SUPPLEMENT

Non-O157 Verotoxin-Producing *Escherichia coli*: A Problem, Paradox, and Paradigm

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The problems associated with identification and characterization of non-O157 verotoxin-producing *Escherichia coli* (VTEC) are discussed. The paradox of VTEC is that most reports of human illnesses are associated with serotypes such as O157:H7, O111:H$^-$ (nonmotile), O26:H11, and O113:H21, which are rarely found in domestic animals. However, those VTEC serotypes commonly found in domestic animals, especially ruminants, rarely cause human illnesses. When they cause human illnesses, the symptoms are similar to those caused by the serotypes *E. coli* O157:H7, O111:H$^-$, O26:H11, and O113:H21. The impact of VTEC on human and animal health is also addressed. The VTEC and their toxicity are considered as a paradigm for emerging pathogens. The question on how such pathogens could arise from a basic commensal population is also addressed. Exp Biol Med 228:333–344, 2003

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Verotoxin-producing *Escherichia coli* (VTEC) also known as Shiga toxin-producing *E. coli* (STEC) have emerged in the past two decades as important causes of morbidity and mortality in humans. With many of the outbreaks of VTEC infections being directly or indirectly associated with food, there have also been extensive economic losses to food processors and suppliers. Worldwide, most of the reported human illness outbreaks have been attributed to the O157:H7 serotype, which was first associated with two major outbreaks in the United States in 1982 (1). This serotype also has been reported as the cause of two major outbreaks in the West Coast of the United States (2) and an outbreak in Japan (3). In the wake of these and many other reported outbreaks due to *E. coli* O157:H7, several selective media have been developed to identify and characterize *E. coli* O157:H7. The earliest of these was based on the realization that *E. coli* O157:H7 strains do not ferment sorbitol, whereas the majority of other *E. coli* do (4). Other media designed specifically to select for these VTEC include CHROMagar O157 (5) and Rainbow agar O157 (6). Therefore, the use of these media and the reports on O157:H7 outbreaks have created the general perception that this serotype is the only VTEC of significant importance.

Although O157 VTEC probably emerged in late 1970s (7, 8), non-O157 VTEC (e.g., serogroups O18, O26, O111, and O128) have been known earlier to produce toxins that have toxic effects on African Green Monkey kidney (Vero) cells in cultures (9). Within three years from publishing this report (9), there were other reports linking VTEC to human illnesses in the United Kingdom (10) and New Zealand (11). In addition, a retrospective study has indicated that these VTEC isolates had been present for some time (12). A reexamination of reports of major human illness outbreaks has also suggested that VTEC may well have been involved. The major outbreak in the North Eastern United States in the 1950s is an example (13). Thus, by the 1980s, it was realized that a newly described group of pathogenic *E. coli* was the cause of many cases of human morbidity and mortality. By the end of the decade, Karmali (14) reviewed the occurrence of VTEC and noted a variety of serotypes.

Since the beginning of the 20th century, it had been realized that strains of *Shigella dysenteriae* Type 1 produce a potent toxin (15, 16), which was known as Shiga toxin. Studies in the 1980s on the nature and properties of the

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toxins involved showed a strong relationship between one of the toxins associated with VTEC and Shiga toxin (17). The antigenically distinct Shiga toxin 2 was described later (18), and its characteristics were evaluated (19). It was soon realized that the hemolytic uremic syndrome (HUS) frequently found in cases associated with outbreaks of VTEC infections was clinically similar to the condition originally described in association with *S. dysenteriae* Type 1 infections (20). As a result, a new group of pathogens (i.e., VTEC or STEC) with the ability to produce one or two toxins (verotoxin 1 [VT1] and verotoxin 2 [VT2]) was established. The problem, however, is that most laboratories around the world have been testing for VTEC O157 strains and totally ignoring other VTEC serotypes that may be as widespread in nature.

The Problem

Because large number of outbreaks of human illnesses were attributed to *E. coli* O157:H7, and due to the availability of several selective media designed for isolation of this serotype, many laboratories around the world have limited their screening of human or animal feces to *E. coli* O157:H7. However, there are over 60 VTEC serotypes that have been associated with human illnesses. Therefore, it seems unrealistic to attempt to screen humans or animals for one serotype such as O157:H7.

Non-O157 VTEC Associated with Human Illnesses. From the first reports on VTEC (9–11), the importance of specific pathogenic VTEC serotypes (i.e., O26:H^- [nonmotile], O26:H11, O55:H7, O111:H^-, and O111:H8) was recognized. In late 1980s, several human illness (i.e., hemorrhagic colitis [HC] and HUS) outbreaks were reported worldwide and were attributed to non-O157:H7 serotypes. These include O4:H^-, O45:H2, O111:H^-, and O145:H^- in the United States (21), O4:H5 and O111:H2 in Australia (22), and O26:H11, O104:H2, O153:H25, and O163:H19 (23) as well as O5:H^-, O55:H7, and O103:H2 (24) in the United Kingdom.

It is worth noting that the number of human illness outbreaks due to VTEC (especially non-O157:H7) infection has increased dramatically in the past decade worldwide. Examples included O111:H^- in Italy (25) and Australia (26, 27), O111:H2 in Germany (28), O103:H2 in France (29), the United States (30), and Germany (31), O145:H5 in Japan (32), O104:H21 in the U.S. (33), and O111:H8 (34) in the United States. Other VTEC strains (e.g., H12, H16, and H30) belonging to the O118 serogroup seem to be continuously emerging as a major cause of human illnesses in many parts of the world (35). However, the question that needs to be addressed is whether these serotypes are newly emerging or have been around in the past without being detected.

Difficulty of Detection of Non-O157 VTEC. The major problem in detecting non-O157 VTEC is that apart from producing verotoxin(s), they do not differ significantly in their biochemical characteristics from typical commensal *E. coli*. The only exception is the possible decreased ability to ferment carbohydrate-like substances (36). However, this characteristic has not been used in developing a medium for VTEC detection in a manner similar to that of sorbitol-MacConkey (SMAC) agar (4). Thus, it is the goal of microbiologists in this field to be able to select non-O157 VTEC by using a specialized medium as the case in selecting most O157 VTEC by using SMAC agar.

Detection Media Based on Enterohemolysin Production. The observations by Beutin *et al.* (37) of a close association between cytotoxicity and production of a newly described hemolysin (i.e., Enterohemolysin; Ehly), led to development of media able to detect EHly-producing *E. coli* and, therefore, potentially VTEC (38). This Ehly can only be observed on media prepared with washed sheep erythrocytes rather than whole sheep blood. The use of these media in Australia has led to the realization that non-O157 VTEC are as important as O157 VTEC (39, 40), and has significantly increased the isolation rate of these food-borne pathogens. However, it should be noted that not all VTEC produce Ehly, and not all Ehly-positive strains are VTEC. The addition of antibiotics (i.e., vancomycin, cefixime, and cefsulodin) to the basic washed sheep blood agar media (41, 42) significantly enhanced the yield of VTEC and this vancomycin-cefixime-cefsulodin blood agar greatly facilitates the detection of the Ehly-positive variants of VTEC. Because exceptions such as the presence of sorbitol-fermenting *E. coli* O157 strains (43) exist, it is important to emphasize the presence of VTEC that do not produce Ehly as well as Ehly-producing *E. coli* that do not produce toxins.

Detection of Toxin Activity. Having been originally described on the basis of inducing toxic effects on Vero cells (9), demonstration of the cytotoxic effects of VTEC on these cells remains the standard method of detection. It is important to emphasize that the effects of the verotoxins (VT1 and VT2) are similar. These toxins can be titrated and, thus, a quantitative measure of the amount present can be assessed. Neutralization studies with specific antibodies can also be performed. Of greatest importance is the fact that using Vero cells is the only means by which a possible third verotoxin will ever be found if such a toxin exists. Methods based on identifying specific antigens or specific base sequences on the genes that code for the toxins are likely to overlook potential new toxins. Although using the cytotoxic effects on Vero cells continues, it is still considered a detection method that is not very easily used in a routine laboratory. It requires a continuous supply of cells, specialized techniques, and time because the cytotoxic effects sometimes require several days to be detected.

Immunological Tests for VTEC. As part of the early investigations of VTEC in the 1980s, their serological diversity was established (17–19). These studies also made available a number of antibodies that can be used for detection. For example, an immunoassay developed by Acheson *et al.* (44) uses the P1 glycoprotein from sheep hydatid cyst fluid to detect verotoxins. Other immunoassays use antibodies produced in one animal species to capture the

toxins and antibodies produced in another to detect the captured antibodies. A number of these methods have been developed commercially and include tests using enzyme-linked immunoassay methods and latex agglutination. Although, in general, these tests are very successful in detecting VTEC, they are relatively expensive. For rapid identification, a commercial latex agglutination technique was developed (45). It is not only reproducible, but is also adaptable to detecting potential VTEC directly from primary isolation media (46).

