SUPPLEMENT

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

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In Spain, as in many other countries, verotoxin-producing *Escherichia coli* (VTEC) strains have been frequently isolated from cattle, sheep, and foods. VTEC strains have caused seven outbreaks in Spain (six caused by *E. coli* O157:H7 and one by *E. coli* O111:H$^-$ [nonmotile]) in recent years. An analysis of the serotypes indicated serological diversity. Among the strains isolated from humans, serotypes O26:H11, O111:H$^-$, and O157:H7 were found to be more prevalent. The most frequently detected serotypes in cattle were O20:H19, O22:H8, O26:H11, O77:H41, O105:H18, O113:H21, O157:H7, O171:H2, and OUT (O untypeable):H19. Different VTEC serotypes (e.g., O5:H$^-$, O6:H10, O91:H$^-$, O117:H$^-$, O128:H$^-$, O128:H2, O146:H8, O146:H21, O156:H$^-$, and OUT:H21) were found more frequently in sheep. These observations suggest a host serotype specificity for some VTEC. Numerous bovine and ovine VTEC serotypes detected in Spain were associated with human illnesses, confirming that ruminants are important reservoirs of pathogenic VTEC. VTEC can produce one or two toxins (VT1 and VT2) that cause human illnesses. These toxins are different proteins encoded by different genes. Another virulence factor expressed by VTEC is the protein intimin that is responsible for intimate attachment of VTEC and effacing lesions in the intestinal mucosa. This virulence factor is encoded by the chromosomal gene *eae*. The *eae* gene was found at a much less frequency in bovine (17%) and ovine (5%) than in human (45%) non-O157 VTEC strains. This may support the evidence that the eae gene contributes significantly to the virulence of human VTEC strains and that many animal non-O157 VTEC strains are less pathogenic to humans. Exp Biol Med 228:345–351, 2003

Key words: *Escherichia coli*; food safety; cytotoxicity; food-borne pathogens; verotoxins

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Verotoxin-producing *Escherichia coli* (VTEC), including O157:H7, have emerged as food-borne pathogens that can cause severe and potentially fatal human illnesses. They are a major cause of gastroenteritis that may be complicated by hemorrhagic colitis (HC) or the hemolytic uremic syndrome (HUS), which is the main cause of acute renal failure in children. Ruminants, especially cattle and sheep, have been implicated as the principal reservoirs of VTEC. Transmission of these food-borne pathogens occurs through consumption of undercooked

meat, unpasteurized dairy products, vegetables, or water contaminated by ruminant feces. Person-to-person transmission has also been documented (1, 2).

The VTEC are capable of producing one or two potent toxins called verotoxins (VT1 and VT2) that have cytotoxic effects on African Green Monkey kidney (Vero) cells in cultures (3). In addition to toxin production, another virulence-associated factor expressed by VTEC is a protein called intimin, which is responsible for intimate attachment of VTEC to the intestinal epithelial cells, causing attaching and effacing lesions in the intestinal mucosa. Intimin is encoded by the chromosomal gene *eae*, which is part of a pathogenicity island termed the locus for enterocyte effacement. Another factor that may also affect virulence of VTEC (4–6) is the enterohemolysin (i.e., enterohemorrhagic *E. coli* hemolysin [EHEC-*HlyA*]).

Although VTEC strains that cause human illnesses belong to a large number of the O:H serotypes, most outbreaks and sporadic cases of HC and HUS have been attributed to strains belonging to *E. coli* O157:H7 (7–10). Infections with non-O157 VTEC types such as O26:H$^-$ (nonmotile), O26:H11, O91:H$^-$, O103:H2, O111:H$^-$, O113:H21, O118:H16, O128:H2, O145:H$^-$, O145:H28, and O146:H21 were associated with severe human illnesses. Severe diarrhea (especially HC) and HUS were also associated with VTEC serotypes carrying the *eae* gene (6). Non-O157 VTEC serotypes that are negative for the *eae* gene have been rarely implicated in severe human illnesses and have been more frequently found among healthy subjects. However, it should be noted that production of intimin is not essential for pathogenesis because a number of sporadic cases of HUS were caused by *eae*-negative non-O157 VTEC strains. For example, VTEC O104:H21 and O113:H21 strains lacking *eae* gene were responsible for human illness outbreaks in the United States and Australia (11–13).

VTEC Infections in Spain

VTEC in Cattle. Our studies indicated that VTEC colonization is widespread among healthy cattle in Spain. Between 1993 and 1995, 1069 healthy cattle were examined for VTEC (14–17). VTEC-positive cattle were found in 95% of the farms tested, and the proportion of VTEC-positive cattle in each farm ranged from 0% to 100%. The overall prevalence rates of VTEC were 37% in calves and 27% in cows. In Spain, VTEC strains having the VT2 gene or both VT1 and VT2 genes were present in similar proportions in calves and cows. In contrast, VTEC strains having the VT1 and *eae* genes were more commonly recovered from calves than from cows. *E. coli* O157:H7 was detected only in eight (0.7%) of the 1069 cattle examined. Interestingly, the majority of *eae*-positive non-O157 VTEC strains and the eight O157:H7 strains were isolated from calves, confirming that young cattle are the most important reservoir of *eae*-positive VTEC strains (15–17).

