International Comparison of Clinical, Bovine, and Environmental
*Escherichia coli* O157 Isolates on the Basis of Shiga Toxin-Encoding
Bacteriophage Insertion Site Genotypes

Joshua H. Whitworth,1 Narelle Fegan,2 Jasmin Keller,2 Kari S. Gobius,2 James L. Bono,3
Douglas R. Call,1 Dale D. Hancock,4 and Thomas E. Besser1*

Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington 99164-7040; Department of
Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164-6610; Microbiology Group, Food Science Australia, P.O. Box 3312, Tingalpa DC, Queensland 4173, Australia; and
U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture,
Clay Center, Nebraska 68933-0166

Received 28 May 2008/Accepted 30 September 2008

*Escherichia coli* O157:H7 genotypes in the bovine reservoir may differ in virulence. The proportion of clinical
genotypes among cattle isolates was weakly (*P* = 0.054) related to the international incidence of *E. coli*
O157:H7-associated hemolytic-uremic syndrome, varied among clinical isolates internationally, and also dif-
fered along the putative cattle-hamburger-clinical case transmission chain.

Infection with enterohemorrhagic *Escherichia coli* serotypes O157:H7 and O157:H− (EHEC-O157) may cause diarrhea,
hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (13, 24). Cattle are considered the principal reservoir of EHEC
(18). EHEC-O157 typically produces Shiga toxins Stx1 and/or
Stx2, encoded by lambdoid bacteriophages (23, 28, 34). In
EHEC-O157 isolates, EDL933 and Sakai, Stx-encoding phages
are inserted in yehV− and wrbA (19, 40). Shaikh and Tarr (44),
however, demonstrated insertion site diversity in Stx-encoding bacteriophages among clinical EHEC-O157 isolates, defining
three predominant clinical genotypes (genotypes 1 to 3). Sub-
sequently, the predominance of genotypes 1 to 3 among a
larger set of U.S. human clinical isolates was confirmed; in
contrast, considerable additional diversity of Stx-encoding bac-
teriophage insertion sites was demonstrated in isolates from
the bovine reservoir (6). Since nonclinical genotypes rep-
resented almost half of the bovine isolates, broad exposure of the human population to these genotypes would be expected.

The frequency of reported EHEC-O157-associated disease varies markedly internationally. For example, the incidence of
EHEC-O157 (infections/100,000 population annually) was re-
ported as 4.1 (Scotland, 2004), 0.9 (United States, 2004), 0.87
(Japan, 2004), 0.13 and 1.6 (Germany, 2004 and 1997 to 2003,
respectively), 0.11 (Republic of Korea, 2003), and 0.08
(Australia, 2004) (2, 9, 14, 15, 27, 31, 38). HUS, an uncom-
mon sequel to EHEC-O157 infection, may be less under-
reported due to its severity (32). The corresponding inci-
dence of EHEC-O157-associated HUS was 0.41 (Scotland),
0.1 (United States), 0.002 to 0.20 (Germany), 0.05 (Republic
of Korea), and 0.01 (Japan and Australia) (1, 2, 10, 14, 15,
20, 37).

To determine whether the proportion of EHEC-O157 ge-
genotypes in the bovine reservoir influences the rates of the
diverse international incidence of EHEC-O157 disease, we
genotyped EHEC-O157 isolates obtained from cattle in sev-
eral countries. Study isolates included non-sorbitol-ferment-
ing, β-glucuronidase-negative EHEC-O157 isolates from cattle
originating from different farms in geographically disseminated
locations within the United States (1994 to 2002), Australia
(1993 to 2003), Japan (1996 to 1997; provided by Masato
Akiba, National Institute of Animal Health, Tsukuba, Ibaraki,
Japan), Scotland (1999; provided by Barti Synge, Scottish Ag-
cultural College, Inverness, United Kingdom), and Korea
(1997; provided by B. Young).

Genotypes of EHEC-O157 isolates were determined by us-
ing a multiplexed variation of a PCR method previously de-
dcribed (6, 44). Multiplex 1 included stx1 (36), the right wrbA-
bacteriophage junction, and the left yehV-bacteriophage junction. Multiplex 2 included stx2 (39), the left wrbA-bacte-
riophage junction, and the right yehV-bacteriophage junction.
EHEC-O157 cells were grown overnight at 37°C in LB broth
with shaking and diluted 1:10 with water for use as a whole-cell
template. The 50-μl reaction mixtures included 2.5 U/μl Taq
polymerase, 2 mM MgCl2, 0.4 mM deoxynucleoside triphos-
phates, 5 μl 10× buffer (Invitrogen, Carlsbad, CA), and 2 μl of
the whole-cell template. Thermocycler (iCycler; Bio-Rad, Her-
ricanes, CA) parameters included one 95°C (5 min) cycle and 35
cycles at 94°C (30 s), 58°C (45 s), and 72°C (90 s), followed by
a final 72°C (10 min) cycle. The assignment of genotypes was
based on the presence or absence of the six PCR products (6).
Controls included *E. coli* DH5α (negative control) and
EDL933 (positive control).