DNA-Based Methods for VTEC Detection. A number of assays based on polymerase chain reaction (PCR) for detection of VTEC have been developed over the years. In a recent review (47), 14 PCR systems that detect and subtype VTEC were summarized. Although different primers were used in most tests, they were generally successful in identification of the toxin genes. Only one system (48) gave nonspecific reaction with all the strains tested. Because there are 13 promising PCR systems available, it should not be difficult to select one that fits specific circumstances of a testing laboratory. However, it should be noted that any of these PCR systems is capable only of detecting the presence of a specific DNA sequence (i.e., toxin genes). This does not necessarily mean that the bacterium is capable of producing the toxins. Because fecal or food samples may contain substances that specifically inhibit the PCR, these samples may require initial processes before amplification. Specific DNA preparation methods or specific tests may have to be performed on cultures obtained from growing *E. coli* in selective or nonselective media.

Summary of the Problem. Because of the increasing number of human illness outbreaks due to non-O157:H7 VTEC, many laboratories have explored potential development and adaptation of some of the techniques discussed above. However, several obstacles, (i.e., time, effort, and cost) are responsible for the slow expansion of using these techniques for routine testing. Even if these tests are used, they will only be able to determine if a non-O157 VTEC is indeed present in a given food, water, or fecal sample. These tests then will require a large range of specific *E. coli* antisera, both "O" and "H" to be able to characterize the VTEC serotype. Such full *E. coli* serotyping is currently only available in a few reference laboratories around the world. At the present time, it is relatively easy to identify *E. coli* O157 in a sample by using SMAC agar and testing the suspect colonies with a commercial serological reagent for O157. Unfortunately, this not only is impossible for many important non-O157 VTEC at present, but also may never be achievable.

The Paradox

Extensive studies worldwide have demonstrated the presence of different VTEC serotypes in the gastrointestinal tract of animals, especially ruminants, without causing them illnesses. Occasionally, the same VTEC serotypes are implicated in illnesses in preweaned ruminants. Also, there appears to be a certain host species-specificity with respect to the VTEC being carried. Of the nonruminants, pigs are the other main group of animals from which VTEC can be isolated. The presence of VTEC in swine is generally associated with sick young animals, and the VTEC infection seems confined to a very restricted group of serotypes (e.g., O138:H14, O138:H$^-$, O139:H1, O141:H4, O141:H$^-$, O149:H10, and O149:H19) that do not appear to be present in ruminants. These porcine non-O157 VTEC serotypes are generally not detected in the gastrointestinal tract of healthy humans.

Ecological Aspects of Non-O157 VTEC in Animals and Humans. Fagan *et al.* (49) demonstrated that 61%, 38%, and 40% of fecal samples from healthy sheep, cattle, and goats, respectively, were VTEC positive. These results are in agreement with those reported in earlier studies (50–52). More recently, a Japanese study (53) showed VTEC prevalence rates of 46%, 66%, and 69% in calves, heifers, and cows, respectively. Most of the VTEC isolates detected in this study belonged to non-O157 serogroups including O8, O26, O84, O113, and O116. As discussed previously, there have been VTEC infections in humans for many years before being considered food-borne pathogens in the 1980s. The contamination of beef during slaughter also was recognized at that time. Apart from individuals closely associated with ruminants at the preharvest (farmers or ranchers) or postharvest (meat packers) levels, it appears that healthy humans do not normally carry VTEC (54, 55). These studies have also shown that the VTEC serotypes isolated from healthy humans were those commonly isolated from healthy ruminants.

The VTEC O26 Serogroup. The O26 VTEC serogroup was among the first VTEC to be reported (10, 11). Of the 1560 reported strains of non-O157 VTEC, 105 (6.7%) belonged to the O26 serogroup. These VTEC O26 strains are summarized in Table I. This summary shows that these O26 strains appear to occur exclusively in cattle, their products, and humans. The ratio of isolations from sick and healthy cattle was 4:3, whereas this ratio was 76:3 in humans. Thus, these VTEC strains should be considered as pathogens for both cattle and humans.

The VTEC O111 Serogroup. Of the O serogroups of VTEC, the O111 is probably the most important. More outbreaks of human illnesses have been attributed to strains belonging to this serogroup than to other serogroups except for O157. Of the 1560 reported strains of non-O157 VTEC, 82 (5.3%) belonged to the O111 serogroup. These VTEC O111 strains are summarized in Table II, which illustrates infection patterns similar to those observed for the serogroup O26 (Table I). The majority of the human isolates (97%) were from individuals with symptoms of illnesses (i.e., diarrhea, HC, or HUS). Of the cattle isolates, only 30% were from sick animals. It is worth noting that the one isolate from wild deer was unique in carrying the H45 antigen.

Table I. Numbers and Distributions of VTEC Strains Belonging to the O26 Serogroup[a]

Serotype	Humans			Cattle		Cattle products		No. of isolates
	Healthy	Sick	Unknown	Healthy	Sick	Milk	Cheese	
O26:H2	–	1	–	–	–	–	–	1
O26:H11	2	45	1	5	12	2	1	68
O26:H12	–	1	–	–	–	–	–	1
O26:H21	1	1	–	1	–	–	–	3
O26:H^{-b}	–	28	1	3	–	–	–	32
Total	3	76	2	9	12	2	1	105

[a] The data were summarized from several reports (32, 41, 59, 60, 76–116).
[b] Nonmotile.

Table II. Numbers and Distributions of VTEC Strains Belonging to the O111 Serogroup[a]

Serotype	Humans			Cattle		Ruminant products		No. of isolates
	Healthy	Sick	Unknown	Healthy	Sick	Beef	Venison	
O111:H2	–	4	–	1	–	–	–	5
O111:H7	–	1	–	–	–	–	–	1
O111:H8	–	8	–	4	1	–	–	13
O111:H11	–	–	–	1	2	–	–	3
O111:H21	–	–	1	–	–	–	–	1
O111:H30	–	1	–	–	–	–	–	1
O111:H45	–	–	–	–	–	–	1	1
O111:H^{-b}	–	44	1	8	3	1	–	57
Total	–	58	2	14	6	1	1	82

[a] The data were summarized from several reports (22, 23, 25, 27, 28, 32, 34, 41, 59, 76–78, 80, 82, 83, 87, 89, 90, 97–99, 103–107, 109, 112–115, 117–127).
[b] Nonmotile.

Table III. Numbers and Distributions of VTEC Strains Belonging to the O5 Serogroup[a]

Serotype	Source	Condition	No. of isolates
O5:H10	Cattle	Healthy	1
O5:H11	Goats	Healthy	1
O5:H^{-b}	Humans	Healthy	2
O5:H$^-$	Humans	Sick	7
O5:H$^-$	Cattle	Healthy	4
O5:H$^-$	Cattle	Sick	4
O5:H$^-$	Goats	Healthy	1
O5:H$^-$	Sheep	Healthy	5
O5:H$^-$	Beef	–	1
O5:H$^-$	Mutton	–	1
O5:H$^-$	Sausage	–	1
Total			28

[a] The data were summarized from several reports (21, 24, 50, 51, 76, 78, 82, 83, 87, 97, 104, 106, 113, 114, 126, 128–135).
[b] Nonmotile.

The VTEC O5 Serogroup. Table III summarizes published reports on VTEC isolates belonging to the O5 serogroup. Of these 28 O5 isolates, nine were from humans, 10 were from cattle, six were from sheep, two were from goats, and one was from swine (sausage) origin. It is worth noting that the number of nonmotile strains was very high (i.e., 93% of total isolates).

The VTEC O91 Serogroup. Table IV summarizes published reports on the number and distribution of VTEC isolates belonging to the O91 serogroup. This table shows that the number of nonmotile strains was high (35% of total isolates). The table also illustrates the presence of several motile strains, particularly those carrying the H antigens 10, 14, and 21. Sheep and pigs appeared to carry the nonmotile O91strain of VTEC, whereas cattle were the source of the motile serotypes, especially O91:H21. It remains to be determined whether this unique variation in VTEC shedding reflects a difference in host specificity.