Between 1993 and 1999, three studies (10, 15–17) on *E. coli* O157:H7 in cattle were conducted in our laboratory. In the first study (15, 16), *E. coli* O157:H7 was recovered from one (0.6%) of 161 calves and none (0%) of the 525 cows examined. In the second study (17), *E. coli* O157:H7 was isolated from seven (2%) of 383 slaughtered cattle. In the third study, *E. coli* O157:H7 was isolated from 55 (12%) of 471 calves (4 to 8 months old) in a feedlot (17). Similar prevalence rates of *E. coli* O157:H7 in cattle were found in the Netherlands (18), Italy (19), Belgium (20), Sweden (21), Czech Republic (22), the United Kingdom (23, 24), United States (25, 26), and Canada (27). According to a recent U.S. Department of Agriculture feedlot survey, the *E. coli* O157:H7 serotype contaminated approximately 50% of feedlot cattle. This prevalence rate was 10-fold higher than previous estimates (28).

In our laboratory, 432 non-O157 VTEC strains isolated from cattle were serotyped (17) and tested for presence of the virulence genes using polymerase chain reaction (PCR). The PCR demonstrated that 99 strains (23%) had the VT1 gene, 232 (54%) had the VT2 gene, and 101 (23%) had both toxin genes. Non-O157 VTEC strains belonged to 65 O serogroups, with 75% of these strains belonging to 24 O serogroups (i.e., O2, O4, O8, O20, O22, O26, O41, O64, O77, O82, O91, O103, O105, O113, O116, O126, O128, O136, O141, O162, O163, O171, O174, and OX177). A correlation was found between the O serogroup and the toxin produced. The majority of VTEC strains of serogroups O26, O64, O103, O128, and O136 had the VT1 gene, whereas the majority of the strains of serogroups O2, O4, O77, O91, O113, O116, O162, O163, O171, and O174 had the VT2 gene. The strains having both toxin genes belonged to the serogroups O20, O22, O82, O105, and O126. The EHEC-*HlyA* and *eae* virulence genes were detected in 244 (56%) and 75 (17%) of the 432 strains tested, respectively. The 432 non-O157 VTEC strains belonged to 112 serotypes. Interestingly, 69% of these strains belonged to one of the 26 serotypes listed in Table I. Of these 26 serotypes, 22 were found to cause human illnesses and 15 have been associated with HUS cases (17). Bovine VTEC isolates in other studies reviewed by the United States were assigned to 126 O serogroups and 357 O:H serotypes (10). The predominate bovine VTEC serotypes in most surveys were O113:H21 in Europe, O26:H11 in North America and Australia, and O45:H8, O45:H$^-$, and O145:H$^-$ in Japan (7, 8, 10).

VTEC in Sheep and Goats. Sheep have been subjected to fewer epidemiological surveys than cattle. Recently, *E. coli* O157:H7 has been detected in sheep and goats (29). This suggests that small ruminants also may represent a source of transmission of VTEC to humans. Fecal swabs from 1300 lambs across 93 flocks in Spain during 1997 were tested for VTEC (30). Of these flocks, 68% were VTEC positive. *E. coli* O157:H7 was isolated from five (0.4%) lambs, whereas non-O157 VTEC were isolated from 462 (36%) lambs. In a following study during

Table I. VTEC Serotypes Most Frequently Found in Spain[ab]

Cattle	Sheep	Food	Human
O2:H27 (7)	**O5:H⁻** (19)	O1:H20 (2)	O1:H7 (2)
O2:H29 (5)	**O6:H⁻** (3)	**O8:H21** (4)	O9:H21 (2)
O4:H4 (11)	O6:H10 (25)	O8:H⁻ (2)	**O26:H11** (13)
O8:H2 (9)	O52:H45 (3)	**O22:H8** (3)	O77:H41 (2)
O20:H19 (18)	**O91:H⁻** (64)	O64:H5 (3)	**O91:H⁻** (3)
O22:H8 (25)	O104:H7 (9)	O77:H41 (2)	**O98:H⁻** (2)
O26:H11 (23)	O110:H⁻ (7)	**O113:H21** (2)	**O103:H2** (2)
O77:H41 (21)	O112:H⁻ (7)	**O157:H7** (5)	**O111:H⁻** (5)
O82:H8 (7)	O117:H⁻ (16)	O171:H2 (2)	O113:H4 (2)
O91:H21 (8)	O123:H⁻ (3)	OUT:H21 (6)	**O113:H21** (3)
O103:H2 (6)	**O128:H⁻** (46)	**OUT:H⁻** (3)	**O118:H16** (2)
O105:H18 (15)	**O128:H2** (14)		**O145:H⁻** (2)
O113:H4 (8)	O136:H20 (11)		O146:H21 (3)
O113:H21 (33)	**O146:H8** (14)		O150:H⁻ (2)
O116:H21 (9)	O146:H21 (27)		**O157:H7** (24)
O128:H⁻[c] (5)	O156:H⁻ (13)		O166:H28 (2)
O156:H⁻ (9)	**O157:H7** (5)		**O174:H⁻** (2)
O157:H7 (9)	O166:H28 (11)		OUT:H4 (2)
O171:H2 (20)	OX176:H4 (9)		OUT:H8 (2)
O174:H⁻ (5)	**OUT:H⁻** (9)		**OUT:H⁻** (3)
O174:H2 (8)	OUT:H21 (16)		
O174:H21 (6)			
OX177:H11 (5)			
OUT:H⁻[d] (12)			
OUT:H2 (5)			
OUT:H19 (18)			

