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**Research Note**

# Characterization of O157:H7 and Other *Escherichia coli* Isolates Recovered from Cattle Hides, Feces, and Carcasses†

**GENEVIEVE A. BARKOCY-GALLAGHER,* TERRANCE M. ARTHUR, MILDRED RIVERA-BETANCOURT, XIANGWU NOU, STEVEN D. SHACKELFORD, TOMMY L. WHEELER, AND MOHAMMAD KOOHMARAIE**

*Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933-0166, USA*

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## ABSTRACT

In a previous study, the seasonal prevalence was reported for *stx*$^+$ *Escherichia coli* O157:H7 in feces and on hides and carcasses of cattle at processing. Overall, 1,697 O157:H7 isolates have now been characterized for the incidence of (i) $eae_{O157}$, *hlyA,* $stx_1$, and $stx_2$ in the recovered isolates and (ii) presumptive rough and presumptive nonmotile isolates. Seven O157:H7 isolates (0.4%) lacked *stx* genes, although they carried *eae* and *hlyA.* All but one of the isolates carried both *eae* and *hlyA.* Approximately two-thirds of the isolates (64% when one isolate per sample was considered) carried both $stx_1$ and $stx_2$. *E. coli* O157:H7 cells that harbored both $stx_1$ and $stx_2$ were more often recovered from hides in the fall (79% of the fall hide isolates) and winter (84% of the winter hide isolates) than in the spring (53%) and summer (59%). Isolates recovered from preevisceration carcasses showed a similar but not statistically significant trend. Twenty-three of the 25 O157:H7 isolates carrying $stx_1$ but not $stx_2$ were recovered during summer. Fifteen presumptive rough and 117 presumptive nonmotile *stx*$^+$ O157:H7 isolates were recovered. Ten (67%) of the presumptive rough isolates were recovered during summer. Ninety-five of the presumptive nonmotile isolates (81%) were recovered during fall. Forty-eight percent of the false-positive isolates (175 of 363) tentatively identified as O157:H7 were O157$^+$ H7$^-$ and lacked $eae_{O157}$, *hlyA,* and *stx.* These data suggest that in beef processing samples (i) there are minor seasonal variations in the prevalence of *stx* genes among *E. coli* O157:H7 isolates, (ii) presumptive rough and presumptive nonmotile *stx*$^+$ O157:H7 isolates are present, (iii) *E. coli* O157:H7 isolates lacking *stx* genes may be rare, and (iv) O157$^+$ H7$^-$ isolates lacking *stx* genes can result in many false-positive results.

*Escherichia coli* O157:H7 is an enteric pathogen that can cause diseases ranging from mild diarrhea to hemolytic uremic syndrome, kidney failure, and death (for reviews, see *(17, 21)*). The Shiga toxins, Stx1 and Stx2, are considered to be the primary *E. coli* O157:H7 virulence factors, and the cells may harbor genes that express one or both of these toxins. However, the Shiga toxins alone may not be sufficient to cause disease. Additional known virulence factors include intimin and enterohemolysin, products of the *eae* and *hlyA* genes, respectively. Other virulence factors also may be involved.

*E. coli* O157:H7 has been declared by the U.S. Food Safety and Inspection Service to be an adulterant in ground beef and nonintact beef products *(2)*. Cattle are considered to be the primary reservoir of *E. coli* O157:H7 that is associated with human disease *(8)*. Previously, we examined the seasonal prevalence of *E. coli* O157:H7 in feces and on hides and carcasses at beef processing plants *(4)*. As with disease cases *(8)*, the prevalence of *E. coli* O157:H7 was high during warmer months and low during winter. The present study was conducted to examine the prevalence of $stx_1$, $stx_2$, or both in the *E. coli* O157:H7 isolates. In addition, the prevalence of *E. coli* O157:H7 isolates lacking *stx* genes and presumptive rough or nonmotile isolates is reported. Finally, some false-positive isolates, which initially had been identified incorrectly as O157:H7, also are described.