The VTEC O128, O153, and O8 Serogroup. Summaries of published reports on the number and distribution of VTEC isolates belonging to the O128 (Table V), O153 (Table VI), and O8 (Table VII) serogroups are presented. Of the 38 VTEC O128 isolates (Table V), *E. coli* O128:H2 was the predominant serotype (i.e., 58% of total isolates). It is interesting that several O128 serotypes (i.e., H2, H7, H8, H10, and H45) have been isolated from humans. Of the 31 VTEC O153 isolates (Table VI), *E. coli* O153:H25 was the predominant serotype (i.e., 48% of total isolates). Of the 35 VTEC O8 isolates (Table VII), *E. coli* O8:H19 was the predominant serotype (i.e., 34% of total isolates).

The VTEC O22, O103, O113, O118, and O145 Serogroups. Table VIII summarizes published reports on the remaining non-O157 VTEC serogroups (O22, O103, O113, O118, and O145) isolated from humans and cattle. Of the 195 reported isolates, 80 were from humans exhibiting VTEC-related illnesses, 64 were from healthy cattle, and 51

Table IV. Numbers and Distributions of VTEC Strains Belonging to the O91 Serogroup[a]

Serotype	Source	Condition	No. of isolates
O91:H7	Cattle	Healthy	1
O91:H10	Humans	Sick	4
O91:H10	Cattle	Healthy	1
O91:H14	Humans	Healthy	3
O91:H14	Humans	Sick	4
O91:H14	Cattle	Healthy	1
O91:H14	Pork	–	1
O91:H21	Humans	Sick	7
O91:H21	Cattle	Healthy	6
O91:H21	Beef	–	3
O91:H21	Pork	–	1
O91:H21	Meat	–	1
O91:H21	Milk	–	1
O91:H21	Cheese	–	1
O91:H40	Humans	Sick	1
O91:H49	Cattle	Healthy	1
O91:H^{-}[b]	Humans	Healthy	1
O91:H^{-}	Humans	Sick	5
O91:H^{-}	Beef	–	2
O91:H^{-}	Sheep	Healthy	6
O91:H^{-}	Mutton	–	1
O91:H^{-}	Meat	–	1
O91:H^{-}	Pigs	Healthy	1
O91:H^{-}	Pigs	Sick	1
O91:H^{-}	Sausage	–	2
Total			57

[a] The data were summarized from several reports (50, 51, 55, 59, 77, 82, 83, 90, 94, 97, 99, 100, 104, 114, 129–132, 134–145).
[b] Nonmotile.

Table V. Numbers and Distributions of VTEC Strains Belonging to the O128 Serogroup[a]

Serotype	Source	Condition	No. of isolates
O128:H2	Humans	Healthy	4
O128:H2	Humans	Sick	12
O128:H2	Humans	Unknown	1
O128:H2	Beef	–	2
O128:H2	Sheep	Healthy	3
O128:H7	Humans	Sick	1
O128:H8	Humans	Sick	2
O128:H10	Humans	Healthy	1
O128:H35	Cattle	Healthy	1
O128:H35	Beef	–	1
O128:H45	Humans	Sick	2
O128:H45	Humans	Unknown	1
O128:H^{-}[b]	Humans	Sick	5
O128:H^{-}	Cattle	Healthy	1
O128:H^{-}	Sheep	Healthy	1
Total			38

[a] The data were summarized from several reports (32, 50, 51, 55, 59, 77, 81–83, 91, 99, 101, 109, 110, 113, 124, 137, 144, 151).
[b] Nonmotile.

were from cattle products (beef or milk). As illustrated in this table, the serotypes listed are more frequently associated with human illnesses.

A Summary of Non-O157 VTEC Isolates. Table IX summarizes the published data (i.e., the 1560 reported

Table VI. Numbers and Distributions of VTEC Strains Belonging to the O153 Serogroup[a]

Serotype	Source	Condition	No. of isolates
O153:H2	Humans	Sick	1
O153:H8	Beef	–	1
O153:H9	Cattle	Healthy	1
O153:H12	Cattle	Healthy	1
O153:H12	Cattle	Sick	2
O153:H19	Cattle	Healthy	2
O153:H21	Cattle	Healthy	2
O153:H25	Humans	Sick	3
O153:H25	Cattle	Healthy	7
O153:H25	Beef	–	3
O153:H25	Milk	–	1
O153:H25	Sheep	Healthy	1
O153:H31	Cattle	Healthy	3
O153:H^{-}[b]	Cattle	Healthy	1
O153:H^{-}	Sheep	Healthy	2
Total			31

[a] The data were summarized from several reports (23, 24, 50, 83, 97, 100, 101, 104, 106, 112, 114, 129, 134, 135, 140, 146–148).
[b] Nonmotile.

Table VII. Numbers and Distributions of VTEC Strains Belonging to the O8 Serogroup[a]

Serotype	Source	Condition	No. of isolates
O8:H2	Humans	Sick	1
O8:H2	Cattle	Healthy	1
O8:H2	Pork	–	1
O8:H8	Cattle	Healthy	1
O8:H9	Cattle	Sick	1
O8:H9	Beef	–	3
O8:H9	Pork	–	1
O8:H9	Cheese	–	1
O8:H11	Pigs	Sick	1
O8:H19	Cattle	Healthy	6
O8:H19	Cattle	Sick	1
O8:H19	Beef	–	4
O8:H19	Pork	–	1
O8:H21	Humans	Sick	1
O8:H25	Humans	Sick	2
O8:H25	Cattle	Healthy	1
O8:H30	Beef	–	1
O8:H35	Cattle	Healthy	1
O8:H^{-}[b]	Humans	Healthy	1
O8:H?	Humans	Sick	3
O8:H?	Beef	–	2
Total			35

[a] The data were summarized from several reports (41, 50, 55, 59, 79, 83, 100–102, 104, 105, 114, 123, 126, 130, 134, 143, 149, 150).
[b] Nonmotile.

isolates) on non-O157 VTEC. This table demonstrates that there are some clear differences between the various VTEC serogroups. Although 48.2% of the VTEC isolates belonged to the more common serogroups (i.e., O5, O8, O22, O26, O91, O103, O111, O118, O128, O145, and O153) associated with human illnesses, only 26.3% of the less common VTEC serogroups were associated with human illnesses. However, it is interesting that only 22.9% of the more common serogroups and 40.7% of the less common VTEC sero-

Table VIII. Numbers and Distributions of VTEC Strains Belonging to the O22, O103, O113, O118, and O145 Serogroup[a]

Serotype	Humans			Cattle		Cattle products		No. of isolates
	Healthy	Sick	Unknown	Healthy	Sick	Beef	Milk	
O22:H8	1	1	–	11	–	10	1	24
O103:H2	5	24	1	12	–	4	1	47
O113:H4	3	2	–	6	–	7	1	19
O113:H21	–	8	–	10	1	6	–	25
O113:H^{-}[b]	2	–	–	6	–	1	–	9
O118:H2	–	3	–	–	–	–	–	3
O118:H12	–	11	–	–	–	–	–	11
O118:H16	–	10	1	4	1	–	–	16
O118:H30	–	2	–	–	–	–	–	2
O118:H^{-}	–	3	1	4	2	–	–	10
O145:H8	–	–	–	2	–	–	–	2
O145:H16	–	1	–	1	–	–	–	2
O145:H25	–	1	–	1	–	–	–	2
O145:H28	–	2	–	1	–	1	–	4
O145:H^{-}	–	12	–	6	–	1	–	19
Total	11	80	3	64	4	30	3	195

[a] The data were summarized from several reports (21, 24, 29, 30, 32, 35, 41, 50, 54, 55, 59, 77, 82, 83, 89, 93, 97–101, 103–110, 112–115, 124, 126, 129, 130–132, 134, 139, 140, 142, 145, 148, 149, 152–162).
[b] Nonmotile.

groups were isolated from healthy cattle. It is still unclear why outbreaks of human illnesses are commonly associated with VTEC serotypes that are found at less frequency in healthy cattle.

Recent studies (56–58) confirmed that ruminant-derived VTEC are potential human pathogens. It is possible that only some variants may actually be capable of causing human illnesses. It has been shown that certain verotoxin subtypes are more likely to be associated with more virulent VTEC serogroups such as O26, O103, O111, O145, and O157 (59), and also there appears to be a relationship between the verotoxin produced and the VTEC serotype (56, 58). Further studies may confirm that certain serotypes are associated with certain hosts and tend to carry unique virulence factors. This appears to be the case with the attaching and effacing (*eae*) genes, which have been associated with many of the more important human pathogenic VTEC serotypes, including O111:H^{-} and O157:H7 (60). However, strains of *E. coli* O113:H21 as well as other VTEC lacking the *eae*-genes have been recently shown (61) to carry another virulence factor, Saa (STEC autoagglutinating adhesin). Because not all VTEC produce either of these factors, it is clear that other virulence factors remain to be identified.