[a] Number of strains are listed between parenthesis.
[b] Serotypes isolated from patients with hemolytic uremic syndrome are in bold.
[c] Nonmotile (H⁻).
[d] O Untypeable (OUT).

2000 and 2001 by our group (Rey J, Blanco JE, Blanco M, Mora A, Blanco J, unpublished data), *E. coli* O157:H7 was isolated from 7 (1%) of the 697 lambs tested. Prevalence rates of non-O157 VTEC were higher in sheep (67%) and goats (56%) than in cattle (21%) in Germany (31). In France, VTEC prevalence rates ranging from 55% to 95% of the goats tested were reported (32). In Australia (33–35), VTEC prevalence rate of 40% was reported for goats, whereas rates ranging from 56% to 68% were reported for sheep. In the United States, Kudva *et al.* (36, 37) detected VTEC strains of 43% of sheep. In the United Kingdom, a survey of 1000 sheep at slaughter revealed a prevalence rate of 2% for *E. coli* O157:H7 (24). In The Netherlands, *E. coli* O157:H7 was also detected in 4% of the 52 ewes and 49 lambs tested (38). In Norway, however, no *E. coli* O157:H7 was found in the 364 sheep tested (39). In contrast, Kudva *et al.* (36, 37) detected *E. coli* O157:H7 at a high rate (31%) in a U.S. sheep flock.

In a recent study in our laboratory, 379 ovine non-O157 VTEC strains were isolated and characterized (30). The PCR demonstrated that 213 strains (56%) had the VT1 gene, five (1%) had the VT2 gene, and 161 (43%) had both toxin genes. Non-O157 VTEC strains belonged to 34 O serogroups. Of these strains, 83% belonged to 13 O serogroups (i.e., O5, O6, O91, O104, O110, O112, O117, O128, O136, O146, O156, O166, and OX176). A correlation was found between the serogroup and the toxin produced. Most strains of the serogroups O6, O104, O110, O112, O117, O136, O156, and OX176 had the VT1 gene, whereas the majority of O91 and O128 strains had both VT1 and VT2 genes. Strains belonging to the serogroups O5, O146, and O166 had the VT1 gene alone or with the VT2 gene. The EHEC-*HlyA* and *eae* virulence genes were detected in 101 (27%) and 18 (5%) of the 379 strains tested, respectively.

Ovine VTEC strains belonging to 53 O serogroups and 105 O:H serotypes have been found in studies reviewed for the United States (10). Only a few VTEC serotypes (i.e., O5:H⁻, O91:H⁻, O128:H2, O146:H8, and O146:H21) have been the most commonly found in sheep in different countries. The predominant VTEC serotype in Germany (31), Spain (30), Australia (35), and the United States (37) was O91:H⁻. A total of 55 (52%) of the 105 ovine VTEC serotypes detected in sheep (10) have been recovered from humans, including 23 serotypes associated with HUS cases (10). In Spain, 16 of the 21 most prevalent ovine serotypes were also found to cause human illnesses, including HUS (eight VTEC serotypes; Table I).

Comparing non-O157 VTEC serotypes from sheep with those from cattle revealed remarkable differences, suggesting a host serotype specificity. For example, eight of the 21 VTEC serotypes more frequently detected in sheep in Spain (i.e., O52:H45, O91:H⁻, O104:H7, O110:H⁻, O112:H⁻, O123:H⁻, O128:H2, and O136:H20) were not among the 357 VTEC serotypes detected in cattle. More

than one-half (54%) of bovine non-O157 VTEC strains had the VT2 gene, whereas only 1% of ovine strains had the VT2 gene. Interestingly, the *eae* gene was found in fewer ovine (5%) than bovine (17%) strains. Because this important virulence gene is present only in a very small proportion (5%) of ovine non-O157 VTEC strains, most ovine strains may be less toxic to humans (30).