## MATERIALS AND METHODS

Previously, we studied the prevalence of *stx*$^+$ *E. coli* O157:H7 in large Midwestern beef processing plants over four seasons *(4)*. Samples were collected from hides, feces, and preevisceration and postintervention (in the cooler) carcasses. During that study, colonies initially were screened using DrySpot O157 latex reagents (Oxoid, Ogdensburg, N.Y.). Up to three presumptive *E. coli* O157:H7 isolates were recovered and characterized per sample, as described elsewhere *(4)*. In brief, isolates were first screened by indirect enzyme-linked immunosorbent assay (ELISA) for the expression of the O157 and H7 antigens. Then, at least one isolate per sample (see below) was (i) subjected to multiplex PCR, as described by Paton and Paton *(24)* to detect $stx_1$, $stx_2$, $eae_{O157}$, and *hlyA*; (ii) confirmed to be *E. coli* by biochemical analyses; and (iii) tested for sorbitol fermentation on sorbitol MacConkey agar (Difco, Becton Dickinson, Sparks, Md.) supplemented with cefixime and potassium tellurite (Dynal, Lake Success, N.Y.). A total of 3,277 potential *E. coli* O157:H7 isolates were recovered,

* Author for correspondence. Tel: 402-762-4228; Fax: 402-762-4149; E-mail: gallagher@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

TABLE 1. *Sample site and* stx *genotype of all* stx$^+$ E. coli *O157:H7 isolates by phenotype*

| Sample site and *stx* genotype | No. of isolates by phenotype (%)[a] | | | |
|---|---|---|---|---|
| | O157$^+$ H7$^+$[b] | Presumptive rough[c] | Presumptive nonmotile[d] | Total *stx*$^+$ O157:H7[e] |
| Feces | | | | |
| *stx*$_1^+$ | 0 (0) | 0 | 0 (0) | 0 |
| *stx*$_2^+$ | 50 (50) | 0 | 0 (0) | 50 (49) |
| *stx*$_1^+$ *stx*$_2^+$ | 51 (50) | 0 | 1 (100) | 52 (51) |
| Total | 101 | 0 | 1 | 102 |
| Hide | | | | |
| *stx*$_1^+$ | 9 (0.9) | 0 (0) | 12 (14) | 21 (1.9) |
| *stx*$_2^+$ | 302 (30) | 9 (100) | 13 (15) | 324 (29) |
| *stx*$_1^+$ *stx*$_2^+$ | 696 (69) | 0 (0) | 59 (70) | 755 (69) |
| Total | 1,007 | 9 | 84 | 1,100 |
| Preevisceration carcass | | | | |
| *stx*$_1^+$ | 2 (0.5) | 0 (0) | 2 (6.2) | 4 (0.9) |
| *stx*$_2^+$ | 172 (40) | 6 (100) | 14 (44) | 192 (41) |
| *stx*$_1^+$ *stx*$_2^+$ | 255 (59) | 0 (0) | 16 (50) | 271 (58) |
| Total | 429 | 6 | 32 | 467 |
| Postintervention carcass | | | | |
| *stx*$_1^+$ | 0 (0) | 0 | 0 | 0 (0) |
| *stx*$_2^+$ | 7 (33) | 0 | 0 | 7 (33) |
| *stx*$_1^+$ *stx*$_2^+$ | 14 (67) | 0 | 0 | 14 (67) |
| Total | 21 | 0 | 0 | 21 |
| All | | | | |
| *stx*$_1^+$ | 11 (0.7) | 0 (0) | 14 (12) | 25 (1.5) |
| *stx*$_2^+$ | 531 (34) | 15 (100) | 27 (23) | 573 (34) |
| *stx*$_1^+$ *stx*$_2^+$ | 1,016 (65) | 0 (0) | 76 (65) | 1,092 (65) |
| Total | 1,558 | 15 | 117 | 1,690 |

[a] The percentage of a given genotype among isolates from a specified sample site is indicated in parentheses.
[b] All isolates that were both O157$^+$ and H7$^+$ by ELISA, including up to three isolates from each positive fall sample.
[c] All isolates that were O157$^+$ and H7$^+$ by ELISA, *stx*$^+$, and carried the *rfbE*$_{O157}$ and *fliC*$_{H7}$ genes.
[d] All isolates that were O157$^+$ and H7$^-$ by ELISA, *stx*$^+$, and carried the *rfbE*$_{O157}$ and *fliC*$_{H7}$ genes.
[e] All isolates considered positive for O157:H7 and carrying at least one *stx* gene, including presumptive rough and nonmotile isolates.

and 1,208 samples were identified as positive for *stx*-containing *E. coli* O157:H7.

