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Invited Review: Prevalence of Shiga Toxin-Producing *Escherichia coli* in Dairy Cattle and Their Products

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ABSTRACT

The main objective of this review was to assess the role of dairy cattle and their products in human infections with Shiga toxin-producing *Escherichia coli* (STEC). A large number of STEC strains (e.g., members of the serogroups O26, O91, O103, O111, O118, O145, and O166) have caused major outbreaks and sporadic cases of human illnesses that have ranged from mild diarrhea to the life-threatening hemolytic uremic syndrome. These illnesses were traced to O157 and non-O157 STEC. In most cases, STEC infection was attributed to consumption of ground beef or dairy products that were contaminated with cattle feces. Thus, dairy cattle are considered reservoirs of STEC and can impose a significant health risk to humans. The global nature of food supply suggests that safety concerns with beef and dairy foods will continue and the challenges facing the dairy industry will increase at the production and processing levels. In this review, published reports on STEC in dairy cattle and their products were evaluated to achieve the following specific objectives: 1) to assemble a database on human infections with STEC from dairy cattle, 2) to assess prevalence of STEC in dairy cattle, and 3) to determine the health risks associated with STEC strains from dairy cattle. The latter objective is critically important, as many dairy STEC isolates are known to be of high virulence. Fecal testing of dairy cattle worldwide showed wide ranges of prevalence rates for O157 (0.2 to 48.8%) and non-O157 STEC (0.4 to 74.0%). Of the 193 STEC serotypes of dairy cattle origin, 24 have been isolated from patients with hemolytic uremic syndrome. Such risks emphasize the importance and the need to develop long-term strategies to assure safety of foods from dairy cattle.
(**Key words:** foodborne pathogen, *Escherichia coli*, dairy cattle, Shiga toxin)

Abbreviation key: ***eae*** = *E. coli* attaching and effacing gene, ***ehxA*** = enterohemolysin gene, **HUS** = hemolytic uremic syndrome, **HUT** = an untypeable H antigen, **H$^-$** = nonmotile, **OR** = a rough O antigen, **OUT** = an untypeable O antigen, **OX3** = the provisional designation for the O174 antigen, **STEC** = Shiga toxin-producing *E. coli*, **Stx1** = Shiga toxin 1, ***stx*$_1$** = Shiga toxin 1 gene, **Stx2** = Shiga toxin 2, ***stx*$_2$** = Shiga toxin 2 gene.

INTRODUCTION

The safety concern about foods of bovine origin emerged 2 decades ago and increased in recent years because of the growing number of human infections with Shiga toxin-producing *Escherichia coli* (**STEC**). These infections result in illnesses (Griffin and Tauxe, 1991) such as mild diarrhea, bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (**HUS**). Hemolytic uremic syndrome can lead to acute or chronic renal failure, especially in children (Siegler et al., 1991). Other illnesses include strokes (Griffin and Tauxe, 1991) and thrombotic thrombocytopenic purpura that is characterized by nervous system abnormalities (Paton and Paton, 2000).

Dairy cattle are considered reservoirs of O157 (Besser and Hancock, 1994; Hancock et al., 1994; Mechie et al., 1997) and non-O157 STEC (Rahn et al., 1997; Conedera et al., 2001; Kobayashi et al., 2001). Contamination of raw milk (Wells et al., 1991; Sandhu et al., 1996; Chiueh et al., 2002), cheese (Clarke et al., 1994; Pradel et al., 2000; Pradel et al., 2001), or ground beef from dairy cattle (Doyle, 1991) poses a significant risk to humans. Raw milk caused a small number of human illness outbreaks (USDA-APHIS-VS, 1997) that were traced to O157 (Martin et al., 1986; Borczyk et al., 1987; Lahti et al., 2002) and non-O157 STEC (CDC, 1995; Bielaszewska et al., 2000; Allerberger et al., 2001). Outbreaks and sporadic cases of illnesses were also traced to consumption of STEC-contaminated cheese (Deschênes et al., 1996; CDC, 2000) and yogurt (Morgan et al., 1993). Culled dairy cows are mainly used for production of ground beef (USDA-APHIS-VS, 1996a) and therefore can impose a significant health risk (Ostroff et al., 1990; Doyle, 1991; Faith et al., 1996). Because of the increased concern with the safety of ground beef and dairy products in recent years, the objective of this review was

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to assess the role of dairy cattle in human infections with STEC.

VIRULENCE FACTORS OF STEC

In addition to *E. coli* O157:H7, >100 STEC serotypes (e.g., members of the O26, O91, O103, O111, O118, O145, and O166 serogroups) are known to cause human illnesses, including HUS (Paton and Paton, 2000). Pathogenic STEC strains are often referred to as enterohemorrhagic *E. coli* and are known to produce 1 or 2 toxins that resemble those of *Shigella dysenteriae* (O'Brien and Holmes, 1987). These are Shiga toxin 1 (**Stx1**) and Shiga toxin 2 (**Stx2**). The STEC strains can also produce other virulence factors such as those responsible for intimate attachment to the intestinal surface (intimin) and for enterocyte damage (enterohemolysin). In addition to *E. coli* O157:H7, members of the O26, O103, and O111 serogroups are considered the most important enterohemorrhagic *E. coli* (Franke et al., 1995). Although Stx1 and Stx2 are different proteins, encoded by the Shiga toxin 1 gene (***stx*$_1$**) and the Shiga toxin 2 gene (***stx*$_2$**), respectively, their biological activities are quite similar (Neill, 1997). It is worth noting that STEC-related illnesses can be caused by an *E. coli* strain that produces Stx1, Stx2, or both toxins (Lior, 1994). Other virulence factors (intimin and enterohemolysin) are encoded by the *E. coli* attaching and effacing (***eae***) and enterohemolysin (***ehxA***) genes. The *eae* and *ehxA* genes are found in virtually all *E. coli* O157 strains (Neill, 1997) and appear to be more common in the non-O157 STEC strains responsible for human illnesses (Beutin et al., 1994). The *eae* and *ehxA* genes, however, are not absolutely required to cause human illnesses because STEC strains lacking these genes were shown to cause human illnesses (Neill, 1997). Therefore, each STEC strain should be considered a potential enterohemorrhagic *E. coli* (Bürk et al., 2002).

HUMAN INFECTIONS WITH STEC FROM DAIRY CATTLE

Most human STEC infections have been traced to consumption of contaminated foods of bovine origin such as ground beef (Griffin and Tauxe, 1991; USDA-APHIS-VS, 1997) and raw milk (Herriott et al., 1994; Lahti et al., 2002). Other infection routes include manure-contaminated vegetables (Cieslak et al., 1993), person to person (Reida et al., 1994), animal to person (Crump et al., 2002), and contaminated water through drinking (Yatsuyanagi et al., 2002) or swimming (Keene et al., 1994). Beef from dairy cattle, raw milk, and some of the dairy products are considered contributing factors to STEC infections (Martin et al., 1986; Steele et al., 1997). Shiga toxin-producing *E. coli* also can spread from the dairy farm environment to humans by touching the animals (Crump et al., 2002). These pathogens were prevalent at high rates on US dairy farms (Zhao et al., 1995) and were widely distributed in the dairy farm environment (Zhao et al., 1995).

Several outbreaks and sporadic cases of human illnesses caused by infection with *E. coli* O157 (O157:H7, O157:**H**$^-$ [nonmotile], or other isolates that were not typed for the H antigen) from dairy cattle (Table 1) emphasized the role of raw milk as an important vehicle of transmission (Reitsma and Henning, 1996). With regard to *E. coli* O157:H7, 2 US outbreaks (Herriott et al., 1994; USDA-APHIS-VS, 1997) were caused by consumption of raw milk in 1992 (9 cases) and 1993 (6 cases) and were traced to 2 dairies that were licensed to sell raw milk (USDA-APHIS-VS, 1994). Drinking raw milk in the US resulted in HUS cases in Wisconsin (Martin et al., 1986), Washington State (Wells et al., 1991), and Oregon (Keene et al., 1997). Raw milk also caused human illnesses in Canada (Borczyk et al., 1987; Wilson et al., 1996) and Finland (Lahti et al., 2002). Fortunately, the number of raw milk drinkers in Western societies is small. It was estimated that 1 to 2% of the US population consume raw milk (USDA-APHIS-VS, 1994). Raw milk consumption, however, has decreased from 429 million kg in 1980 to 230 million kg in 1990 (USDA, 1992). These statistics may explain why the number of STEC outbreaks caused by raw milk consumption has been small.

Outbreaks caused by *E. coli* O157-contaminated dairy products are limited. For example, the only reported yogurt outbreak occurred in the UK in 1991 (Morgan et al., 1993) and involved 16 people, of which 5 had HUS. Only one cheese outbreak was reported in the US (Wisconsin) and involved 55 people. In addition to the 4 cheese outbreaks that occurred in the UK (Table 1), 2 cheese outbreaks were also reported in France (Anonymous, 1994) and the UK (Curnow, 1994). Other contaminated dairy products (butter and unpasteurized cream) were the cause of O157 infection in one (Reid, 2001) and 7 (CDSC, 1998) adults, respectively, in the UK.

Although many human illness outbreaks were traced to ground beef (USDA-APHIS-VS, 1997), the beef source (beef or dairy cattle) was not known in most cases. This is because ground beef is produced mainly from culled dairy and beef cattle and includes low-value cuts from finished steers or heifers. Cows culled because of health, age, or production reasons are a significant component of the hamburger consumed in the US; 6 million culled dairy cows enter the food chain as ground beef annually (Troutt and Osburn, 1997; National Cat-

Table 1. Human infections with *Escherichia coli* O157[1] from dairy cattle.

