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

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The antiterminator Q gene of bacteriophage 933W (Q933) 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 Q933-positive isolates than Q933-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 stx2-gene variant dubbed stx2 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<sup>R</sup> (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 stx2 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 stx2-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<sub>933</sub>) or 2.5 (Q<sub>21</sub>) mmol MgCl<sub>2</sub>, 200 µmol/L each deoxynucleoside triphosphate, 1 U *Taq* polymerase, 0.6 pg/µL of primer 595 (5′-CCGAGGAAAAACCCAGTAACAG-3′) (9), and 0.6 pg/µL of either primer Q<sub>933</sub> (5′-CGGAGGGATTGTGAAGGC-3′; sense) (9) or primer Q<sub>21</sub> (5′-GAAATCTCTCAATGCTCGTTG-3′; antisense), 2.5 or 55°C (Q<sub>21</sub>) 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. Repeat 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<sub>induced</sub>−OD<sub>noninduced</sub>). *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<sub>933</sub>-595 and Q<sub>21</sub>-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
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 Q₉₃₃ 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 Q₉₃₃ gene target was more commonly identified in human disease–associated strains of E. coli O157 than from strains of bovine origin. Amplification of the Q₉₃₃ target, either alone or in combination with amplification of the Q₂₁ target from the same isolate, was identified in 60 (9%) of 66 (55/66 alone and 5/66 in combination with Q₂₁; 1 isolate amplified neither target) compared to 40 (44%) of 91 (32/91 alone, and 8/91 in combination with Q₂₁) 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 Q₉₃₃ 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 Q₂₁ 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

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

| Country of origin | No. tested | Q₉₃₃ | Q₂₁ | Both |
|------------------|------------|------|-----|------|
| 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     |
|-----------------------|----------|--------------|
| OD_{600nm} noninduced | Q_{933}  | 0.442        |
|                       | Q_{21}   | 0.170        |
| OD_{600nm} induced    | Q_{933}  | 1.228        |
|                       | Q_{21}   | 0.165        |
| Fold increase in OD   | Q_{933}  | 2.2          |
| 600nm after induction | Q_{21}   | 0.9          |

*(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_{2vd} 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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**References**

1. Karmali MA, Arbus GS, Petric M, Patrick ML, Roscoe M, Shaw J, et al. Hospital-acquired *Escherichia coli* O157:H7 associated haemolytic uraemic syndrome in a nurse [letter]. Lancet. 1988;1:526.

2. O’Loughlin EV, Robins-Browne RM. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. Microbes Infect. 2001;3:493–507.

3. Kimura N, Watanabe M, Komatsubara A. [Verotoxin producing ability of verotoxin-producing *Escherichia coli* strains isolated from fecal specimens of healthy persons is lower than that of patients]. Kansenshogaku Zasshi. 2000;74:849–51.

4. Pradel N, Boukhors K, Berin 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. 2001;67:2460–8.

5. Wagner PL, Neely MN, Zhang X, Acheson DW, Waldor MK, Friedman DL. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. J Bacteriol. 2001;183:2081–5.

6. Baker DR, Moxley RA, Francis DH. Variation in virulence in the gnotobiotic pig model of O157:H7 *Escherichia coli* strains of bovine and human origin. Adv Exp Med Biol. 1997;412:53–8.

7. Kim J, Niefeldt J, Benson AK. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. Proc Natl Acad Sci U S A. 1999;96:13288–93.

8. McNally A, Roe AJ, Simpson S, Thomson-Carter FM, Hoey DE, Currie C, et al. Differences in levels of secreted locus of enterocyte effacement proteins between human disease-associated and bovine *Escherichia coli* O157. Infect Immun. 2001;69:5107–14.

9. Unkmeir A, Schmidt H. Structural analysis of phage-borne stx genes and their flanking sequences in shiga toxin-producing *Escherichia coli* and *Shigella dysenteriae* type 1 strains. Infect Immun. 2000;68:4856–64.

