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Reviews

Escherichia coli O157:H7: Animal Reservoir and Sources of Human Infection

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Abstract

This review surveys the literature on carriage and transmission of enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 in the context of virulence factors and sampling/culture technique. EHEC of the O157:H7 serotype are worldwide zoonotic pathogens responsible for the majority of severe cases of human EHEC disease. EHEC O157:H7 strains are carried primarily by healthy cattle and other ruminants, but most of the bovine strains are not transmitted to people, and do not exhibit virulence factors associated with human disease. Prevalence of EHEC O157:H7 is probably underestimated. Carriage of EHEC O157:H7 by individual animals is typically short-lived, but pen and farm prevalence of specific isolates may extend for months or years and some carriers, designated as supershedders, may harbor high intestinal numbers of the pathogen for extended periods. The prevalence of EHEC O157:H7 in cattle peaks in the summer and is higher in postweaned calves and heifers than in younger and older animals. Virulent strains of EHEC O157:H7 are rarely harbored by pigs or chickens, but are found in turkeys. The bacteria rarely occur in wildlife with the exception of deer and are only sporadically carried by domestic animals and synanthropic rodents and birds. EHEC O157:H7 occur in amphibian, fish, and invertebrate carriers, and can colonize plant surfaces and tissues via attachment mechanisms different from those mediating intestinal attachment. Strains of EHEC O157:H7 exhibit high genetic variability but typically a small number of genetic types predominate in groups of cattle and a farm environment. Transmission to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with manure, animals, or infected people.

Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) cause hemorrhagic colitis and are often associated with devastating or life-threatening systemic manifestations. The most severe sequelae, the hemolytic uremic syndrome (HUS), results from Shiga toxins (Stxs) produced by the bacteria in the intestine and act systemically on sensitive cells in the kidneys, brain, and other organs (Gyles, 2007). Although most EHEC strains produce Stxs, EHEC O157:H7 are especially virulent and are responsible for the majority of HUS cases of bacterial etiology worldwide (Gyles, 2007; Serna and Boedeker, 2008). In this review, findings on transmission of EHEC O157:H7 within the animal reservoir and the farm environment are presented in context of virulence characteristics and the culture techniques used, to complement the reviews that typically rarely include the methodological details and do not take into account virulence of the isolates (Hussein and Bollinger, 2005; Muniesa *et al.*, 2006). It is well documented that the *E. coli* strains of the O157:H7 serotype differ widely in their ability to cause human disease, colonize animal carriers, and survive in the environment (Dorn and Angrick, 1991; Roldgaard *et al.*, 2004; Marouani-Gadri *et al.*, 2009b; Vanaja *et al.*, 2009), and it is likely that these characteristics are influenced by virulence factors. Also, the findings on EHEC prevalence should not be generalized without regard to methodology, since probability of detection of *E. coli* O157:H7 is highly dependent upon the specific approaches to sampling and culturing. To provide a complete view of EHEC circulation, the review includes data on carriage of EC O157:H7 by animals whose role in transmission of the pathogen remains undetermined. Some related topics were recently reviewed elsewhere and are not included here: decontamination and sterilization of carcasses (LeJeune and Wetzel, 2007); decontamination of produce and suppression of the pathogen by food additives (Erickson and Doyle, 2007); the impact of diet on EHEC O157 carriage by cattle (Callaway *et al.*, 2009); and preslaughter measures aiming to reduce the pathogen load in cattle via herd management, vaccination, probiotics, and bacteriophages (LeJeune and Wetzel, 2007).

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EHEC and Human Disease

HUS and other devastating manifestations of EHEC infection are caused by Stxs, extremely potent cytotoxins that enter host cells expressing toxin receptors and block protein synthesis by irreversibly damaging ribosomal RNA (Endo *et al.*, 1988). EHEC are a subgroup of Stx-producing *E. coli* (STEC) that together comprise hundreds of O:H serotypes and are commonly carried by healthy wild and domesticated ruminant animals (Beutin *et al.*, 1993; Cerqueira *et al.*, 1999; Kaddu-Mulindw *et al.*, 2001). Ruminants are not sensitive to Stxs due to an absence of vascular Stx receptors (Pruimboom-Brees *et al.*, 2000), and the widespread carriage of stx genes by *E. coli* colonizing ruminant animals has not been satisfactorily explained; hypotheses include a modulation of immune respone by Stxs (Hoffman *et al.*, 2006) and antiviral activity of STEC (Ferens *et al.*, 2006). Non-EHEC STEC can be pathogenic to humans with the disease severity highly dependent on serotype and a combination of virulence factors (reviewed in Gyles, 2007; Hussein, 2007), but the *Stx* genes are not necessarily associated with morbidity, and STEC may be carried asymptomatically by humans (Koch *et al.*, 2001; Stephan and Schumacher, 2001; Friedrich *et al.*, 2003; Jenkins *et al.*, 2003). Non-O157 STEC were found in 5.6% (90/1602) of healthy workers at a slaughter company in Korea (Hong *et al.*, 2009) and O157 STEC in 1.1% (4/350) of farm workers in Italy (Silvestro *et al.*, 2004).

While the attention devoted to EHEC O157:H7 is justified by the pathogenicity, low infectious dose, and ability of the bacteria to survive in extra-intestinal environments, a number of non-O157:H7 EHEC cause severe human disease and are often implicated in HUS, and their animal reservoirs and modes of transmission are not well understood (Karch *et al.*, 2005). Almost half (47%) of 424 HUS isolates collected in Germany from 1996 to 2003 were of a canonical O157:H7 serotype, 17% were sorbitol-fermenting O157:NM, and the rest were O26:H11/NM, O145:H25/H28/NM, O111:H8/NM, O103:H2, and others (Karch *et al.*, 2005). Non-O157:H7 EHEC lack the biochemical characteristics differentiating them from nonpathogenic *E. coli*, and thus present a special detection challenge and are perhaps insufficiently investigated.

Identification and Detection of EHEC O157:H7

Almost all EHEC O157 display delayed (negative) fermentation of D-sorbitol with >99% of the sorbitol-negative H7 strains of the O157 serotype (al-Saigh *et al.*, 2004) β-glucuronidase deficient and resistant to several wide-spectrum antibiotics and antimicrobial agents (Ratnam *et al.*, 1988). The bacteria are commonly identified by plating on sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC) (Chapman *et al.*, 1991; Zadik *et al.*, 1993) and confirmed serologically and biochemically as pale colonies positive for O157 and H7. CT-SMAC may be supplemented with 4-methylumbelliferyl-beta-D-glucuronide that is converted by most non-O157:H7 *E. coli* to UV-fluorescing 4-methylumbelliferone (Fujisawa *et al.*, 2000), or with light-visible 8-hydroxyquinoline-beta-D-glucuronide (Reinders *et al.*, 2000). On this medium, EHEC O157:H7 appear pale and nonfluorescent under UV light. Selective plating will occasionally generate false-positives and false-negatives because rare O157:H7 isolates may be sorbitol-fermenting and glucuronidase positive (Sanchez *et al.*, 2009b). Moreover, non-O157 *E. coli* may be unable to ferment sorbitol, whereas sorbitol-negative isolates obtained by selective plating may be able to process the sugar. Among 139 sorbitol-negative isolates of 38 *E. coli* serogroups from food and cattle feces, only 14.6% were O157; however, 39.2% of 139 fermented sorbitol in broth, and exclusion of these increased the percentage of O157 serotype to over 24% (Manna *et al.*, 2009). Serotyping may generate false-negatives since H7 and O157 antigens may not be expressed; out of 100 meat isolates, 42 were H7-negative by latex agglutination but H7 positive by polymerase chain reaction (Narang *et al.*, 2009). An O157-negative rough phenotype can be identified by phage testing (Rozand and Feng, 2009; Rump *et al.*, 2009). In some parts of the world, especially in Europe, sorbitol-fermenting nonmotile isolates of O157:H- emerged, characterized by a higher incidence of progression to HUS than canonical O157:H7 (Buvens *et al.*, 2009; Orth *et al.*, 2009).

Direct plating on CT-SMAC can enumerate viable EHEC O157 at densities >100 colony forming units (CFU)/g sample but may fail to detect highly stressed bacteria unable to survive in a selective medium (Brashears *et al.*, 2001). The detection limit can be lowered by enrichment, that is, incubation in broth before plating and/or concentration by immunomagnetic separation (IMS) with anti-O157 beads (Chapman *et al.*, 1994), but recovery is highly dependent on technique and still likely to underestimate a true prevalence of *E. coli* O157:H7 (Vidovic *et al.*, 2007). The probability of detection can be greatly increased by nonselective enrichment in broth (e.g., buffered peptone water, or McConkey broth) without antimicrobials other than novobiocin as fully viable bacteria of some strains may not grow in selective media; however, a disadvantage of nonselective medium is a possibility of competitive exclusion of O157:H7 strains by other strains of *E. coli*, better able to grow in nonrestrictive conditions. Thus, fresh fecal samples from 43 cattle farms yielded 89 and 127 isolates with selective and nonselective enrichment, respectively, and the latter yielded more isolates associated with severe disease, although a few strains were isolated with selective enrichment only (Foster *et al.*, 2003). Low prevalence or EHEC O157 may thus reflect scarcity of the bacteria, but can also result from subjecting samples to exacting storage and culture conditions. For example, a year-long survey of two feedlots in Alberta yielded surprisingly few (namely, 2) isolates of EHEC O157:H7 by direct plating swabs transported in a chilled selective medium onto CT-SMAC (Van Donkersgoed *et al.*, 2005). A more recent prevalence survey of 21 Alberta feedlots employed nonselective overnight enrichment culture at 42°C followed by IMS, and revealed the presence of *E. coli* O157 in up to 15% of cattle and 57% of pens (Van Donkersgoed *et al.*, 2009). Since the results of studies on EHEC O157:H7 prevalence are critically dependent on processing of samples and identification procedures, the methodological details are specified in this review where relevant.