Country	Year	Cases (no.)	Age	Illness (no.)	Infection route	Reference
US						
WI	1986	2	<13 mo	HUS[2]	Raw milk	Martin et al., 1986
WA	1986	37	Adults	HC[3] (all), HUS (1), and TTP[4] (3)	Ground beef	Ostroff et al., 1990
	1990	2	Adults	HUS	Raw milk	Wells et al., 1991
OR	1993	14	Adults	Diarrhea	Raw milk	Keene et al., 1997
WI	1998	55	15 mo to 90 yr	Abdominal pain/bloody diarrhea	Cheese	CDC, 2000
PA	2000	47	<10 yr	Abdominal pain/bloody diarrhea (all) and HUS (8)	Visiting a dairy farm	Crump et al., 2002
		4	Adults	Abdominal pain/bloody diarrhea		
WA	2000	5	2 to 14 yr	Abdominal pain/bloody diarrhea (all) and HUS (1)	Visiting a dairy farm	Georgia Division of Public Health, 2002
Canada (Ontario)	1986	60	<5 yr	Abdominal pain/diarrhea (all) and HUS (3)	Raw milk	Borczyk et al., 1987
		14	Adults	Abdominal pain/diarrhea		
Canada (Alberta)	1987	15	Elderly	Abdominal pain/bloody diarrhea	Ground beef	Todd et al., 1993
UK (England)	1991	11	<10 yr	Diarrhea or HC (all) and HUS (5)	Yogurt	Morgan et al., 1993
		5	Adults	Diarrhea or HC		
Canada (Ontario)	1992	1	6 mo	No signs of illness	Raw milk or animal/manure contact	Wilson et al., 1996
UK (England)	1993	4	Children	Abdominal pain (all) and HUS (3)	Raw milk	Wall et al., 1996[5]
		3	Adults	Abdominal pain		
UK (Scotland)	1994	3	Children	Abdominal pain/bloody diarrhea (2) and HUS (1)	Cheese	Reid, 2001[5]
		19	Adults	Abdominal pain/bloody diarrhea		
UK (England)	1996	3	Adults	Abdominal pain/diarrhea	Raw milk	CDSC, 1996[5]
Finland	1997	4	<11 yr	Bloody diarrhea (all) and HUS (2)	Raw milk or animal/manure contact	Lahti et al., 2002
		2	<11 yr	Diarrhea		
		1	A child	No signs of illness		
		1	An adult	No signs of illness		
Czech Republic	1998	2	<6 yr	Bloody diarrhea and HUS	Visiting a dairy farm	Bielaszewska et al., 2000[6]
Finland	1998	1	An adult	Bloody diarrhea	Raw milk	Lahti et al., 2002
UK (England)	1998	7	Adults	Abdominal pain/diarrhea (all) and HUS (4)	Cream	CDSC, 1998[5]
UK (Scotland)	1998	1	3 yr	Abdominal pain/diarrhea	Raw milk	Reid, 2001
		1	12 yr	HUS	Cheese	
		1	An adult	Abdominal pain/diarrhea	Butter	
		3	Adults	Abdominal pain/diarrhea (all) and HUS/death (1)	Cheese	
UK (England)	1999	60	Adults	Abdominal pain/diarrhea	Raw milk	CDSC, 1999a[5]
	1999	3	Adults	Abdominal pain/diarrhea	Cheese	CDSC, 1999b[5]
	2000	1	A child	HUS	Raw milk	International Association of Milk, Food, and Environmental Sanitarians, 2000[5]
		3	Adults	Bloody diarrhea		
Austria	2001	1	6 yr	Bloody diarrhea and HUS	Raw milk	Allerberger et al., 2001[6]

[1]All isolates were *E. coli* O157:H7 unless indicated otherwise.

[2]HUS = Hemolytic uremic syndrome.

[3]HC = Hemorrhagic colitis.

[4]TTP = Thrombotic thrombocytopenic purpura.

[5]The *E. coli* O157 isolates were not typed for the H antigen.

[6]A nonmotile *E. coli* O157 isolate.

Table 2. Human infections with non-O157 Shiga toxin-producing *Escherichia coli* from dairy cattle.

Country	Year	Cases (no.)	Age	Illness (no.)	Serotype	Infection route	Reference
US (MT)	1994	4	Adults	Abdominal pain/bloody diarrhea	O104:H21	Raw milk	CDC, 1995
Canada (Ontario)	1992	2	<2 yr	No signs of illness	O5:H^{-1} or O7:H4	Raw milk or animal/manure contact	Wilson et al., 1996
		6	Adults	No signs of illness	O80:H$^-$, O91:H14, O103:H2, O119:H25, O132:H$^-$, or O146:H21		
France	1992-1993	4	<15 mo	HUS[2] (all) and bloody diarrhea (3)	Unknown	Cheese	Deschênes et al., 1996
Germany	1997	1	2 yr	HUS	OUT[3]:H8	Raw milk	Gallien et al., 1997

[1]H$^-$ = A nonmotile *E. coli* isolate.
[2]HUS = Hemolytic uremic syndrome.
[3]OUT = An untypeable O antigen.

tlemen's Beef Association, 2001). Culled dairy cows account for 17% of the ground beef produced in the US (Troutt and Osburn, 1997). In New York State alone, culled dairy cows contribute 4.8 million kg of hamburger annually (Segelken, 1996). In general, it is difficult to trace a specific ground beef outbreak to dairy or beef cattle. The only 2 known dairy beef outbreaks are summarized in Table 1.

Contact with dairy farm environments through touching the cattle or their manure resulted in severe cases of *E. coli* O157:H7-related illnesses (HUS) in the US (Crump et al., 2002; Georgia Division of Public Health, 2002). The illnesses were associated with farm visitors, and no symptoms were reported for farm residents or employees (Crump et al., 2002). Visitors who washed their hands also did not become ill, indicating a protective effect of hand washing. Two severe cases attributable to visiting a dairy farm were also reported in the Czech Republic and were caused by *E. coli* O157:H$^-$ (Bielaszewska et al., 2000). As shown in Table 1, responses to infection varied from no signs of illness (Wilson et al., 1996; Lahti et al., 2002) to bloody diarrhea and HUS (Lahti et al., 2002) and reflected the immunity status of the affected individuals (mostly children). In general, previous exposure to farm animals is known to decrease the risk of infection with STEC (Wilson et al., 1997). In a study of numerous environmental sites on dairy farms (Blanco et al., 2001), evidence of STEC was found in composite samples of calf feeders (19%), calf-barn surfaces (18%), cow feeders (15%), and cow-barn surfaces (11%). Therefore, contaminated farm environments may remain sources of STEC infection for several months.

Infections with non-O157 STEC from dairy cattle are summarized in Table 2. Most of those were traced to consumption of raw milk, as was the case with the US outbreak (CDC, 1995). The remaining raw milk outbreaks occurred in Canada (Wilson et al., 1996) and Germany (Gallien et al., 1997). Although *E. coli* O5:H$^-$ and O103:H2 serotypes were not shown to cause any signs of illness (Table 2), they are known to cause HUS (WHO, 1998). Several outbreaks were also attributed to consumption of cheeses (those made from unpasteurized milk) in Sweden, the UK, the US (Adams and Motarjemi, 1999), and France (Deschênes et al., 1996). Brie cheese was responsible for the outbreaks in Sweden and the US, whereas local cheeses were responsible for the remaining ones. Contact with dairy cattle manure was also the cause of Canadian outbreaks of STEC (O5:H$^-$ and O7:H4) infections (Wilson et al., 1996).

STEC SEROTYPES FROM MILK AND DAIRY PRODUCTS

Studies on contamination of milk or its products with *E. coli*, in general, have been limited. Testing raw milk and cheese samples (500 and 739, respectively) revealed low levels (<20 cfu/g) of *E. coli* contamination (Coia et al., 2001). Detection of STEC in raw milk, milk filters, and cheeses (Table 3) was reported in Canada, Belgium, Germany, the UK, and the US (WHO, 1998). Padhye and Doyle (1991) reported that *E. coli* O157:H7 was detected in raw milk and cheese. Two STEC serotypes (O91:H21 and an untypeable O antigen [**OUT**]:H$^-$) and members of the O91 and O113 serogroups were isolated from raw milk and cheese (Table 3). None of these isolates is known to cause human illnesses attributable to the consumption of dairy foods. However, *E. coli* O91:H21 and OUT:H$^-$ were isolated from patients with HUS and bloody diarrhea, respectively (WHO, 1998).

Table 3. Shiga toxin-producing *Escherichia coli* from raw milk, milk filters, and cheese.

Source	Serotype or serogroup	Reference
Raw milk	O1:H20, O26:H11, O91:H21, O113:H21, O121:H7, O136:H12, O136:H16, O142:H38, O163:H19, OUT[1]:H8, OUT:H21, and OUT:H^{-}[2]	Steele et al., 1997
	O5:H^{-} and O7:H4	Wilson et al., 1996
	O6:H34	Clarke et al., 1994; Sandhu et al., 1996; Wilson et al., 1996
	O21:H21, O30:H8, O114:H4, and OUT:H^{-}	WHO, 1998
	O22:H8	Wells et al., 1991; Sandhu et al., 1996; Wilson et al., 1996
	O91:H21, O156:H25, and OUT:H7	Clarke et al., 1994
	O103:H2	Wells et al., 1991; Clarke et al., 1994; Sandhu et al., 1996; Wilson et al., 1996
	O104:H21	CDC, 1995
	O113:H4	Sandhu et al., 1996; Wilson et al., 1996
	O157	CDSC, 1996; Wall et al., 1996; CDSC, 1999a; Reid, 2001
	O157:H7	Martin et al., 1986; Borczyk et al., 1987; Padhye and Doyle, 1991; Wells et al., 1991; Abdul-Raouf et al., 1996; Wilson et al., 1996; Keene et al., 1997; Lahti et al., 2002
	O157:H^{-}	Klie et al., 1997; Allerberger et al., 2001
	OUT:H2	Clarke et al., 1994; Wilson et al., 1996
	OUT:H8	Gallien et al., 1997
	OUT:HUT[3]	Chiueh et al., 2002
Milk filters	O26:H11	Wells et al., 1991; Sandhu et al., 1996; Wilson et al., 1996
	O43:H2	Clarke et al., 1994
	O44:H^{-} and OUT:H19	WHO, 1998
	O153:H25	Wells et al., 1991; Sandhu et al., 1996
	OUT:H8	Clarke et al., 1994; Wilson et al., 1996
Cheese	O26:H32 and OUT:H^{-}	WHO, 1998
	O91, O113, and OX3[4]	Pradel et al., 2000
	O91:H21	Pradel et al., 2001
	O116:H^{-}	Clarke et al., 1994
	O157	CDSC, 1999b; Reid, 2001
	O157:H7	Padhye and Doyle, 1991; CDC, 2000

[1]OUT = An untypeable O antigen.

[2]H^{-} = A nonmotile *E. coli* isolate.

[3]HUT = An untypeable H antigen.

[4]OX3 = Provisional designation for the O174 antigen.

Interestingly, the *E. coli* OUT:H8 found in milk filters (Table 3) is known to cause HUS (Table 2). Of *E. coli* isolates (O6:H10, O26:H32, O116:H^{-}, and OUT:H^{-}) found in Canadian cheeses (WHO, 1998), 2 serotypes (O26:H32 and OUT:H^{-}) are known to cause HUS (WHO, 1998). Recently, several STEC strains (members of the O8, O103, O156, and O157 serogroups) were isolated from raw milk tanks in the UK (McKee et al., 2003). Although these isolates had 1 (stx_1 in O103 and stx_2 in O8 and O156) or 2 (stx_2 and *eae* in O157) virulence factors, only members of the O103 and O157 serogroups are known to cause human illnesses (WHO, 1998).