10. Arber W, Enquist L, Hohn B, Murray K, Murray N. Experimental methods. In: Hendrix R, Roberts J, Stahl F, Weisberg R, editors. Lambda II. Cold Springs Harbor (NY): Cold Spring Harbor Laboratory; 1983.

11. Schmidt H, Scheef, J, Morabito S, Caprioli A, Wieler LH, Karch H. A new Shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. Appl Environ Microbiol. 2000;66:1205–8.
12. Dorn CR, Angrick E. Serotype O157:H7 *Escherichia coli* from bovine and meat sources. J Clin Microbiol. 1991;29:1225–31.

13. Locking M. HUS rates in Scotland. Glasgow: Scottish Centre for Infection & Environmental Health; 2002.

14. Elliott EJ, Robins-Browne RM, O’Loughlin EV, Bennett-Wood V, Bourke J, Henning P, et al. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. Arch Dis Child. 2001;85:125–31.

15. Karch H, Russmann H, Schmidt H, Schwarzkopf A, Heesemann J. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. J Clin Microbiol. 1995;33:1602–5.

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### Appendix 1. Source of human isolates used in this study

| FAHRP ID | Source ID | Country | Year | Clinical signs and symptoms | References or source |
|----------|-----------|---------|------|----------------------------|----------------------|
| 6        | FRIK 528  | USA     | 1998 | Diarrhea                   | 1                    |
| 7        | FRIK 579  | USA     | 1998 | Diarrhea                   | 1                    |
| 8        | 93-001    | USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 9        | ATCC 35150| USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 16       | 91671     | USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 17       | ATCC 43889| USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 18       | NE 037    | USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 19       | NE 15     | USA     | 1999 | Hemorrhagic colitis        | 2                    |
| 39       | E29962    | UK      | 1991 | NR                         | 3                    |
| 54       | CL56      | Canada  | 1991 | NR                         | 3                    |
| 60       | E32511    | USA     | 2002 | HUS                        | 4                    |
| 58       | EDL 933   | USA     | 1982 | Hemorrhagic colitis        | 5                    |
| 126      | 02 5225   | USA     | 2002 | NR                         | Washington<sup>b</sup> |
| 127      | 02 4857   | USA     | 2002 | NR                         | Washington           |
| 128      | 02 6776   | USA     | 2002 | NR                         | Washington           |
| 129      | 02 6579   | USA     | 2002 | NR                         | Washington           |
| 130      | 02 6546   | USA     | 2002 | NR                         | Washington           |
| 131      | 02 6722   | USA     | 2002 | NR                         | Washington           |
| 132      | 02 6598   | USA     | 2002 | NR                         | Washington           |
| 133      | 02 6696   | USA     | 2002 | NR                         | Washington           |
| 134      | 02 6791   | USA     | 2002 | NR                         | Washington           |