One O157$^+$ H7$^+$ isolate per sample was arbitrarily selected as the "primary" isolate for complete characterization. Also, because of concerns that the relative prevalence of the different genotypes might be misrepresented by choosing only one isolate per sample, PCR data were collected for all isolates recovered in one sampling period (fall). If an O157$^+$ H7$^+$ isolate did not appear to harbor an *stx* gene on the basis of the initial multiplex PCR result, it was subjected to an alternative multiplex PCR that detected *stx*$_1$, *stx*$_2$, *rfbE*$_{O157}$, *fliC*$_{H7}$, and *eae*$_{O157}$ *(18)*; all available additional isolates from the same sample were also analyzed. In addition, all isolates that were ELISA-positive for only the H7 antigen (O157$^-$ H7$^+$) or only the O157 antigen (O157$^+$ H7$^-$) were retested and subjected to both multiplex PCR assays *(18, 24)*. The O157$^-$ H7$^+$ isolates were considered to be presumptive rough O157:H7 strains when *rfbE*$_{O157}$, *fliC*$_{H7}$, and at least one *stx* gene were detected by PCR. The O157$^+$ H7$^-$ isolates were considered to be presumptive nonmotile O157:H7 strains when *fliC*$_{H7}$, *rfbE*$_{O157}$, and at least one *stx* gene were detected by PCR. The remaining O157$^+$ H7$^+$ isolates, a total of 1,224, were not fully characterized. Statistical analyses were performed as described elsewhere *(4)*.

## RESULTS AND DISCUSSION

Recently, we reported the seasonal prevalence of *stx*$^+$ *E. coli* O157:H7 at three large Midwestern beef processing plants *(4)*. A total of 1,690 *stx*$^+$ *E. coli* O157:H7 isolates from these samples now have been completely characterized, including at least one isolate per positive sample, all isolates recovered during fall, and all presumptive rough and nonmotile isolates (Table 1). All of the fully characterized positive isolates were sorbitol nonfermenting and harbored both *eae* and *hlyA,* with the exception of one strain lacking *hlyA* that was found by examining all of the fall isolates.

The *stx* genotypes of one positive isolate per sample are summarized in Table 2. Most of these primary isolates were both O157$^+$ and H7$^+$ according to the ELISA results. However, the only available isolates from some samples did not express one of these antigens. For those samples, the selected isolate was positive by PCR for *rfbE*$_{O157}$, *fliC*$_{H7}$, and at least one *stx* gene. Therefore, 7 of the presumptive rough isolates and 30 of the presumptive nonmotile isolates

TABLE 2. *Sample site and* stx *genotype of primary* stx$^+$ E. coli *O157:H7 isolates by season*

| Sample site and *stx* genotype | No. of primary isolates (%)[a] | | | | |
|---|---|---|---|---|---|
| | Spring | Summer | Fall | Winter | Total |
| Feces | | | | | |
| $stx_1^+$ | 0 (0)[b] | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| $stx_2^+$ | 3 (27) A[c] | 19 (51) A | 12 (57) A | 0 (0) A | 34 (49) |
| $stx_1^+$ $stx_2^+$ | 8 (73) A | 18 (49) A | 9 (43) A | 1 (100) A | 36 (51) |
| Total | 11 | 37 | 21 | 1 | 70 |
| Hide | | | | | |
| $stx_1^+$ | 0 (0) | 7 (3.0) | 0 (0) | 1 (1.0) | 8 (1.0) |
| $stx_2^+$ | 106 (47) A | 90 (38) A | 47 (21) B | 15 (16) B | 258 (33) |
| $stx_1^+$ $stx_2^+$ | 119 (53) A | 139 (59) A | 176 (79) B | 81 (84) B | 515 (66) |
| Total | 225 | 236 | 223 | 97 | 781 |
| Preevisceration carcass | | | | | |
| $stx_1^+$ | 0 (0) | 3 (2.3) | 0 (0) | 0 (0) | 3 (0.9) |
| $stx_2^+$ | 55 (47) AB | 61 (47) A | 28 (31) B | 1 (25) AB | 145 (42) |
| $stx_1^+$ $stx_2^+$ | 63 (53) AB | 66 (51) A | 62 (69) B | 3 (75) AB | 194 (57) |
| Total | 118 | 130 | 90 | 4 | 342 |
| Postintervention carcass | | | | | |
| $stx_1^+$ | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| $stx_2^+$ | 1 (11)[b] | 0 (0) | 2 (67) | 0 (0) | 3 (20) |
| $stx_1^+$ $stx_2^+$ | 8 (89)[b] | 3 (100) | 1 (33) | 0 (0) | 12 (80) |
| Total | 9 | 3 | 3 | 0 | 15 |
| All | | | | | |
| $stx_1^+$ | 0 (0) | 10 (2.4) | 0 (0) | 1 (1.0) | 11 (0.9) |
| $stx_2^+$ | 150 (41) A | 170 (42) A | 89 (26) B | 16 (16) B | 425 (35) |
| $stx_1^+$ $stx_2^+$ | 213 (59) A | 226 (56) A | 248 (74) B | 85 (83) B | 772 (64) |
| Total | 363 | 406 | 337 | 102 | 1,208 |