DAIRY CATTLE AS RESERVOIRS OF STEC

Although STEC are not host specific, they are more prevalent in ruminants than in other animals (Riemann and Cliver, 1998). In addition, human illnesses caused by STEC infection have been traced mostly to cattle (Dean-Nystrom et al., 1997). Therefore, cattle (Martin et al., 1986; Wilson et al., 1992; Chapman et al., 1993), including dairy cows, heifers, and calves (Hancock et al., 1994; Zhao et al., 1995; Besser et al., 1997), have been considered reservoirs of STEC. Zhao et al. (1995) estimated that 22 to 50% of the US dairy farms are contaminated with *E. coli* O157:H7. Higher estimates should be considered for STEC prevalence when taking into account other serotypes. The wide distribution of STEC on dairy farms was documented (Rahn et al., 1998) in the high prevalence rates reported for cows (58 of 274; 21.2%) and calves (68 of 135; 50.4%). In a recent study of 3 Japanese dairy farms (Ezawa et al., 2004), the prevalence rates of *E. coli* O157 were 33.7, 18.5, and 0% for heifers, cows, and calves, respectively. The high prevalence rate in heifers was attributed to their close contact during grazing. Other studies (Wells et al., 1991, 1992) illustrated the presence of a wide range of STEC serotypes in dairy cattle at different stages of production. Testing 1266 fecal samples from cows, heifers, and calves from 22 farms, a stockyard, and a packing plant (Wells et al., 1991) revealed the presence of *E. coli* O157:H7, O157:H^{-}, and 27 other non-

O157 serotypes (O10:H$^-$, O15:H27, O22:H8, O22:H40, O25:H$^-$, O26:H11, O45:H2, O45:H$^-$, O76:H21, O84:H2, O84:H$^-$, O103:H2, O103:H$^-$, O111:H$^-$, O116:H21, O121:H7, O145:H$^-$, O153:H25, O163:H19, O171:H2, **OX3** [the provisional designation for the O174 antigen]:H21, OX3:H$^-$, **OR** [a rough O antigen]:H2, OR:H8, OR:H$^-$, OUT:H$^-$, and OUT:**HUT** [an untypeable H antigen]). Except for no detection of *E. coli* O157:H7, a similar distribution of non-O157 STEC was found when 1790 fecal samples from dairy cows and calves were tested (Wilson et al., 1992). The STEC strains included O2:HUT, O3:HUT, O4:H7, O4:H16, O4:H$^-$, O6:HUT, O8:H8, O8:H16, O8:H$^-$, O8:HUT, O9:H$^-$, O11:HUT, O15:H$^-$, O22:H1, O22:H8, O26:H$^-$, O32:H7, O32:H16, O32:H$^-$, O32:HUT, O40:HUT, O43:H2, O43:H6, O43:H12, O82:H$^-$, O87:H$^-$, O103:H2, O103:H6, O103:H12, O103:H16, O103:H39, O103:HUT, O106:HUT, O109:H16, O111:H$^-$, O113:H$^-$, O117:H$^-$, O117:HUT, O119:H16, O121:H7, O121:HUT, O146:H$^-$, O153:H7, O153:H32, O153:H$^-$, O153:HUT, O163:HUT, OX3:H7, OX3:H16, OX3:H21, OX3:H39, OX3:H$^-$, OX3:HUT, OX8 [a provisional designation for a new O antigen]:HUT, OUT:H2, OUT:H3, OUT:H6, OUT:H7, OUT:H8, OUT:H12, OUT:H16, OUT:H21, OUT:H40, OUT:H$^-$, and OUT:HUT. Similar trends were also found in Japan (Tada et al., 1992; Furuhata et al., 1999), where members of the O26, O111, O145, and O157 serogroups (Tada et al., 1992) and 84 non-O157 STEC serotypes (Furuhata et al., 1999) were detected in dairy cattle feces. Furuhata et al. (1999) indicated that different serotypes were associated with specific geographical locations or climates and suggested that certain STEC serotypes may have resistance to hot or cold climates. With regard to sex effects on STEC shedding by dairy cattle, only one study was found (Montenegro et al., 1990). Various STEC serotypes (O3:H$^-$, O10:H21, O22:H8, O39:H40, O75:H8, O82:H8, O82:H40, O91:H10, O104:H21, O105:H18, O113:H21, O116:H21, O126:H20, O126:H21, O136:H12, O139:H8, O156:H21, O157:H7, OR:H18, OUT:H16, and OUT:H29) were recovered from 17 and 9% of the 47 cows and 212 bulls tested, respectively. To better define dairy cattle as reservoirs of STEC, published reports on STEC prevalence in dairy cattle were evaluated.

PREVALENCE OF STEC IN DAIRY CATTLE

Prevalence rates of STEC in dairy cattle are summarized in Table 4. With 2 exceptions (Mechie et al., 1997; Bonardi et al., 1999), the prevalence rates of *E. coli* O157:H7 were based on one-time sampling of cattle feces. In these 2 studies, multiple samples (monthly for 15 mo or 1 yr, respectively) were tested. In Canada (Jackson et al., 1998), a very high prevalence rate (59 of 95; 62.1%) was found in a dairy farm that was associated with a child infection with *E. coli* O157:H7. In the US, *E. coli* O157:H7 prevalence rates ranged from 0.2 to 8.4% for cows, from 1.6 to 3.0% for heifers, and from 0.4 to 40.0% for calves. In Canada, Italy, Japan, and the UK, the corresponding ranges of *E. coli* O157:H7 prevalence rates were 0.3 to 16.1%, 10.0 to 14.1%, and 1.7 to 48.8%, respectively. These data emphasized the significant impact of animal age on epidemiology of *E. coli* O157:H7 as illustrated in the higher prevalence rates for younger (2- to 24-mo old) than for older cattle. Testing dairy cattle on 10 Dutch farms (Heuvelink et al., 1998) revealed prevalence rates of *E. coli* O157:H7 ranging from 0.8 to 22%, with calves (4- to 12-mo old) having the highest rate (21%). High prevalence rates were also reported for young beef cattle (Hancock et al., 1997c) and were attributed to the greater susceptibility to colonization for calves and heifers than for cows (Hancock et al., 1998). Prevalence rates of *E. coli* O157:H7 among dairy calves also appear to be affected by age. For example, the prevalence rate increased from 1.4% (6 of 423) before weaning to 4.8% (25 of 518) after weaning (Garber et al., 1995). Testing a large number of calves from 60 dairy herds in Washington State revealed that *E. coli* O157:H7 was more prevalent in weaned (7 of 1083; 0.65%) than in unweaned (0 of 649; 0%) calves (Hancock et al., 1994).

Factors that may affect the prevalence of *E. coli* O157:H7 in dairy cows were evaluated (Hancock et al.1994; Fitzgerald et al., 2003). In the study by Hancock et al. (1994), similar prevalence rates were reported for lactating (2 of 1273; 0.16%) and dry (1 of 477; 0.21%) cows. In contrast, a higher prevalence rate (43% vs. 22%) was reported for lactating cows than for dry cows (Fitzgerald et al., 2003). The time of fecal sampling was shown (Fitzgerald et al., 2003) to affect the number of dry cows shedding *E. coli* O157:H7 (28% for a.m. vs. 17% for p.m.) but not the lactating cows (average, 43%). Parity and DIM did not influence the number of cows shedding *E. coli* O157:H7 (Fitzgerald et al., 2003). The higher prevalence rates reported by Fitzgerald et al. (2003) could be attributed to the hot weather during the time of fecal sampling (August) in New Mexico. In the study by Hancock et al. (1994), however, the reported prevalence rates were average values of fecal testing of different dairy farms over a 1-yr investigation in Washington State.

Prevalence rates of *E. coli* O157:H7 in culled dairy cows have been variable. For example, prevalence rates ranging from 0.5% in Canada (Clarke et al., 1994) to 16.1% in the UK (Chapman et al., 1997) were reported. The 16.1% prevalence rate (Chapman et al., 1997) was much higher than the rates (1 to 2%) reported for younger dairy cows in the UK (Chapman et al., 1993)

or in the US (Hancock et al., 1994). Prevalence rates for culled dairy cows ranged from 0.9 to 3% in New York State (USDA-APHIS-VS, 1996b). Based on data from 91 dairy farms, Garber et al. (1999) showed a higher prevalence rate (2.8% vs. 0.9%) for *E. coli* O157:H7 in culled cows than in those in production. Rice et al. (1997) tested fecal samples from culled dairy cows on the farm (205 cows from 19 herds) and at slaughter (103 cows from 15 herds) in Idaho, Oregon, and Washington State and reported that 3.4 and 3.9% of the cows on the farm and at slaughter, respectively, were positive for *E. coli* O157:H7. Dairy cattle with downer cow syndrome are those suffering from assorted maladies (e.g., mastitis, calving paralysis, and milk fever) and/or injuries (e.g., during transport) that render them immobile to various degrees (Correa et al., 1993). If their condition does not improve, they are culled from the production herd (Faith et al., 1996; Troutt and Osburn, 1997) and enter the food chain as ground beef. As a result, downer dairy cows harboring STEC at slaughter can be a health risk to humans. This potential risk was recently evaluated at 2 packing plants in Wisconsin over a 6-mo (May to October) period (Byrne et al., 2003). The prevalence rate was higher for downer (10 of 203; 4.9%) than for healthy (3 of 201; 1.5%) cows that were harvested at the same time and were from similar geographical locations.

Prevalence rates of non-O157 STEC in dairy cattle are also summarized in Table 4. Multiple sampling (monthly over a 1-yr period) of cattle feces was used in 2 studies (Hancock et al., 1997a; Conedera et al., 2001) but one-time sampling was used in the others. Worldwide, the prevalence rates ranged from 0.4 to 52.0% for cows, from 1.7 to 74.0% for heifers, and from 1.3 to 68.7% for calves. Non-O157 STEC were more prevalent in dairy cattle than *E. coli* O157 (Table 4). In addition to detecting strains that belonged to 39 O serogroups, a total of 77 STEC serotypes were isolated from dairy cattle feces (Table 4). Of these, 15 (O2:H29, O22:H8, O26:H11, O103:H2, O103:H$^-$, O105:H18, O111:H8, O111:H$^-$, O113:H21, O145:H$^-$, O153:H25, O163:H19, OX3:H21, OUT:H2, and OUT:H25) are known to cause HUS and 20 (O7:H4, O15:H27, O22:H40, O22:H$^-$, O25:H$^-$, O45:H2, O45:H$^-$, O70:H11, O84:H2, O91:H$^-$, O113:H2, O113:H4, O119:H$^-$, O125:H$^-$, O146:H21, O171:H2, OR:H$^-$, OUT:H18, OUT:H21, and OUT:H$^-$) are known to cause other illnesses (WHO, 1998).

In contrast to the *E. coli* O157:H7 data, there was no clear age effect on the prevalence of non-O157 STEC (Table 4). Interestingly, Nielsen et al. (2002) reported

Table 4. Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in dairy cattle.

		Cows		Heifers[1]		Calves[2]		
Country	Year	no.	%	no.	%	no.	%	Reference
US								
WA and WI	Not reported	1/662	0.2	12/394	3.0	5/210	2.3	Wells et al., 1991[3]
WI	Not reported	13/154	8.4	32/168	19.0			Wells et al., 1991[4]
AL, CA, CO, CT, GA, OR, IA, ID, IL, IN, MA, ME, MI, MN, NC, NE, NH, NY, OH, PA, RI, TN, VA, VT, WA, and WI						28/6894	0.4	USDA-APHIS-VS, 1994[3]
CA[5]	1993					7/85	8.2	Zhao et al., 1995[3]
FL[5]						1/12	8.3	
MD[5]						1/56	1.8	
NE[5]						2/21	9.5	
NY[5]						3/80	3.8	
OH[5]						1/27	3.7	
TN[5]						1/43	2.3	
WI[5]						3/32	9.4	
CA[6]						1/50	2.0	
MN[6]						2/5	40.0	
NY[6]						4/54	7.4	
OH[6]						1/8	12.5	
VT[6]						1/28	3.6	
WA[6]						3/42	7.1	
WA[5]	1993	3/1750	0.17			7/1732	0.4	Hancock et al., 1994[3]
WA[6]	1993–1994	20/4762	0.4	58/3483	1.7	13/1040	1.3	Hancock et al., 1997a[7]
ID,[5] OR,[5] and WA[5]	1994	19/4505	0.4	72/4419	1.6	6/1385	0.4	Besser and Hancock, 1994[3]
WA[5]	1995	46/545	8.4			10/116	8.6	Besser et al., 1997[3]
NV	1999			2/23[8]	9.5			Thran et al., 2001

Continued

Table 4 (Continued). Prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in dairy cattle.

