Human *Escherichia coli* O157:H7 Genetic Marker in Isolates of Bovine Origin

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The antiterminator *Q* gene of bacteriophage 933W (Q_{933}) was identified upstream of the *stx2* gene in 90% of human disease–origin *Escherichia coli* O157:H7 isolates and in 44.5% of bovine isolates. Shiga toxin production was higher in Q_{933}-positive isolates than Q_{933}-negative isolates. This genetic marker may provide a useful molecular tool for epidemiologic studies.

Escherichia coli O157 is recognized worldwide as an important cause of diarrheal disease, which in some patients is followed by hemolytic uremic syndrome and death (1). A primary virulence factor of this pathogen is the prophage-encoded Shiga toxin (2). Greater Shiga toxin production per bacterium is associated with increasing severity of human disease (3,4). Because of its location in the phage genome, the *stx*-gene variant dubbed stx_2 is under similar regulatory control as other phage late-genes, as it is governed by the interaction of the transcription antiterminator Q with the late promoter P_R' (5).

Although cattle and other ruminants appear to be the natural reservoir for *E. coli* O157 and other Shiga toxin–producing *E. coli* (STEC), only a small fraction of STEC serotypes routinely present in cattle are frequently isolated from human patients. Mounting evidence suggests that considerable genetic, phenotypic, and pathogenic diversity exists among these pathogens (6–8). Furthermore, genetic subtypes or lineages of *E. coli* O157 do not appear to be equally distributed among isolates of bovine and human origin (7). The purpose of this study was to examine the distribution of specific sequences upstream of the stx_2 gene among *E. coli* O157:H7 of human and bovine origin, along with corresponding magnitudes of Shiga toxin production.

The Study

A total of 158 stx_2-encoding *E. coli* O157:H7 isolates were assayed, 91 isolates of bovine origin and 67 originally isolated from ill persons (see online Appendixes 1 and 2; http://www.cdc.gov/ncidod/EID/vol10no8/03-0784_app1.htm and http://www.cdc.gov/ncidod/EID/vol10no8/03-0784_app2.htm). All isolates demonstrated unique banding patterns on pulsed-field gel electrophoresis (PFGE). For polymerase chain reaction (PCR) analysis, 5 µL of DNA obtained from boiled stationary-phase bacteria was added to a 50-µL PCR master mix containing a final concentration of 1.5 (Q_{933}) or 2.5 (Q_{21}) mmol $MgCl_2$, 200 µmol/L each deoxynucleoside triphosphate, 1 U *Taq* polymerase, 0.6 pg/µL of primer 595 (5′-CCGAAGAAAAACCCAGTAACAG-3′) (9), and 0.6 pg/µL of either primer Q_{933} (5′-CGGAGGGGATTGTTGAAGGC-3′;Q_{Stxf}) (9) or primer Q_{21} (5′-GAAATCCTCAATGCCTCGTTG-3′; this study). PCR consisted of an initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 30 s, 52°C (Q_{933}) or 55°C (Q_{21}) for 1 min, and 72°C for 1 min; and a final 10-min extension step at 72°C. *E. coli* strain 933 or FAHRP88 was used as a positive control and master mix alone as a negative control. All PCR products were separated by gel electrophoresis (100 V) in 1% agarose gels, stained with ethidium bromide, and visualized by using UV illumination.

Shiga toxin production was determined by using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Premiere EHEC, Meridian Diagnostics, Cincinnati, OH). Briefly, log-phase cells from Luria-Bertani broth enrichments were diluted to 0.6 optical density (OD) at 600 nm, subsequently pelleted, resuspended in phosphate-buffered saline, and induced by exposure to UV light (240 nm) for 3 s (10). A 1:9 volume of a 10x concentrate of brain heart infusion broth was added to each culture and shaken at 37°C for 2.5 h. Replicate cultures that were not exposed to UV light (noninduced controls) were maintained at 4°C. Two hundred microliters of each induced and noninduced enrichment was subsequently used as the specimen in the EHEC ELISA, as described (11). OD results were recorded for each isolate both with and without UV induction. The relative change in Shiga toxin production after induction was calculated for each isolate; $(OD_{induced})/OD_{noninduced})$. *E. coli* O157 (EDL933) and a toxin-negative control isolate were assayed as positive and negative controls each time the assay was repeated.

