the relationships between the proven and postulated factors involved in enterohemorrhagic *Escherichia coli* (EHEC) transmission. Integrating and understanding the interplay of these factors involving humans, animals, and the environment will facilitate One Health approaches to prevent and control zoonotic transmission of EHEC.

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 (Figure 1) (Crump et al. 2002; Grant et al. 2008; Swerdlow et al. 1992; Varma et al. 2003).

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 (Davies et al. 2005; Keene et al. 1994; Michino et al. 1999; Ryan et al. 1986; Shukla et al. 1995; Spika et al. 1986). Irrigation water can also contaminate produce (Solomon et al. 2002). EHEC O157 survival and replication in a soil protozoan (*Acanthamoeba polyphaga*) suggests a potential environmental reservoir for transmission (Barker et al. 1999). The infective dose in humans has been estimated at 4 to 24 organisms, similar to that of *Shigella* spp. (Strachan et al. 2001).

Infected individuals are highly contagious and may be considered a public health hazard (Ahn et al. 2009). 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 (Snedeker et al. 2009).

Reservoir Hosts of EHEC

A reservoir host is "an organism in which a parasite that is pathogenic for some other species lives and multiplies without damaging its host."[2] The reservoir of EHEC O157 generally includes ruminant animals, particularly cattle, since they periodically or seasonally ubiquitously shed EHEC O157 at prevalences 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 (Hussein 2007; Renter and Sargeant 2002). One study evaluating previously published reports in beef

[2]Definition from the National Library of Medicine Medline Plus Medical Dictionary (www.nlm.nih.gov/medlineplus/mplusdictionary.html), accessed May 5, 2010.

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 (Hussein 2007). 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%) (Hussein and Sakuma 2005).

Specific strain types of EHEC O157 can exist stably on a particular farm for up to several years (Besser et al. 1997; Carlson et al. 2009; Cobbaut et al. 2008; LeJeune et al. 2004a; Rahn et al. 1997; Renter et al. 2003). 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 North America as well as in many other regions of the world have seen a strong seasonal pattern of shedding, with prevalence peaking in summer and early autumn (Fernández et al. 2009; Hancock et al. 2001; Milnes et al. 2009; Rhoades et al. 2009). An exception to this seasonal pattern was observed in Scotland, where a late autumn peak shedding coincided with the movement of animals off summer pastures and into winter housing (Synge et al. 2003).

Another strong pattern is relatively higher-prevalence shedding in subadult cattle, aged 2 months (weaning) to 2 years (first calving), compared to either younger or older animals (Cobbold and Desmarchelier 2000; Hancock et al. 2001; Renter et al. 2004). 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 (Hancock et al. 1998b); seasonal variation in day length affecting hormone levels, with effects on the intestinal environment (Edrington et al. 2008); and seasonal presence of increased numbers of young, high shedders (Cobbold and Desmarchelier 2000; Hancock et al. 2001; Renter et al. 2004).

Microbiology of EHEC O157 Infection of Cattle

Both cattle and sheep are well-characterized hosts of EHEC O157 but, while both have been repeatedly linked to human infection, cattle have received much more research attention. Numerous epidemiologic studies over the past 20 years have described the bovine EHEC O157 reservoir (for reviews, Hancock et al. 2001; LeJeune and Wetzel 2007; Renter and Sargeant 2002; Sargeant et al. 2007).

Diverse analytical methods have detected differences in the strain compositions of EHEC O157 populations in cattle compared to clinical isolates. Such methods include octamer-based genomic scans (Kim et al. 1999), whole genome PCR scanning (Ohnishi et al. 2002), *stx-Q* alleles (LeJeune et al. 2004b), a *tir* polymorphism (Bono et al. 2007), and the integration sites of Stx-encoding bacteriophages (Besser et al. 2007). The latter demonstrates considerably larger diversity among the bovine isolates as well.

Given the presumed biology of this zoonotic agent in cattle and other animal populations, these differences suggest that the reservoir(s), which probably account for most of the total population of EHEC O157 at any given time, have a large group of diverse strain types that differ in their infectivity or virulence for humans, thereby accounting for the (lower) diversity among clinical isolates. This variability is the expected result of a "source-sink" population structure,[3] with human clinical infection (where secondary infections are unusual and transient) representing a sink (Sokurenko et al. 2006). An alternative view is that this model reflects the pattern of an "accidental pathogen," in which a subset of the diverse reservoir population acquires the particular combination of virulence factors necessary to produce human infection and/or disease (Rendón et al. 2007).