| 135      | 02 6829   | USA     | 2002 | NR                         | Washington           |
| 136      | 02 6755   | USA     | 2002 | NR                         | Washington           |
| 137      | 02 6644   | USA     | 2002 | NR                         | Washington           |
| 138      | 06 781    | USA     | 2002 | Diarrhea                   | Idaho<sup>c</sup>    |
| 139      | 06 852    | USA     | 2002 | NR                         | Idaho                |
| 140      | 06 854    | USA     | 2002 | Watery diarrhea, vomiting  | Idaho                |
| 141      | 06 856    | USA     | 2002 | Diarrhea                   | Idaho                |
| 142      | 06 855    | USA     | 2002 | NR                         | Idaho                |
| 143      | 06 886    | USA     | 2002 | Diarrhea, abdominal pain   | Idaho                |
| 144      | 06 889    | USA     | 2002 | Abdominal pain             | Idaho                |
| 145      | 06 988    | USA     | 2002 | Gastrointestinal bleeding  | Idaho                |
| 146      | 07 004    | USA     | 2002 | Bloody stool               | Idaho                |
| 147      | 07 007    | USA     | 2002 | Bloody stool               | Idaho                |
| 148      | 07 023    | USA     | 2002 | Bloody stool               | Idaho                |
| 149      | 07 085    | USA     | 2002 | NR                         | Idaho                |
| 150      | 07 147    | USA     | 2002 | NR                         | Idaho                |
| 151      | 07 154    | USA     | 2002 | NR                         | Idaho                |
| 152      | O2191230  | USA     | 2002 | Diarrhea                   | Ohio<sup>d</sup>     |
| 153      | O2191229  | USA     | 2002 | Diarrhea                   | Ohio                 |
| 154      | O2191231  | USA     | 2002 | Diarrhea                   | Ohio                 |
| 155      | O2191294  | USA     | 2002 | Diarrhea                   | Ohio                 |
| O157:H7 Genetic Marker, Appendix 1 | CDC EID |
|---|---|
| 156 | O2190819 USA 2002 Diarrhea Ohio |
| 157 | O2190864 USA 2002 Diarrhea Ohio |
| 158 | O2191309 USA 2002 Diarrhea Ohio |
| 159 | O2191311 USA 2002 Diarrhea Ohio |
| 160 | O2191313 USA 2002 Diarrhea Ohio |
| 161 | O2191361 USA 2002 Diarrhea Ohio |
| 162 | O2191602 USA 2002 Diarrhea Ohio |
| 163 | O2191624 USA 2002 Diarrhea Ohio |
| 164 | O2191541 USA 2002 Diarrhea Ohio |
| 165 | O2191546 USA 2002 Diarrhea Ohio |
| 166 | O2191423 USA 2002 Diarrhea Ohio |
| 167 | O2191509 USA 2002 Diarrhea Ohio |
| 168 | O2191363 USA 2002 Diarrhea Ohio |
| 169 | O2191364 USA 2002 Diarrhea Ohio |
| 170 | O2191365 USA 2002 Diarrhea Ohio |
| 171 | O2191366 USA 2002 Diarrhea Ohio |
| 172 | O2190889 USA 2002 Diarrhea Ohio |
| 173 | O2190893 USA 2002 Diarrhea Ohio |
| 174 | O2191176 USA 2002 Diarrhea Ohio |
| 175 | O2191177 USA 2002 Diarrhea Ohio |
| 176 | O2191623 USA 2002 Diarrhea Ohio |
| 177 | O2191625 USA 2002 Diarrhea Ohio |
| 178 | O2191645 USA 2002 Diarrhea Ohio |
| 179 | O2191675 USA 2002 Diarrhea Ohio |
| 180 | O2191765 USA 2002 Diarrhea Ohio |
| 181 | O2191831 USA 2002 Diarrhea Ohio |