E. coli O157 isolates were classified on the basis of the presence or absence of bands of the predicted size on the Q_{933}-595 and Q_{21}-595 PCR reactions (Figure). A chi-square test was used to determine whether different PCR genotypes were equally distributed among isolates of bovine and human origin. Likewise, a chi-square test was used to assess the equality of distribution of PCR genotypes among bovine isolates from different countries. One-way analysis of variance for nonparametric data (Kruskal-Wallis test) was used to identify differences in

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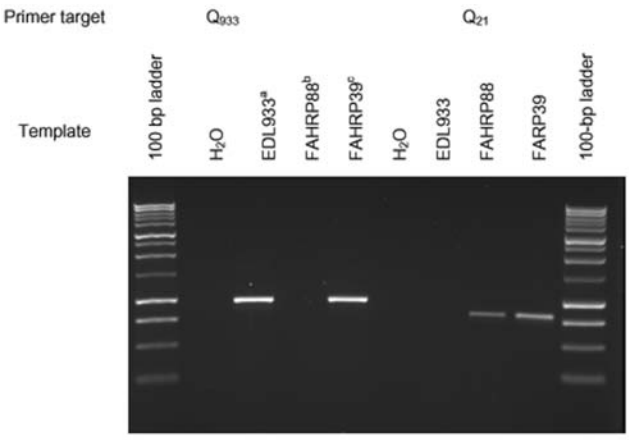

Figure. Ethidium bromide–stained gel of the amplification products obtained from Q933-595 and Q21-595 polymerase chain reactions. [a]EDL933, human isolate (ATCC43895). Obtained from the STEC Center, Michigan State University. [b]FAHRP88, isolated from Ohio dairy cow. [c]FAHRP39, human isolate (E29962) (12).

ranked-transformed toxin production among noninduced and induced *E. coli* O157 isolates as well as to determine significant differences in the percent increase in toxin following induction.

Previously, Kim et al. described a nonrandom distribution of *E. coli* O157 subtypes among cattle and humans by using an octamer-based genome-scanning method (7). We tested several of the isolates that had been previously characterized. Nine had been previously identified as belonging to the lineage I genotype and seven isolates as belonging to the lineage II genotype. We found that all nine lineage I isolates consistently amplified the Q_{933} target, regardless of species of origin. All four bovine isolates classified as lineage II by Kim et al. amplified the Q_{21} target. One lineage II human isolate (NE015) amplified the Q_{933} target, and another lineage II isolate (NE037) produced no amplicons in either PCR reaction. One human isolate classified as lineage II (ATCC 43889) amplified both target sequences, presumably because of polylysogeny.

The distribution of the specific *Q*-gene alleles found upstream of the prophage *stx* region among bovine isolates may have a geographic component. The distribution of *E. coli* O157 phage genotypes collected from healthy cattle from diverse geographic areas is consistent with the variable incidences of human disease in different countries (Table 1). For example, six (75%) of eight Scottish bovine isolates examined amplified the Q_{933} target, the same target that is frequently present in human isolates of human disease origin. Scotland reports some of the highest incidence rates of human *E. coli* O157–related diseases and hemolytic uremic syndrome (13). In contrast, none of the seven Australian *E. coli* O157 bovine isolates amplified the 1750-bp fragment. Contrary to the situation in Scotland and the United States, *E. coli* O157 infection of humans is rarely reported in Australia (14).

Conclusions

The Q_{933} gene target was more commonly identified in human disease–associated strains of *E. coli* O157 than from strains of bovine origin. Amplification of the Q_{933} target, either alone or in combination with amplification of the Q_{21} target from the same isolate, was identified in 60 (9%) of 66 (55/66 alone and 5/66 in combination with Q_{21}; 1 isolate amplified neither target) compared to 40 (44%) of 91 (32/91 alone, and 8/91 in combination with Q_{21}) of bovine isolates ($p < 0.001$). Furthermore, these genetic subtypes were nonrandomly distributed among the *E. coli* O157 isolates of bovine origin obtained from different countries ($p < 0.05$) (Table 1).