The Paradigm

The Concept of the Paradigm. All warm-blooded animals as well as humans carry *E. coli* in their intestines, with numbers as high as 10^9/g of feces (62). Thus, it can be

Table IX. A Summary of Serogroup Distribution of Non-O157 VTEC Strains Isolated from Humans, Cattle, Sheep, and Other Animals

Serogroup	Humans			Cattle		Sheep	Other animals	Total
	Healthy	Sick	Unknown	Healthy	Sick	Healthy		
O5	2	7	–	5	4	–	5	28
O8	1	7	–	10	–	–	15	35
O26	3	76	2	9	12	–	3	105
O91	4	21	–	10	–	6	16	57
O111	–	58	2	14	6	–	2	82
O128	5	22	2	2	–	4	3	33
O153	–	4	–	17	3	3	4	31
O22, O103, O118, and O145	11	80	3	64	4	–	33	195
Total	26	275	9	131	31	18	81	571
Percentage	4.6	48.2	1.6	22.9	5.4	3.2	14.2	
Other groups	72	260	8	403	4	45	197	989
Percentage	7.3	26.3	0.8	40.7	0.4	4.6	19.9	
Grand total	98	535	17	534	35	63	278	1560
Percentage	6.3	34.3	1.1	34.2	22.4	4.0	17.8	

easily calculated that 10^{21} *E. coli* of human origin are shed daily into the environment. *E. coli* usually colonize in human infants within 4 days. Infants acquire their *E. coli* either from their mothers' feces at birth or more rarely from the environment (63). *E. coli* are continuously ingested with food and replace part of the intestinal *E. coli* population (64). This creates the great variety of *E. coli* types present in human feces at any time (65). These various *E. coli* types are in constant flux and competition with each other and with other bacterial species in the human intestine. *E. coli* are also able to survive outside the intestines and have been isolated from most nonmarine aqueous environments, including pristine samples in tropical rain forests and water reservoirs designed for human use (66).

In the 1940s, it was realized that certain *E. coli* types can cause severe diarrhea, especially in infants (67). In the 1970s, Rowe *et al.* (68) indicated that a group of *E. coli* was responsible for a large number of the cases of travelers' diarrhea. The pathogens were shown to produce one or two enterotoxins and were named the enterotoxigenic *E. coli* (ETEC). Occasional outbreaks of food-borne diarrhea in the developed countries were also attributed to these ETEC (69). The VTEC emerged since the early 1980s (9) as cases of outbreaks of human illnesses were reported.

The healthy human colonic *E. coli* community is diverse, and a clear distinction can be drawn between the resident and transient *E. coli* serotypes. Only special selective methodology will be able to characterize the transient types of *E. coli*. Changes in life patterns such as travel or eating out appear to cause a greater change in the colonic *E. coli* community than regular eating at home (64, 70, 71).

The changes in *E. coli* population in ruminants such as cattle and sheep are most probably similar to those occurring in humans. For example, grazing cattle and sheep will continuously be recontaminated with the *E. coli* strains that were shed by other members of their herds or flocks, respectively. It has been shown that during slaughter, dressing, and processing of ruminant carcasses, fecal *E. coli* can contaminate the meat surfaces (72).

For almost two decades, most VTEC studies concentrated only on *E. coli* O157:H7. A recent study (73), however, has shown that although a single outbreak was predominantly due to *E. coli* O111:H$^-$, other VTEC serotypes (e.g., O157:H7) were also involved. If only standard screening methods had been used, this outbreak would have been attributed to *E. coli* O157:H7. When VTEC isolates were recently compared over a 5-year period in Australia, it was found that VTEC other than O157:H7 were the major causes of human illnesses (40).

The concept of the VTEC paradigm is that out of commensal *E. coli*, there can emerge pathogenic variants that cause human illnesses. These pathogens differ from their commensal counterparts in their carriage of the virulence factors. There will also be strains among these commensal *E. coli* that carry some of the virulence factors, but cannot cause human illnesses.

When is a VTEC not a pathogen? The characteristics that are required for a commensal *E. coli* to establish itself within the human intestines and to compete with other *E. coli* as well as other bacterial species or types are obviously of great advantage to VTEC. The verotoxins have been demonstrated to be very potent toxins, with LD_{50} levels for mice in the order of 10^{-9} g (74). Also, many VTEC produce a variety of virulence factors that make them potent human pathogens. Despite being such potent human pathogens, they appear to be virtually harmless to ruminants, in which they are able to spread from animal to animal, from herd to herd, and across continents. The VTEC serotypes with a global distribution include O5:H$^-$, O26:H11, O91:H$^-$, O113:H21, O116:H21, O123:H$^-$, and O128:H2. Although VTEC comprise only a small proportion of the ruminant *E. coli* community, they have a selective advantage (75). This suggests that the factors providing VTEC with a competitive advantage in one niche (ruminants) may be the same factors that make them into pathogens in another host (humans).

Future Considerations

Our methods of producing, distributing, and handling food have changed extraordinarily in the past century. Many of these processes have created appropriate niches for food-borne pathogens. Such pathogens will continue to be difficult to identify, they will merge among the commensal groups, and will require extensive microbiological experience to be identified. It should be considered that more pathogenic *E. coli* will continue to emerge. There are already numbers of such variants, including the enteroaggregative *E. coli*, the diffuse adherent *E. coli*, the cytotoxic necrotizing factor-producing *E. coli*, the cytolethal distending toxin-producing *E. coli*, and probably many others. Therefore, pathogenic *E. coli* can be seen as a paradigm of potential future food-borne pathogens and probably other pathogens as well. The current heavy reliance on very specific tests based either on the use of specific nucleic acid sequences or monoclonal antibodies will become a severe disadvantage in detecting such newly emerging pathogens. Only the experienced and well-trained microbiologist with a keen eye, an open mind, and a strong sense of curiosity will be able to observe such new developments. It is in the hands of these scientists that the challenges of the twenty-first century should be placed.

1. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells GJ, Davis BR, Herbert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med **308**:681–685, 1983.
2. Centers for Disease Control and Prevention. Update: multistate outbreak of *Escherichia coli* O157 infections in hamburgers: Western United States 1992-1993. Morbid Mortal Wkly Rep **42**:258–263, 1993.
3. Watanabe H, Wada A, Inagaki Y, Itoh K, Tamura K. Outbreaks of enterohaemorrhagic *Escherichia coli* O157:H7 infection by two different genotype strains in Japan. Lancet **348**:381–382, 1996.