		Cows		Heifers[1]		Calves[2]		
Country	Year	no.	%	no.	%	no.	%	Reference
Japan	1991			1/10	10.0	30/92	32.6	Tada et al., 1992[3]
	1992–1994	7/387	1.8					Miyao et al., 1998[3]
		94/387[9]	24.3					Miyao et al., 1998
Canada	1992–1993	4/886	0.45			10/592	1.7	USDA-APHIS-VS, 1997[3]
	1992–1993	3/291	1.0			4/115	3.5	Rahn et al., 1997[10]
		1/291	0.3			2/115	1.7	
						2/115	1.7	
	1993	26/291[11]	8.9			19/115[12]	16.5	Rahn et al., 1997
UK	1993–1994	80/3131	2.6	25/177	14.1	44/336	13.1	Mechie et al., 1997[3]
Canada	1996	133/886	15.0			289/592	48.8	Sandhu et al., 1996[3]
Italy	1996–1997			138/186	74.0	39/186	21.0	Conedera et al., 2001[7]
Spain	1996–1997	5/197[13]	2.5	23/114[14]	20.2	8/101[15]	7.9	Orden et al., 2002
Germany	1996–1998	131/726	18.0					Zschöck et al., 2000
Italy	1997–1998	23/137	16.1					Bonardi et al., 1999[3]
Japan	1997–1998	5/27	18.5	28/83	33.7			Ezawa et al., 2004[7]
	1998	45/183[16]	24.6	28/88[17]	31.8	19/87[18]	21.8	Kobayashi et al., 2001
	1998–1999	21/106[19]	19.8	3/7[20]	42.9	57/83[20]	68.7	Shinagawa et al., 2000
Denmark	1999	23/160	14.4	24/76	31.6	41/176	23.3	Nielsen et al., 2002[7]
Brazil	1999–2000	33/63[21]	52.0	33/58[22]	57.0	53/122[23]	44.0	Moreira et al., 2003
Denmark	2000–2001	11/452	2.4	51/499	10.2	34/572	5.9	Rugbjerg et al., 2003[7]

[1]6 mo to 2 yr old.

[2]<6 mo old.

[3]Studies tested only for *E. coli* O157:H7.

[4]The prevalence rate for the heifers included all animals that were <2 yr old. The cattle shed STEC isolates belonging to various serotypes (O10:H$^-$ [a nonmotile isolate], O15:H27, O22:H8, O22:H40, O25:H$^-$, O26:H11, O45:H2, O45:H$^-$, O76:H21, O84:H2, O84:H$^-$, O103:H2, O103:H$^-$, O111:H$^-$, O116:H21, O121:H7, O145:H$^-$, O153:H25, O157:H$^-$, O163:H19, O171:H2, OX3 [the provisional designation for the O174 antigen]:H21, OX3:H$^-$, OR [a rough O antigen]:H2, OR:H8, OR:H$^-$, OUT [an untypeable O antigen]:H$^-$, and OUT:HUT [an untypeable H antigen]).

[5]Control herds (herds in which *E. coli* O157 was not previously isolated).

[6]Case herds (herds in which *E. coli* O157 was previously isolated).

[7]The *E. coli* O157 isolates were not typed for the H antigen.

[8]One heifer shed STEC isolates belonging to *E. coli* O26:H$^-$, and the other heifer shed an untypeable isolate.

[9]The cows shed STEC isolates belonging to various serotypes (O2:H25, O2:H29, O16:H2, O16:H21, O22:H8, O22:H$^-$, O42:H25, O45:H8, O45:H$^-$, O70:H8, O70:H11, O74:H19, O74:H52, O74:HUT, O84:H8, O84:H$^-$, O87:H8, O105:H18, O109:H$^-$, O113:H21, O113:H$^-$, O119:H16, O132:H2, O136:H1, O136:H16, O145:H25, O145:H$^-$, O146:H21, O153:H19, O153:H25, O153:HUT, O156:H25, OUT:H7, OUT:H8, OUT:H16, OUT:H18, OUT:H19, OUT:H$^-$, and OUT:HUT) with O45:H8, O45:H$^-$, and O145:H$^-$ being the most frequently isolated serotypes.

[10]The prevalence data as illustrated in the 3 consecutive rows were based on 3 fecal samplings (0, 1, and 3 mo, respectively) of the same herd. No animals tested positive at more than one sampling time.

[11]The cows shed STEC isolates belonging to the serotypes O2:H29, O15:H7, O98:H25, O111:H8, O113:H2, O119:H$^-$, O153:H25, O156:H$^-$, OUT:H21, OUT:H25, and OUT:H$^-$.

[12]The calves shed STEC isolates belonging to the serotypes O7:H4, O8:H9, O26:H11, O45:H2, O103:H$^-$, O113:H4, O153:H25, O156:H$^-$, OUT:H2, OUT:H4, OUT21, and OUT:H$^-$.

[13]Each cow shed a STEC isolate that belonged to a different serogroup (O84, O87, O110, O113, and O136).

[14]The serogroups of the STEC isolates and the number of heifers shedding these isolates were O2 (3), O5 (4), O8 (5), O21 (1), O22 (5), O74 (1), O91 (1), O98 (2), O113 (4), O116 (1), O172 (4), O174 (3), O175 (4), and OUT (11).

[15]The serogroups of the STEC isolates and the number of calves shedding these isolates were O4 (2), O8 (2), O23 (3), O103 (3), O111 (5), and O174 (1).

[16]The serogroups of the STEC isolates and the number of cows shedding these isolates were O8 (1), O15 (4), O22 (2), O26 (4), O38 (2), O55 (1), O73 (1), O84 (4), O88 (1), O103 (2), O104 (1), O111 (2), O113 (3), O116 (2), O125 (1), O136 (2), O153 (2), O157 (1), O158 (1), O163 (1), and OUT (7).

[17]The serogroups of the STEC isolates and the number of heifers shedding these isolates were O1 (1), O2 (1), O8 (4), O22 (2), O26 (1), O38 (1), O84 (1), O103 (1), O113 (2), O116 (3), O119 (2), O123 (1), O136 (1), and OUT (7).

[18]The serogroups of the STEC isolates and the number of calves shedding these isolates were O8 (3), O26 (4), O28 (1), O84 (1), O113 (3), O116 (1), O136 (1), O163 (1), and OUT (4).

[19]The serogroups of the STEC isolates were O26, O111, and O157.

[20]The STEC isolates were untypeable.

[21]STEC isolates that belonged to the serotypes O91:H$^-$ and O157:H$^-$.

[22]The heifers (12 to 24 mo old) shed STEC isolates that belonged to the O157:H$^-$ serotype.

[23]The calves (<12 mo old) shed STEC isolates belonged to the serotypes O29:H$^-$, O91:H$^-$, O112:H$^-$, O119:H$^-$, O125:H$^-$, and O157:H$^-$.

variations in prevalence of non-O157 STEC within Danish dairy calves. A higher prevalence rate (8.6% vs. 0.7%) was found for older (2 to 6 mo) than for younger (<2 mo) calves. Rugbjerg et al. (2003) also reported a higher prevalence rate (11.8% vs. 2.1%) in older (3 to 4 mo) than in younger (1 to 2 mo) calves.

PREVALENCE OF STEC IN RAW MILK

Prevalence of STEC in raw milk was determined in a limited number of studies focusing on *E. coli* O157:H7 in bulk tanks (Wells et al., 1991; Murinda et al., 2002). In the US, a low prevalence rate (2 of 268; 0.75%) was reported in Tennessee (Murinda et al., 2002) and a relatively higher rate (1 of 23; 4.3%) was reported in Wisconsin and Washington State (Wells et al., 1991). Similar (2 of 35; 5.7%) and higher (6 of 37; 16.2%) prevalence rates were reported in the UK (Mechie et al., 1997) and Canada (Cardinal, 1993), respectively. A 2-yr study of bulk milk in the UK (McKee et al., 2003) revealed a lower prevalence rate for STEC, in general, than those reported for *E. coli* O157:H7 (Wells et al., 1991; Cardinal, 1993; Mechie et al., 1997). The presumed route of STEC transmission to raw milk is fecal contamination during milking. This could be eliminated by improving sanitation during the milking process, as shown in a Taiwanese study (Chiueh et al., 2002). Raw milk samples from 407 cows tested negative for STEC, although 8 cows in the production herd tested positive (Chiueh et al., 2002).

DAIRY CATTLE AS TRANSIENT CARRIERS OF STEC

Prevalence rates of STEC in cattle have significant fluctuations over time (Hancock et al., 1998). Repeated fecal testing of cattle herds demonstrated that STEC are, at least occasionally, present on most farms (Hancock et al., 1997a, b). In general, cattle have not been reported as long-term carriers of STEC, and fecal shedding has not been associated with any cattle disease (Garber et al., 1995; Hancock et al., 1997b,c). Studies with dairy cattle showed the transient presence of *E. coli* O157:H7 (Hancock et al., 1994; 1997a). In an initial study (Hancock et al., 1994), *E. coli* O157:H7 was found on 5 of the 60 dairy farms that were sampled once. In a following study (Hancock et al., 1997a), 8 of the initially negative farms were sampled monthly for 3 to 12 mo, and *E. coli* O157:H7 was detected in 4 of those farms. It was concluded, therefore, that repeated sampling is needed to establish an accurate prevalence status of STEC.

Fecal shedding of STEC by dairy cattle has been shown to follow a seasonal trend. In the US, the highest prevalence rates of *E. coli* O157:H7 in dairy cattle were in warm weather (Hancock et al., 1997a,b; Garber et al., 1999), which is consistent with the timing of most human illness outbreaks (USDA-APHIS-VS, 1997). Monthly fecal sampling of dairy cattle in Idaho, Oregon, and Washington State (Hancock et al., 1997b) over a 6-mo period (July to December) revealed a sharp decline in *E. coli* O157:H7 prevalence in cold weather (November and December). An investigation of *E. coli* O157 on 9 dairy herds for 13 mo revealed prevalence rates that were several times higher in warm (June to October) than in cold (December to March) weather (Hancock et al., 1997a). The prevalence of *E. coli* O157:H7 peaked at 2.6% in June and was lowest (0%) in December. In another study covering 20 states (CA, FL, ID, IL, IN, IA, KY, MI, MN, MO, NM, NY, OH, OR, PA, TN, TX, VT, WA, and WI), Garber et al. (1999) tested 4361 fecal samples from cows on 91 dairy farms over a 6-mo period (February to July) and reported a higher prevalence rate (52.9% vs. 7.0%) in the summer than in the spring. Herds in the Southern states had a higher prevalence rate (61.9% vs. 12.9%) than those in the Northern states. Hancock et al. (1997b) observed a significant correlation between multiple fecal testing of dairy herds and prevalence of *E. coli* O157:H7 and reported that some herds had tendencies to have higher or lower prevalence rates that were somewhat stable over time.