EHEC O157 can be isolated from all levels of the bovine gastrointestinal tract at necropsy, but uniquely specifically colonizes the most distal few centimeters of the intestine, the rectoanal junction (RAJ) (Naylor et al. 2003). The specific colonization of this site is evident in (1) the higher sensitivity of culture using swabs taken at the RAJ (Rice et al. 2003), (2) the ability to visualize surface microcolonies of EHEC O157 adherent to the epithelium at the RAJ, with attaching and effacing lesions (Naylor et al. 2005), and (3) the greatly increased ratio of EHEC O157 to total *E. coli* at the RAJ compared to other levels of the bovine gastrointestinal tract (Naylor et al. 2005). This unique colonization site is consistent with the suggestion that fly transmission may be important in the dissemination of EHEC O157 among animals (Ahmad et al. 2007), since the resulting 1000X higher concentration of the agent on the surface compared to the interior of the feces produced by infected cattle (Naylor et al. 2003) would greatly increase its availability to fly vectors.

An important feature of bovine infection/colonization with *E. coli* in general, that is also probably true for EHEC O157 specifically, is the role of cattle in amplifying these bacteria. Experimental infection of cattle with EHEC O157 typically entails administration of single oral doses (10^9–10^{10} CFU) of the bacterium and results in an initial period of relatively very high fecal shedding (e.g., more than 10^5 CFU/g in the first few days after challenge) that often accounts for most of the animals' EHEC O157 fecal shedding during the experimental infection (Cray and Moon 1995). When corrected for the fecal volume, it is clear that a very high degree of amplification of the challenge dose of EHEC O157 has occurred. This high shedding level is not maintained but soon drops to that of a natural infection (<10^4 CFU/g).

The initial high rate of shedding is not unique to EHEC O157 but rather may be a common feature of any or even most *E. coli* strains, since oral doses of other *E. coli* strains

[3]Evolutionary "source-sink" model refers to the evolution of bacterial pathogens associated with continuous switching between permanent (source) and transient (sink) habitats (Sokurenko et al. 2006).

similarly result in predominant shedding of the inoculated strain within 24 hours after the challenge dose (Daniels et al. 2009). Therefore, cattle (and perhaps other herbivores), which typically have lower total *E. coli* fecal density than other species, may have a unique ability to temporarily amplify ingested *E. coli* strains.

The amount of research on cattle as EHEC reservoirs is logical and appropriate considering their implication as the most frequent animal source of human infection, but the lack of data for other animal reservoirs could limit the ability to develop methods to reduce human exposure to EHEC O157. For example, it is possible that human infection is due primarily to the efficient ability of cattle to amplify EHEC O157 after exposure to other animal or environmental reservoirs. The seasonal variation in fecal shedding of EHEC O157 by cattle is consistent with this possibility, and these factors together suggest that efforts to identify other reservoirs of EHEC O157 external to cattle can contribute to the development of more effective measures to contain the spread of EHEC infection.

EHEC O157 in Nonruminant Animals on Cattle Farms

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 (Trevena et al. 1996). 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 (Sancak et al. 2004) 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 (Roopnarine et al. 2007).

Swine also are potential reservoirs of O157:H7, susceptible to both direct (contact) and indirect (aerosol) transmission (Cornick and Vukhac 2008). Feral swine that shared rangeland with livestock gained access to adjacent crop fields and were identified as vectors of O157:H7 in the 2006 outbreak linked to consumption of fresh spinach (Jay et al. 2007). This outbreak highlighted the complex ecology of agricultural animals and wildlife and zoonotic transmission of EHEC O157.

STEC have also been isolated from rabbits (Blanco et al. 1996; Kim et al. 1997; Pohl et al. 1993), which are considered both vectors and reservoir hosts of EHEC (Bailey et al. 2002; García and Fox 2003; Leclercq and Mahillon 2003; Pritchard et al. 2001; Scaife et al. 2006). Although a study of fecal samples from domestic rabbits in the Netherlands did not detect EHEC O157 in any of the samples (Assies et al. 2007), in the United Kingdom wild rabbits were implicated as vectors in an outbreak that included cases of hemorrhagic diarrhea and one case of HUS in visitors to a petting zoo (Pritchard et al. 2001). In this outbreak, the rabbits appeared to have carried the pathogen from a farm with cattle shedding EHEC O157 to the adjacent petting zoo by consumption of contaminated pasture (Pritchard et al. 2001). Recent studies have found that Dutch belted rabbits are natural and experimental animal models of EHEC infection and Stx-induced disease including enteric and renal lesions (García and Fox 2003; García et al. 2002, 2006, 2008).