4. March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with haemorrhagic colitis. J Clin Microbiol **23**:869–872, 1986.
5. Bettelheim KA. Reliability of CHROMagar O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. J Appl Microbiol **85**:425–428, 1998.
6. Bettelheim KA. Studies of *Escherichia coli* cultured on Rainbow agar O157 with particular reference to Enterohaemorrhagic *Escherichia coli* (EHEC). Microbiol Immunol **42**:265–269, 1998.
7. Ørskov F, Ørskov I, Villar JA. Cattle as a reservoir of verotoxin-producing *Escherichia coli* O157:H7. Lancet **ii**:276, 1987.
8. Feng P, Lampel KA, Karch H, Whittam TS. Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. J Infect Dis **177**:1750–1753, 1998.
9. Konowalchuk J, Speirs JL, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun **18**:775–779, 1977.
10. Wade WG, Thom BT, Evans N. Cytotoxic Enteropathogenic *Escherichia coli*. Lancet **ii**:1235–1236, 1979.
11. Wilson MW, Bettelheim KA. Cytotoxic *Escherichia coli* serotypes. Lancet **i**:201, 1980.
12. Bettelheim KA, Wilson MW. The enterotoxigenicity of strains of *Escherichia coli* isolated from the faeces of healthy people and cattle. J Hyg (Camb) **88**:121–123, 1982.
13. Belnap WD, O'Donnell JJ. Epidemic gastroenteritis due to *Escherichia coli* O-111. J Pediatr **47**:178–193, 1955.
14. Karmali MA. Infection by verocytotoxin-producing *Escherichia coli*. Clin Microbiol Rev **2**:15–38, 1989.
15. Conradi H. Ueber lösliche, durch aseptische autolyse erhaltene Giftstoffe von Ruhr- und Typhus-Bazillen. Dtsche Med Wchschr **29**:26–28, 1903.
16. Neisser M, Shiga K. Ueber freie Receptoren von Typhus- und Dysenteriebazillen und über das Dysenterietoxin. Dtsche Med Wchschr **29**:61–62, 1903.
17. O'Brien 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.
18. Strockbine NA, Marques LRM, Newland JW, Smith HW, Holmes RK, O'Brien AD. Two toxin-converting phages from *Escherichia coli* O157:H7 strain 933 encode antigenically distinct toxins with similar biological activities. Infect Immun **53**:135–140, 1986.
19. O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. Microbiol Rev **51**:206–220, 1987.
20. von Gasser C, Gautier E, Steck A, Siebenmann RE, Oechslin R. Hämolytisch-urämischessyndrome: beilaterale nierenrindennekrosen bei akuten erworbenen hämolytischen anämien. Schweiz Med Wchschr **85**:905–909, 1955.
21. Tzipori S, Wachsmuth KI, Smithers J, Jackson C. Studies in gnotobiotic piglets on non-O157:H7 *Escherichia coli* serotypes isolated from patients with hemorrhagic colitis. Gastroenterology **94**:590–597, 1988.
22. Gunzburg S, Gracey M, Forbes D, Hewitt I, Bettelheim KA. Haemolytic-uraemic syndrome and verotoxigenic *Escherichia coli*. Med J Aust **149**:54–55, 1988.
23. Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol **25**:237–243, 1988.
24. Dorn CR, Scotland SM, Smith HR, Willshaw GA, Rowe B. Properties of verocytotoxin-producing *Escherichia coli* of human and animal origin belonging to serogroups other than O157:H7. Epidemiol Infect **103**:83–95, 1989.
25. Caprioli A, Luzzi I, Rosmini F, Resti C, Edefonti A, Perfumo F, Farina C, Goglio A, Gianviti A, Rizzoni G. Community-wide outbreak of hemolytic-uremic syndrome associated with non-O157 verocytotoxin-producing *Escherichia coli*. J Infect Dis **169**:208–211, 1994.
26. Cameron S, Walker C, Beers M, Rose N, Anear E. Enterohaemorrhagic *Escherichia coli* outbreak in South Australia associated with consumption of mettwurst. Comm Dis Intell **19**:70–71, 1995.
27. Paton AW, Ratcliff RM, Doyle MR, Seymour-Murray J, Davos D, Lancer JA, Paton JC. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. J Clin Microbiol **34**:1622–1627, 1996.
28. Morabito S, Karch H, Mariani-Kurkdjian P, Schmidt H, Minelli F, Bingen E, Caprioli A. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J Clin Microbiol **36**:840–842, 1998.
29. Mariani-Kurkdjian P, Denamur E, Milon A, Picard B, Cave H, Lambert-Zechovsky N, Loirat C, Goullet P, Sansonetti P, Elion J. Identification of a clone of *Escherichia coli* O103:H2 as a potential agent of hemolytic uremic syndrome in France. J Clin Microbiol **31**:296–301, 1993.
30. Tarr PI, Fouser LS, Stapleton AE, Wilson RA, Kim HH, Vary JC, Clausen CR. Hemolytic-uremic syndrome in a six-year-old girl after a urinary tract infection with Shiga-toxin-producing *Escherichia coli* O103:H2. N Engl J Med **335**:635–638, 1996.
31. Karch H, Geitz C, Schmidt H. Increased Incidence of Infections with EHEC O103:H2. Notiziaro dell'Istituto Superiore di Sanita **10**(3):2, 1997.
32. Kudoh Y, Kai A, Obata H, Kusunoki J, Monma C, Shingaki M, Yanagawa Y, Yamada S, Mtsushita S, Itoh T, Ohta K. Epidemiological Surveys on Verocytotoxin-Producing *Escherichia coli* Infections in Japan. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp53–56, 1994.
33. Centers for Disease Control and Prevention. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21: Helena, Montana, 1994. Morbid Mortal Wkly Rep **44**:501–503, 1995.
34. Bergmire-Sweat D, Marengo L, Pendergrass P, Hendricks K, Garcia M, Drumgoole R, Baldwin T, Kingsley K, Walsh B, Lang S, Prine L, Busby T, Trujillo L, Perrotta D, Hathaway A, Jones B, Jaiyeola A. *Escherichia coli* O111:H8 outbreak among teenage campers: Texas, 1999. J Am Med Assoc **283**:2517, 2000.
35. Wieler LH, Busse B, Steinruck H, Beutin L, Weber A, Karch H, Baljer G. Enterohemorrhagic *Escherichia coli* (EHEC) strains of serogroup O118 display three distinctive clonal groups of EHEC pathogens. J Clin Microbiol **38**:2162–2169, 2000.
36. Bettelheim KA. Biochemical studies on enterohaemorrhagic *Escherichia coli* (EHEC). In: Kay D, Fricker C, Eds. Coliforms and *E. coli* Problem or Solution. Gateshead, Tyne, and Wear, UK: Royal Society of Chemistry, Atheneum Press, pp243–248, 1997.
37. Beutin L, Montenegro MA, Ørskov I, Ørskov F, Prada J, Zimmermann S, Stephan R. Close association of verocytotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. J Clin Microbiol **27**:2559–2564, 1989.
38. Bettelheim KA. Identification of enterohaemorrhagic *Escherichia coli* by means of their production of enterohaemolysin. J Appl Bacteriol **79**:178–180, 1995.
39. Bettelheim KA. A spectrum of verocytotoxigenic *Escherichia coli*. J Med Microbiol **47**:1037–1038, 1998.
40. Bettelheim KA. Enterohaemorrhagic *Escherichia coli* O157:H7: a red herring? J Med Microbiol **50**:201–202, 2001.
41. Lehmacher A, Meier H, Aleksic S, Bockemühl J. Detection of hemolysin variants of Shiga toxin-producing *Escherichia coli* by PCR and culture on vancomycin-cefixime-cefsulodin blood agar. Appl Environ Microbiol **64**:2449–2453, 1998.
42. Hornitzky MA, Bettelheim KA, Djordjevic SP. The detection of Shiga toxin-producing *Escherichia coli* in diagnostic bovine faecal samples using vancomycin-cefixime-cefsulodin blood agar and PCR. FEMS Microbiol Lett **198**:17–22, 2001.
43. Karch H, Bielaszewska M. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H$^-$ strains: epidemiology, pheno-