EHEC isolates are commonly matched by patterns generated by pulsed-field gel electrophoresis (PFGE) of genomic DNA digests. The resulting multitude of genetic patterns may be misleading, as shown by the results of oral challenge of cattle with a single strain of the EHEC O157 that yielded 12 different PFGE profiles resulting from five chromosomal deletions (Yoshii *et al.*, 2009). In general, genetic analysis of EHEC O157:H7 relatedness is hampered by a high degree of variation that may reflect widespread genetic drift of unclear practical significance. For example, highly related lineages

and subpopulations of the pathogen exhibit varied expression of genes of the locus of enterocyte effacement due to genomic instability of a prophage-dense region adjacent to the *pch* sequences (Yang *et al.*, 2009). Also, nonparental PFGE patterns of <89% similarity to cognate strain were observed among randomly selected progeny of a single colony of EC O157:NM (Bielaszewska *et al.*, 2006).

Our current understanding of the ecology of *E. coli* O157:H7 (O157) comes from numerous studies that span several decades and used different sample/culture technique combinations with different sensitivities and criteria to distinguish the bacteria. To fully convey the information, we differentiate specific categories of *E. coli*, defined by serotypic and metabolic characteristics, and virulence factors (Table 1). The term "clinical isolate" when used in reference to the bacteria cultured from animal or environmental samples is used to denote a genetic identity (usually determined by PFGE patterns) between such isolate and the bacteria cultured from a human patient. Included are some relevant surveys for STEC, and unless otherwise indicated, studies were within the United States.

Animal Reservoir of EHEC

EHEC O157:H7 prevalence paradox

Results of sampling for the presence of EHEC O157:H7 are paradoxical: the bacteria occur worldwide, but in many prevalence studies are found infrequently. The reasons for this discrepancy are complex, and may reflect either scarcity of the bacteria in excreta and environment resulting from the sporadic nature of EHEC O157 carriage and low numbers of the bacteria residing in colonized animals, or insufficiently sensitive sampling and culturing approaches. For example, nonenrichment testing of 425 calves in Argentina yielded only two isolates of EC O157:H7 (Chinen *et al.*, 2003), whereas another study of preslaughter cattle using enrichment found EHEC O157:H7 in 4.1% of fecal samples, a result congruent with a high incidence of HUS in the country (Masana *et al.*, 2010). In Norway, only <0.1% of cattle, sheep, and pigs from >800 farms carried EC O157:H7 detected by selective enrichment (Johnsen *et al.*, 2001), but 4.6% (23/504) of cattle imported to the country tested positive with nonselective enrichment (Vold *et al.*, 2001). Farm prevalence of EHEC O157 was 18% (88/481) in Scotland in a study employing IMS and a rigorous sampling pattern) (Chase-Topping *et al.*, 2007), and the highest prevalence was noted in Japan, where up to a third of animals were positive for the pathogen on 3/4 farms; in this study, large fecal samples (10 g) were subjected to 18 h nonselective enrichment culture at 42°C (Ezawa *et al.*, 2004a). However, some studies yielded very low prevalence data despite using enrichment and IMS. A two-country study found EC O157 in 4/50 farms in Ohio (5/750 animals), but in 0/50 farms ($n = 680$) in Norway (LeJeune *et al.*, 2006a); in Greece, only one goat isolate of EC O157:H7 was found at 25 goat, sheep, and cattle farms (Dontorou *et al.*, 2004); in Australia, 1/505 dairy cows from >200 farms harbored two EC O157 isolates, but the samples were analyzed after prolonged refrigeration (Hallaran and Sumner, 2001); in Taiwan, EC O157:H7 were found in 4/3062 cattle in 2/78 herds (Lin *et al.*, 2001); in France, a representative survey of abattoir cattle did not find STEC O157, although 18.1% fecal samples were stx^+ by polymerase chain reaction, and STEC were found in 67/154; (Rogerie *et al.*, 2001). A nationwide survey in Sweden found EHEC O157 in 1.2% (37/3071) cattle from >2000 farms (Albihn *et al.*, 2003). Among Scandinavian countries, an exceptionally high STEC O157 prevalence was noted using nonselective enrichment and IMS in Denmark, in 10/60 farms, and 21% (88/412) of tested animals (Nielsen *et al.*, 2002). Low prevalence results usually reflect a reduced probability of detecting EHEC O157 due to one or more aspects of methodology, including single sampling visits, small numbers of tested animals/farm, absence of comprehensive farm surveys, and selective or no enrichment applied to stored samples. On the other hand, cattle farms in the negative countries were typically much smaller operations compared to positive countries. Correlation between herd/farm size and EHEC prevalence was not studied specifically, but surveys of large cattle operations usually detect the organism. Thus, a 1-visit, selective enrichment culture fecal sampling of 15 animals/pen in 73 feedlots in the United States (>7500 head) found EHEC O157 in 10.2% ($n = 10{,}622$) of animals in 70/73 lots, 52% of pens, and up to 14/15 animals sampled (Sargeant *et al.*, 2003); sampling of other large feedlots found average prevalence of 1%–2% (Berg *et al.*, 2004) detecting the organism at all visits.

Prevalence studies of EC O157:H7 are associated with two major caveats: first, the bacteria may be harbored extraintestinally with little correlation to fecal shedding (Boqvist *et al.*, 2009), and second, intestinal carriage is usually intermittent or short-term (Shere *et al.*, 1998). The bacteria were much more prevalent among all age groups on the ears than in

Table 1. The Serotypic, Biochemical, and Virulence Characteristics of the Categories of *Escherichia coli* Differentiated in the Review

	Category				
	EC O157	*EC O157:H7*	*EHEC O157*	*EHEC O157:H7*	*STEC* E. coli
O157 antigen	Yes	Yes	Yes	Yes	?
H7 antigen	?	Yes	?	Yes	?
Growth on selective media[a]	Yes	Yes	?	Yes	?
stx	?	?	Yes	Yes	Yes
eae, *tir*, *hly*, *ehx*, or 92-Kb plasmid	?	?	Yes[b]	Yes[b]	?

[a]Standard selective media used to isolate typical *E. coli* O157:H7.
[b]One or more of the virulence factors detected in an isolate.
? = Undetermined or not reported.
EHEC, enterohemorrhagic *Escherichia coli*; STEC, Shiga toxin–producing *E. coli.*

fecal samples in a survey in Sweden (Boqvist *et al.*, 2009); hide prevalence of EC O157:H7 was as high as 92% on belly swipes of feedlot cattle (Kalchayanand *et al.*, 2009). STEC O157 were isolated from the oral cavity or hides of 130/139 feedlot cattle, including 50 cattle that were fecal-negative (Keen and Elder, 2002). Possibly, extra-intestinal prevalence is reduced by high insolation; in a survey of 13 dairy herds in Louisiana, STEC O157:H7 were found in 0%, 0.7%, and 25.2% of samples from oral cavity, skin, and feces, respectively (Dunn *et al.*, 2004b), whereas hide prevalence was 10 times higher than fecal prevalence (60.6% vs. 5.9%) in a study performed at three midwestern beef plants found (Barkocy-Gallagher *et al.*, 2003).

EHEC O157:H7 in domesticated ruminant animals

The major animal carriers are healthy domesticated ruminants, primarily cattle (Gyles, 2007) and, to a lesser extent, sheep and possibly goats (La Ragione *et al.*, 2009). Genetic analysis of 31 strains of EHEC O157:H7 of different phage types revealed 4,084 open reading frames in all strains and 1,751 that were variably present or absent, indicating that the strains form at least two distinct lineages, with 11 distinct genetic regions present in $\geq$80% of strains of lineage I and absent from $\geq$92% of lineage II; strains of lineage I are more often associated with human disease than the strains of lineage II that typically occur in cattle and other ruminants (Zhang *et al.*, 2007). A number of studies performed in different countries compared PFGE profiles and virulence factors of bovine and human isolates and concluded that the majority of bovine isolates of EHEC O157 do not occur, or are underrepresented, in people (Chapman *et al.*, 2001; Galland *et al.*, 2001; Lahti *et al.*, 2001b; Cho *et al.*, 2006). For example, a comparison of 63 bovine isolates and 86 human isolates in Denmark revealed that human isolates associated with severe disease constituted a minor fraction of the bovine strains, and that little overlap existed between the two sets in a number of phage types (Roldgaard *et al.*, 2004); also, analysis of phage insertion points revealed greater genetic diversity of bovine isolates compared to human ones (Besser *et al.*, 2007). Although bovine strains of EHEC O157 share numerous virulence factors with human strains, including *stxs* and *eae*, and may be considered potential human pathogens (Heuvelink *et al.*, 1998), bovine isolates generally harbor fewer virulence factors while exhibiting greater tolerance for adverse conditions (Vanaja *et al.*, 2009). This contention is supported by the finding that the isolates from healthy cattle were less virulent in gnotobiotic pigs than human clinical isolates (Baker *et al.*, 2007). Non-O157 STEC isolated from healthy cattle also harbor relatively few virulence factors; 77% of 222 STEC isolates from slaughtered cattle in France presented only one of *stx*, *eae*, or *ehx* genes, and only 3/222 presented these 3 virulence genes together (Rogerie *et al.*, 2001).