No significant association was observed between the propor-
tion of clinical genotypes among isolates from the international
bovine reservoirs and the respective international incidences
of EHEC-O157 disease (*r* [Spearman’s rho statistic] = 0.50, *P* =

---

1 Corresponding author. Mailing address: Department of Veteri-
nary Microbiology and Pathology, College of Veterinary Medicine,
Washington State University, P.O. Box 647040, Pullman, WA 99164-
7040. Phone: (509) 335-6075. Fax: (509) 335-8529. E-mail: tbesser
@vetmed.wsu.edu.

2 Published ahead of print on 10 October 2008.
TABLE 1. Genotypes of Stx-encoding bacteriophage insertion sites from an international group of clinical, bovine, and environmental isolates of EHEC-O157

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Presence or absence of PCR products&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. (％) of isolates from indicated country and source:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence of stx&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;, stx&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;, yehV&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;-left, yehV&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;-right, wrbA&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;-left, and wrbA&lt;sub&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;/sub&gt;-right shown in concatenated code. 1, present; 0, absent.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td>Japan</td>
</tr>
<tr>
<td></td>
<td>Bovine</td>
<td>Human</td>
</tr>
<tr>
<td>1 001100 21 (35.0)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3 (20.0)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>2 011111 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>3 111111 2 (3.3)</td>
<td>4 (26.7)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>4 001001 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5 011000 2 (3.3)</td>
<td>2 (13.3)</td>
<td>14 (31.8)</td>
</tr>
<tr>
<td>6 111000 15 (25.0)</td>
<td>2 (13.3)</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>7 111011 0 (0)</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>8 101100 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>9 000000 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>10 001000 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>11 000100 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>12 010011 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>13 010101 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>14 100110 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>15 110000 5 (8.3)</td>
<td>2 (13.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>16 111010 12 (20.0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>17 010010 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>18 111101 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>19 001000 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>20 000100 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>21 011011 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>22 111011 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>23 111110 0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Genotypes 1 to 3 (clusters 1 to 3) (44) and 4 to 16 (6).
<sup>b</sup> Presence or absence of PCR products for stx<sub><sup>c</sup></sub>, stx<sub><sup>c</sup></sub>, yehV<sub><sup>c</sup></sub>-left, yehV<sub><sup>c</sup></sub>-right, wrbA<sub><sup>c</sup></sub>-left, and wrbA<sub><sup>c</sup></sub>-right shown in concatenated code. 1, present; 0, absent.
<sup>c</sup> Sorbitol-fermenting, β-glucuronidase-positive EHEC-O157:H<sub>1</sub>--; all other columns are for non-sorbitol-fermenting, β-glucuronidase-negative Escherichia coli O157:H<sub>7</sub>.
<sup>d</sup> Beef, retail ground beef; sewage, untreated municipal sewage.
<sup>e</sup> Percentage of total number of isolates for the column.

FIG. 1. The proportion of bovine isolates with clinical genotypes (genotypes 1 to 3, unfilled bars) among isolates from cattle in the specified countries and the incidence (cases per 100,000 population, filled bars) of EHEC-O157 HUS are shown. Error bars indicate 95% confidence intervals.
Differences in EHEC-O157 genotypes among clinical and bovine reservoir isolates have been previously reported, including those detected by Octamer Based Genomic Scanning (OBGS; lineage I versus lineage II), with the bacteriophage antterminator allele Q933 (presence versus absence) and a polymorphism in \(\text{tir}^{255} \text{T versus } A\), and by phage typing (21/28 versus others from Scotland) (8, 17, 25, 26, 29, 31). Some of these genotypes may be correlated; for example, both Stx insertion typing and OBGS classify most Australian isolates into genotypes less associated with clinical disease (lineage II and nonclinical genotypes, respectively). The biologic basis of the differential representation of these genotypes in cattle and in human disease remains largely unexplained.

In summary, while the proportion of clinical genotypes in the bovine reservoir tended to correlate with the incidence of HUS in human populations, this tendency was too weak to provide a satisfying explanation for the magnitude of the differences in the international incidences of HUS and other EHEC-O157-related diseases. Assuming that the incidence estimates for EHEC-O157 disease are accurate, then other factors, such as the prevalence of EHEC-O157 in cattle, genotype-related differences in fecal shedding by cattle, survival in food products and environmental niches, and infectivity and virulence, as well as differences in food preparation practices and dietary composition, may contribute significantly to the differing international incidences of EHEC-O157 disease.

This project was funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract number N01-AI-30055. Liz Ossian provided excellent technical support for this work.

REFERENCES

3. Ansai, S. E., K. A. Darling, and C. W. Kaspar. 1999. Survival of \(\text{Escherichia coli}\) \(\text{O157:H7}\) in ground-beef patties during storage at \(2{\text{ to }} 2.5\) and then \(2{\text{ degrees C}}\), and \(2{\text{ to 20 degrees C}}\). J. Food Prot. 62:1243–1247.