VTEC in Foods. Raw beef products collected in Spain between 1995 and 1998 were examined for VTEC. VTEC were detected in 57 (13%) of the 455 meat samples tested. *E. coli* O157:H7 was isolated from five (1%) samples and non-O157 VTEC was isolated from 54 (12%) samples. Chapman *et al.* (40) found *E. coli* O157:H7 in 36 (1%) of 3216 samples of beef products in the United Kingdom. Heuvelink *et al.* (41) also detected *E. coli* O157:H7 in six (1%) of 571 samples of raw minced beef in The Netherlands. Tarr *et al.* (42), however, did not detect *E. coli* O157:H7 in a large number (1400) of ground beef samples in Seattle (WA). In contrast, Doyle and Schoeny (43) reported a 31% prevalence rate of *E. coli* O157:H7 in beef samples in Calgary, Canada. According to a USDA survey (28), almost 90% of the ground beef sampled since September 1999 has been contaminated with *E. coli* O157:H7. The frequency of non-O157 VTEC occurrence in Spain (12%) was similar to those (i.e., 11%, 9%, 11%, and 15%) reported in other countries such as Canada (44), Thailand (45), the United Kingdom (46), and Sweden (47), respectively.

We have characterized 60 non-O157 VTEC strains isolated from beef products in Spain (Table I). The PCR demonstrated that 16 (27%) strains had the VT1 gene, 36 (60%) had the VT2 gene, and eight (13%) had both toxin genes. Non-O157 VTEC strains belonged to 29 O serogroups (i.e., O1, O2, O4, O6, O8, O15, O20, O21, O22, O26, O39, O42, O54, O64, O75, O77, O88, O103, O110, O112, O113, O116, O118, O120, O146, O156, 171, O174, and OX178) and 41 O:H serotypes. Of these serotypes, 11 (i.e., O8:H21, O22:H8, O26:H11, O26:H$^-$, O103:H2, O103:H$^-$, O112:H2, O113:H21, O118:H16, O174:H21, and OUT [O untypeable]:H$^-$) were associated with HUS cases. The EHEC-*HlyA* and *eae* virulence genes were detected (Blanco M, Blanco JE, Mora A, Rey J, Blanco J, unpublished data) in 21 (35%) and eight (13%) of the 60 strains tested, respectively.

VTEC in Humans. The presence of VTEC in patients with diarrhea or other gastrointestinal problems was examined (48). VTEC were isolated from stool samples of 126 (2.5%) of the 5054 patients investigated. *E. coli* O157:H7 was detected in 24 patients (0.5%), whereas non-O157 VTEC were detected in 104 patients (2.1%). Our findings suggested that VTEC are a significant cause of human illnesses in Spain. Our data also confirmed that human infections with non-O157 VTEC were more common than those with O157:H7 strains in Europe (49). Non-O157 VTEC were isolated at higher rates than O157 strains in several studies in Germany. Examples of these prevalence rates include 7% vs 3% (50), 1% vs 0.4% (51), and 2% vs 0.4% (52). In other parts of Europe, similar trends were reported. Examples of the prevalence rates for non-O157 versus O157 VTEC include 3% vs 0% in France (53), 1% vs 0% in Switzerland (54), and 0.7% vs 0.2% in Belgium (55). In Denmark, non-O157 VTEC strains were found three times more often than O157 strains (56).

A total of 102 non-O157 VTEC strains from human stool samples in Spain were recently characterized (48). The PCR demonstrated that 42% of the strains had the VT1 gene, 33% had the VT2 gene, and 25% had both toxin genes. These VTEC strains belonged to 40 O serogroups and 65 O:H serotypes, including 20 (i.e., O1:H7, O1:H$^-$, O20:H19, O26:H11, O26:H$^-$, O46:H31, O84:H$^-$, O91:H21, O91:H$^-$, O98:H$^-$, O103:H2, O111:H$^-$, O113:H21, O118:H16, O128:H2, O145:H$^-$, O172:H$^-$, O174:H$^-$, OX177:H$^-$, and OUT:H$^-$) associated with HUS cases. The most frequently found serotypes are listed in Table I. The VTEC serotypes found in Spain were similar to those found in other countries. *E. coli* O26:H11 was the most common serotype among humans in Spain. This serotype has been the first or second (after O103:H2) non-O157 VTEC most frequently isolated from humans in Germany (51, 57), Belgium (55), Denmark (56), and Finland (58). The EHEC-*HlyA* and *eae* virulence genes were detected in 63% and 45% of the 102 strains tested (48), respectively. The majority of *eae*-positive strains belonged to the VTEC serotypes O26:H11 (13 strains) and O157:H7 (24 strains). The majority of patients with *E. coli* O157:H7 infection had diarrhea or HC, whereas a child developed HUS.