typic and molecular characteristics, and microbiological diagnosis. J Clin Microbiol **39**:2043–2049, 2001.
44. Acheson DWK, Keusch GT, Lightowlers M, Donohue-Rolfe A. Enzyme-linked immunosorbent assay for Shiga toxin and Shiga-like toxin II using P1 glycoprotein from hydatid cysts. J Infect Dis **161**:134–137, 1990.
45. Chart H, Willshaw GA, Cheasty T. Evaluation of a reversed passive latex agglutination test for the detection of verocytotoxin (VT) expressed by strains of VT-producing *Escherichia coli*. Lett Appl Microbiol **32**:370–374, 2001.
46. Bettelheim KA. Development of a rapid method for the detection of verocytotoxin-producing *Escherichia coli* (VTEC). Lett Appl Microbiol **33**:31–35, 2001.
47. Bastian SN, Carle I, Grimont F. Comparison of 14 PCR systems for the detection and subtyping of *stx* genes in Shiga-toxin-producing *Escherichia coli*. Res Microbiol **149**:457–472, 1998.
48. Paton AW, Paton JC, Goldwater PN, Manning PA. Direct detection of *Escherichia coli* Shiga-like toxin genes in primary fecal cultures by polymerase chain reaction. J Clin Microbiol **31**:3063–3067, 1993.
49. Fagan PK, Hornitzky MA, Bettelheim KA, Djordjevic SP. Detection of Shiga-like toxin (*stx1* and *stx2*), intimin (*eaeA*), and enterohemorhagic *Escherichia coli* (EHEC) hemolysin EHEC *hlyA*) genes in animal feces by multiplex PCR. Appl Environ Microbiol **65**:868–872, 1999.
50. 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.
51. 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.
52. Sidjabat-Tambunan H, Bensink J. Verotoxin-producing *Escherichia coli* from faeces of sheep, calves and pigs. Aust Vet J **75**:292–293, 1997.
53. Kobayashi H, Shimada J, Nakazawa M, Morozumi T, Pohjanvirta T, Pelkonen S, Yamamoto K. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl Environ Microbiol **67**:484–489, 2001.
54. Stephan R, Hoelzle LE. Characterization of Shiga toxin type 2 variant B-subunit in *Escherichia coli* strains from asymptomatic human carriers by PCR-RFLP. Lett Appl Microbiol **31**:139–142, 2000.
55. Stephan R, Ragettli S, Untermann F. Prevalence and characteristics of verotoxin-producing *Escherichia coli* (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. J Appl Microbiol **88**:335–341, 2000.
56. 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.
57. Pradel N, Boukhors K, Bertin Y, Forestier C, Martin C, Livrelli V. 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, 2001.
58. Ramachandran V, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP. The common ovine Shiga toxin 2-containing *Escherichia coli* serotypes and human isolates of the same serotypes possess a Stx2d toxin type. J Clin Microbiol **39**:1932–1937, 2001.
59. Piérard D, Stevens D, Moriau L, Lior H, Lauwers S. Isolation and virulence factors of verocytotoxin-producing *Escherichia coli* in human stools. Clin Microbiol Infect **3**:531–540, 1997.
60. Sperandio V, Kaper JB, Bortolini MR, Neves BC, Keller R, Trabulsi LR. Characterization of the locus of enterocyte effacement (LEE) in different enteropathogenic *Escherichia coli* (EPEC) and Shiga-toxin producing *Escherichia coli* (STEC) serotypes. FEMS Microbiol Lett **164**:133–139, 1998.
61. Paton AW, Srimanote P, Woodrow MC, Paton JC. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infect Immun **69**:6999–7009, 2001.
62. Mitsuoka T, Hayakawa K. Die Faecalflora bei Menschen. I. Mitteilung: Die Zusammensetzung der Faecalflora der verschiedenen Altersgruppen. Zbl Bakt Parasit Hyg I Abt Orig A **223**:333–342, 1972.
63. Bettelheim KA, Lennox-King SMJ. The acquisition of *Escherichia coli* by newborn babies. Infection **4**:174–179, 1976.
64. Shooter RA, Bettelheim KA, Lennox-King SMJ, O'Farrell S. *Escherichia coli* serotypes in the faeces of healthy adults over a period of several months. J Hyg (Camb) **78**:95–98, 1977.
65. Bettelheim KA, Faiers M, Shooter RA. Serotypes of *Escherichia coli* in normal stools. Lancet **ii**:1224–1226, 1972.
66. Niemi RM, Niemi JS. Bacterial pollution of waters in pristine and agricultural lands. J Environ Qual **20**:620–627, 1991.
67. Bray J. Isolation of antigenically homogeneous strains of *Bact. coli neapolitanum* from summer diarrhea of infants. J Pathol Bacteriol **63**:239–247, 1945.
68. Rowe B, Taylor J, Bettelheim KA. An investigation of travelers' diarrhea. Lancet **i**:1–5, 1970.
69. Caprioli A, Falbo V, Ruggeri FM, Bisichia R, Casalino M, Donelli G. Comparison among enterotoxigenic strains of *Escherichia coli* isolated in Italy and Somalia. Eur J Epidemiol **4**:251–255, 1988.
70. Bettelheim KA, Cooke EM, O'Farrell SM, Shooter RA. The effect of diet on intestinal *Escherichia coli*. J Hyg (Camb) **79**:43–45, 1977.
71. Majed NI, Bettelheim KA, Shooter RA, Moorhouse E. The effect of travel on faecal *Escherichia coli* serotypes. J Hyg (Camb) **8**:481–487, 1978.
72. Bettelheim KA. The isolation of *Escherichia coli* from a sheep slaughtering line in an abattoir. Comp Immun Microbiol Infect Dis **4**:93–100, 1981.
73. Goldwater PN, Bettelheim KA. *Escherichia coli* O group serology of an haemolytic uraemic syndrome (HUS) epidemic. Scand J Infect Dis **32**:385–394, 2000.
74. Tesh VL, Burris JA, Owens JW, Gordon VM, Wadolkowski EA, O'Brien AD, Samuel JE. Comparison of the relative toxicities of Shiga-like toxin type I and type II for mice. Infect Immun **61**:3392–3402, 1993.
75. Bettelheim KA. Ecology of verotoxigenic *Escherichia coli*. Aust Vet J **75**:912, 1997.
76. Bielaszewska M, Janda J, Blahova K, Feber J, Potuzník V, Souckova A. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome in the Czech Republic. Clin Nephrol **46**:42–44, 1996.
77. Bockemühl J, Aleksic S, Karch H. Serological and biochemical properties of Shiga-like toxin (Verotoxin)-producing strains of *Escherichia coli*, other than O-group 157, from patients in Germany. Zbl Bakt **276**:189–195, 1992.
78. Bielaszewska M, Janda J, Blahova K, Srámkova L, Havlík J, Potuzník V. Verocytotoxin-producing *Escherichia coli* in children with hemolytic uremic syndrome and diarrhea in the Czech Republic. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp37–40, 1994.
79. Blanco M, Blanco JE, Blanco J, Mora A, Prado C, Alonso MP, Mourino M, Madrid C, Balsalobre C, Juarez AV. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet Microbiol **54**:309–319, 1997.
80. Bokete TN, Whittam TS, Wilson RA, Clausen CR, Ocallahan CM, Moseley SL, Fritsche TR, Tarr PI. Genetic and phenotypic analysis of *Escherichia coli* with enteropathogenic characteristics isolated from Seattle children. J Infect Dis **175**:1382–1389, 1997.
81. Brooks HJL, Bettelheim KA, Todd B, Holdaway MD. Non-O157 vero cytotoxin producing *Escherichia coli*: aetiological agents of di-