[a] One isolate per positive sample is included. The percentage of a given genotype among isolates from a specified sample site is indicated in parentheses.

[b] Statistical analyses were not performed for $stx_1^+$ isolates and postintervention isolates because of the low number of isolates in each group.

[c] Within a row, values for an organism with the same letter are not significantly different ($P > 0.05$).

(see below) were included in Table 2. Overall, the majority of the primary isolates harbored both $stx_1$ and $stx_2$ (Figure 1A, lane 2). In comparison, the relative genotype proportions for the fall isolates did not change significantly when all 791 of the isolates (up to 3 isolates each from 337 samples) were analyzed (data not shown; $P > 0.05$; also see Table 1). Other studies have reported similar proportions of $stx_1^+$, $stx_2^+$, and $stx_1^+$ $stx_2^+$ genotypes in *E. coli* O157:H7 cells isolated from cattle *(11, 12, 23, 25)* and human clinical cases *(17, 22, 23)*. For example, among 343 *E. coli* O157:H7 isolates recovered from beef processing sites, Elder et al. *(11)* found that 1.4, 41.2, and 57.4% carried $stx_1$, $stx_2$, and both $stx_1$ and $stx_2$, respectively. Ostroff et al. *(22)* examined isolates from 93 human clinical cases and found that 3% carried $stx_1$, 20% carried $stx_2$, and 76% carried both $stx_1$ and $stx_2$.

When one isolate per sample was considered, the proportion of *E. coli* O157:H7 isolates harboring both $stx_1$ and $stx_2$ was higher on hides during fall and winter than during spring and summer ($P > 0.05$; Table 2). The inverse was true for $stx_2^+$ cells. A similar trend was evident for isolates from preevisceration carcasses, but the number of isolates recovered during winter was too low for adequate comparison. These small differences are not likely to be biologically relevant and suggest that seasonal variations have only minor effects on carriage rates of $stx_2$ only or $stx_1$ and $stx_2$ together. Twenty-three of 25 $stx_1^+$ strains (lacking $stx_2$; e.g., Fig. 1A, lane 1) were from 21 cattle and carcasses sampled from 10 lots during summer (data not shown). Fourteen of the $stx_1^+$ isolates were presumed to be nonmotile (see below; Fig. 1A, lane 4). The low prevalence of $stx_1^+$ among *E. coli* O157:H7 isolates differs from the 47% prevalence of $stx_1^+$ isolates among non-O157 Shiga toxin–producing *E. coli* (STEC) recovered from beef carcass samples *(3)*. It has been suggested that cells that carry $stx_1$ are less virulent than those that carry either $stx_2$ or both types of *stx* genes *(6, 9, 10, 22, 30)*. The significantly higher prevalence of $stx_2^+$ and both $stx_1^+$ $stx_2^+$ genotypes among O157:H7 strains, compared with non-O157 STEC, suggests that a larger percentage of O157:H7 strains may be more highly virulent, which may, in turn, contribute to the higher proportion of O157:H7 strains associated with disease in the United States.