Bacteriophage Typing and Virulence Genes of Human and Animal *E. coli* O157:H7 Isolates. A total of 141 *E. coli* O157:H7 strains isolated in Spain were characterized by bacteriophage typing and PCR of virulence genes (59). A total of 47 strains were obtained from humans, 87 from animals (82 bovine and five ovine), and seven from raw beef products. Eighteen phage types (PT) were detected and included PT2, PT4, PT8, PT14, PT21, PT23, PT26, PT27, PT28, PT31, PT32, PT34, PT39, PT45, PT50, PT51, PT54, and PT63. The numbers of VTEC strains carrying these phages were 37, 1, 30, 11, 9, 4, 2, 1, 1, 1, 2, 4, 5, 2, 1, 1, 11, and 2, respectively. The distribution of strains among PT was hyperbolic with five types (i.e., PT2, PT8, PT14, PT21, and PT54) accounting for 70% of the total. The PT2 and PT8 were most frequently found in human (55%) and bovine (46%) strains. The PCR demonstrated that two (1%) strains had the VT1 gene, 82 (58%) had the VT2 gene, and 57 (41%) had both toxin genes. The majority of VTEC strains with PT2 (36 of 37) had the VT2 gene, whereas the majority of strains with PT8 (25 of 30) had both toxin genes. All 141 *E. coli* O157:H7 strains had the *eae* and EHEC-*hlyA* genes. The PT found in Spain were similar to those found in other countries. The predominant PT among human VTEC O157:H7 strains isolated in Europe were PT2, PT4, PT8, PT14, PT24/PT28, and PT32 (60,

Table II. Human Illness Outbreaks in Spain Attributed to VTEC

Year	No. of cases	Subjects	VTEC serotype	Toxin gene	Phage type	Reference
1986	3	Tourists	O157:H7	VT2		64
1994		Tourists	O157:H7	VT2	PT2	63
1995	13	Children at a summer camp	O111:H^{-b}	VT1		66
1997	14 (3 with HUS[a])	Tourists	O157:H7	VT2	PT2	65
1999	8 (1 with HUS)	Children in a nursery	O157:H7			67
1999	2 (1 with HUS)		O157:H7			67
2000	158 (6 with HUS)	Children in five schools	O157:H7	VT2	PT2	H. Pañella, J.M. Oliva, M.D. Ferrer (personal communication)

[a] Hemolytic uremic syndrome
[b] Nonmotile (H$^-$).

61). The PT14 has emerged as the predominant type in Canada (62).

VTEC Human Illness Outbreaks in Spain. According to our data (Table II), VTEC strains have caused seven human illness outbreaks in Spain in recent years. Six outbreaks have been caused by *E. coli* O157:H7 and one by *E. coli* O111:H$^-$. Three outbreaks of HC were caused by *E. coli* O157:H7 (PT2) affected only foreign tourists (63–65). In March 1997, an outbreak of *E. coli* O157:H7 (PT2) infection occurred among tourists returning from the Canary Islands. The cause of such infection was attributed to the use of well water contaminated with the pathogen (65). The major human illness outbreak in Spain occurred in September 2000 and involved five schools (one in Barcelona city and four in surrounding towns). In this outbreak, 158 (mostly children) developed symptoms of gastrointestinal illnesses. Of these, 53 tested positive for *E. coli* O157:H7 (PT2) and six developed HUS. The cause of such outbreak was attributed to serving sausage (mostly pork and contained other meat sources) in the five schools (Pañella H, Oliva JM, Ferrer MD, personal communication).

Conclusions

Our investigations of ruminant and human fecal samples as well as food samples of animal origin revealed that O157 and non-O157 VTEC are widely distributed in Spain. The VTEC serotypes found in Spain were similar to those found in other countries. Our data emphasized the importance of the virulence genes in the causation of human illnesses and suggested that small ruminant (e.g., sheep and goats) strains of VTEC may be less pathogenic to humans. It is advisable to carry out a careful surveillance of VTEC infections in the population and to consider adequate control measures.