Although the majority of bovine strains of EHEC O157 are not transmitted to humans, there is little doubt that cattle are the main source of human EHEC infections (Dorn and Angrick, 1991; Blanco *et al.*, 1996; Heuvelink *et al.*, 1996; Besser *et al.*, 1997; Toth *et al.*, 2009). Outbreaks have been linked to consumption of undercooked beef or unpasteurized dairy products (Anonymous, 1993, 1997, 2002); in 350 U.S. outbreaks between 1982 and 2002, ground beef was the vehicle in 75/183 foodborne outbreaks (Rangel *et al.*, 2005). Isolates from beef products and from cattle were matched to the outbreak isolates (Willshaw *et al.*, 1994; Meng *et al.*, 1995; Anonymous, 2002), and to isolates from clinical cases (Paros *et al.*, 1993; Tuttle *et al.*, 1999; Proctor *et al.*, 2002; Hussein, 2007) and specifically to HUS (Heuvelink *et al.*, 1998). Carriage of clinical EC O157:H7 isolates by cattle may simply reflect a high probability of pathogen transmission from cattle to people as a consequence of the predominance of beef and dairy cattle among domesticated animals, and the voluminous output of bovine manure. EHEC is a special case of zoonotic STEC infections, and there is a clear association of cattle density and the occurrence of all STEC-related gastroenteritis in humans; in Ontario, there was a correlation with cattle density, but not with presence of sheep or goats (Valcour *et al.*, 2002), and in Germany the risk increased by 68% per additional 100 cattle/km^2 (Frank *et al.*, 2008). However, studies that measured intestinal prevalence of EHEC O157 in cattle and sheep at slaughter consistently show higher prevalence in cattle. In the United Kingdom, the bacteria were found in 4.7% of cattle and 1.7% of sheep (Paiba *et al.*, 2002), in 15.7% of cattle versus 2.2% of sheep (Chapman *et al.*, 1997), and in 4.7% of cattle and 0.7% of sheep (Milnes *et al.*, 2008); in Holland, abattoir survey revealed EC O157 in 10.6% cattle and 4.0% sheep (Heuvelink *et al.*, 1998). This relationship was also true when overall prevalence of the pathogen was very low; in Norway, EHEC O157:H7 was found in 0.19% of cattle (3/1541) and 0/665 sheep (Johnsen *et al.*, 2001). In seasons of low prevalence when EC O157:H7 are typically rarely found in sheep and other farm animals, cattle are most likely to carry the bacteria (Chapman *et al.*, 1997; Hancock *et al.*, 1997; Kudva *et al.*, 1997).

Typical EC O157:H7 are prevalent in bovines other than *Bos taurus*. Fecal prevalence of the bacteria in farmed bison was greater than in cattle and ranged from 17% to 83% across days (Reinstein *et al.*, 2007); high prevalence was noted in water buffalo in Bangladesh (Islam *et al.*, 2008) and 11 EC O157:H7 strains were isolated from 300 healthy water buffalo in Turkey (Seker *et al.*, 2009). Small ruminants can be a significant source of human EHEC infection (La Ragione *et al.*, 2009), and EHEC O157:H7 were identified in sheep (Chapman *et al.*, 1997; Kudva *et al.*, 1997; Heuvelink *et al.*, 1998; Lenahan *et al.*, 2007) and matched to clinical strains (Chapman *et al.*, 2000b). In Italy, most adult sheep tested in a slaughterhouse were positive for virulent O157 STEC (Franco *et al.*, 2009); 8/13 STEC strains isolated from ovine dairy products in Mexico were O157 (Caro *et al.*, 2007); EC O157:H7 were isolated from pelts and carcasses in a lamb-processing plants (Kalchayanand *et al.*, 2007). EHEC O157:H7 were found in 8% of beef, and 2% of lamb and goat meat samples in Ethiopia (Hiko *et al.*, 2008), and were isolated from goats in Spain (Orden *et al.*, 2008). STEC O157 was more often found in goats (9%) than in cows (7%) in Bangladesh (Islam *et al.*, 2008). Some ruminants appear remarkably free of EHEC O157; a prevalence survey of slaughtered animals, using enrichment and IMS, failed to detect the bacteria in semidomesticated free-range reindeer ($n = 1387$) (Lahti *et al.*, 2001a), and none were found in 192 llamas at 22 farms in California (Rulofson *et al.*, 2001). No STEC nor anti-Stx antibodies were detected in fecal and serum samples from 400 camels in five east African countries (El-Sayed *et al.*, 2008).

Carriage of EHEC O157:H7 by nonruminant farm animals

Pigs routinely carry STEC, and can be colonized by EC O157; inoculated pigs harbored EC O157:H7 for up to 2

months (Booher *et al.*, 2002), and passed the bacteria to naïve penmates by direct and indirect contact (Cornick and Helgerson, 2004; Cornick and Vukhac, 2008). However, EHEC O157:H7 can be highly pathogenic in swine and are rarely found in these animals; a 2-year multifarm survey in the United States did not yield any isolates (Richards *et al.*, 2006) and in another 13-state survey no H7 or *hly933* were detected among 106 isolates of EC O157 from healthy pigs (Feder *et al.*, 2007); in Canada EC O157 were found in 3% of samples from 10 farms, but no EC O157:H7 (Farzan *et al.*, 2009). In the United Kingdom, an abattoir survey yielded EHEC O157 from 15.7% of cattle and 2.2% of sheep, but only from 0.4% of pigs; while almost all of 752 cattle isolates and all 22 sheep isolates were *stx*+ with a 92 Kb plasmid, these virulence factors were absent in swine isolates (Chapman *et al.*, 1997), that are typically deficient in major virulence factors. Only 8.8% (283/3,218) of fecal *E. coli* isolates from piglets with edema disease in Germany were *stx*+, and all 283 lacked genes for intimin, EHEC hemolysin, and subtilase cytotoxin (Barth *et al.*, 2007). In France, all 48 STEC swine isolates were non-O157:H7 and *stx2*+ *eae*$^-$ *ehx*$^-$ (Bouvet *et al.*, 2002) and in Italy 1/150 pigs carried five strains of EC O157, 4/5 *stx* and *eae* negative (Bonardi *et al.*, 2003). The prevalence of EC O157:H7 in a Swedish survey of five swine abattoirs was <0.01% (2/2446) and although a swine herd on a farm with 8–12% of cattle positive for EC O157:H7 was positive for 11 months, the bacteria did not persist in pigs transferred to a cattle-free farm (Eriksson *et al.*, 2003). In Switzerland, STEC were found in 22% of pigs, but EC O157 only in 7.5% and 30/31 isolates were non-H7 sorbitol positive (Kaufmann *et al.*, 2006). None of the 23 STEC strains and 76 isolates from swine/pork samples (meat, feces, or carcasses) in Germany was associated with human disease (von Muffling *et al.*, 2007). In contrast with the prevailing results, a 2-year U.S. study of four swine farms found EC O157:H7 in 8.9% of rectal swabs (Doane *et al.*, 2007), but the study did not report any morbidity, and the bacteria could be nonvirulent. High prevalence of EC O157 in swine feces was noted in South Africa (9.5%, 23/76), but none of 20 isolates harbored *stx* genes and only 5/20 were *eae*+ (Ateba and Bezuidenhout, 2008). Potentially pathogenic EHEC O157:H7 were isolated from 2% of slaughter pigs (6/305) (Feder *et al.*, 2003), and feral swine in California carried an EHEC O157:H7 isolate matched to the strain from a major spinach-mediated outbreak (Jay *et al.*, 2007).

The relative scarcity of EHEC in swine contrasts with more frequent isolation of EHEC O157 from pork. Typical EC O157:H7 were found in 1.5% of pork samples in Canada (Doyle and Schoeni, 1987), 1% of swine carcasses in New Zealand (Wong *et al.*, 2009), and in 1/50 swine meat samples in Greece (Dontorou *et al.*, 2003). However, a literature search revealed only one small outbreak of EHEC O157 infection traced to consumption of pork meat and salami in Italy (Conedera *et al.*, 2007). In contrast to cattle, swine may be an unlikely source of human infection, due to their sensitivity to virulent EHEC, that would result in curing or culling morbid animals, thus limiting transmission of the pathogen.

EHEC O157:H7 is rare in chickens, but frequently isolated from turkeys. Chickens ($n = 1000$) harbored no EC O157 in the United Kingdom (Chapman *et al.*, 1997) or in Holland ($n = 501$, pooled fecal samples), but 1/6 of the EC O157 isolates from turkeys ($n = 459$) was virulent (Heuvelink *et al.*, 1999). Fecal samples from seven species of domestic animals revealed no *stx* genes in chicken feces (Beutin *et al.*, 1993), and surveys of farms with multiple animal types indicate that the prevalence of EC O157 in chickens is low, even if the bacteria are carried by the neighboring cattle. In the United States, EC O157:H7 was found in 3.6% of beef cattle and 7.5% of turkeys but only in 0.9% of chicken (Doane *et al.*, 2007); 1%–6% of bovine fecal samples in each of 12 large cattle farms contained sorbitol-negative EC O157, but only 0.5% (1/200) of pooled samples from poultry was positive (Hancock *et al.*, 1998). In Korea, up to 6.7% of cattle were positive for EC O157 but only one pig (0.3%) and no chickens were positive (Jo *et al.*, 2004). However, a survey of a Norwegian farm found EHEC O157:H7 of the same PFGE type in cattle, sheep, and chickens (Wasteson *et al.*, 2005), and a beef isolate was PFGE-matched to a chicken isolate in Argentina (Chinen *et al.*, 2009). Typical EC O157:H7 were found in 1.5% (4/263) of the poultry samples from retail outlets in Wisconsin (Doyle and Schoeni, 1987). Interestingly, chickens inoculated with EC O157:H7 became colonized and shed for up to 11 months (Schoeni and Doyle, 1994), indicating a possibility that poor transmission, rather than host incompatibility, creates a barrier to infection.