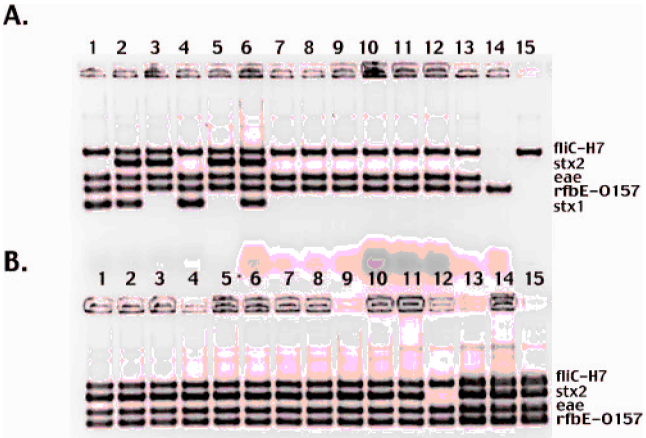


FIGURE 1. *Representative results of multiplex PCR assay for* $stx_1$, $stx_2$, $rfbE_{O157}$, $fliC_{H7}$, *and* eae *(18). (A) Lane 1, O157$^+$ H7$^+$ strain 712HR1 ($stx_1^+$ $rfbE_{O157}^+$ eae$^+$ $fliC_{H7}^+$); lane 2, O157$^+$ H7$^+$ strain 712FR1 ($stx_1^+$ $rfbE_{O157}^+$ eae$^+$ $stx_2^+$ $fliC_{H7}^+$); lane 3, O157$^+$ H7$^+$ strain 756AC1 ($rfbE_{O157}^+$ eae$^+$ $stx_2^+$ $fliC_{H7}^+$); lane 4, presumptive nonmotile O157$^+$ strain 955HR1 ($stx_1^+$ $rfbE_{O157}^+$ eae$^+$ $fliC_{H7}^+$); lane 5, presumptive nonmotile O157$^+$ strain 946HC1 ($rfbE_{O157}^+$ eae$^+$ $stx_2^+$ $fliC_{H7}^+$); lane 6, presumptive nonmotile O157$^+$ strain 946HR3 ($stx_1^+$ $rfbE_{O157}^+$ eae$^+$ $stx_2^+$ $fliC_{H7}^+$); lanes 7 through 13, O157$^+$ H7$^+$ strains lacking* stx *($rfbE_{O157}^+$ eae$^+$ $fliC_{H7}^+$); lane 14, O157$^+$ H7$^-$ strain 909FR1 ($rfbE_{O157}^+$); and lane 15, O157$^-$ H7$^-$ strain 738HR3 ($fliC_{H7\text{-}like}^+$). (B) Lanes 1 through 15, presumptive rough strains ($rfbE_{O157}^+$ eae$^+$ $stx_2^+$ $fliC_{H7}^+$). Strain 161EHC1 (lane 12) appears to have lost the* $stx_2$ *gene.*

**O157$^+$ H7$^+$ isolates lacking *stx*.** The seven O157$^+$ H7$^+$ isolates that lacked *stx* (Fig. 1A, lanes 7 through 13 and Table 3) were the only ones recovered from three samples, one fecal and two preevisceration carcass samples, acquired during summer. These isolates may have spontaneously lost the *stx* genes *(13)*. For example, a presumptive rough strain appears to have lost the $stx_2$ gene during routine culture and storage at −70°C (Fig. 1B, lane 12 and data not shown). It is possible that the prevalence of *stx*-lacking isolates has been underestimated, because not all of the O157$^+$ H7$^+$ isolates recovered during spring, summer, and winter were tested for *stx* genes. However, no *E. coli* O157:H7 isolates lacking *stx* were found among all of the fall isolates, which suggests that these strains may be rare. *E. coli* O157:H7 isolates lacking *stx* have been recovered from clinical and cattle fecal samples *(13, 26, 28, 31)*. Although the Shiga toxins are considered to be the primary virulence factors of STEC *(21)*, it has been suggested that O157:H7/H$^-$ strains do not require *stx* genes to cause disease *(1, 28)*. For example, some isolates may express an alternative virulence factor(s), such as cytolethal distending toxin *(19)*.

