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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

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

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 im-

plicated 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 O91 strain 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 viru-

lence 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 10⁹/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 LD₅₀ 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.

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