Other carriers of EHEC O157:H7 in the farm environment

EC O157 are occasionally found in domestic animals, synanthropic rodents, and birds. Fecal samples from cats, rodents, or birds were negative on a dairy farm positive for EHEC O157:H7 (Rahn *et al.*, 1997), but another 3-visit survey found sorbitol-negative EC O157 in 1%–6% of bovine fecal samples and in 1/90 from horses, 2/65 from dogs, and none from cats (0/33), or mice/rats (0/300) (Hancock *et al.*, 1998). In Norway, EHEC O157 were carried by 20% of feedlot cattle and 4/10 rats, but droppings of pigeons and house sparrows were negative (Cizek *et al.*, 1999). Interestingly, inoculated domestic pigeons shed EC O157 for 2–3 weeks, whereas shedding by rats was 2 or 10 days, depending on dose, although the bacteria survived >36 weeks in their feces (Cizek *et al.*, 2000). Pigeons rarely carry EC O157:H7, but a strain PFGE-identical to a cattle isolate was found in 1/99 samples at a dairy farm (Shere *et al.*, 1998). In circumstances facilitating transmission of EHEC (crowding, intense movement) the bacteria can be harbored by nonhabitual carriers. Thus, PFGE-indistinguishable EHEC O157 with the same virulence factors were isolated from pigs, sheep, and goats of several breeds, other animals, and compost at an inner-city exposition farm in the United Kingdom (Chapman *et al.*, 2000a). STEC O157 were found in 19/31 public displays of animals in the United Kingdom and risk was correlated with the number of animals and the presence of young cattle and adult pigs (Pritchard *et al.*, 2009).

Wildlife

Wildlife does not constitute a significant source of EHEC O157; rather, sporadic isolation of the bacteria likely reflects environment-mediated transmission from humans and animal reservoirs. EHEC O157 were found only sporadically in wildlife other than deer (Rice *et al.*, 2003). Only 1/521 scat samples contained the bacteria, whereas 1% of range cattle feces were positive (Renter *et al.*, 2003). Carriage was noted in white-tailed deer (*Odocoileus virginianus*) in the United States, where 2.4% of fecal samples collected on cattle pastures at a

period of low prevalence in cattle contained EHEC O157 (Sargeant *et al.*, 1999), and a deer survey revealed EC H7 only in samples from white-tailed deer (Renter *et al.*, 2001). Extensive analysis of feces of a number of wildlife ruminant and bird species ($n = 2228$) found five isolates of EC O157:H7 in 0.8% of samples from white-tailed deer and one in pooled bird feces (0.3%) (Rice *et al.*, 2003). In the southeastern United States, 4.3% of cattle were positive for EC O157:H7 and rectal feces of 3/469 deer killed in the vicinity of cattle pastures yielded isolates, but none matched to cattle isolates and no deer were positive (0/140) on the same site the next year (Fischer *et al.*, 2001). EC O157 were found in 1.25% of cattle and 2.22% of sheep but not in white-tailed deer cograzing with livestock (Branham *et al.*, 2005); in other studies, prevalence was <1% in a captive deer herd (Rice *et al.*, 2003; Dunn *et al.*, 2004a). In Spain, EHEC O157:H7 from deer feces was phagetyped to a human isolate (Sanchez *et al.*, 2009b) and EC O157:H7 were found in 1.5% (3/206) of red deer (*Cervus elaphus*) (Garcia-Sanchez *et al.*, 2007). Inoculated deer shed EC O157:H7 for >26 days and passed the strain to naive penmates (Fischer *et al.*, 2001). Fermented deer sausage was a vehicle for O157:H7 transmission in Missouri (Ahn *et al.*, 2009). High prevalence of 3.3% (7/212) of EHEC O157:H7 was found in wild boars in Spain with one isolate PFGE-identical to a clinical isolate (Sanchez *et al.*, 2009a).

A clinical isolate of EHEC O157 was found in rook feces (Ejidokun *et al.*, 2006) and a 3-year composite of wild bird feces in Scotland yielded a strain with virulence factors and a phage type associated with severe human disease (Foster *et al.*, 2006). In the United Kingdom, STEC O157 were found in 0.9% of fresh fecal samples from wild birds (mostly gulls) at a landfill and 2.9% at an intertidal zone (Wallace *et al.*, 1997); notably, seafood can contain EC O157 (Sehgal *et al.*, 2008).

Nonmammalian carriers

EHEC O157 can be carried by amphibians and fish, as well as invertebrates, such as insects and mollusks. Fish caught near a site of slaughter of zebu (*Bos indicus*) in central Africa harbored EC O157:H7 (Tuyet *et al.*, 2006) and American bullfrogs (*Rana catesbeiana*) are suitable hosts for EC O157:H7 (Gray *et al.*, 2007). EC O157 survived on a slug for 14 days; viable bacteria were shed in feces and persisted there for up to 3 weeks (Sproston *et al.*, 2006). EC O157:H7, identical by PFGE pattern and virulence genes to human isolates, were found in dung beetles (Xu *et al.*, 2003). EHEC O157 were isolated from house flies at cattle (Hancock *et al.*, 1998, Iwasa *et al.*, 1999) and turkey farms (Szalanski *et al.*, 2004). Flies fed four EHEC strains transmitted the bacteria to all 11 exposed calves; all 8 calves singly exposed to flies fed nalidixic acid-resistant EC O157:H7 shed high numbers of the bacteria for 11 days (Ahmad *et al.*, 2007). EHEC can multiply in fly mouthparts and accumulate in crops, resulting in efficient dispersal for >3 days after feeding (Kobayashi *et al.*, 1999). Houseflies carried GFP-tagged O157:H7 from manure to spinach plants (Talley *et al.*, 2009). Black dump flies associated with poultry carried EC O157:H7 in turkey facilities in Arkansas (Szalanski *et al.*, 2004). Flies developing in bovine manure were shown to carry EHEC O157:H7 and deposit the bacteria on leafy vegetables (Talley *et al.*, 2009). The extent of transmission of EHEC O157:H7 mediated by animal vectors is not clear, but it may account for transfer of the bacteria without direct contact between naïve and colonized carriers. Insects may be especially important, as shown by the results of U.S. surveys of 29 county and three state agricultural fairs for EC O157:H7, that found the bacteria in 11.4% of cattle ($n = 1407$), 1.2% of swine ($n = 1102$), 3.6% of sheep and goats ($n = 364$), and in 5.2% of 154 fly pools; at some fairs, isolates from cattle, swine, and flies shared indistinguishable subtypes (Keen *et al.*, 2006).

Transmission of EHEC O157:H7

Extra intestinal survival of EC O157

A conceptual distinction should be made between mere retention of viability by the bacteria in nonsupportive or hostile environments that may provide a conduit for transmission, versus the ability of the bacteria to multiply and persist for extended periods in habitats that may form environmental reservoirs. EHEC survive well and multiply in raw manure and bedding, but do not survive proper composting or waste treatment. EC O157:H7 inoculated in moist bovine manure initially declined, but subsequently grew to a maximum in 5 days at 37°C (Delazari *et al.*, 1998); EHEC O157:H7 human strain 932 survived best at 22° and 37°C for up to 70 days (Wang *et al.*, 1996), and EC O157 survived up to 18 weeks at 15°C, and 14 weeks at 25°C; the best survival occurred with high concentrations of inocula (Fukushima *et al.*, 1999). In a 3-month cattle study, EC O157:H7 were frequently cultured from cedar chip bedding that supported growth of the bacteria when moistened with bovine urine, and samples from the ambient environment were positive when the animals did not carry the bacteria (Davis *et al.*, 2005). Survival in bovine manure may be strongly influenced by diet; manure from cattle fed bromegrass hay supported a dosed streptomycin-resistant EC O157:H7 for 28 days when animals were fed hay for <1 month, but the bacteria survived for >120 days when feed was consistent for >1 month; the impact of diet was attributed to phenolic acids common in forage plants and the fact that ruminants fully adapted to hay diet metabolize the antimicrobial phenolic acids in the rumen. The bacteria also survived up to 45 days longer in manure from corn silage-fed compared to hay-fed cattle (Wells *et al.*, 2005). However, EC O157:H7 survival at −10°C was only marginally better in manure from corn fed steers and no difference between corn and barley was found at 4° and 22°C; the bacteria survived better at 22°C for >2 months (Bach *et al.*, 2005). Decline of antibiotic resistant EC O157:H7 was similar in bovine feces and soil, but the bacteria were detectable in soil for up to 99 days (Bolton *et al.*, 1999). In ovine manure the bacteria survived best at or below 23°C for up to 21 months, yielding from 10^2 to 10^6 CFU/g and genetically similar isolates 12 months apart, but ovine manure aerated by periodic mixing remained culture positive for only 4 months, and survival was >100 days at −20°C, but proper composting killed all EHEC O157:H7 (Kudva *et al.*, 1998). Some discrepancy of EHEC survival time in manure may be related to the origin of the bacterial inoculum; passage of EC O157:H7 through bovine gastro-intestinal tract (GIT) extended manure survival of the bacteria by up to 10 weeks (Scott *et al.*, 2006b).