These limited data suggest that the distribution of *E. coli* O157 strains in cattle may differ between countries or regions, thereby providing an explanation for geographic differences in the incidence of human *E. coli* O157 infection. More isolates from cattle need to be analyzed with these methods to better characterize the *E. coli* O157 in the bovine reservoir of each country.

A positive reaction with the Q_{933} target was significantly associated with higher OD results on the Shiga toxin ELISA (both noninduced and induced) and higher-fold increases in toxin production following induction than isolates amplifying the Q_{21} target alone ($p < 0.0001$) (Table 2). Despite these differences, we did not identify any clinical associations between the magnitude of Shiga toxin production and severity of human disease could be identified in this study. Other, non–Shiga toxin–related virulence factors and host susceptibility are also believed to play

Table 1. Distribution of polymerase chain reaction results from bovine *Escherichia coli* O157 isolates based on geographic origin[a]

		Q allele		
Country of origin	No. tested	933	21	Both
		N (%)	N (%)	N (%)
USA	46	20 (44)	25 (54)	1 (2)
Scotland	8	– (0)	2 (25)	6 (75)
Australia	7	– (0)	7 (100)	– (0)
Japan	17	3 (18)	14 (82)	– (0)
Total	78	23 (29)	48 (62)	7 (9)

[a]–, not detected. Percentages are read across rows, not down columns. Significant difference in proportion of *Q* alleles isolated from different countries ($p < 0.05$, chi-square test for homogeneity).

Table 2. Shiga toxin production by *Escherichia coli* O157:H7 by *Q* allele

Assay	*Q* allele	Response		
		Median	Minimum	Maximum
OD_{600nm} noninduced	Q_{933}	0.442	0.153	2.814
	Q_{21}	0.170	0.120	0.413
OD_{600nm} induced	Q_{933}	1.228	0.172	2.896
	Q_{21}	0.165	0.084	1.210
Fold increase in OD_{600nm} after induction [a]	Q_{933}	2.2	0.3	7.7
	Q_{21}	0.9	0.4	5.1

[a] $(OD_{induced})/(OD_{noninduced})$. The maximum and minimum optical density readings at 600 nm listed in each row are not necessarily from the same isolate; therefore, the maximum -and minimum-fold increase cannot be calculated directly from the table.

essential roles in the outcome of clinical STEC infections. The Q_{933}-negative isolates obtained from human disease might have lost this Q_{933}-containing prophage by the time of isolation, or these isolates might have been recovered from patients also infected with STEC containing Q_{933}-type prophage (15). Whether specific *Q*-gene alleles directly correlate with the magnitude of Shiga-toxin production or whether other (unstudied) factors within the phage lytic cascade genetically linked to specific *Q* alleles instead are responsible for the magnitude of toxin production is not known.

The antiterminator Q, the protein product of the *Q* gene, and $P_{R'}$, the late promoter, are reputed to be involved in regulating phage late-genes and, because of the location of $P_{R'}$ in prophage genome, of Shiga toxin production as well (5). In *E. coli* O157 phage 933W (GenBank no. 9632466) and *E. coli* O157 stx_{2vhd} (GenBank no. 15718404), the 359-bp sequence immediately upstream of the stx_2 gene is nearly identical (>95% nucleotide identity). However, further upstream of this area of identity, DNA sequences differ significantly. In *E. coli* O157 933W, this gene is identified as the antiterminator *Q* gene. In contrast, in *E. coli* O157 stx_{2vhd} this area is occupied by a gene with >95% sequence identity with the antiterminator *Q* gene of bacteriophage 21 (gi 4539472). The *Q* gene of bacteriophage 21 does not share DNA sequence homology with the *Q* gene of bacteriophage 933W, and only 36% predicted amino acid homology. Since the *Q* gene is reputed to play an important role in regulating toxin production, our results provide a plausible explanation (differential regulation of Shiga toxin production) of why certain *E. coli* O157 genotypes are more commonly isolated from human patients (7).

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