\(^a\)FAHRP, Food Animal Health Research Program, Ohio State University; NR, not reported; HUS, hemolytic uremic syndrome.

\(^b\)Washington State Department of Health isolates.

\(^c\)Idaho Department of Health and Welfare isolates.

\(^d\)Ohio Department of Health isolates.

**Appendix 1 References**

1. Gouveia S, Proctor ME, Lee M-S, Luchansky JB, Kaspar CW. Genomic comparisons and Shiga toxin production among *Escherichia coli* O157:H7 Isolates from a day care center outbreak and sporadic cases in southeastern Wisconsin. *J Clin Microbiol.* 1998;36:727–33.

2. Kim J, Nietfeldt J, Benson A. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. *PNAS.* 1999;96:13288–93.

3. Dorn CR, Angrick E. Serotype O157:H7 *Escherichia coli* from bovine and meat sources. *J Clin Microbiol.* 1991;29:1225–31.

4. Schmitt CK, McKee ML, O'Brien AD. Two copies of Shiga-like toxin II-related genes common in enterohemorrhagic *Escherichia coli* strains are responsible for the antigenic heterogeneity of the O157:H- strain E32511. *Infect Immun.* 1991;59:1065–73. Obtained from the STEC Center, Michigan State University.

5. Perna NT, Mayhew GF, Posfai G, Elliott S, Donnenberg MS, Kaper JB, et al. Genome sequence of enterohemorrhagic *Escherichia coli* O157:H7. *Nature.* 2001;409:529–33.
## Appendix 2. Source of bovine isolates used in this study

| FAHRPa ID | Source ID | Country | Year | References or source |
|-----------|-----------|---------|------|----------------------|
| 1         | FRIK 1986 | USA     | 1991 | 1                    |
| 2         | FRIK 1997 | USA     | 1991 | 1                    |
| 3         | FRIK 1994 | USA     | 1991 | 1                    |
| 4         | FRIK 2002 | USA     | 1991 | 1                    |
| 5         | FRIK 1987 | USA     | 1991 | 1                    |
| 10        | FRIK 920  | USA     | 1998 | 2                    |
| 11        | FRIK 1054 | USA     | 1998 | 2                    |
| 12        | FRIK 1540 | USA     | 1998 | 2                    |
| 13        | FRIK 1988 | USA     | 1998 | 1                    |
| 22        | LCDC 87-2930 | Canada | 1991 | 3                     |
| 27        | OARDC1 | USA | 2002 | FAHRP |
| 29        | OARDC2 | USA | 2002 | FAHRP |
| 31        | OARDC3 | USA | 2002 | FAHRP |
| 35        | P673 | UK | 1987 | 4                     |
| 37        | P277 | UK | 1987 | 4                     |
| 47        | c1526-77 | Argentina | 1991 | 3                     |
| 50        | CDC B9253-DMS1 | USA | 1991 | 3                     |
| 51        | A39 | Canada | 1991 | 3                     |
| 52        | A43 | Canada | 1991 | 3                     |
| 56        | LCDC 87-2924 | Canada | 1991 | 3                     |
| 57        | LCDC 87-1799 | Canada | 1991 | 3                     |
| 62        | CDC B6830-MS1/0 | USA | 1991 | 3                     |
| 63        | CDCB7205-MS1/0 | USA | 1991 | 3                     |
| 64        | CDC B8038-MS1/0 | USA | 1991 | 3                     |
| 65        | 8832 | USA | 2002 | 5                     |
| 66        | EC66 | USA | 2002 | FAHRP |
| 67        | EC 67 | USA | 2002 | FAHRP |
| 82        | 8833 | USA | 2002 | 5                     |
| 83        | EC 83 | USA | 2002 | FAHRP |
| 84        | EC 84 | USA | 2002 | FAHRP |
| 85        | 8834 | USA | 2002 | 5                     |
| 87        | EC87 | USA | 2002 | FAHRP |
| 88        | EC88 | USA | 2002 | FAHRP |
| 93        | EC 93 | USA | 2002 | FAHRP |
| 94        | EC94 | USA | 2002 | FAHRP |
| 95        | EC95 | USA | 2002 | FAHRP |