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1. Doyle MP, Zhao T, Meng J, Zhao S. *Escherichia coli* O157:H7. In: Doyle MD, Beuchat LR, Montville TJ, Eds. Food Microbiology, Fundamentals and Frontiers. Washington, DC: ASM Press, pp171–191, 1997.
2. Johnson RP, Wilson JB, Michel P, Rahn K, Renwick SA, Gyles CL, Spika JS. Human infection with verocytotoxigenic *Escherichia coli* associated with exposure to farms and rural environments. In: Stewart CS, Flint HJ, Eds. *Escherichia coli* O157 in Farm Animals. Wallingford, UK: CABI Publishing, pp147–168, 1999.
3. Konowalchuk J, Speirs JL, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun **18**:775–779, 1977.
4. ÓBrien AD, LaVeck GD, Thompson MR, Formal SB. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J Infect Dis **146**:763–769, 1982.
5. Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect Immun **66**:1055–1061, 1995.
6. Oswald E, Schmidt H, Morabito S, Karch H, Marchès O, Caprioli A. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. Infect Immun **68**:64–71, 2000.
7. World Health Organization. Zoonotic non-O157 shiga toxin-producing *Escherichia coli* (STEC). Report of a WHO Scientific Working Group Meeting, Berlin, Germany. http://www.who.int/emc-documents/zoonoses/whocsraph988c.htm, 1998.
8. Bettelheim KA. Serotypes of verotoxin-producing *Escherichia coli* reported in the literature apart from those belonging to serogroup O157. http://www.sciencenet.com.au/bacteriologyhome.htm, 2000.
9. Blanco J, Blanco M, Blanco JE, Mora A, Alonso MP, González EA, Bernárdez MI. O:H serotypes of human verocytotoxigenic *E. coli*. http://www.lugo.usc.es/ecoli/SEROTIPOSHUM.htm, 2001.
10. Blanco J, Blanco M, Blanco JE, Mora A, Alonso MP, González EA, Bernárdez MI. Epidemiology of verocytotoxigenic *Escherichia coli* (VTEC) in ruminants. In: Duffy G, Garvey P, McDowell DA, Eds. Verocytotoxigenic *E. coli*. Trumbull, CT: Food and Nutrition Press, pp113–148, 2001.
11. Beutin L. *Escherichia coli* O157 and other types of verocytotoxigenic *E. coli* (VTEC) isolated from humans, animals and food in Germany. In: Stewart CS, Flint HJ, Eds. *Escherichia coli* O157 in Farm Animals. Wallingford, UK: CABI Publishing, pp121–145, 1999.
12. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of shiga toxin-producing *Escherichia coli* and disease in humans. J Clin Microbiol **37**:497–503, 1999.
13. Paton AW, Woodrow MC, Doyle RM, Lanser JA, Paton JC. Molecular characterization of a shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. J Clin Microbiol **37**:3357–3361, 1999.
14. Blanco M, Blanco JE, Blanco J, González EA, Alonso MP, Mass H, Jansen WH. Prevalence and characteristics of human and bovine verotoxigenic *Escherichia coli* strains isolated in Galicia (north-western Spain). Eur J Epidemiol **12**:13–19, 1996.
15. Blanco M, Blanco JE, Blanco J, González EA, Mora A, Prado C,