EHEC O157 has been isolated from the colon contents of 40% of rats (*Rattus norvegicus*) trapped in a cattle fattening barn (Cizek et al. 1999). Another study noted that STEC isolates from a rat and a bird (*Sturnus vulgaris*) were identical in serotype, virulence profile, and pulsed field gel electrophoresis (PFGE) type to cattle isolates from the farms where the rat and the bird were sampled (Nielsen et al. 2004). The reported frequency of rodent sightings in the pen or alley areas was one of the factors significantly associated with the presence of EHEC O157 in feedlot-cattle water tanks (Sargeant et al. 2004).

EHEC O157 in Wildlife

Numerous reports cite the shedding of EHEC O157 among wild ruminants, particularly deer: 16.2% of 43 deer (species not reported; Asakura et al. 1998), 0.2% of 1608 white-tailed deer (*Odocoileus virginianus*) (Renter et al. 2001), 1.8% of 108 white-tailed deer (Rice et al. 1995), 9.4% of 32 deer (species not reported; Keene et al. 1997), 11.1% of 9 deer (species not reported; Cody et al. 1999), 2.4% of 212 white-tailed deer (Sargeant et al. 1999), 1.5% of 206 red deer (*Cervus elaphus*) (García-Sánchez et al. 2007), 0.6% of 469 white-tailed deer (Fischer et al. 2001), and 30% of 10 deer (species not reported; Chapman et al. 1997). However, others have failed to find EHEC O157 in wild ruminants: deer sharing pastures with EHEC O157–positive cattle and sheep were negative (Branham et al. 2005), and an examination of 1387 reindeer fecal samples and 421 reindeer meat samples did not reveal EHEC O157 isolates (Lahti et al. 2001).

Studies of EHEC O157 shedding in wild animals are few and of very limited geographic scope. Adesiyun (1999) examined fecal samples from 271 animals in the wild, 175 wild animals in captivity, and 373 animals in zoos, all in Trinidad, and did not find any that were shedding EHEC O157. Beutin and colleagues (2007) examined 219 meat samples from various species, including wildlife, in Germany and failed to identify any contaminated with EHEC O157, although they identified other serotypes of STEC and, interestingly, found

that the *stx* gene content of STEC isolates differed significantly among species. Harrison and colleagues (2006) sampled 25 carcasses, including an unspecified number of Roosevelt elk (*Cervus elaphus roosevelti*), and failed to identify any contaminated with EHEC O157. Wahlström and colleagues (2003) sampled 791 wild animals, including geese, deer, hares, moose, wild boar, and gulls, shot by hunters in Sweden and identified a single wild boar (*Sus scrofa*) shedding EHEC O157. Jijón and colleagues (2007) tested 71 fecal samples from diverse species at an Ohio wildlife rehabilitation center but did not isolate EHEC O157. And Miller and colleagues (2009) cultured feces from 240 sea otters (*Enhydra lutris*) in California and failed to find any shedding EHEC O157.

A limited number of experimental studies have evaluated the ability of wildlife species to carry EHEC O157. For example, Fischer and colleagues (2001) demonstrated that white-tailed deer orally inoculated with 10^8 CFU EHEC O157 shed the agent for several weeks and could transmit it horizontally to other deer. Gray and colleagues (2007) exposed tadpole and metamorph bullfrogs (*Rana catesbeiana*) to EHEC O157 and demonstrated persistence of the agent in over half of the metamorphs for at least 2 weeks. However, the metamorphs were housed in stagnant water, and it seems likely that the persistence of EHEC O157 was in the stagnant water environment as well as in the frogs.

EHEC O157 in Birds

Research groups have reported human EHEC infections associated with birds shedding EHEC O157, birds shedding the pathogen unrelated to human disease, and the susceptibility of birds to experimental colonization with EHEC O157. Ejidokun and colleagues (2006) reported on two sibling children infected with EHEC O157 where a PFGE matching strain was subsequently isolated from rooks' feces collected from feed troughs; other environmental and fecal (cattle and rabbits) samples from the farm were all culture negative. Hancock and colleagues (1998a) sampled pooled bird feces on dairy cattle farms and found 1 of 200 positive for EHEC O157. Dipineto and colleagues (2006) examined four Italian layer farms for EHEC O157 shedding by hens and found that two were positive on three different sampling dates, whereas there was no shedding on the other two farms. Cumulatively, 3.6% of 720 hens were EHEC O157 positive. Szalanski and colleagues (2004) reported identification of EHEC O157, based on detection of rfb_{O157} and $fliC_{H7}$ genes by polymerase chain reaction (PCR), from more than 10% of 174 turkey fecal samples from both brooder and finisher birds on two farms. Best and colleagues (2003) challenged specific pathogen–free chicks with 10^5 CFU EHEC O157 and reported fecal amplification (10^8 CFU/g within 24 hours) and persistent fecal shedding (10^7 CFU/g at 92 days post challenge). Wallace and colleagues (1997) cultured bird feces from urban landfills and intertidal sediments and reported EHEC O157 prevalences of 0.9% and 2.9% respectively, with three positive samples from landfills and ten from intertidal areas. In contrast, Makino and colleagues (2000) did not isolate EHEC O157 from cultures of 50 fecal specimens from seagulls, although they identified two STEC of other serotypes.