| 96        | EC96 | USA | 2002 | FAHRP |
| 97        | EC97 | USA | 2002 | FAHRP |
| 98        | EC98 | USA | 2002 | FAHRP |
| 99        | EC99 | USA | 2002 | FAHRP |
| 100       | EC100 | USA | 2002 | FAHRP |
| 102       | EC102 | USA | 2002 | FAHRP |
| 103       | EC103 | USA | 2002 | FAHRP |
| No. | Origin     | Country | Year | Marker |
|-----|------------|---------|------|--------|
| 104 | EC104      | USA     | 2002 | FAHRP  |
| 113 | 8837       | USA     | 2002 | FAHRP  |
| 115 | EC115      | USA     | 2002 | FAHRP  |
| 116 | EC116      | USA     | 2002 | FAHRP  |
| 117 | EC117      | USA     | 2002 | FAHRP  |
| 120 | EC120      | USA     | 2002 | FAHRP  |
| 122 | EC122      | USA     | 2002 | FAHRP  |
| 182 | 757        | USA     | 1994 | FAHRP  |
| 183 | 817        | USA     | 1994 | FAHRP  |
| 185 | 1104       | USA     | 1994 | FAHRP  |
| 186 | 1119       | USA     | 1994 | FAHRP  |
| 187 | 1124       | USA     | 1994 | FAHRP  |
| 188 | 1136       | USA     | 1994 | FAHRP  |
| 189 | 1273       | USA     | 1996 | FAHRP  |
| 190 | 3735       | USA     | 1996 | FAHRP  |
| 191 | 4048       | USA     | 1996 | FAHRP  |
| 192 | 7407       | Japan   | 1996 | FAHRP  |
| 193 | 7409       | Japan   | 1996 | FAHRP  |
| 194 | 7416       | Japan   | 1996 | FAHRP  |
| 195 | 7420       | Japan   | 1996 | FAHRP  |
| 196 | 7421       | Japan   | 1996 | FAHRP  |
| 197 | 7423       | Japan   | 1996 | FAHRP  |
| 198 | 7433       | Japan   | 1996 | FAHRP  |
| 199 | 7436       | Japan   | 1996 | FAHRP  |
| 200 | 7439       | Japan   | 1996 | FAHRP  |
| 201 | 7460       | Japan   | 1996 | FAHRP  |
| 202 | 7469       | Japan   | 1996 | FAHRP  |
| 203 | 7478       | Japan   | 1996 | FAHRP  |
| 204 | 7484       | Japan   | 1996 | FAHRP  |
| 205 | 7488       | Japan   | 1996 | FAHRP  |
| 206 | 7495       | Japan   | 1996 | FAHRP  |
| 207 | 7500       | Japan   | 1996 | FAHRP  |
| 208 | 7505       | Japan   | 1996 | FAHRP  |
| 209 | 7622       | Scotland| 1996 | FAHRP  |
| 210 | 7630       | Scotland| 1999 | FAHRP  |
| 211 | 7632       | Scotland| 1999 | FAHRP  |
| 213 | 7637       | Scotland| 1999 | FAHRP  |
| 214 | 7638       | Scotland| 1999 | FAHRP  |
| 217 | 7648       | Scotland| 1999 | FAHRP  |
| 218 | 7649       | Scotland| 1999 | FAHRP  |
| 219 | 7653       | Scotland| 1999 | FAHRP  |
| 220 | 8176       | Australia| 1999 | FAHRP  |
| 221 | 8177       | Australia| 1996 | FAHRP  |
| 222 | 8179       | Australia| 1997 | FAHRP  |
| 223 | 8182       | Australia| 1997 | FAHRP  |
| 224 | 8183       | Australia| 1997 | FAHRP  |
| 225 | 8184       | Australia| 1998 | FAHRP  |
| 226 | 8185       | Australia| 1999 | FAHRP  |
Appendix 2 References

1. Kim J, Nietfeldt J, Benson A. *Octomer-based genome scanning distinguishes a unique subpopulation of* *Escherichia coli* *O157:H7 strains in cattle.* PNAS. 1999;96:13288–93.
2. Shere JA, Bartlett KJ, Kaspar CW. *Longitudinal study of* *Escherichia coli O157:H7 dissemination on four dairy farms in Wisconsin.* Appl Environ Microbiol. 1998;64:1390–9.
3. Dorn CR, Angrick E. *Serotype O157:H7 Escherichia coli from bovine and meat sources.* J Clin Microbiol. 1991;29:1225–31.
4. Huck LG, Dorn CR, Angrick EJ. *DNA probe for detection of serogroup O157 enterohemorrhagic Escherichia coli.* Int J Food Microbiol. 1995;25:277–87.
5. Davis MA, Hancock DD, Besser TE, Rice DH, Hovde CJ, Digiacomo R, et al. *Correlation between geographic distance and genetic similarity in an international collection of bovine faecal Escherichia coli O157:H7 isolates.* Epidemiol Infect. 2003;131:923–30.