Treatment of dairy wastewater in an artificial wetland resulted in a 2-log reduction of EC O157:H7 numbers (Ibekwe *et al.*, 2002). Survival was poor in water from waste lagoons independent of circulation and EC O157:H7 failed to establish even after four sequential inoculations (Ravva *et al.*, 2006).

EC O157:H7 survived in raw chicken manure for 6–22 weeks at 4°C, multiplied at 20°C (Himathongkham and Riemann, 1999), and declined at 37°C (Himathongkham *et al.*, 2000); drying and exposing manure to ammonia reduced numbers by 8 logs (Himathongkham and Riemann, 1999). EHEC persisted on wood and galvanized steel for >28 days, but were quickly eliminated by desiccation and heat (Williams *et al.*, 2005); survival of EC O157 was better in straw and on hide than on concrete or metal, and in fecal versus broth inoculum (Small *et al.*, 2003). Ability to survive drying on concrete was compared among 123 isolates of EC O157 obtained from human patients, cattle and sheep, and food samples; isolates from human disease were on average more sensitive to drying over a 24-h period, but some strains of phage types associated with severe disease (PT 4, 21/28, and 32) survived better than strains of other phage types (Avery and Buncic, 2003). However, the ability to withstand antimicrobial interventions did not differ between strains of EC O157:H7 with genetic polymorphism associated with human disease and strains not found in humans (Arthur *et al.*, 2008). The prophage-encoded antiterminator Q gene upstream of *stx2* was associated with resistance of EC O157 to inactivation by high pressure, heat, and UV and gamma radiation (Malone *et al.*, 2007), and EC O157:H7 repaired UV-induced damage within 30 min versus 2 h required by nonpathogenic *E. coli* (Zimmer-Thomas *et al.*, 2007).

Samples of farm water inoculated with EC O157:H7 yielded viable bacteria for >1 month at 15°C (McGee *et al.*, 2002) and infectivity to 10-week old calves was retained after >6 months in simulated water trough sediments (LeJeune *et al.*, 2001). EHEC O157 survived for 8 and 16 days in water samples from two dairy farms (Rice and Johnson, 2000), but EC O157:H7 survived 5–7 weeks in rain/river water and 38 weeks in tap water (Randall *et al.*, 1999).

EC O157:H7 biofilms on stainless chips defeated washing with commonly used concentrations of detergents (Silagyi *et al.*, 2009) and were not completely inactivated by NaClO (Ueda and Kuwabara, 2007); incomplete removal of biofilm may habituate the bacteria to antimicrobials and promote increased acid resistance (Skandamis *et al.*, 2009). Proteins of the autotransporter family are involved in biofilm formation by EHEC O157 (Wells *et al.*, 2009b). Bovine EC O157:H7 isolate grown in meat and produce broths formed similar biofilms on steel and glass, but spinach broth stimulated much greater production of autoinducer 2 (Silagyi *et al.*, 2009). Genetic profiles of 48 EC O157 isolates from animal feces (cattle, goats, sheep, chickens, and pigs) and freshwater biofilms collected downstream of the farm revealed that only cattle isolates clustered with the biofilm isolates and 2/4 of the cattle isolates were within a 0.975 value of Simpson's diversity index, indicating clonal identity (Cooper *et al.*, 2007). The O157 plasmid is required for a hyper-adherence phenotype (Lim *et al.*, 2009). Attachment of EC O157:H7 isolates from a meat-processing plant to polyurethane increased in the presence of *Staphylococcus* spp. and *Bacillus* spp. bacteria (Marouani-Gadri *et al.*, 2009a). Substituting a nonpathogenic derivative of EHEC for the parent may produce misleading results in studies of adherence (Marouani-Gadri *et al.*, 2009b).

The presence of highly genetically similar strains at distant sites, not involved in exchange of animals, suggests that the environment can provide vehicles for effective transmission. Ninety-two EC O157:H7 isolates from 20 dairy farms in Ohio were divided into 50 PFGE subtypes and indistinguishable types were located in eight pairs of farms, 9–50 km apart, in cattle and wild bird feces and bedding (Wetzel and LeJeune, 2006). In Norway, PFGE-identical EC O157:H7 isolates were found on two cattle farms <10 km apart, and on a distant swine farm (>50 km) (Johnsen *et al.*, 2001). A detailed study of 69 isolates of EC O157:H7 from four countries and continents yielded statistically significant but low correlation between geographic distance and genetic similarity (Dice index of 6-enzyme PFGE profiles) showing that increased distance reduced but did not eliminate transmission (Davis *et al.*, 2003a). PFGE-similar EC O157:H7 found in cattle feed and feces suggested feed as a possible vehicle (Davis *et al.*, 2003b).

Long-term survival of EHEC in the environment may be contingent upon the presence of animals carriers. In Holland, EC O157:H7-infected calves transmitted the bacteria to naïve calves present at the same pasture; however, naïve animals introduced to the pasture the next year did not shed the pathogen, indicating that a pasture did not function as a reservoir for the pathogen (Schouten *et al.*, 2009).

EHEC in the farm environment

Farm surveys may reveal large numbers of EHEC strains, but typically a small number of genetic types predominate, and sometimes these strains persist on the farm independently of animal carriers. A 20-pen feedlot EC O157:H7 survey yielded 56 PFGE patterns, but 4 closely related ones comprised ~60% of 230 isolates and were found in all pens throughout the sampling period despite massive turnover of the cattle population (LeJeune *et al.*, 2004). In an 11-month study of range cattle, 235 isolates formed 79 unique PFGE patterns; 54/79 were found once, but 7 most frequent were identified in 124/235 isolates, and PFGE-identical isolates occurred up to 10 months apart (Renter *et al.*, 2003). A 1-year survey of Alberta dairies yielded 65 isolates of EC H7 grouped into 23 PFGE 2-enzyme profiles; 60/65 belonged to three patterns with >90% homology (Stanford *et al.*, 2005). In a long-term study of EC O157:H7 carriage by feedlot cattle, 132 isolates were divided into 32 PFGE types; one type accounted for 53% of all isolates, and showed high adherence to Caco-2 cells (Carlson *et al.*, 2009). Persistent and dominant strains may be those that can easily colonize cattle and survive in the farm environment; the pen prevalence may be continuous for 5 months, and a given strain may persist on a farm for >2 years (Shere *et al.*, 1998). A survey of an empty feedlot yielded EC O157 isolates of different PFGE profiles, but a strain first identified 5 days postcattle entry spread to all pens (Sanderson *et al.*, 2006), whereas 95 EC O157:H7 isolates from four adjacent pens differed by 0–2 bands in PFGE profiles, clustered within 80% similarity, and were shed by over half of 69 cattle within 3 months (Scott *et al.*, 2006a). EHEC O157 can circulate among cattle, extending group prevalence; each of weekly tested 100 steers assigned to 10 pens harbored EC O157:H7 at least once, with >1 positive animal each week, and 1–8 positive animals/pen (Khaitsa *et al.*, 2003). It is likely that EHEC reciprocally circulates between cattle and the environment, but data on direction of transmission are scarce. Water tanks in large cattle feedlots were five times more likely to harbor EHEC O157 if a pen was positive for the bacteria, but the direction of spread was not determined (Sargeant *et al.*, 2003).

Movement of large groups of cattle may transmit EHEC independent of environment and promote strains that are not easily transmitted. A survey of four cattle feedlots (>35,000 head, up to 250/pen) yielded 57 highly genetically diverse isolates of EC O157:H7, rarely found in consecutive samplings, indicating that most isolates originated from incoming cattle and were stably associated with groups of penmates, as 3/5 isolates from one lot were similar, and 2/3 originated from the same pen, when tested 3 weeks apart (Galland *et al.*, 2001). Likewise, 19 EHEC O157 isolates from 1448 cattle brought from 16 farms at separate geographic locations in Finland belonged to 10 different PFGE profiles (Lahti *et al.*, 2001b). Transfer of animals from a farm characterized by a high prevalence of EHEC may reduce carriage; hide and fecal prevalence in feedlot cattle was 18% and 9.5% 2 weeks before transport and 4.5% and 5.5% at slaughter, respectively (Barham *et al.*, 2002).

An experimental system used to study the spread of EHEC by natural circumstance involves "Trojan" carriers, whereby dosed EHEC-positive animals are introduced to negative penmates. Typically, the bacteria are rapidly transmitted. Within 24 h of placement of individual EC O157:H7-shedding animals in six pens, the bacteria were present in environmental samples, within 48 h on hides of 20/30 animals, and from days 3–23 in fecal samples of 15/30 calves (McGee *et al.*, 2004). Calves located in adjacent or across pens started shedding within 8 days of introduction of a positive calf to a room (Shere *et al.*, 2002). However, this experimental model does not guarantee transmission and may result in highly variable and inconsistent carriage in potential recipients (Sheng *et al.*, 2004).