arrhoea in children in Dunedin. New Zealand Comp Immun Microbiol Infect Dis **20:**163–170, 1997.
82. Beutin L, Zimmermann S, Gleier K. Human infections with Shiga toxin-producing *Escherichia coli* other than serogroup O157 in Germany. Emerg Infect Dis **4:**635–639, 1998.
83. Clarke RC, Wilson JB, Read SC, Renwick S, Rahn K, Johnson RP, Alves D, Karmali MA, Lior H, McEwen SA, Spika J, Gyles CL. Verocytotoxin-producing *Escherichia coli* (VTEC) in the food chain: preharvest and processing perspectives. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp17–24, 1994.
84. Caprioli A, Luzzi I. VT2-producing *E. coli* O26:H11. Notiziaro dell'Istituto Superiore di Sanita **8:**4, 1995.
85. Czirok E, Herpay M. National surveillance of VTEC infection (Hungary). Notiziaro dell'Istituto Superiore di Sanita **8:**2, 1995.
86. Cobbold R, Desmarchelier P. A longitudinal study of Shiga-toxigenic *Escherichia coli* (STEC) prevalence in three Australian dairy. Vet Microbiol **71:**125–137, 2000.
87. Dorn CR, Francis DH, Angrick EJ, Wilgohs JA, Wilson RA, Collins JE, Jenke BH, Shawd SJ. Characteristics of verocytotoxin-producing *Escherichia coli* associated with intestinal colonization and diarrhea in calves. Vet Microbiol **36:**149–159, 1993.
88. Enami M, Nakasone N, Honma Y, Kakinohana S, Kudaka J, Iwanaga M. Expression of type I pili is abolished in verotoxin-producing *Escherichia coli* O157. FEMS Microbiol Lett **179:**467–472, 1999.
89. Fey PD, Wickert RS, Rupp ME, Safranek TJ, Hinrichs SH. Prevalence of non-O157:H7 Shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg Infect Dis **6:**530–533, 2000.
90. Goldwater PN, Bettelheim KA. The role of enterohaemorrhagic *E. coli* serotypes other than O157:H7 as causes of disease. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp57–60, 1994.
91. Giammanco A, Maggio M, Giammanco G, Morelli R, Minelli F, Scheutz F, Caprioli A. Characteristics of *Escherichia coli* strains belonging to enteropathogenic *E. coli* serogroups isolated in Italy from children with diarrhea. J Clin Microbiol **34:**689–694, 1996.
92. Farina C, Goglio A, Conedera G, Minelli F, Caprioli A. Antimicrobial susceptibility of *Escherichia coli* O157 and other enterohaemorrhagic *Escherichia coli* isolated in Italy. Eur J Clin Microbiol Infect Dis **15:**351–353, 1996.
93. Huppertz HI, Rutkowski S, Busch DH, Eisebit R, Lissner R, Karch H. Bovine colostrum ameliorates diarrhea in infection with diarrheagenic *Escherichia coli*, Shiga toxin-producing *E. coli*, and *E. coli* expressing intimin and hemolysin. J Pediatr Gastroenteriol Nutr **29:**452–456, 1999.
94. Keskimäki M, Saari M, Heiskanen T, Siitonen A. Shiga toxin-producing *Escherichia coli* in Finland from 1990 through 1997: prevalence and characteristics of isolates. J Clin Microbiol **36:**3641–3646, 1998.
95. Ludwig K, Petric M, Blanchette V, Karmali M. Isolated thrombocytopenic purpura associated with infection due to verocytotoxin (Shiga toxin)-producing *Escherichia coli* serotype O26:H11. Clin Infect Dis **27:**660–661, 1998.
96. Mackenzie AMR, Lebel P, Orrbine E, Rowe PC, Hyde L, Chan F, Johnson W, McLaine PN. Sensitivities and specificities of premier *E. coli* O157 and premier EHEC enzyme immunoassays for diagnosis of infection with verotoxin (Shiga-like toxin)-producing *Escherichia coli*. J Clin Microbiol **36:**1608–1611, 1998.
97. Meng JH, Zhao SH, Doyle MP. Virulence genes of Shiga toxin-producing *Escherichia coli* isolated from food, animals and humans. Int J Food Microbiol **45:**229–235, 1998.
98. Miyamoto H. Statistical and Epidemiological Analyses on Food Poisonings and Infections and Applications by Microbiological Analyses, Project Research on Application of DNA and Phage Typing, etc. as Epidemiological Markers and Development of New Rapid Typings in Big Outbreak of Bacterial Food Poisonings. National Reports on "Emerging and Reemerging Infectious Diseases" 1997–1999; Ministry of Health and Welfare, Japan, 2000.
99. Piérard D, Stevens D, Moriau L, Lior H, Lauwers S. Three years PCR screening for VTEC in human stools in Brussels. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp33–36, 1994.
100. 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.
101. Read SC, Clarke RC, Martin A, De Grandis SA, Hii J, Gyles CL. Polymerase chain reaction for detection of verocytotoxigenic *Escherichia coli* isolated from animal and food sources. Mol Cell Probes **6:**153–161, 1992.
102. Smith HR, Scotland SM, Willshaw GA, Wray C, McLaren IM, Cheasty T, Rowe B. Verocytotoxin production and the presence of VT genes in *Escherichia coli* strains of animal origin. J Gen Microbiol **134:**829–834, 1988.
103. Scheutz F. VTEC isolated from Danish patients, 1986–1994. Notiziaro dell'Istituto Superiore di Sanita **8:**3, 1995.
104. Sandhu KS, Clarke RC, McFadden K, Brouwer A, Loouie M, Wilson J, Lior H, Gyles CL. Prevalence of the *eaeA* gene in verotoxigenic *Escherichia coli* strains from dairy cattle in Southwest Ontario. Epidemiol Infect **116:**1–7, 1996.
105. Schmidt H, von Maldeghem J, Frosch M, Karch H. Antibiotic susceptibilities of verocytotoxin-producing *Escherichia coli* O157 and non-O157 strains isolated from patients and healthy subjects in Germany during 1996. Antimicrob Chemother **42:**548–550, 1998.
106. Sandhu KS, Clarke RC, Gyles CL. Virulence markers in Shiga toxin-producing *Escherichia coli* isolated from cattle. Can J Vet Res **63:**177–184, 1999.
107. Schmidt H, Geitz C, Tarr PI, Frosch M, Karch H. Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. J Infect Dis **179:**115–123, 1999.
108. Schurman RD, Hariharan H, Heaney SB, Rahn K. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in beef cattle slaughtered on Prince Edward Island. J Food Protect **63:**1583–1586, 2000.
109. Tamura K, Sakazaki R, Murase M, Kosako Y. Serotyping and categorisation of *Escherichia coli* strains isolated between 1958 and 1992 from diarrhoeal diseases in Asia. J Med Microbiol **45:**353–358, 1996.
110. Thomas A, Cheasty T, Frost JA, Chart H, Smith HR, Rowe B. Vero cytotoxin producing *Escherichia coli*, particularly serogroup O157, associated with human infections in England and Wales: 1992–1994. Epidemiol Infect **117:**1–10, 1996.
111. Takeda Y. Enterohemorrhagic *Escherichia coli* infection in Japan. Pediatr Int. **41:**198–201, 1999.
112. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN Downes FP, Martin ML, Griffin PM, Ostroff SM, Potter ME, Tauxe RV, Wachsmuth IK. Isolation of *Escherichia coli* O157:H7 and other Shiga-like toxin-producing *E. coli* from dairy cattle. J Clin Microbiol **29:**985–989, 1991.
113. Wieler LH, Vieler E, Erpenstein C, Schlapp T, Steinrück H, Bauerfeind R, Byomi A, Baljer G. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of eae and other genes. J Clin Microbiol **34:**2980–2984, 1996.
114. Wilson JB, Clarke RC, Renwick SA, Rahn K, Johnson RP, Karmali MA, Lior H, Alves D, Gyles CL, Sandhu KS, McEwen SA, Spika JS.

Vero cytotoxigenic *Escherichia coli* infection in dairy farm families. J Infect Dis **174:**1021–1027, 1996.