Fernández L, Rio M, Ramos J, Alonso MP. Prevalence and characteristics of *Escherichia coli* serotype O157:H7 and other verotoxin-producing *E. coli* in healthy cattle. Epidemiol Infect **117**:251–257, 1996.
16. Blanco M, Blanco JE, Blanco J, Mora A, Prado C, Alonso MP, Mouriño M, Madrid C, Balsalobre C, Juárez A. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet Microbiol **54**:309–319, 1997.
17. Blanco M, Blanco JE, Mora A, González EA, Blanco J. Serotypes and virulence genes of verocytotoxigenic *E. coli* (VTEC) isolated from cattle in Spain. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 3: Pathogenicity and Virulence of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, p183, 2000.
18. Heuvelink AE, Van Den Biggelaar FLAM, Zwartkruis-Nahuis JTM, Herbes RG, Huyben R, Nagelkerke N, Melchers WJG, Monnens LAH, De Boer E. Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. J Clin Microbiol **36**:3480–3487, 1998.
19. Bonardi S, Maggi E, Bottarelli A, Pacciarin ML, Ansuini A, Vellini G, Morabito S, Caprioli A. Isolation of verocytotoxin-producing *Escherichia coli* O157:H7 from cattle at slaughter in Italy. Vet Microbiol **67**:203–211, 1999.
20. De Zutter L, Uradzinski J, Pierard D. Prevalence of enterohemorrhagic *E. coli* O157 in Belgian slaughter cattle. Abstracts of the Second International Symposium of the European Study Group on Enterohemorrhagic *Escherichia coli*. Acta Clin Bel **54**:48, 1999.
21. Eriksson E, Engvall A, Gunnarsson A. Effects of pooling of samples and cold storage of pre-enrichment broth on sensitivity for detecting *E. coli* O157 in cattle faeces. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 1: Methods for Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, pp36–40, 1999.
22. Cizek A, Alexa P, Smola J. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and rats from a large-scale farm. Abstracts of the Second International Symposium of the European Study Group on Enterohemorrhagic *Escherichia coli*. Acta Clin Bel **54**:47, 1999.
23. Synge BA, Hopkins GF. Studies of verocytotoxigenic *E. coli* O157 in cattle in Scotland and association with human cases. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. New York: Elsevier Science, pp65–68, 1994.
24. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect **119**:245–250, 1997.
25. Sanderson MW, Gay JM, Hancock DD, Gay CC, Fox LK, Besser TE. Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. J Clin Microbiol **33**:2616–2619, 1995.
26. Laegreid WW, Elder RO, Keen JE. Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning. Epidemiol Infect **123**:291–298, 1999.
27. Van Donkersgoed J, Graham T, Gannon V. The prevalence of verotoxins, *Escherichia coli* O157, and *Salmonella* in the feces and rumen of cattle at processing. Can Vet J **40**:332–338, 1999.
28. Hoyle B. Renewed concerns over *E. coli* O157:H7 in ground beef. ASM News **66**:331–332, 2000.
29. Mainil J. Shiga/verocytotoxins and Shiga/verotoxigenic *Escherichia coli* in animals. Vet Res **30**:235–257, 1999.
30. Rey J, Alonso JM, Blanco JE, Blanco M, Mora A, González EA, Blanco J. Serotypes and virulence genes of verocytotoxigenic *E. coli* (VTEC) isolated from sheep in Spain. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 3: Pathogenicity and Virulence of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, p185, 2000.
31. Beutin L, Geier D, Steinrück H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J Clin Microbiol **31**:2483–2488, 1993.
32. Bastian S, Carle I, Grimont F, Grimont PAD. Diversity of shiga toxin producing *E. coli* in herds of dairy cows and goats. Abstracts of the Second International Symposium of the European Study Group on Enterohemorrhagic *Escherichia coli*. Acta Clin Bel **54**:49, 1999.
33. Sidjabat-Tambunan H, Bensink JC. Verotoxin-producing *Escherichia coli* from the faeces of sheep, calves and pigs. Aust Vet J **75**:292–293, 1997.
34. Fagan PK, Hornitzky MA, Bettelheim KA, Djordjevic SP. Detection of shiga-like toxin (*stx*1 and *stx*2), intimin (*eae*A), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl Environ Microbiol **65**:868–872, 1999.
35. Djordjevic SP, Hornitzky MA, Bailey G, Gill P, Vanselow B, Walker K, Bettelheim KA. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. J Clin Microbiol **39**:2017–2021, 2001.
36. Kudva IT, Hatfield PG, Hovde CJ. *Escherichia coli* O157:H7 in microbial flora of sheep. J Clin Microbiol **34**:431–433, 1996.
37. Kudva IT, Hatfield PG, Hovde CJ. Characterization of *Escherichia coli* O157:H7 and other shiga toxin-producing *E.coli* serotypes isolated from sheep. J Clin Microbiol **35**:892–899, 1997.
38. Heuvelink AE, Van Den Biggelaar FLAM, De Boer E, Herbes RG, Melchers WJG, Huis 'T Veld JHJ, Monnens LAH. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and Sheep. J Clin Microbiol **36**:878–882, 1998.
39. Johnsen G, Berget OI, Wasteson Y, Herikstad H. *E. coli* O157:H7 in faeces from cattle, sheep and pigs in the south west part of Norway during 1998. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 3: Pathogenicity and virulence of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, p186, 2000.
40. Chapman PA, Siddons CA, Cerdan Malo AT, Harkin MA. A one year study of *Escherichia coli* O157 in raw beef and lamb products. Epidemiol Infect **124**:207–213, 2000.
41. Heuvelink AE, Zwartkruis-Nahuis JTM, Beumer RR, De Boer E. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in The Netherlands. J Food Prot **62**:1115–1122, 1999.
42. Tarr PI, Tran TN, Wilson RA. *Escherichia coli* O157:H7 in retail ground beef in Seattle: results of a one-year prospective study. J Food Prot **62**:133–139, 1999.
43. Doyle MP, Schoeni JL. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl Environ Microbiol **53**:2394–2396, 1987.
44. Read SC, Gyles CL, Clarke RC, Lior H, McEwen S. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork, and chicken in southwestern Ontario. Epidemiol Infect **105**:11–20, 1990.
45. Suthienkul O, Brown E, Seriwatana J, Tienthongdee S, Sastravaha S, Echeverria P. Shiga-like-toxin-producing *Escherichia coli* in retail meats and cattle in Thailand. Appl Environ Microbiol **56**:1135–1139, 1990.
46. Willshaw GA, Smith HR, Roberts D, Thirlwell J, Cheasly T, Rowe B. Examination of raw beef products for the presence of vero cytotoxin producing *Escherichia coli*, particularly those of serogroup O157. J Appl Bacteriol **75**:420–426, 1993.
47. Lindqvist R, Antonsson AK, Norling B, Persson L, Ekström ACL, Fäger U, Eriksson E, Löfdahl S, Norberg P. The prevalence of verocytotoxin-producing *Escherichia coli* (VTEC) and *E. coli* O157:H7 in beef in Sweden determined by PCR assays and an immuno-magnetic serparation (IMS) method. Food Microbiol **15**:591–601, 1998.
48. Blanco JE, Blanco M, Mora A, Alonso MP, Coira A, Rodríguez A, Blanco J. Serotypes and virulence genes of verocytotoxigenic *E. coli* (VTEC) isolated from human patients in Spain. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 3: Pathogenicity and Virulence of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, p191, 2000.