EHEC O157 in Flies and Other Insects

Various species of flies can transmit EHEC O157. Kobayashi and colleagues (1999) studied contamination of flies in an investigation of a nursery-associated EHEC O157 outbreak and reported detection of the agent in fly intestines, excretion by contaminated flies for a 3-day period, and retention of viable pathogens in the flies' crops for 4 days. They further noted EHEC O157 adherent to the mouthparts of culture-positive flies, suggesting a biological rather than just a mechanical association.

Not surprisingly, various species of flies on farms show contamination with EHEC O157. Hancock and colleagues (1998a) isolated the bacterium from 2 of 60 pooled fly samples from feedlots and dairy farms. Heuvelink and colleagues (1998) also isolated EHEC O157 from stable flies (*Stomoxys calcitrans*) on Dutch dairy farms. Iwasa and colleagues (1999) reported five flies positive for cultures of 310 collected from four farms. Szalansky and colleagues (2004) determined that 0.4–1.3% of pools of flies of two different species (*Musca domestica* and *Hydrotaea aenescens*) on a turkey farm were PCR positive for EHEC O157 markers, and Keen and colleagues (2006) demonstrated a 5.2% EHEC O157 carriage rate in flies sampled at agricultural fairs.

An example of the important role of flies in dissemination of EHEC O157 is their ability to transmit contamination from one spinach plant to another (Talley et al. 2009). Janisiewicz and colleagues (1999) similarly noted that fruit flies (*Drosophila melanogaster*) could spread EHEC O157 contamination to fresh-cut apple tissue. Ahmad and colleagues (2007) showed that eight cattle exposed to contaminated flies became colonized with and shed EHEC O157, whereas eight other cattle not exposed to the flies remained culture negative.

The relatively frequent detection of EHEC O157 in flies, the apparent biological association with flies noted by Kobayashi and colleagues (1999), and the predilection of EHEC O157 for the RAJ colonization site, which results in enriched fecal surface contamination, are all consistent with the possible coevolution of this bacterium to use cattle hosts and fly vectors for transmission.

While flies are a common insect vector for EHEC O157, a Chinese study isolated the bacterium from the intestine of 4 of 113 dung beetles (*Catharsius molossus*) and found that its PFGE pattern and virulence genes were identical to those in ten strains isolated from humans with diarrhea in the same geographic region (Xu et al. 2003).

EHEC O157 in the Environment

EHEC O157 has the capability for replication and prolonged survival in environmental niches. Keen and colleagues (2006) tested 689 environmental samples from a total of 20

fairgrounds 10 months or more after the end of the fair, and demonstrated the persistence of EHEC O157 in four beef cattle barns on three fairgrounds. Similarly, others have reported prolonged survival on fairground premises—42 weeks (Ohio; Bopp et al. 2003), 5 months (North Carolina; Durso et al. 2007), 46 days (Texas; Durso et al. 2005)—and on spinach leaves (14+ days; Mitra et al. 2009), where the bacterium showed better survival than on the surrounding soil.

Similarly consistent with long-term environmental persistence are observations that specific strains of EHEC O157 may be associated with individual farm premises for periods of at least several years, despite seasonal variation that may make the agent undetectable for several months each year. It is clearly impossible to rule out long-term low-level animal colonization as the mechanism by which this strain persistence occurs, but the sensitivity of current diagnostic techniques and the year-long stability of both dietary factors and body temperatures suggest that the persistence from year to year may in fact be in the environment rather than in colonized cattle.

For humans, contaminated drinking water and recreational waters are associated with EHEC O157 infection. Among animals, cattle water troughs contaminated with EHEC O157 are associated with increased EHEC O157 fecal prevalence in cattle (Hancock et al. 2001). Renter and colleagues (2003) identified the agent in 0.2% of water sources for pastured cattle, and Sanderson and colleagues (2005) isolated it from 25% of water sources for feedlot cattle. Sargeant and colleagues (2004) isolated EHEC O157 from 13% of cattle water sources and reported that positive troughs were also associated with increased water opacity, use of fly traps on the farm (a likely indicator of high fly density), and frequency of rodent sightings on the premises.