115. Verweyen HM, Karch H, Allerberger F, Zimmerhackl LB. Enterohemorrhagic *Escherichia coli* (EHEC) in pediatric hemolytic-uremic syndrome: A prospective study in Germany and Austria. Infection **27:**341–347, 1999.
116. Zhang WL, Bielaszewska A, Liesegang H, Tschäpe H, Schmidt H, Bitzan M, Karch H. Molecular characteristics and epidemiological significance of Shiga toxin-producing *Escherichia coli* O26 strains. J Clin Microbiol **38:**2134–2140, 2000.
117. Piérard D, Etterijck R, van Breynaert J, Moriau L, Lauwers S. Results of screening for verocytotoxin-producing *Escherichia coli* in faeces in Belgium. Europ J Clin Microbiol Infect Dis **9:**198–201, 1990.
118. Pryor W, Hodson H, McIntyre P, Bettelheim KA. Toxigenic *Escherichia coli* in uraemic haemolytic syndrome. Med J Aust **152:**221–222, 1990.
119. Allerberger F, Rossboth D, Dierich MP, Aleksic S, Schmidt H, Karch H. Prevalence and clinical manifestations of Shiga toxin-producing *Escherichia coli* infections in Austrian children. Eur J Clin Microbiol Infect Dis **15:**545–550, 1996.
120. Banatvala N, Debeukelaer MM, Griffin PM, Barrett TJ, Greene KD, Green JH, Wells JG. Shiga-like toxin producing *Escherichia coli* O111 and associated hemolytic-uremic syndrome: a family outbreak. Pediatr Infect Dis J **15:**1008–1011, 1996.
121. Huppertz HI, Busch D, Schmidt H, Aleksic S, Karch H. Diarrhea in young children associated with *Escherichia coli* non-O157 organisms that produce Shiga-like toxin. J Pediatr **128:**341–346, 1996.
122. Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. Diag Microbiol Infect Dis **26:**69–72, 1996.
123. Karch H, Huppertz HI, Bockemühl J, Schmidt H, Schwarzkopf A, Lissner R. Shiga toxin-producing *Escherichia coli* infections in Germany. J Food Protect **60:**1454–1457, 1997.
124. Strockbine N, Sowers E, Greene K, Hayes P, Griffin P, Wells J. Characterization of Shiga toxin-producing non-O157 *Escherichia coli* from the United States, 1983–1997 [abstract]. VTEC '97 3rd International Symposium and Workshop on Shiga Toxin (Verocytotoxin)-Producing *Escherichia coli* Infections. Baltimore (MD): Lois Joy Galler Foundation for Hemolytic Uremic Syndrome, Inc.; 1997. p67. Abstract nr **235/III.**
125. Asakura H, Makino S, Shirahata T, Tsukamoto T, Kurazono H, Ikeda T, Takeshi K. Detection and genetical characterization of Shiga toxin-producing *Escherichia coli* from wild deer. Microbiol Immun **42:**815–822, 1998.
126. Gallien P, Karch H, Much C, Steinruck H, Lehmann S, Timm M, Richter H, Perlberg KW, Protz D. Subtyped *eae*-genes in Shiga toxin-producing *Escherichia coli* (STEC): occurrence in raw or undercooked food samples and comparison of isolates from faecal samples and stool samples. Fleischwirtschaft **80:**84–89, 2000.
127. Hornitzky MA, Bettelheim KA, Djordjevic SP. The isolation of enterohaemorrhagic *Escherichia coli* O111:H$^-$ from Australian cattle. Aust Vet J **78:**636–637, 2000.
128. Hall GA, Chanter N, Bland AP. Comparison in gnotobiotic pigs in lesions caused by verotoxigenic and non-verotoxigenic *Escherichia coli.* Vet Pathol **25:**205–210, 1985.
129. Willshaw GA, Scotland SM, Smith HR, Rowe B. Properties of verocytotoxin-producing *Escherichia coli* of human origin of O groups other than O157. J Infect Dis **166:**797–802, 1992.
130. Willshaw GA, Smith HR, Roberts D, Thirlwell J, Cheasty T, Rowe B. Examination of raw beef products for the presence of verocytotoxin-producing *Escherichia coli*, particularly those of serogroup O157. J Appl Bacteriol. **75:**420–426, 1993.
131. Wilson JB, Clarke RC, Renwick SA, Rahn K, Johnson D, Alves D, Karmali MA, Lior H, McEwen SA, Spika J. Verocytotoxigenic *Escherichia coli* infection on dairy farms in Southern Ontario. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp61–64, 1994.
132. Sidjabat-Tambunan H, Bensink J, Bettelheim KA. Isolation of verocytotoxin-producing *Escherichia coli* from mutton carcasses. Aust Vet J **76:**364–365, 1998.
133. Starr M, Bennett-Wood V, Bigham AK, Dekoning-Ward TP, Bordun AM, Lightfoot D, Bettelheim KA, Jones CL, Robins-Browne RM. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli*: case report and review. Clin Infect Dis **27:**310–315, 1998.
134. Todd ECD, Szabo RA, MacKenzie JM, Martin A, Rahn K, Gyles C, Gao A, Alves D, Yee AJ. Application of a DNA hybridization-hydrophobic-grid membrane filter method for detection and isolation of verotoxigenic *Escherichia coli.* Appl Environ Microbiol **65:**4775–4780, 1999.
135. Bettelheim KA, Bensink JC, Sidjabat-Tambunan H. Serotypes of verocytotoxin-producing (Shiga toxin-producing) *Escherichia coli* isolated from healthy sheep. Comp Immun Microbiol Infect Dis **23:**1–7, 2000.
136. Piérard D, Van Damme L, Stevens D, Moriau L, Lauwers S. Detection of verocytotoxin-producing *Escherichia coli* in meat in Belgium. In: Karmali MA, Goglio AG, Eds. Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections (Excerpta Medica International Congress Series 1072). Amsterdam, The Netherlands: Elsevier Science, pp77–80, 1994.
137. Beutin L, Geier D, Zimmermann S, Aleksic S, Gillespie HA, Whittam TS. Epidemiological relatedness and clonal types of natural populations of *Escherichia coli* strains producing Shiga toxins in separate populations of cattle and sheep. Appl Environ Microbiol **63:**2175–2180, 1997.
138. Blanco M, Blanco JE, Gonzalez EA, Mora A, Jansen W, Gomes TAT, Zerbini LF, Yano T, Decastro AFP, Blanco J. Genes coding for enterotoxins and verotoxins in porcine *Escherichia coli* strains belonging to different O:K:H serotypes: relationship with toxic phenotypes. J Clin Microbiol **35:**2958–2963, 1997.
139. Gallien P, Richter H, Klie H, Timm M, Karch H, Perlberg KW, Steinrück H, Riemer S, Djuren M, Protz D. Detection of STEC and epidemiological investigations in surrounding of a HUS patient. Berlin Münch Tierarzt Wchscht **110:**342–346, 1997.
140. Richter H, Klie H, Timm M, Gallien P, Steinruck H, Perlberg KW, Protz D. Verotoxin-producing *E. coli* (VTEC) in faeces from cattle slaughtered in Germany. Berlin München Tierarzt Wchsch **110:**121–127, 1997.
141. Bonnet R, Souweine B, Gauthier G, Rich C, Livrelli V, Sirot J, Joly B, Forrestier C. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with hemolytic uremic syndrome in adults. J Clin Microbiol **36:**1777–1780, 1998.
142. Baker M, Bennett J, Nicol C. Emergence of verotoxigenic *Escherichia coli* (VTEC) in New Zealand. New Zealand Publ Hlth Rpt **6:**9–12, 1999.
143. Gallien P, Much C, Perlberg KW, Protz D. Subtyping of stx-genes in Shiga toxin-producing *Escherichia coli* (STEC): presence in raw foods and correlation to other factors. Fleischwirtschaft **79:**99–103, 1999.
144. McCluskey BJ, Rice DH, Hancock DD, Hovde CJ, Besser TE, Gray S, Johnson RP. Prevalence *of Escherichia coli* O157 and other Shiga toxin-producing *E. coli* in lambs at slaughter. J Vet Diag Invest **11:**563–565, 1999.
145. Timm M, Klie H, Richter H, Gallien P, Perlberg KW, Lehmann S, Protz D. Detection and prevalence of verotoxin-producing *Escherichia coli* (VTEC) in raw sausages. Berlin Münch Tierarzt Wchscht **112:**385–389, 1999.
146. Tokhi AM, Peiris JSM, Scotland SM, Willshaw GA, Smith HR, Roberts D, Cheasty T. A longitudinal study of verocytotoxin-producing *Escherichia coli* cattle calves in Sri Lanka. Epidemiol Infect **110:**197–208, 1993.
147. Asakura H, Makino S, Shirahata T, Tsukamoto T, Kurazono H, Ikeda

T, Takeshi K. Detection and long-term existence of Shiga toxin (Stx)-producing *Escherichia coli* in sheep. Microbiol Immun **42**:683–688, 1998.

148. Miyao Y, Kataoka T, Nomoto T, Kai A, Itoh T, Itoh K. Prevalence of verotoxin-producing *Escherichia coli* harbored in the intestine of cattle in Japan. Vet Microbiol **61**:137–143, 1998.
149. Heuvelink AE, Wernars K, de Boer E. Occurrence of *Escherichia coli* O157 and other verocytotoxin-producing *E. coli* in retail raw meats in The Netherlands. J Food Protect **59**:1267–1272, 1996.
150. Stephan R, Kühn K. Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovine coli mastitis and their antibiotic resistance patterns. Zbl Veterinärm B **46**:423–427, 1999.
151. Bettelheim KA, Bowden DS, Doultree JC, Catton MG, Chibo D, Ryan NJP, Wright PJ, Gunesekere IC, Griffith JM, Lightfoot D, Hogg GG, Bennett-Wood V, Marshall JA. Combined infection of Norwalk-like virus and verotoxin-producing bacteria associated with a gastroenteritis outbreak. J Diarrh Dis Res **17**:34–36, 1999.
152. Bettelheim KA, Brown JE, Lolokha S, Echeverria P. Serotypes of *Escherichia coli* that hybridized with DNA probes for genes encoding Shiga-like Toxin I, Shiga-like Toxin II and serogroup O157 enterohemorrhagic *E. coli* fimbriae isolated from adults with diarrhea in Thailand. J Clin Microbiol **28**:293–295, 1990.
153. Suthienkul O, Brown JE, 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.
154. Thomas A, Chart H, Cheasty T, Smith HR, Frost JA, Rowe B. Verocytotoxin-producing *Escherichia coli* particularly serogroup O157 associated with human infections in the United Kingdom. Epidemiol Infect **110**:591–600, 1993.
155. Cerqueira AMF, Tibana A, Guth BEC. High occurrence of Shiga-like toxin-producing *Escherichia coli* isolated from raw beef products in Rio de Janeiro City. Brazil J Food Protect **60**:177–180, 1997.
156. Klie H, Timm M, Richter H, Gallien P, Perlberg KW, Steinrück H. Detection and occurrence of verotoxin-forming and/or Shiga toxin-forming *Escherichia coli* (VTEC and/or STEC) in milk. Berlin Münch Tierarzt Wchscht **110**:337–341, 1997.
157. Beutin L, Müller W. Cattle and verotoxigenic *Escherichia coli* (VTEC), an old relationship. Vet Rec **142**:283–284, 1998.
158. Goldwater PN, Giles N, Bettelheim KA. An unusual case of microangiopathic haemolytic anaemia associated with enterohaemorrhagic *Escherichia coli* O113:H21 infection, a verocytotoxin-2/Shiga toxin-2 producing serotype. J Infect **37**:302–304, 1998.
159. Rahn K, Renwick SA, Johnson RP, Wilson JB, Clarke RC, Alves D, McEwen SA, Lior H, Spika J. Follow-up study of verocytotoxigenic *Escherichia coli* infection in dairy farm families. J Infect Dis **177**:1139–1140, 1998.
160. Hashimoto H, Mizukoshi K, Nishi M, Kawakita T, Hasui S, Kato Y, Ueno Y, Takeya R, Okuda N, Takeda T. Epidemic of gastrointestinal tract infection including hemorrhagic colitis attributable to Shiga toxin 1-producing *Escherichia coli* O118:H2 at a junior high school in Japan. Pediatrics **103**:E21–E25, 1999.
161. Paton AW, Woodrow MC, Doyle MR, 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.
162. Stephan R, Untermann F. Virulence factors and phenotypical traits of verotoxin-producing *Escherichia coli* strains isolated from asymptomatic human carriers. J Clin Microbiol **37**:1570–1572, 1999.