In a 1-yr study, similar seasonal effects on *E. coli* O157:H7 prevalence in dairy cows, heifers, and calves were detected in Canada, Denmark, Italy, and the UK. In the UK (Mechie et al., 1997), testing of 3593 fecal samples showed the highest prevalence rates (average, 8.3%) to occur from May to July and the lowest rate (0%) to occur from December to April. In Denmark (Rugbjerg et al., 2003), testing of 1706 fecal samples also showed a higher prevalence rate in summer than in winter (9.9% vs. 2.9%). In Northern Italy (Bonardi et al., 1999), testing of 450 fecal samples revealed a higher prevalence rate (17.5% vs. 2.9%) in the warm months (April to October) than in the cold months (November to January). In the same region (Conedera et al., 2001), testing of 650 fecal samples showed higher prevalence rates in August and September (average, 20%) than in January and February (average, 15%). In Canada (Van Donkersgoed et al., 1999), testing of 1247 fecal samples from culled cows at slaughter also showed *E. coli* O157:H7 to be most prevalent from June to August. Except for one investigation (Thran et al., 2001), no studies evaluating prevalence of non-O157 STEC over time were found. Testing fecal samples from 23 heifers over 1 yr resulted in detecting STEC only during winter at a prevalence rate of 9.5% (Thran et al., 2001). Interestingly, the STEC isolates belonged to 2 non-O157 serotypes (O26:H$^-$ and OUT:HUT).

Table 5. Virulence factors[1] in *Escherichia coli* O157[2] from dairy cattle.

Toxin genes		Other genes		
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Reference
+	–	ND[3]	ND	Wells et al., 1991[4]; Faith et al., 1996
		+		Sandhu et al., 1996
			+	Byrne et al., 2003
–	+	ND	ND	Montenegro et al., 1990; Ostroff et al., 1990[4]; Wells et al., 1991; Tada et al., 1992[4]; Faith et al., 1996; Wilson et al., 1996; Cerqueira et al., 1999; Furuhata et al., 1999[4]; Conedera et al., 2001
		+		Sandhu et al., 1996; Wilson et al., 1996; Heuvelink et al., 1998[5]; Bonardi et al., 1999[5]
			+	Allerberger et al., 2001[4,6]; Lahti et al., 2002[6]; Murinda et al., 2002; Byrne et al., 2003
			–	Bielaszewska et al., 2000[6]; Lahti et al., 2002[6]
+	+	ND	ND	Wells et al., 1991[2,6]; Tada et al., 1992; Faith et al., 1996; Conedera et al., 2001
		+		Sandhu et al., 1996; Heuvelink et al., 1998[5]; Jung et al., 2000
			+	Kobayashi et al., 2001[5]; Murinda et al., 2002; Byrne et al., 2003
			–	Byrne et al., 2003

[1]The virulence factors include Shiga toxin 1 (*stx*$_1$), Shiga toxin 2 (*stx*$_2$), *E. coli* attaching and effacing (*eae*), and enterohemolysin (*ehxA*) genes.

[2]All isolates were *E. coli* O157:H7 unless indicated otherwise.

[3]ND = Not determined.

[4]Studies did not test for presence of the toxin genes but examined their expression (e.g., cytotoxicity).

[5]The *E. coli* O157 isolates were not typed for the H antigen.

[6]A nonmotile *E. coli* O157 isolate.

PATHOGENICITY OF STEC FROM DAIRY CATTLE

A few studies evaluated distribution of the virulence genes among STEC isolates from dairy cattle (Sandhu et al., 1996; Wilson et al., 1996; Byrne et al., 2003). For example, Wilson et al. (1996) reported the presence of the *eae* gene in 37% of O157:H7 and non-O157:H7 STEC isolates. Sandhu et al. (1996) also showed that the *eae* gene was more frequently found (42% vs. 18%) in STEC from calves than from cows. In the same study, 35% of *E. coli* O157:H7 strains were positive for the *eae* gene. Of the *eae*-positive isolates, 73% produced only Stx1, suggesting a strong association between certain O serogroups (O5, O26, O69, O84, O103, O111, O145, and O157) and the *eae* gene. It was concluded, therefore, that Stx1 production is more frequently associated with *eae*-positive than with *eae*-negative STEC strains (Sandhu et al., 1996). In another study, 57 *E. coli* O157:H7 isolates were tested for the presence of the *stx*$_1$, *stx*$_2$, *eae*, and *ehxA* genes (Byrne et al., 2003). Results showed 67% of the isolates to have the 4 genes, whereas the remaining isolates had various combinations of 3 genes at different rates (19% had *stx*$_1$, *eae*, and *ehxA*; 9% had *stx*$_2$, *eae*, and *ehxA*; and 5% had *stx*$_1$, *stx*$_2$, and *eae*). The presence of these virulence factors was evaluated for O157 (Table 5) and non-O157 STEC (Table 6) isolates from dairy cattle.

With regard to *E. coli* O157 (Table 5), some isolates had *stx*$_1$, *stx*$_2$, or both genes. The ability of this serotype, as well as other non-O157:H7 STEC strains, to cause human illnesses depends on the production of Stx1, Stx2, or both toxins (Karmali et al., 1985). All *E. coli* O157:H7 isolates that were tested for the presence of the *eae* gene gave positive results (Table 5). Except for one isolate (Byrne et al., 2003), all of the tested ones also were positive for the *ehxA* gene. Beutin et al. (1994) investigated human infections with O157 and non-O157 STEC (O26:H11, O111:H8, and O145:H$^-$) and reported that they had the *eae* and *ehxA* genes at high rates (92 and 88%, respectively). Beutin et al. (1994) also reported that *E. coli* O157:H7 was responsible for the most severe illnesses (hemorrhagic colitis and HUS). The data in Table 5, therefore, suggest a high level of pathogenicity for several *E. coli* O157:H7 isolates of dairy cattle origin (Murinda et al., 2002; Byrne et al., 2003).

Table 6 shows that 44 STEC serotypes and members of 2 O serogroups (O87 and O88) had only *stx*$_1$, whereas 69 serotypes and members of 11 O serogroups (O1, O8, O15, O21, O28, O104, O111, O158, O172, O174, and O175) had only *stx*$_2$. Additionally, 41 serotypes and members of 6 O serogroups (O23, O38, O55, O73, O123, and O125) had both genes. Although isolates from 4 serotypes had only *stx*$_1$ (OUT:H$^-$ and OUT:HUT) or *stx*$_2$ (OUT:H8 and OUT:H21), the isolates from each of the remaining serotypes exhibited different toxin genotypes. For example, isolates from each of the O22:H$^-$, O98:H$^-$, O113:H21, O145:H21, O153:H25, O153:H$^-$, and OUT:H7 serotypes had *stx*$_1$ or *stx*$_2$. Isolates from each of the O105:H18 and O111:H$^-$ serotypes had *stx*$_1$ or both *stx*$_1$ and *stx*$_2$, whereas those from each of the O22:H16, O45:H8, O45:H9, O45:HUT, O82:H8, O116:H21, O153:H19, O153:HUT, O157:H$^-$, OUT:H16,

Table 6. Virulence factors[1] in non-O157 Shiga toxin-producing *Escherchia coli* from dairy cattle.

Toxin genes		Other genes			
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Serotype[2] or serogroup	Reference
+	–	ND[3]	ND	O3:H^{-}[4], O10:H21, **O105:H18**, and O136:H12	Montenegro et al., 1990
				O10:H^{-}, **O26:H11**, O45:H2, O45:H^{-}, O76:H21, O84:H2, **O103:H2**, **O103:H^{-}**, **O111:H^{-}**, O121:H7, **O153:H25**, **OUT[5]:H^{-}**, and OUT:HUT[6]	Wells et al., 1991[7]
				O1:H7, **O5:H^{-}**, **O26:H11**, **O26:H^{-}**, O98:H25, **O98:H^{-}**, **O103:H2**, **O103:H^{-}**, O104:H32, **O111:H^{-}**, **O113:H21**, O116:H16, O145:H8, O145:H11, O145:H16, O153:H^{-}, and OUT:HUT	Tada et al., 1992[7]
				O22:H^{-}, O70:H11, O74:H52, **O113:H21**, O136:H1, **O145:H25**, O156:H25, OUT:H7, and **OUT:H^{-}**	Miyao et al., 1998[7]
				OUT:H^{-}	Cerqueira et al., 1999
				O18:H25, **O26:H11**, **O26:H^{-}**, **O103:H2**, **O103:H^{-}**, O105:H8, **O105:H18**, **O111:H^{-}**, O126:H^{-}, O153:H2, and **OUT:H^{-}**, OUT:HUT	Furuhata et al., 1999[7]
				O26:H^{-}	Thran et al., 2001
		+		**O5:H^{-}**, **O103:H2**, and O119:H25	Wilson et al., 1996
				O5, O84, O87, O98, and O103	Orden et al., 2002
			+	O26 and O103	Kobayashi et al., 2001
				O26	Zschöck et al., 2000
			–	O26	Kobayashi et al., 2001
		–	ND	O91:H41 and O146:H21	Wilson et al., 1996
				O26:H46, **O26:H^{-}**, and **OUT:H^{-}**	Jung et al., 2000
				OUT and O136	Orden et al., 2002
			+	O26, O84, O88, and O136	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O26	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
–	+	ND	ND	**O22:H8**, O39:H40, O75:H8, O82:H8, **O91:H10**, **O113:H21**, O116:H21, O126:H20, O126:H21, O139:H8, OUT:H16, and OUT:H29	Montenegro et al., 1990
				O22:H8, O116:H21, **O145:H^{-}**, **O163:H19**, O171:H2, OX3[8]:H21, OX3:H^{-}, OR[9]:H2, OR:H8, and OR:H^{-}	Wells et al., 1991
				O76:H8, O84:H^{-}, **O98:H^{-}**, O113:H^{-}, O145:H19, **O145:H^{-}**, OUT:H21, and OUT:HUT	Tada et al., 1992
				O2:H25, **O2:H29**, O16:H2, O16:H21, **O22:H8**, O22:H^{-}, O42:H25, O45:H8, O45:H^{-}, O74:H19, O74:HUT, O84:H^{-}, O109:H16, O109:H^{-}, **O113:H21**, O113:H^{-}, O132:H2, O136:H16, **O145:H^{-}**, O146:H21, O153:H19, **O153:H25**, O153:HUT, OUT:H7, OUT:H8, OUT:H16, OUT:H19, **OUT:H^{-}**, and OUT:HUT	Miyao et al., 1998
				O22:H16, OUT:H18, and OUT:H28	Cerqueira et al., 1999
				O2:H^{-}, O2:H12, **O2:H29**, O45:H9, O45:H^{-}, O45:HUT, O82:H8, O82:HUT, O116:H21, O124:H19, O126:H19, O126:H^{-}, O132:H2, O132:H9, O132:H^{-}, O153:H19, O153:H42, O153:H^{-}, O163:H9, OUT:H19, OUT:H21, OUT:H49, **OUT:H^{-}**, and OUT:HUT	Furuhata et al., 1999
				OUT:HUT	Thran et al., 2001
		+		O80:H^{-}	Wilson et al., 1996
				O172	Orden et al., 2002
			+	O111	Kobayashi et al., 2001
			–	O104	Kobayashi et al., 2001
		–	ND	O7:H4 and O132:H^{-}	Wilson et al., 1996
				OUT:H21 and **OUT:H^{-}**	Jung et al., 2000
				O2, O8, O21, O22, O74, O91, O113, O116, O174, O175, and OUT	Orden et al., 2002
			+	O28, O113, O116, O153, and O163	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O1, O2, O15, O113, and O158	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
				OUT:H7 and OUT:H21	Chiueh et al., 2002
+	+	ND	ND	O82:H8, O82:H40, O104:H21, O116:H21, O156:H21, and OR:H18	Montenegro et al., 1990
				O15:H27 and **O22:H8**	Wells et al., 1991
				O111:H^{-}	Tada et al., 1992

Continued

Table 6 (Continued). Virulence factors[1] in non-O157 Shiga toxin-producing *Escherchia coli* from dairy cattle.