**O157$^-$ H7$^+$ isolates.** Twenty-two O157$^-$ H7$^+$ isolates were identified on the basis of ELISA, despite colony screening with DrySpot *E. coli* O157 latex reagents. Fifteen of these isolates did not ferment sorbitol on ctSMAC agar and carried the $rfbE_{O157}$ gene in addition to $fliC_{H7}$, $stx_2$, $eae_{O157}$, and *hlyA* (Fig. 1B, lanes 1 through 15). Therefore, they were considered to be presumptive rough *stx*$^+$ *E. coli* O157:H7 (Table 1). However, further analyses are necessary to confirm the absence of O antigen *(15)*. Positive DrySpot reactions were obtained using pure cultures of these isolates, and 14 of them also produced a weak positive band on ImmunoCard STAT! *E. coli* O157 devices (Meridian Bioscience, Inc., Cincinnati, Ohio). The apparent discrepancy in DrySpot, ImmunoCard, and ELISA results may reflect differences in antibody specificity *(15)*. These 15 isolates were recovered from three preevisceration carcasses and seven hides from cattle in six lots sampled over one summer and one winter plant visit. Other rough *stx*$^+$ O157:H7 isolates have been recovered from beef and human clinical cases in other countries *(15, 32)*, which suggests that they can survive processing and cause disease.

TABLE 3. *Sample site and phenotype of all negative isolates originally presumed to be* stx$^+$ E. coli *O157:H7*[a]

| Sample type | No. of isolates by ELISA-based phenotype and relevant genotype | | | | |
|---|---|---|---|---|---|
| | O157$^-$ H7$^+$ (*rfbE* not O157)[b] | O157$^+$ H7$^-$ (*fliC* not H7)[b] | O157$^-$ H7$^-$ | O157$^+$ H7$^+$, no *stx*[d] | Total |
| Feces | 0 | 94 | 10 | 1 | 105 |
| Hide | 4 | 19 | 97 | 0 | 120 |
| Preevisceration carcass | 3 | 55 | 67 | 6 | 131 |
| Postintervention carcass | 0 | 7 | 0 | 0 | 7 |
| Total | 7 | 175 | 174 | 7 | 363 |

[a] A total of 3,277 isolates were recovered; 1,690 isolates were determined to be *stx*$^+$ *E. coli* O157:H7, and 1,224 O157$^+$ H7$^+$ isolates were not fully characterized.
[b] These isolates did not carry *stx, eae,* or *hlyA* genes.
[c] The genotype of these isolates was not determined.
[d] These isolates did carry $eae_{O157}$ and *hlyA*.

The remaining seven *E. coli* isolates classified as O157$^-$ H7$^+$ fermented sorbitol (recovered from ntRainbow agar) and initially appeared to carry the $fliC_{H7}$ gene but not $rfbE_{O157}$, $stx_1$, $stx_2$, $eae_{O157}$, or *hlyA* (e.g., Fig. 1A, lane 15 and Table 3). A supplementary PCR–restriction-fragment length polymorphism analysis revealed that the *fliC* gene in these strains was similar, but not identical, to the $fliC_{H7}$ gene of *E. coli* O157:H7 (data not shown *(16)*). When pure cultures were tested, four of these isolates were DrySpot *E. coli* O157$^+$, and one agglutinated both the test and the control latex. These observations illustrate some of the possibilities surrounding false-positives, including the O157$^-$ H7$^-$ (ELISA-based) isolates (Table 3). Isolates may be misidentified because of cross-reactivity with the detection antibodies, false reactions from sampling crowded growth on the plates *(29)*, or a lack of attention to controls. However, overall DrySpot screening has been shown to be ~90% accurate for isolates from these samples *(4)*.