A recent study compared prevalence of EHEC O157:H7 among 180 farms of different types (dairy, beef, mixed, and veal) in Belgium and found higher farm prevalence in dairy farms (61%) than in beef farms (23%), with an intermediate prevalence in mixed farms (44%); pen prevalence was also higher at dairy farms (Cobbaut *et al.*, 2009). Since this is the first comparative study of this kind, which rigorously applied the same sampling and culturing techniques to all surveyed farms, it is not yet possible to conclude what factors may be responsible for the noticed differences. Individual prevalence of EC O157:H7 at slaughter was higher in downer dairy cattle (10/204, or 4.9%) than in healthy cattle (3/201, or 1.5%), indicating a possibility that exhausting physiological stress imposed by milking may play a role. However, in contrast to beef cattle, dairy cattle are moved several times daily between pens and milking parlors, and this may greatly facilitate dissemination and transmission of the bacteria.

Clustered distribution of EHEC O157 in cattle

Most cattle pass EC O157 at <100 CFU/g of feces, that is, close to the lower limit of detection by IMS, and it is likely that studies underestimate the true prevalence (LeJeune *et al.*, 2006b). Moreover, EHEC present in the bovine GIT may not necessarily be excreted with every bowel evacuation and are unevenly distributed in feces; when 120 fresh fecal pats from two feedlots were sampled multiple times, prevalence increased from 8.2% with one sample/pat to a plateau of 20% with five samples/pat (Echeverry *et al.*, 2005). However, rare individuals may harbor high numbers of EC O157:H7 and/or for long duration. A summertime, 9-visit survey of abattoir cattle ($n = 589$) in Scotland found EHEC O157 in rectal feces of 44 cattle, with 27/44 at CFU/g <log 2, 13/44 at 2–4 logs, and 4/44 at 4–6 logs; thus, 1/10 carriers excreted >96% of total EHEC O157 (Omisakin *et al.*, 2003). The concept of "supershedders" posits that some cattle are predisposed to carry exceptionally high numbers of EHEC ($>10^3$–10^4 CFU/g) and are primarily responsible for spreading the pathogen at the farm (Cobbold *et al.*, 2007); the term "supershedder" has not been strictly defined, and is used to denote animals shedding relatively high numbers of the bacteria for a given herd. Among 160 steers randomly assigned to 20 pens in a feedlot, five cattle were identified as persistently EC O157:H7-colonized supershedders; their penmates showed above average prevalence, and yielded isolates similar in PFGE profile, whereas negative animals were five times more likely not to share a pen with a supershedder (Cobbold *et al.*, 2007). Presence of supershedders secreting >200 CFU/g feces was associated with high pen hide prevalence (Arthur *et al.*, 2009). U.K. farms harboring high-shedding cattle exhibited high prevalence of low-level shedding, consistent with a possibility of higher-level transmission (Chase-Topping *et al.*, 2007); thus, 78% of 952 farms were negative for EC O157, but at 2% of farms 90%–100% of fecal pats were positive (Matthews *et al.*, 2006). Weekly survey of an Alberta feedlot from May to January revealed greater fecal and hide prevalence in the spring/summer than in fall/winter; the presence of supershedders increased the probability of fecal and hide carriage by penmates in all seasons (Stephens *et al.*, 2009).

Age-dependent carriage

Preweaning calves rarely carry EHEC O157, whereas the prevalence is much higher in postweaning calves and heifers than in older cattle (Wells *et al.*, 1991; Cray and Moon, 1995; Garber *et al.*, 1995; Zhao *et al.*, 1995; Hancock *et al.*, 1997), and heifers shed at higher CFU/g feces than other cattle carriers (Hancock *et al.*, 1997, Shere *et al.*, 1998); in Japan, overall prevalence was 5%–10%, but 32%–46% among heifers ($n > 400$) (Ezawa *et al.*, 2004b). In Italy, 3.6% of adult cattle but not veal calves (0/437) carried intestinal EHEC O157 at slaughter (Conedera *et al.*, 1997); in another study 16% (58/360) of beef and dairy cows but none of veal calves (0/90) were positive for *stx*+ EC O157 (Bonardi *et al.*, 1999). Three cohorts of veal calves, reared from 1 to 20 weeks on milk replacer, were tested for STEC and O157 weekly; 68% of fecal samples, and 62/62 calves had fecal cultures positive for STEC by Stx ELISA, but only 2/1151 fecal samples from 2/62 calves were positive for virulent EC O157:H7 (Cristancho *et al.*, 2008). EHEC can, however, colonize preweaned calves; animals 13–30 days old at challenge and fed milk replacer shed the inoculated EC O157:H7 strain for up to 58 days, whereas adult cows shed only for up to 2 weeks (Wray *et al.*, 2000). EHEC shedding on eight cattle farms in Denmark was higher among postweaned than nonweaned calves, and it was reduced among calves 1–4 months old if they suckled colostrum or stayed >2 days with their dams after birth (Rugbjerg *et al.*, 2003), whereas calves inoculated with EC H7 and fed milk replacer exhibited somewhat increased shedding on days 6 and 10, compared to milk-fed controls (Alali *et al.*, 2004). In England and Wales within-herd prevalence of STEC O157 in cattle from 29/75 positive farms was 1%–51% and the shedding

was the lowest among calves <2 months old, and highest in calves 2–6 months (Paiba *et al.*, 2003). EHEC O157 were found in fresh fecal samples collected in abattoirs in Holland over 2 successive years from 10% of the adult cows (27/270), but only once each year from veal calves (1/183, 1/214) (Heuvelink *et al.*, 1998). Similar to cattle, in 378 weaned and 265 suckling lambs tested for EC O157 all five isolates were found in weaned lambs (Battisti *et al.*, 2006), and colostrum-deprived lambs inoculated with EC O157:H7 shed more bacteria than conventionally reared animals (La Ragione *et al.*, 2006).

Low prevalence of EC O157:H7 in calves may be related to the presence of protective antibodies in colostrum and milk, as indicated by results of some studies with colostrum-deprived animals, or to EHEC-adverse microbiota and other factors of immature GIT. High prevalence in heifers, but not in young bulls, indicates a role of sex and may involve hormonal shifts associated with pregnancy and/or lactation. Heifers also exhibit high rate of intramammary bacterial infections for as yet unexplained reasons (Nickerson *et al.*, 1995; Fox, 2009) and may be similarly susceptible to intestinal carriage of EHEC.

Seasonal prevalence

In temperate climates, EHEC O157 prevalence in cattle peaks between late spring and early fall. EC O157:H7 were enumerated in fecal samples and rope swabs in Alberta dairies for 1 year and occurred 15-fold more often from June to September than in other periods (Stanford *et al.*, 2005); in 91 Colorado dairies sampled from February to July, STEC O157 were 7-fold more likely to occur on/after May 1st (Garber *et al.*, 1999). In Sweden, peak prevalence coincided with the period of pasture grazing from May to September (Albihn *et al.*, 2003). In Finland, prevalence of EC O157 in abattoir cattle peaked in July and none were found in November and December (Lahti *et al.*, 2001b). In Holland, a 1-year survey of slaughter cattle registered the highest prevalence of EC O157:H7 in summer (Van Donkersgoed *et al.*, 1999), whereas no EC O157 were isolated between December and April in 1-visit year-long survey of 678 dairy farms (Schouten *et al.*, 2004). In Nebraska, carriage of EC O157:H7 by feedlot cattle approached 100% in most pens during fall and then declined sharply to <20% by March; in most pens the hide prevalence rose rapidly to an overall 63% in April (Arthur *et al.*, 2009).

The seasonal differences are also apparent in countries featuring mild winters. In northern Italy, up to 17% of abattoir cattle carried STEC O157 in spring and summer, but only up to 2.9% in winter (Bonardi *et al.*, 1999), and in a 15-month farm survey, peak prevalence in heifers was eightfold higher than the low and occurred in July and August (Conedera *et al.*, 2001). In a 15-month postoutbreak U.K. survey of a cattle farm, EC O157:H7 were found in 4.3% (153/3593) of rectal swabs, with the peak prevalence in May/July and briefly in November after transfer of cattle into a building, with no shedding from December to May (Mechie *et al.*, 1997); in another survey a decrease of CFU/g digesta and feces occurred in the winter (Laven *et al.*, 2003). Consistent with peak summer prevalence, 0/32 wild rabbits caught from January to March in proximity to cattle farms in the United Kingdom carried EC O157, but 8/97 were positive from May 30 to August 5 (Scaife *et al.*, 2006). A year-long survey of 93 abattoirs in the United Kingdom showed peak carriage of EC O157:H7 from June to August (Milnes *et al.*, 2009).