Toxin genes		Other genes			
stx$_1$	*stx*$_2$	*eae*	*ehxA*	Serotype[2] or serogroup	Reference
				O45:H8, O45:H$^-$, O70:H8, O84:H8, O87:H8, O105:H18, OUT:H8, OUT:H16, OUT:H18, and **OUT:H$^-$**	Miyao et al., 1998
				O22:H16, O82:H8, OUT:H21, and OR:H19	Cerqueira et al., 1999
				O28ac[10]:H25, O45:H9, O45:H28, O45:HUT, O88:H25, **O91:H21**, **O111:H$^-$**, O126:H$^-$, O153:H19, O158:H16, O163:H9, O163:H49, OUT:H9, OUT:H11, and OUT:HUT	Furuhata et al., 1999
		+		O111	Orden et al., 2002
		–	ND	O111:HUT, O153:HUT, and **OUT:H2**	Jung et al., 2000
				O22 and O23	Orden et al., 2002
			+	O22, O38, O123, O125, and O163	Kobayashi et al., 2001
				OUT:HUT	Vernozy-Rozand et al., 2002
			–	O22, O38, O55, and O73	Kobayashi et al., 2001
				OUT:H51	Chiueh et al., 2002

[1]The virulence factors include Shiga toxin 1 (*stx*$_1$), Shiga toxin 2 (*stx*$_2$), *E. coli* attaching and effacing (*eae*), and enterohemolysin (*ehxA*) genes.

[2]The serotypes in bold are known (WHO, 1998; Blanco et al., 2003) to cause hemolytic uremic syndrome.

[3]ND = Not determined.

[4]H$^-$ = A nonmotile *E. coli* isolate.

[5]OUT = An untypeable O antigen.

[6]HUT = An untypeable H antigen.

[7]Studies did not test for presence of the toxin genes but examined their expression (e.g., cytotoxicity).

[8]OX3 = Provisional designation for the O174 antigen.

[9]OR = A rough O antigen.

[10]Reflecting a certain antigenic relationship within the O28 serogroup in which "a" represents the common factor and "c" represents a specific factor (Lior, 1994).

OUT:H18, OUT:H21, OUT:H$^-$, and OUT:HUT serotypes had *stx*$_2$ or both *stx*$_1$ and *stx*$_2$. Finally, isolates from each of the serotypes O45:H$^-$ and O126:H$^-$ had *stx*$_1$, *stx*$_2$, or both genes. Table 6 shows that 20 STEC serotypes (in bold) commonly isolated from dairy cattle are known to cause HUS (WHO, 1998; Blanco et al., 2003). Several other serotypes (O1:H7, O7:H4, O15:H27, O22:H16, O22:H$^-$, O45:H2, O45:H$^-$, O70:H11, O80:H$^-$, O82:H8, O84:H2, O104:H21, O116:H21, O126:H21, O126:H$^-$, O132:H$^-$, O145:H16, O146:H21, O153:H2, O171:H2, OR:H$^-$, OUT:H18, OUT:H19, and OUT:H21) in Table 6 are also known to cause diarrhea, bloody diarrhea, or hemorrhagic colitis (WHO, 1998).

Based on potential toxicity by having *stx*$_1$ and/or *stx*$_2$ genes, it is clear from Table 6 that it is not necessary for an isolate to have and express both genes to cause human illnesses. With regard to other virulence genes (*eae* and *ehxA*), unfortunately, the 20 STEC serotypes in Table 6 that are known to cause HUS were not evaluated for presence of the *ehxA* gene. Of these serotypes, 5 (O5:H$^-$, O26:H$^-$, O103:H2, OUT:H2, and OUT:H$^-$) were evaluated for presence of the *eae* gene, and only 2 (O5:H$^-$ and O103:H2) tested positive. The ability of these *eae*-positive isolates to produce a functional intimin, however, was not reported. Investigating STEC pathogenicity (HUS cases) as affected by the presence of *eae* and *ehxA* genes (Karch et al., 1997) revealed that most STEC isolates were *eae*-positive. Several STEC strains involved in severe human illnesses (e.g., HUS), however, have lacked this gene or did not express a functional intimin (Keskimäki et al., 1997). This suggests that STEC strains lacking the *eae* gene can colonize the small intestine by expressing additional adherence factors. Studies of STEC in HUS patients showed that both *eae* and *ehxA* genes were found in virtually all *E. coli* O157 strains (Beutin et al., 1994) but were more variably present among the non-O157 strains (Neill, 1997). The data in Tables 5 and 6 support this conclusion. Although the *eae* and *ehxA* genes appear to be more common in the STEC strains associated with human illnesses, they are not absolutely required for pathogenicity, as strains lacking these genes have been shown to cause human illnesses (Neill, 1997).

CONCLUSIONS

The role of dairy cattle and their products in human infections with STEC was evaluated. These pathogens cause human illnesses ranging from mild diarrhea to the life-threatening HUS. Most STEC outbreaks were traced to the consumption of ground beef or dairy products. Contact with dairy farm environments by urban visitors also caused outbreaks of human illnesses. Long-term and short-term testing of dairy cattle feces world-

wide showed high prevalence rates for O157 (ranging from 0.2 to 48.8%) and non-O157 STEC (ranging from 0.4 to 74.0%). Shiga toxin-producing *E. coli* were also isolated from raw milk, milk filters, and dairy products such as yogurt and soft cheeses. Dairy cattle, therefore, are considered reservoirs of STEC. The data summarized in this review showed 193 STEC serotypes to derive from dairy cattle origin. Of these serotypes, 24 have been isolated from HUS patients. Considering the wide distribution of STEC on dairy farms, the high prevalence rates reported, and the isolation of several serotypes of high virulence from dairy cattle or their products, long-term strategies to assure safety of foods from dairy cattle should be developed. These strategies should focus on establishing educational programs to bring awareness of the STEC problem to dairy farmers, processors, and consumers. Developing and implementing pre- and postharvest control methods to effectively decrease STEC carriage by dairy cattle and to eliminate contamination of their products during processing are essential steps toward sustaining a competitive dairy industry.

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REFERENCES

Abdul-Raouf, U. M., M. S. Ammar, and L. R. Beuchat. 1996. Isolation of *Escherichia coli* O157:H7 from some Egyptian foods. Int. J. Food Microbiol. 29:423–426.

Adams, M., and Y. Motarjemi. 1999. Basic Food Safety for Health Workers. World Health Organization, Geneva, Switzerland.

Allerberger, F., M. Wagner, P. Schweiger, H.-P. Rammer, A. Resch, M. P. Dierich, A. W. Friedrich, and H. Karch. 2001. *Escherichia coli* O157 infections and unpasteurised milk. Eurosurveillance 6:147–151.

Anonymous. 1994. Two clusters of haemolytic uraemic syndrome in France. Commun. Dis. Rep. Wkly. 4:1.

Besser, T. E., and D. D. Hancock. 1994. Surveillance for *Escherichia coli* O157:H7: Seasonal variation and duration of infection in cattle. Pages FS13–FS16 in Proc. 8th Int. Congr. Anim. Hygiene. Int. Soc. Anim. Hygiene, St. Paul, MN.

Besser, T. E., D. D. Hancock, L. C. Pritchett, E. M. McRae, D. H. Rice, and P. I. Tarr. 1997. Duration of detection of fecal excretion of *Escherichia coli* O157:H7 in cattle. J. Infect. Dis. 175:726–729.

Beutin, L., S. Aleksic, S. Zimmermann, and K. Gleier. 1994. Virulence factors and phenotypical traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. Med. Microbiol. Immunol. 183:13–21.

Bielaszewska, M., S. Herbert, L. Almut, P. Rita, R. Wolfgang, T. Helmut, C. Alois, J. Jan, B. Kveta, and K. Helge. 2000. Cattle can be a reservoir of sorbitol-fermentation Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains and a source of human disease. J. Clin. Microbiol. 38:3470–3473.

Blanco, J., M. Blanco, J. E. Blanco, A. Mora, M. P. Alonso, E. A. González, and M. I. Bernárdez. 2001. Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants. Pages 113–148 in Verocytotoxigenic *Escherichia coli*. G. Duffy, P. Garvey, and D. McDowell, ed. Food and Nutrition Press Inc., Trumbull, CT.

Blanco, J., M. Blanco, J. E. Blanco, A. Mora, E. A. González, M. I. Bernárdez, M. P. Alonso, A. Coira, A. Rodriguez, J. Rey, J. M. Alonso, and M. A. Usera. 2003. Verotoxin-producing *Escherichia coli* in Spain: Prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp. Biol. Med. 228:345–351.

Bonardi, S., E. Maggi, A. Bottarelli, M. L. Pacciarini, A. Ansuini, G. Vellini, S. Morabito, and A. Caprioli. 1999. Isolation of Verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. Vet. Microbiol. 67:203–211.

Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. C. Duncan. 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157:H7. Lancet i:98.

Bürk, C., I. G. B. Braumiller, H. Becker, and E. Märtlbauer. 2002. Nuclease fluorescence assay for the detection of verotoxin genes in raw milk. Lett. Appl. Microbiol. 35:153–156.

Byrne, C. M., I. Erol, J. E. Call, C. W. Kaspar, D. R. Buege, C. J. Hiemke, P. J. Fedorka-Cray, A. K. Benson, F. M. Wallace, and J. B. Luchansky. 2003. Characterization of *Escherichia coli* O157:H7 from downer and healthy dairy cattle in the upper Midwest region of the United States. Appl. Environ. Microbiol. 69:4683–4688.

Cardinal, P. 1993. Isolation of *Escherichia coli* O157:H7 and detection of verotoxigenic *E. coli* in food and cattle feces in Quebec in 1990–1991. Pages 141–147 in *Escherichia coli* O157:H7 and Other Verotoxigenic *E. coli* in Foods. E. C. D. Todd and J. M. MacKenzie, ed. Food Directorate, Health Protection Branch, Health and Welfare Canada, and the Canadian Meat Council, Ottawa, Canada.