49. Caprioli A, Tozzi AE. Epidemiology of Shiga toxin-producing *Escherichia coli* infections in continental Europe. In: Kaper JB, OBrien AD, Eds. *Escherichia coli* O157:H7 and Other Shiga Toxin-Producing *E. coli* strains. Washington, DC: American Society for Microbiology, pp38–48, 1998.
50. Gunzer F, Böhm H, Rüssmann H, Bitzan M, Aleksic S, Karch H. Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. J Clin Microbiol **30:**1807–1810, 1992.
51. Karch H, Huppertz HI, Bockemühl J, Schmidt H, Schwarzkopf A, Lissner R. Shiga toxin-producing *Escherichia coli* infections in Germany. J Food Prot **60:**1454–1457, 1997.
52. Pulz M. Epidemiology and current importance of enterohaemorrhagic *Escherichia coli* (EHEC) in North Bavaria (1996). Gesundheitswesen **59:**656–660, 1997.
53. Pradel N, Livrelli V, De Champs C, Palcoux JB, Reynaud A, Scheutz F, Sirot J, Joly B, Forestier C. 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, 2000.
54. Burnens AP, Boss P, Orskov F, Orskow I, Schaad UB, Müller F, Heinzle R, Nicolet J. Occurrence and phenotypic properties of verotoxin producing *Escherichia coli* in sporadic cases of gastroenteritis. Eur J Clin Microbiol Infect Dis **11:**631–634, 1992.
55. Piérard D, Stevens D, Moriau L, Lior H, Lauwers S. Isolation and virulence factors of verocytotoxin-producing *Escherichia coli* in human stool samples. Clin Microbiol Infect **3:**531–540, 1997.
56. Scheutz F, Olesen B, Gerner-Smidt P. Verocytotoxigenic *E. coli* (VTEC) from Danish patients 1997-1999. Part 1: Characterised by serotypes and virulence factors (abstract). Proceedings of the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections; 2000; Kyoto, Japan. p98. Abstract nr 207.
57. Beutin L, Zimmermann S, Gleier K. Association between serotypes, virulence markers and disease in a group of 679 verocytotoxin-producing *Escherichia coli* (VTEC) strains isolated from human patients in Germany (1997-1999). In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 5: Epidemiology of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, pp5–11, 2001.
58. Keskimäki M, Saari M, Heiskanen T, Siitonen A. Shiga toxin-producing *Escherichia coli* in Filand from 1990 through 1997: Prevalence and characteristics of isolates. J Clin Microbiol **36:**3641–3646, 1998.
59. Mora A, Usera MA, Blanco M, Blanco JE, Alonso MP, Prats G, Stirrat A, Carter FM, Blanco J. Bacteriophage typing and virulence genes of verocytotoxigenic *E. coli* (VTEC) O157:H7 strains isolated in Spain. In: Duffy G, Garvey P, Coia J, Wasteson Y, McDowell DA, Eds. Verocytotoxigenic *E. coli* in Europe, 3: Pathogenicity and Virulence of Verocytotoxigenic *E. coli*, Concerted Action CT98-3935. Dublin, UK: The National Food Centre, p189, 2000.
60. Cheasty T, Allerberger F, Beutin L, Caprioli A, Heuvelink A, Karch H, Lofdahl S, Pièrard D, Scheutz F, Siitonen A, Smith H. VTEC O157 phage types isolated from 15 European countries: 1997-1999 (abstract). Proceedings of the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections; 2000; Kyoto, Japan. p126. Abstract nr 263.
61. Saari M, Cheasty T, Leino K, Siitonen A. Phage types and genotypes of shiga toxin-producing *Escherichia coli* O157 in Finland. J Clin Microbiol **39:**1140–1143, 2001.
62. Ahmed R, Ali A, Demezuk W, Woodward D, Clark C, Khakhria R, Rodgers F. Emergence of new molecular and phage typing variants of *E. coli* O157:H7 in Canada. Proceedings of the 4th International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections; 2000; Kyoto, Japan. Abstract nr 224, p106.
63. Willshaw GA, Cheasty T, Frost JA, Threlfall EJ, Rowe B. Antimicrobial resistance of O157 VTEC in England and Wales. EVC news 5, Notiziario dellIstituto Superiore di Sanità **9**(11 Suppl 3):3–4, 1996.
64. Smith HR, Rowe B, Gross RJ, Fry NK, Scotland SM. Haemorrhagic colitis and vero-cytotoxin-producing *Escherichia coli* in England and Wales. Lancet. **1:**1062–1064, 1987.
65. Pebody RG, Furtado C, Rojas A, McCarthy N, Nylen G, Ruutu P, Leino T, Chalmers R, de Jong B, Donnelly M, Fisher I, Gilham C, Graverson L, Cheasty T, Willshaw G, Navarro M, Salmon R, Leinikki P, Wall P, Bartlett C. An international outbreak of vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists: a challenge for the European infectious disease surveillance network. Epidemiol Infect **123:**217–223, 1999.
66. Blanco JE, Blanco M, Molinero ME, Peiró E, Mora A, Blanco J. Brote de gastroenteritis asociado con un *Escherichia coli* verotoxigénico (ECVT) O111:H$^-$ VT1$^+$ *eae*$^+$. Alimentaria **275:**109–113, 1996.
67. Gomariz M, Robertson MT, Serrano E, Iglesias L, Pérez-Trallero E. Estudio de dos brotes de *E. coli* O157:H7 en Gipuzkoa. Enferm. Infecc Microbiol Clín **18**(S1):84, 2000.