In warm climates, prevalence of EHEC in cattle may be less season dependent; in an October to December survey of a herd in Florida ($n = 296$), 3% of fecal samples were culture positive for EC O157:H7, and 9% of cows were culture positive at least once (Riley *et al.*, 2003); in Lousiana, 5/13 dairy herds and 3%–34% cattle were positive for EC O157:H7, prevalence in spring (13.3%) was higher than in summer (10.5%), and dropped further in fall and winter; interestingly, in variance to most other studies, fecal prevalence was higher than hide prevalence (Dunn *et al.*, 2004b). Only a few exceptions to high summer prevalence were noted: in the United Kingdom, STEC O157 were isolated from ~1% of 1356 herds with no regard to season (Richards *et al.*, 1998), but the samples were from cases of bovine gastrointestinal disease and so not random or systematic. In Japan, summer prevalence peaked in the first year, was lower in second, and disappeared in the third year; secondary peaks between January and March were also noted (Ezawa *et al.*, 2004b). EHEC were detected in 6.3% (81/1281) of the bovine fecal samples in Belgium, and 0.7% (4/551) samples in Poland, and positive samples occurred in August to October (Tutenel *et al.*, 2002). It is possible that seasonal preference may be absent in large or high-density cattle operations, that may foster environment-independent EHEC transmission. Thus, prevalence of EC O157:H7 in a large beef feedlot in Kansas was 9.2% (82/891 representative samples) with no difference among season and the highest prevalence of 18.1% in February (Alam and Zurek, 2006); no seasonal preference was found during a 1-year survey on a finishing cattle farm where animals were housed indoors (Lahti *et al.*, 2003). A caveat that applies to longitudinal surveys is that most test only fecal samples, but peak seasonal prevalence in feces does not necessarily coincide with hide contamination (Barkocy-Gallagher *et al.*, 2003). Since viable extra-intestinal bacteria may be present at the farm even when fecal samples are negative, and the intestinal recolonization can occur at any time, the longitudinal studies of EC O157:H7 prevalence should include nonfecal samples.

It is not known what climate-associated factors contribute to peak summer prevalence of EHEC; a hypothesis that day length impacts bovine hormones was tested by exposing cattle to additional 5 h of artificial lighting/day, and increased prevalence of EC O157:H7 was observed, compared to controls; 43 days after cessation of lighting, prevalence decreased to control levels (Edrington *et al.*, 2006), and cattle treated with exogenous thyroid hormone shed the same numbers of EC O157 as controls in winter, and marginally less in summer (Edrington *et al.*, 2007). Acyl-homoserine-lactone (AHL) autoinducer produced by non-EHEC appear to enhance EHEC colonization of the bovine GIT, and the rumen samples from feedlot cattle were negative for AHLs in the winter (Edrington *et al.*, 2009). EHEC harbors SdiA, a regulator that senses AHLs and is necessary for efficient EHEC colonization of cattle; when AHLs are prominent within the bovine rumen, SdiA-AHL chemical signaling upregulated acid resistance genes to help EHEC pass through the acidic abomasum; AHLs are absent in other areas of the GI tract and lack of SdiA-AHL chemical signaling upregulates the LEE for efficient bacterial colonization of the recto-anal junction mucosa (Hughes *et al.*, 2010). One study found association between seasonal prevalence and diet; fecal and hide prevalence of EC

O157:H7 was higher in feedlot cattle fed wet distillers grains with solubles during periods of seasonally dependent low pathogen prevalence (Wells *et al.*, 2009a). In a survey of five dairy farms in Argentina, prevalence of *stx1* increased and *stx2* decreased in the summer (Fernandez *et al.*, 2009).

Sources of Human Infection

Undercooked contaminated ground beef and other meats

Ground beef is a particularly efficient transmission vehicle of EHEC due to the ease of cross-contamination, dispersion of the bacteria throughout the substrate, and poor efficiency of dry heat as a sterilizing agent, whereas bacteria contaminating the surface of a meat slab are unlikely to survive heat exposure. In the first U.S. outbreak in which EHEC was recognized as a class of human pathogens, 17/19 nursing home residents with hemorrhagic colitis ate hamburgers, but only 28/67 healthy residents (Ryan *et al.*, 1986), and indirect evidence pointed to ground meat in a number of other outbreaks (Belongia *et al.*, 1991). A multistate outbreak was traced to hamburgers distributed by a restaurant chain (1993), and isolates with identical toxin profiles and plasmid traits were found in patients (O'Brien *et al.*, 1993). PFGE-identical EC O157:H7 were isolated from 26 patients and 27 samples of incriminated ground meat (Barrett *et al.*, 1994); direct evidence implicated undercooked hamburgers in a number of other outbreaks (Roberts *et al.*, 1995; Shefer *et al.*, 1996; Cieslak *et al.*, 1997). One- third of the meat samples from randomly selected stores in Bologna (northern Italy) contained *E. coli*, and 3/149 were positive for STEC O157 (Stampi *et al.*, 2004). In a cluster of EC O157:H7–associated HUS and gastroenteritis cases, PFGE-identical isolates were found in ground meat samples in processing plant, in grocery store, and patients (Vogt and Dippold, 2005). Hamburgers prepared at home were also implicated (Mead *et al.*, 1997). Contaminated equipment, such as meat grinders, was linked to food-borne EC O157:H7 infections (Banatvala *et al.*, 1996). Sausages, dry-cured salami, and other food items containing uncooked meat were shown to transmit EC O157:H7 (1995) (Williams *et al.*, 2000; MacDonald *et al.*, 2004; Sartz *et al.*, 2008), and pork salami transmitted a severe EC O157 infection (Conedera *et al.*, 2007).

Unpasteurized dairy products

Uncooked/unpasteurized cow and goat milk was a vehicle of infection in several clusters of HUS caused by EC O157:H7 (Wells *et al.*, 1991; Bielaszewska *et al.*, 1997) and EHEC O157:H7 were found in sheep dairy products (Caro *et al.*, 2007). PFGE-identical isolates were obtained from patients and the implicated dairy (Keene *et al.*, 1997; Denny *et al.*, 2008), and an outbreak was traced to a dairy not licensed to distribute raw milk (Anonymous, 2007b), and to a faulty pasteurizer at a small dairy farm (Clark *et al.*, 1997). EC O157:H7 can survive and sometimes grow in different cheeses made from unpasteurized milk (Jordan and Maher, 2006; Schlesser *et al.*, 2006; Wahi *et al.*, 2006), and Gouda cheese was a vehicle (Honish *et al.*, 2005); STEC were detected in cheese after 12-month ripening (Caro and Garcia-Armesto, 2007). EHEC O157:H7 were found in 2/268 bulk tank milk samples and on 8/30 dairy farms in Tennessee (Murinda *et al.*, 2002), and 4.3% (36/859) of samples of bulk tank milk from U.S. dairies were positive for virulence factors associated with O157:H7 (*eaeA*, *tir*, and *stx*) (Karns *et al.*, 2007). Although STEC may be routinely found in dairy products, relatively few outbreaks were traced to this vehicle; in France only one O5 isolate among 40 STEC strains from milk and cheese samples had a virulence gene panel identical to HUS strains (Pradel *et al.*, 2008) and non-O157 STEC were found in cheese samples in Switzerland (Stephan *et al.*, 2008).

Contaminated fresh fruits and vegetables

Produce provides a variety of vehicles for transmission of EHEC as the bacteria can attach to intact or processed fruits and vegetables as well as survive in fruit juice. EC O157:H7 survived for 20 days in apple cider (Besser *et al.*, 1993) and outbreaks were traced to this vehicle (Hilborn *et al.*, 2000). The bacteria can grow in apple tissue and defeat decontamination procedures (Janes *et al.*, 2005), although pasteurization is protective (Mak *et al.*, 2001), as are some additives (Uljas and Ingham, 1999; Comes and Beelman, 2002; Knight and McKellar, 2007). The ability of EHEC O157:H7 to survive and grow in produce is highly dependent on the plant species and specific conditions; a cocktail of four strains grew on cut mangoes and papayas at 23°C, but only on papayas at 12°C; the bacteria survived for at least 180 days on both substrates (Strawn and Danyluk, 2009). EHEC O157:H7 interact with plants in complex ways, throughout the plant growing cycle, processing, and storage. Major outbreaks were linked to lettuce (Ackers *et al.*, 1998), including a multistate outbreak (Hilborn *et al.*, 1999); sprouts (Michino *et al.*, 1999) and spinach (Anonymous, 2006) are implicated in numerous HUS cases (Grant *et al.*, 2008). Internalization of the bacteria by growing spinach was rare (1/120 plants), but surface contamination was common once plants had grown for 3 weeks and the pathogen survived throughout the cultivation period when introduced into the growth medium at 10^7 CFU/mL level (Pu *et al.*, 2009). EHEC O157:H7 were shown to adhere to and penetrate roots (Wachtel *et al.*, 2002), although typically produce-mediated outbreaks were linked to foliage contaminated by irrigation/spray water (Wachtel *et al.*, 2002; Solomon *et al.*, 2003). The natural microflora on lettuce and spinach may be inhibitory (Johnston *et al.*, 2009); EC O157:H7 were not detected on lettuce grown for 50 days in organic manure inoculated with 10^4 CFU/g, although the bacteria persisted in soil for 8 weeks; *Pseudomonas fluorescence*, an *in vitro* inhibitor of EC O157:H7, was isolated from the plants (Johannessen *et al.*, 2005). However, lettuce grown in contaminated soil internalized EC O157:H7 as indicated by detection of the pathogen from surface-sterilized plants (Mootian *et al.*, 2009). In the absence of mechanical damage, heat and drought stress do not promote internalization of O157:H7 by plants (Zhang *et al.*, 2009). EC O157:H7 sprinkled on growing spinach survived and multiplied for 14 days (Mitra *et al.*, 2009) and repeated exposure to contaminated water increased bacterial load (Solomon *et al.*, 2003). The bacteria grew better on young romaine lettuce than on middle-leaf (Brandl and Amundson, 2008) and could be transferred to produce from contaminated ice and from contaminated product (Kim and Harrison, 2008).