CDC. 1995. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21-Helena, Montana, 1994. Centers for Disease Control and Prevention. Morbid. Mortal. Wkly. Rep. 44:501–503.

CDC. 2000. Outbreak of *Escherichia coli* O157:H7 infection associated with eating fresh cheese curds—Wisconsin, June 1998. Centers for Disease Control and Prevention. Morbid. Mortal. Wkly. Rep. 49:911–913.

CDSC. 1996. VTEC O157 infection in West Yorkshire associated with the consumption of raw milk. Communicable Disease Surveillance Centre. Commun. Dis. Rep. Wkly. 6:181.

CDSC. 1998. *Escherichia coli* O157 infection and unpasteurized cream in England. Communicable Disease Surveillance Centre. Eurosurveillance Wkly. 2:981022.

CDSC. 1999a. VTEC O157 phage type 21/28 infection in North Cumbria. Update. Commun. Dis. Rep. CDR Wkly. 9:105.

CDSC. 1999b. *Escherichia coli* O157 associated with eating unpasteurised cheese – Update. Commun. Dis. Rep. CDR Wkly. 9:131–134.

Cerqueira, A. M. F., B. E. C. Guth, R. M. Joaquim, and J. R. C. Andrade. 1999. High occurrence of Shiga toxin-producing *Escherichia coli* (STEC) in healthy cattle in Rio de Janeiro State, Brazil. Vet. Microbiol. 70:111–121.

Chapman, P. A., C. A. Siddons, A. T. Cerdan Malo, and M. A. Harkin. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol. Infect. 119:245–250.

Chapman, P. A., D. J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infection in man. Epidemiol. Infect. 111:439–447.

Chiueh, L., F. Liu, and D. Y. Shih. 2002. Prevalence of Shiga toxin-producing *Escherichia coli* in feed and raw milk of domestic cattle and sheep. J. Food Drug Anal. 10:39–46.

Cieslak, P. R., T. J. Barrett, and P. M. Griffin. 1993. *Escherichia coli* O157:H7 infection from a manured garden. Lancet 342:367.

Clarke, R. C., J. B. Wilson, S. C. Read, S. Renwick, K. Rahn, R. P. Johnson, D. Alves, M. A. Karmali, H. Lior, S. A. McEwen, J. Spika, and C. L. Gyles. 1994. Verocytotoxin-producing *Escherichia coli* (VTEC) in the food chain: Preharvest and processing perspectives. Pages 17–24 in Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. M. A. Karmali and A. G. Goglio, ed. Elsevier Science, Amsterdam, The Netherlands.

Coia, J. E., Y. Johnston, N. J. Steers, and M. F. Hanson. 2001. A survey of the prevalence of *Escherichia coli* O157 in raw meats,

raw cow's milk and raw-milk cheeses in south-east Scotland. Int. J. Food Microbiol. 66:63–69.
Conedera, G., P. A. Chapman, S. Marangon, E. Tisato, P. Dalvit, and A. Zuin. 2001. A field survey of *Escherichia coli* O157 ecology on a cattle farm in Italy. Int. J. Food Microbiol. 66:85–93.
Correa, M. T., H. N. Erb, and J. M. Scarlett. 1993. Risk factors for downer cow syndrome. J. Dairy Sci. 76:3460–3463.
Crump, J. A., A. J. Langer, R. Gage, M. Baysinger, G. Withers, D. M. Toney, S. B. Hunter, M. Hoekstra, S. K. Wong, P. M. Griffin, and T. J. Van Gilder. 2002. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N. Engl. J. Med. 347:555–560.
Curnow, J. 1994. *E. coli* O157:H7 phage type 28 infections in Grampian. Commun. Dis. Environ. Health Scotland Wkly. Rep. 28:1.
Dean-Nystrom, E. A., B. T. Bosworth, W. C. Cray, Jr., and H. W. Moon. 1997. Pathogenecity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. Infect. Immun. 65:1842–1848.
Deschênes, G., C. Casenave, F. Grimont, J. C. Desenclos, S. Benoit, M. Collin, S. Baron, P. Marian, P. A. Grimont, and H. Nivet. 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurised cheese. Pediatr. Nephrol. 10:203–205.
Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289–302.
Ezawa, A., F. Gocho, K. Kawata, T. Takahashi, and N. Kikuchi. 2004. High prevalence of enterohemorrhagic *Escherichia coli* (EHEC) O157 from cattle in selected regions of Japan. J. Vet. Med. Sci. 66:585–587.
Faith, N. G., J. A. Shere, R. Brosch, K. W. Arnold, S. E. Ansay, M. S. Lee, J. B. Luchansky, and C. W. Kaspar. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. Appl. Environ. Microbiol. 62:1519–1525.
Fitzgerald, A. C., T. S. Edrington, M. L. Looper, T. R. Callaway, K. J. Genovese, K. M. Bischoff, J. L. McReynolds, J. D. Thomas, R. C. Anderson, and D. J. Nisbet. 2003. Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and Salmonella in dairy cattle. Lett. Appl. Microbiol. 37:392–398.
Franke, S., D. Harmsen, A. Caprioli, D. Pierard, L. H. Wieler, and H. Karch. 1995. Clonal relatedness of Shiga-like toxin-producing *Escherichia coli* O101 strains of human and porcine origin. J. Clin. Microbiol. 33:3174–3178.
Furuhata, K., S. Sakata, T. Okamoto, S. Yamamoto, M. Honda, A. Kai, T. Ito, M. Hara, K. Tabuchi, and M. Fukuyama. 1999. Prevalence and serotypes of verocytotoxin-producing *Escherichia coli* (VTEC) isolates from dairy cattle. Kansenshougaku Zasshi 73:445–450.
Gallien, P., H. Richter, H. Klie, M. Timm, H. Karch, K. W. Perlberg, H. Steinrück, S. Riemer, M. Djuren, and D. Protz. 1997. Detection of STEC and epidemiological investigations of a HUS patient. Berl. Münch. Tierärztl. Wochenschr. 110:342–346.
Garber, L. P., S. J. Wells, D. D. Hancock, M. P. Doyle, J. Tuttle, J. A. Shere, and T. Zhao. 1995. Public veterinary medicine: Food safety and handling—Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. JAVMA 207:46–49.
Garber, L., S. Wells, L. Schroeder-Tucker, and K. Ferris. 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. J. Food Prot. 62:307–312.
Georgia Division of Public Health. 2002. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits-Pennsylvania and Washington, 2000. Available: http://www.ph.dhr.state.ga.us/epi/news/apr01/042401.shtml. Accessed Dec. 18, 2003.
Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60–98.
Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. 113:199–207.
Hancock, D. D., T. E. Besser, and D. H. Rice. 1998. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. Pages 85–91 in *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* Strains. J. B. Kaper and A. B. O'Brien, ed. ASM Press, Washington, DC.
Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997a. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. Epidemiol. Infect. 118:193–195.
Hancock, D. D., D. H. Rice, D. E. Herriott, T. E. Besser, E. D. Ebel, and L. V. Carpenter. 1997b. Effects of farm manure handling practices on *Escherichia coli* O157:H7 prevalence in cattle. J. Food Prot. 60:363–366.
Hancock, D. D., D. H. Rice, L. A. Thomas, D. A. Dargatz, and T. E. Besser. 1997c. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J. Food Prot. 60:462–465.
Herriott, D. E., D. D. Hancock, W. E. Keene, and D. H. Rice. 1994. Raw milk associated *E. coli* O157:H7 outbreaks in Oregon. Pages FS17–FS21 in Proc. 8th Int. Congr. Anim. Hygiene. Int. Soc. Anim. Hygiene, St. Paul, MN.
Heuvelink, A. E., F. L. A. M. Van den Biggelaar, J. T. M. Zwartkruis-Nahuis, R. G. Herbes, R. Huyben, N. Nagelkerke, W. J. G. Melchers, L. A. H. Monnens, and E. de Boer. 1998. Occurrence of verotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. J. Clin. Microibiol. 36:3480–3487.
International Association of Milk, Food, and Environmental Sanitarians. 2000. Outbreaks of VTEC O157 infection linked to consumption of unpasteurized milk. Assoc. Milk Food Environ. San. 20:627.
Jackson, S. G., R. B. Goodbrand, R. P. Johnson, V. G. Odorico, D. Alves, K. Rahn, J. B. Wilson, M. K. Welch, and R. Khakhria. 1998. *Escherichia coli* O157:H7 diarrhoea associated with well water and infected cattle on an Ontario farm. Epidemiol. Infect. 120:17–20.
Jung, B., S. Jung, H. Park, G. Cho, and B. Kim. 2000. Characteristics of verotoxin non-producing *Escherichia coli* O157 and verotoxin-producing *E. coli* isolated from healthy cattle. Korean J. Vet. Res. 40:525–531.
Karch, H., H. I. Huppertz, J. Bockemuhl, H. Schmidt, A. Schwarzkopf, and R. Lissner. 1997. Shiga toxin-producing *Escherichia coli* infections in Germany. J. Food Prot. 60:1454–1457.
Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior. 1985. The association between idiopathic hemolytic-uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. 151:775–782.
Keene, W. E., K. Hedberg, D. E. Herriott, D. D. Hancock, R. W. McKay, T. J. Barrett, and D. W. Fleming. 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. J. Infect. Dis. 176:815–818.
Keene, W. E., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N. Engl. J. Med. 331:579–584.
Keskimäki, M., R. Ikäheimo, P. Kärkkäinen, F. Scheutz, Y. Ratiner, R. Puohiniemi, and A. Siitonen. 1997. Shiga toxin-producing *Escherichia coli* serotype OX3:H21 as a cause of hemolytic-uremic syndrome. Clin. Infect. Dis. 24:1278–1279.
Klie, H., M. Timm, H. Richter, P. Gallien, K. W. Perlberg, and H. Steinruck. 1997. Detection and occurrence of verotoxin-forming and/or Shigatoxin producing *Escherichia coli* (VTEC and/or STEC) in milk. Berl. Munch. Tierärztl. Wochenschr. 110:337–341.
Kobayashi, H., J. Shimada, M. Nakazawa, T. Morozumi, T. Pohjanvirta, S. Pelkonen, and K. Yamamoto. 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl. Environ. Microbiol. 67:484–489.
Lahti, E., M. Eklund, P. Ruutu, A. Siitonrn, L. Rantala, P. Nuorti, and T. Honkanen-Buzalski. 2002. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. Eur. J. Clin. Microbiol. Infect. Dis. 21:189–195.
Lior, H. 1994. Classification of *Escherichia coli*. Pages 31–72 in *Escherichia coli* in Domestic Animals and Humans. C. L. Gyles, ed. CAB Int., Wallingford, UK.
Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, J. P. Davis, J. Arnoldi, and J. Tilleli. 1986. Isolation of *Escherichia coli* O157 from dairy cattle