LeJeune and colleagues (2001) showed that model water troughs contaminated with EHEC O157 via feces from infected cattle remained contaminated for more than 180 days despite continuous water turnover at 2 volumes per day. Chlorination of input water resulted in only a small decrement of EHEC O157 contamination. The infectiousness of the EHEC O157 persistent in the water was demonstrated by the infection of naïve calves allowed to drink from the troughs 8 months later. Thus, water troughs are one possible environmental reservoir that enables EHEC O157 to persist from year to year despite the annual wintertime dearth of colonized cattle.

Logically, if the presence of colonized cattle depends on the availability of environmental reservoirs (water, soil, insect, or other locus) of EHEC O157, perhaps cattle should be considered an amplifying vehicle rather than or in addition to their status as a reservoir. If so, further efforts to identify and characterize environmental reservoirs of EHEC O157 may lead to novel control measures.

One Health Approaches to Diagnosis, Treatment, Prevention, and Control

Current recommendations for EHEC diagnosis in humans by clinical laboratories, if followed, could allow for earlier diagnosis and better responses to infection (Gould et al. 2009a). In order to detect O157:H7 as well as non-O157 EHEC strains, stools should be cultured on selective and differential agar such as sorbitol-MacConkey (SMAC) agar and simultaneously assayed with a test that detects Shiga toxins or the genes that encode them (Gould et al. 2009a). Typical O157:H7 strains do not ferment sorbitol and appear as colorless colonies on SMAC agar whereas most non-O157 strains ferment sorbitol and appear as pink colonies on SMAC agar.

No specific treatments are available for HUS in humans. Supportive therapy includes intravenous fluids and volume expansion (Ake et al. 2005), but antibiotic use is contraindicated in suspected or confirmed cases of O157:H7 infection because of the possibility of increased risk of HUS by induction of Stx-encoding bacteriophages (Ahn et al. 2009; Zhang et al. 2000). Intervention strategies in humans consist of vaccines, Gb3 receptor analogues, and monoclonal antibodies against Stx (Bitzan 2009; Orth et al. 2008; Tzipori et al. 2004). Prevention of EHEC O157 infection is the best approach to avoid HUS; recommendations to minimize zoonotic risks associated with animals in public settings are available from the National Association of State Public Health Veterinarians (Ahn et al. 2009; NASPHV et al. 2009). Hand washing is the most important step for reducing the risk of EHEC O157 and non-O157 transmission (NASPHV et al. 2009; Weese 2010, in this issue, makes the same point about methicillin-resistant *Staphylococcus aureus*).

The investigation of the 2006 nationwide outbreak of EHEC O157 in humans, linked to consumption of bagged spinach, demonstrated that the strain was isolated from feral swine, domestic cattle, surface water, sediment, and soil. It thus clearly illustrated the relevance of the One Health concept (Jay et al. 2007), a strategy to better understand and address the contemporary health issues created by the convergence of human, animal, and environmental domains (King et al. 2008).

We propose a combination of interventions for EHEC prevention and control that can address the pathways detailed in Figure 1. For example, control of zoonotic EHEC on farms should primarily target the main source of the organism, the animal reservoir (Fairbrother and Nadeau 2006); methods are available to reduce the risk of EHEC disease in humans at the level of the farm, transport, processing unit, distributor, and retailer/preparer/consumer (Khanna et al. 2008). Preslaughter interventions to reduce the shedding of EHEC O157 in the feces of weaned domestic ruminants consist of probiotics, vaccination, antimicrobials, sodium chlorate, bacteriophages, and other feed additives (Sargeant et al. 2007). Vaccine strategies can decrease the level of EHEC O157 shedding for the purpose of reducing zoonotic risk (Potter et al. 2004).

A coordinated multidisciplinary effort toward understanding and integrating the epidemiology, pathogenesis, and pathophysiology of EHEC will facilitate the development of novel strategies to prevent, control, and treat zoonotic EHEC infection and disease.

Conclusions

EHEC O157 is an important food- and waterborne pathogen of humans that colonizes and is shed in the feces of many animal species. Non-O157 EHEC strains encompass many serotypes that are also prevalent in the animal reservoir. 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. Cattle (and probably other animal species) fit all definitions of a reservoir host, but the instability of cattle colonization coupled with the evidence of stable environmental contamination of EHEC O157 suggest that this zoonotic disease is not associated with simple transmission from a reservoir host, but instead is involved with a complex environmental-host ecology that directly affects the likelihood of EHEC O157 zoonotic transmission.

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