EHEC O157:H7 grew on spinach stored at 8°C or above (Luo *et al.*, 2009), survived refrigeration for up to 15 days (Beuchat, 1999), and increased expression of virulence factors during cold storage (Carey *et al.*, 2009). Exposure of the

bacteria to lettuce lysates resulted in activation of genes associated with resistance to environmental stress, attachment, and virulence, and DNA repair, imparting increased resistance to hydrogen peroxide and calcium hypochlorate (Kyle *et al.*, 2010). The density of the bacteria inoculated on intact, damaged, and exposed to phytopathogen lettuce leaves decreased over 10 days, but more bacteria survived on damaged leaves (Aruscavage *et al.*, 2008). EC O157:H7 load increased 4–11-fold on damaged lettuce leaves and only 2-fold on intact ones, and leaves affected by soft rot (*Erwinia chrysanthemi*) harbored 27 more bacteria than healthy ones (Brandl, 2008). In some major EHEC outbreaks, pathogen was carried on spinach or other produce harvested in the proximity of cattle farms (Jay *et al.*, 2007), and a spinach outbreak strain grew better in autoclaved bovine manure than two other strains (Kim *et al.*, 2009a).

Colonization of spinach and lettuce leaves by EC O157:H7 involves flagella and type III secretion (Xicohtencatl-Cortes *et al.*, 2009); more specifically, the bacteria attach to leaves via filamentous type III secretion that is independent of effector protein translocation (Shaw *et al.*, 2008). Attachment to plant cells occurs by different mediators than to mammalian cells and requires polysaccharides cellulose, poly-beta-1,6-*N*-acetyl-D-glucosamine, and colanic acid (Matthysse *et al.*, 2008).

Food products subjected to fermentation or harboring adversarial microorganisms can reduce the viability of EHEC O157:H7. All 40 isolates of *Lactococcus lactis* from Greek raw goat milk cheese inhibited EC O157:H7 growth (Psoni *et al.*, 2007). EHEC O157:H7 declined by over 2 logs in fermented sausage (Glass *et al.*, 1992) and up to 3.5 logs in soudjouk sausage (Porto-Fett *et al.*, 2008; Hwang *et al.*, 2009). However, fermented products may provide a good vehicle for infection if curing conditions are inadequate; a large outbreak in Sweden was traced to improperly processed sausage (Sartz *et al.*, 2008).

Environment-mediated transmission

Manure is a good vehicle of EHEC and large outbreaks were associated with public events held on grazing areas presumably strewn with manure, especially in rainy weather. Isolates of EHEC O157 from music festival attendees were matched to a cattle herd (Crampin *et al.*, 1999). A scouting event held in Scotland on a muddy field grazed by sheep resulted in an 8% attack rate (18/226), with PFGE-identical isolates from patients, the field, and sheep feces (Strachan *et al.*, 2001); culturable bacteria were isolated for 15 weeks from soil (Ogden *et al.*, 2002). Bioluminescent EC O157:H7 leached from bovine and ovine feces subjected to stimulated rain fall and were more metabolically active in ovine leachate (Williams *et al.*, 2008). EC H7 survived for >2 months in garden soil fertilized with manure (Mukherjee *et al.*, 2006), and four PFGE-identical isolates were obtained from an implicated cow and three people visiting a meadow strewn with manure (Grif *et al.*, 2005). A comparison of 183 cases of sporadic EC O157 human infection with 545 matched controls implicated exposure to animal feces as the strongest risk factor (Locking *et al.*, 2001).

EHEC can grow in sterile fresh water at low carbon source concentration (Vital *et al.*, 2008), and waterborne origin was implicated in a number of sporadic cases and outbreaks of STEC O157 infection (Chalmers *et al.*, 2000) and EC O157:H7 (Muniesa *et al.*, 2006). EHEC outbreaks were associated with swimming in lakes (Keene *et al.*, 1994; Ackman *et al.*, 1997) and pools (Verma *et al.*, 2007), and consumption of water from a farm well (Jackson *et al.*, 1998) or a private water supply (Mannix *et al.*, 2007). PFGE-identical isolates were obtained from a campsite water, sheep feces, and patients (Licence *et al.*, 2001). In Australia, 10% (10/104) of surface water samples contained *E. coli* with 1+ virulence genes, and 6/10 were biochemically identical to human isolates of EHEC O157 (Ahmed *et al.*, 2007). EHEC with similar PFGE profiles, and the same virulence factors were isolated from patients, water samples, and duck feces (Samadpour *et al.*, 2002); EC O157:H7 remained viable for 5 days in beach sand either dry or intermittently wetted with sea water (Williams *et al.*, 2007). Identical and closely related strains of EC O157 were located in California within 135 m from a point source at low water level in an affected stream and up to 32 km at high water level (Cooley *et al.*, 2007). Nontoxigenic bacteria are used as indicators of pathogen contamination potential, but migration potential of toxigenic and nontoxigenic strains may differ (Castro and Tufenkji, 2007). The presence of EC O157 in local recreational water was not related to fecal indicator bacteria (Duris *et al.*, 2009), but the presence of bovine-specific species of Bacteroidales was a good predictor for contamination by EC O157 (Walters *et al.*, 2007), although EC O157:H7 were rarely detected in the absence of indicator *E. coli* within a large geographic setting, such as a river basin in Canada (Wilkes *et al.*, 2009). Interestingly, EC O157:H7 do not survive well in the Ganges river water due to heat-labile noncellular antimicrobials, presumably colicins or antimicrobial peptides (Nautiyal, 2009).

Direct transmission

Even brief physical human–animal contact can transmit EHEC, and hand-washing is the single most important prevention step to reduce transmission resulting from human–animal contact (Anonymous, 2007a). Children are at most risk, as highlighted by an HUS case in a visitor to a petting zoo (Heuvelink *et al.*, 2002) and EHEC O157 diarrhea in children visiting an inner-city exposition farm (Chapman *et al.*, 2000a). In Finland, five EC O157:H7 cases in children living on or visiting a farm were traced to five different dairy farms and isolates PFGE-identical to clinical ones (Lahti *et al.*, 2002). Attendees of agricultural fairs are at increased risk of EHEC infection due to the proximity of animals, concession stands, and people (Crump *et al.*, 2003). In 2004–2005, large outbreaks of pediatric EC O157:H7 infections occurred among visitors to petting zoos at agricultural fairs (Anonymous, 2005). In a study of STEC transmission in Germany, acquisition of isolates from 202 human cases analyzed risk factors by comparing age- and region-matched controls; in children <3 years, touching a ruminant was the greatest risk source (Werber *et al.*, 2007).

Person-to-person transmission of EHEC may contribute to outbreaks from a primary source (Parry *et al.*, 1998; Seto *et al.*, 2007) and may reach 14% (Rangel *et al.*, 2005) or higher; secondary cases occurred in 20/89 households with STEC O157 infection in the United Kingdom (Werber *et al.*, 2008), and significant interpersonal spread was noted in 18-case O157 outbreak in Ireland, with identical/close PFGE patterns in isolates from patients and a rectal animal swab (Mannix *et al.*,

2007). Asymptomatic infected people may be an unappreciated source of the pathogen (Gilbert *et al.*, 2008).

Conclusions

1. Detection of EC O157:H7 is a multistep process that should start with definition of the identifying criteria and end with serological confirmation of suspected colonies. A successful outcome of prevalence survey requires capture of the bacteria by sampling, retention of viability by the organisms during storage, and their ability to avoid a competitive exclusion during culture. Probability of capture of EC O157:H7 in complex environment (e.g., farm) can be increased by multiple (combined) and repeated sampling, stratified by time, season, location, age, and sex of carriers, body site, and form of excreta. Storage may reduce viability of the bacteria, and recovery may require a prolonged incubation in enrichment broth.
2. A conceptual distinction should be made between comparative (different farms/environments) and longitudinal (same farm over time) studies of EC O157:H7 prevalence. The former should employ identical sampling and culturing methods for all surveyed entities, whereas the latter should maximize probability of detection by extensive sampling and culture techniques supportive of recovery of stressed or injured bacteria.
3. Cattle and possibly small domesticated ruminants constitute a primary animal reservoir of EC O157:H7, although most cattle isolates are not highly virulent and do not occur in people. Distribution of EC O157:H7 in cattle is highly clustered, and most of the bacteria are excreted by a small number of carriers ("supershedders") that may harbor large intestinal loads of the pathogen for extended periods. Prevalence of EC O157:H7 in cattle is strongly season dependent and tends to be higher in spring and summer than in fall and winter; the season effect may be reduced in large cattle operations and in climate featuring mild winters. Prevalence is very low in young calves, but highest in heifers. Hide prevalence may not coincide with intestinal prevalence.
4. EHEC O157:H7 may be harbored by pigs and poultry, other domestic and synanthropic animals, ruminant and nonruminant wildlife, and nonmammalian vectors. However, these animals are unlikely to support the continuous existence of the bacteria in the absence of canonical carriers. They can, however, provide a conduit for transmission of the bacteria, and may be responsible for the appearance of the genetically matching isolates at distant geographic locations.
5. EHEC O157:H7 can be transmitted to humans by contaminated undercooked meat and dairy products, produce and fruit juice, drinking and surface water, and, to lesser extent, by contact with animals or manure, and person-to-person contact. The bacteria can colonize plants by mechanisms distinct from those mediating colonization of mammalian tissues, survive for extended time in water and soil, and multiply in manure and other substrates. Survival and transmission can be aided by formation of biofilm, and extraintestinal survival can be influenced by virulence characteristics.

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