associated with two cases of haemolytic uremic syndrome. Lancet ii:1043.
McKee, R., R. H. Madden, and A. Gilmour. 2003. Occurrence of verocytotoxin-producing *Escherichia coli* in dairy and meat processing environments. J. Food Prot. 66:1576–1580.
Mechie, S. C., P. A. Chapman, and C. A. Siddons. 1997. A fifteen-month study of *Escherichia coli* O157:H7 in a dairy herds. Epidemiol. Infect. 118:17–25.
Miyao, Y., T. Kataoka, T. Nomoto, A. Kai, T. Itoh, and K. Itoh. 1998. Prevalence of verotoxin-producing *Escherichia coli* harbored in the intestine of cattle in Japan. Vet. Microbiol. 61:137–143.
Montenegro, M. A., M. Bülte, T. Trumpf, S. Aleksiá, G. Reuter, E. Bulling, and R. Helmuth. 1990. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J. Clin. Microbiol. 28:1417–1421.
Moreira, C. N., M. A. Pereira, C. S. Brod, D. P. Rodrigues, J. B. Carvalhal, and J. A. G. Aleixo. 2003. Shiga toxin-producing *Escherichia coli* (STEC) isolated from healthy dairy cattle in southern Brazil. Vet. Microbiol. 93:179–183.
Morgan, D., C. P. Newman, D. N. Hutchinson, A. M. Walker, B. Rowe, and F. Majid. 1993. Verotoxin producing *Escherichia coli* O157 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181–187.
Murinda, S. E., L. T. Nguyen, S. J. Ivey, B. E. Gillespie, R. A. Almeida, F. A. Draughon, and S. P. Oliver. 2002. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in East Tennessee. J. Food Prot. 65:752–759.
National Cattlemen's Beef Association. 2001. Cattle and Beef Industry Statistics. National Cattlemen's Beef Association, Centennial, CO.
Neill, M. A. 1997. Overview of Verotoxigenic *Escherichia coli*. J. Food Prot. 60:1444–1446.
Nielsen, E. M., C. Teqtmeier, H. J. Anderson, C. Grønbæk, and J. S. Andersen. 2002. Influence of age, sex and herd characteristics on the occurrence of verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. Vet. Microbiol. 88:245–257.
O'Brien, A. D., and R. K. Holmes. 1987. Shiga and Shiga-like toxins. Microbiol. Rev. 51:206–220.
Orden, J. A., D. Cid, J. A. Ruiz-Santa-Quireria, S. García, S. Martínez, and R. de la Fuente. 2002. Verotoxin-producing *Escherichia coli* (VTEC), enteropathogenic *E. coli* (EPEC) and necrotoxigenic *E. coli* (NTEC) isolated from healthy cattle in Spain. J. Appl. Microbiol. 93:29–35.
Ostroff, S. M., P. M. Griffin, R. V. Tauxe, L. D. Shipman, K. D. Greene, J. G. Wells, J. H. Lewis, P. A. Blake, and J. M. Kobayashi. 1990. A statewide outbreak of *Escherichia coli* O157:H7 infections in Washington State. Am. J. Epidemiol. 132:239–247.
Padhye, N. V., and M. P. Doyle. 1991. Rapid procedure for detecting enterohemorrhagic *Escherichia coli* O157:H7 in food. Appl. Environ. Microbiol. 57:2693–2698.
Paton, J. C., and A. W. Paton. 2000. Shiga toxigenic *Escherichia coli* infections. Sci. Med. May/June:28–37.
Pradel, N., K. Boukhors, Y. Bertin, C. Forestier, C. Martin, and V. Livrelli. 2001. Heterogeneity of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients, cattle, and food samples in central France. Appl. Environ. Microbiol. 67:2460–2468.
Pradel, N., V. Livrell, C. de Champs, J. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. 38:1023–1031.
Rahn, K., S. A. Renwick, R. P. Johnson, J. B. Wilson, R. C. Clarke, D. Alves, S. McEwen, H. Lior, and J. Spika. 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. Epidemiol. Infect. 119:251–259.
Rahn, K., S. A. Renwick, R. P. Johnson, J. B. Wilson, R. C. Clarke, D. Alves, S. A. McEwen, H. Lior, and J. Spika. 1998. Follow-up study of verocytotoxigenic *Escherichia coli* infection in dairy farm family. J. Infect. Dis. 177:1138–1139.
Reid, T. M. S. 2001. A case study of cheese associated *E. coli* O157 outbreaks in Scotland. Pages 201–212 in Verocytotoxigenic *Escherichia coli*. G. Duffy, P. Garvey, and D. McDowell, ed. Food and Nutrition Press Inc., Trumbull, CT.
Reida, P., M. Wolff, H. W. Pohls, W. Kuhlmann, A. Lehmacher, S. Aleksic, H. Karch, and J. Bockemuhl. 1994. An outbreak due to enterohemorrhagic *Escherichia coli* O157:H7 in a children day care center characterized by person-to-person transmission and environmental contamination. Zentralbl. Bakteriol. 281:534–543.
Reitsma, C. J., and D. R. Henning. 1996. Survival of enterohemorrhagic *Escherichia coli* O157:H7 during the manufacture and curing of Cheddar cheese. J. Food Prot. 59:460–464.
Rice, D. H., E. D. Ebel, D. D. Hancock, T. E. Besser, D. E. Herriott, and L. V. Carpenter. 1997. *Escherichia coli* O157 in cull dairy cows on farm and at slaughter. J. Food Prot. 60:1386–1387.
Riemann, H. P., and D. O. Cliver. 1998. *Escherichia coli* O157:H7. Pages 41–48 in The Veterinary Clinics of North America—Food Animal Practice: Microbial Food Borne Pathogens. L. Tollefson, ed. W. B. Saunders Co., Philadelphia, PA.
Rugbjerg, H., E. M. Nielsen, and J. S. Andersen. 2003. Risk factors associated with faecal shedding of verocytotoxin-producing *Escherichia coli* O157 in eight known-infected Danish dairy herds. Prev. Vet. Med. 58:101–113.
Sandhu, K. S., R. C. Clarke, K. McFadden, A. Brouwer, M. Louie, J. Wilson, H. Lior, and C. L. Gyles. 1996. Prevalence of the eaeA gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. Epidemiol. Infect. 116:1–7.
Segelken, R. 1996. *E. coli* screen aims to keep bacteria out of hamburger. Available: http://www.news.cornell.edu/releases/May96/beefQA.hrs.html. Accessed Jan. 19, 2004.
Shinagawa, K., M. Kanehira, K. Omoe, I. Matsuda, D. Hu, D. A. Widiasih, and S. Sugii. 2000. Frequency of Shiga toxin-producing *Escherichia coli* in cattle at a breeding farm and at a slaughterhouse in Japan. Vet. Microbiol. 76:305–309.
Siegler, R. L., M. K. Milligan, T. H. Burningham, R. D. Christofferson, S. Y. Changand, and L. B. Jorde. 1991. Long term renal outcome and prognostic indicators in the hemolytic-uremic syndrome. J. Pediatr. 118:195–200.
Steele, M. L., W. B. McNab, C. Poppe, M. W. Griffiths, S. Chen, S. A. Degrandis, L. C. Fruhner, C. A. Larkin, J. A. Lynch, and J. A. Odumeru. 1997. Survey of Ontario bulk raw milk for foodborne pathogens. J. Food Prot. 60:1341–1346.
Tada, H., S. Itami, Y. Yamamoto, K. Kobayashi, M. Taguchi, and M. Nakazawa. 1992. Detection of verotoxin-producing *Escherichia coli* using polymerase chain reaction from dairy cattle. Kasnenshougaku Zasshi 66:1383–1389.
Thran, B. H., H. S. Hussein, M. R. Hall, and S. F. Khaiboullina. 2001. Occurrence of verotoxin-producing *Escherichia coli* in dairy heifers grazing an irrigated pasture. Toxicology 159:159–169.
Todd, E., R. Szabo, and J. MacKenzie. 1993. Cultural and enzyme-linked antibody methods to detect *Escherichia coli* O157:H7. Pages 39–52 in *Escherichia coli* O157:H7 and Other Verotoxigenic *E. coli* in Foods. E. C. D. Todd and J. M. MacKenzie, ed. Food Directorate, Health Protection Branch, Health and Welfare Canada, and the Canadian Meat Council, Ottawa, Canada.
Troutt, H. F., and B. I. Osburn. 1997. Meat from dairy cows: Possible microbiological hazards and risks. Rev. Sci. Technol. Off. Int. Epizoot. 16:405–414.
USDA. 1992. Agricultural Statistics 1992. Agricultural Statistics Board, USDA, Washington, DC.
USDA-APHIS-VS. 1994. *Escherichia coli* O157:H7 in US Dairy Calves. January Report, National Animal Health Monitoring System. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.
USDA-APHIS-VS. 1996a. Economic opportunities for dairy cow culling management options. Info Sheet. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.
USDA-APHIS-VS. 1996b. New York cull cow study on *Escherichia coli* O157:H7. APHIS Report of FY 1995. Accomplishments in Animal Production Food Safety. USDA Animal and Plant Health

Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.

USDA-APHIS-VS. 1997. An update: *Escherichia coli* O157:H7 in humans and cattle. USDA Animal and Plant Health Inspection Service, Veterinary Service, Centers for Epidemiology and Animal Health, Fort Collins, CO.

Van Donkersgoed, J., T. Graham, and V. Gannon. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. Can. Vet. J. 40:332–338.

Vernozy-Rozand, C., M. P. Montet, F. Lequerrec, E. Serillon, B. Tilly, C. Bavai, S. Ary-Gueniot, J. Bouvet, C. Mazuy-Cruchaudet, and Y. Richard. 2002. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in slurry, farmyard manure and sewage sludge in France. J. Appl. Microbiol. 93:473–478.

Wall, P. G., R. J. McDonnell, G. K. Adak, T. Cheasty, H. S. Smith, and B. Rowe. 1996. General outbreaks of verocytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. Commun. Dis. Rep. CDR Rev. 6:R26–R33.

Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and K. I. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. 29:985–989.

WHO. 1998. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific Working Group Meeting. World Health Organization, Geneva, Switzerland.

Wilson, J. B., R. C. Clarke, S. A. Renwick, K. Rahn, R. P. Johnson, M. A. Karmali, H. Lior, D. Alves, C. L. Gyles, K. S. Sandhu, S. A. McEwen, and J. S. Spika. 1996. Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. J. Infect. Dis. 174:1021–1027.

Wilson, J. B., R. P. Johnson, R. C. Clarke, K. Rahn, S. A. Renwick, D. Alves, M. A. Karmali, P. Michel, E. Orrbine, and J. S. Spika. 1997. Canadian perspectives on verotoxin-producing *Escherichia coli* infection. J. Food Prot. 60:1451–1453.

Wilson, J. B., S. A. McEwen, R. C. Clarke, K. E. Leslie, R. A. Wilson, D. Waltner-Toews, and C. L. Gyles. 1992. Distribution and characteristics of verotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. Epidemiol. Infect. 108:423–439.

Yatsuyanagi, J., S. Saito, and I. Ito. 2002. A case of hemolytic-uremic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O121 infection caused by drinking water contaminated with bovine feces. Jpn. J. Infect. Dis. 55:174–176.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61:1290–1293.

Zschöck, M., H. P. Hamann, B. Kloppert, and W. Wolter. 2000. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: Prevalence and virulence properties. Lett. Appl. Microbiol. 31:203–208.