**O157$^+$ H7$^-$ isolates.** Altogether, 292 *E. coli* O157$^+$ H7$^-$ isolates were recovered on the basis of ELISA reactivity. These isolates were not passaged in semisolid media to enhance motility. All of these isolates were tested for the presence of the $fliC_{H7}$ gene, in addition to $rfbE_{O157}$, *stx,*

$eae_{O157}$, and *hlyA*. Of these, 117 were genetically O157:H7 (positive for $rfbE_{O157}$ and $fliC_{H7}$), sorbitol nonfermenting, and positive for at least one *stx* gene, *eae,* and *hlyA* (e.g., Fig. 1A, lanes 4 through 6). These 117 isolates therefore were considered to be presumptive nonmotile *stx*$^+$ *E. coli* O157:H7. The majority (65%) carried both $stx_1$ and $stx_2$ genes. To the best of our knowledge, these presumably nonmotile isolates are capable of causing disease *(7, 14, 16, 20).*

The remaining 175 *E. coli* O157$^+$ H7$^-$ isolates did not carry $fliC_{H7}$, $eae_{O157}$, or *hlyA* (e.g., Fig. 1A, lane 14 and Table 3). These strains also did not possess either *stx* gene and therefore could not be considered STEC or enterohemorrhagic *E. coli*. They formed colonies on ntRainbow agar that looked similar to those of O157:H7 isolates but fermented sorbitol on ctSMAC agar. Many of them appeared unusually large when grown to the midlog phase in tryptic soy broth without glucose (Difco) and examined by phase-contrast microscopy for motility (data not shown). Sorbitol-fermenting *stx*$^+$ and *stx*-lacking *E. coli* O157:H7/H$^-$ strains have been isolated from disease outbreaks and cattle in Germany and elsewhere *(5, 19, 27, 28)*. The disease-associated strains lacking *stx* appear to have alternative virulence factors and are likely to be enteropathogenic *E. coli (27)*. Tests for alternative virulence factors were not performed, because *stx*$^+$ *E. coli* O157:H7 cells were targeted.

The O157$^+$ H7$^-$ isolates lacking *stx* caused a number of samples to be incorrectly identified as positive, because they reacted with DrySpot *E. coli* O157 screening tests. For example, 20% of the preevisceration carcasses sampled during one spring plant visit were identified incorrectly as positive because of these strains. With experience, it was noted that these nontarget strains formed whiter colonies on ntRainbow than O157:H7 strains. Thus, these isolates were increasingly excluded during the selection process. As a consequence, the majority of them (115 of 175) were recovered during the first (spring) sampling season. However, the phenotypic difference in the colonies is subtle, and even experienced laboratory personnel were not always able to correctly exclude these isolates. This group of isolates constituted just under one-half (48%) of the false-positive isolates recovered (Table 3). Therefore, these strains present a significant potential problem, particularly for inexperienced personnel, in situations where false-positives cannot be tolerated. The data suggest that, in those situations, complete analyses are critical before any conclusions can be drawn.

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## REFERENCES

1. Allerberger, F., M. P. Dierich, U. Gruber-Moesenbacher, A. Liesegang, R. Prager, G. Hartmann, W. Rabsch, H. Tschape, and H. Karch. 2000. Nontoxigenic sorbitol-fermenting *Escherichia coli* O157:H$^-$ associated with a family outbreak of diarrhoea. *Wien. Klin. Wochenschr.* 112:846–850.
2. Anonymous. 1999. Beef products contaminated with *Escherichia coli* O157:H7. U.S. Department of Agriculture, Food Safety and Inspection Service. Code of Federal Regulations. *Fed. Regist.* 64: 2803–2805.
3. Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie. 2002. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* 68:4847–4852.
4. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* spp. in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
5. Bielaszewska, M., H. Schmidt, A. Liesegang, R. Prager, W. Rabsch, H. Tschäpe, A. Cízek, J. Janda, K. Bláhová, and H. Karch. 2000. Cattle can be a reservoir of sorbitol-fermenting Shiga toxin–producing *Escherichia coli* O157:H$^-$ strains and a source of human diseases. *J. Clin. Microbiol.* 38:3470–3473.
6. Boerlin, P., S. A. McEwen, F. Boerlin-Petzold, J. B. Wilson, R. P. Johnson, and C. L. Gyles. 1999. Associations between virulence factors of Shiga toxin–producing *Escherichia coli* and disease in humans. *J. Clin. Microbiol.* 37:497–503.
7. Bopp, C. A., K. D. Greene, F. P. Downes, E. G. Sowers, J. G. Wells, and I. K. Wachsmuth. 1987. Unusual verotoxin-producing *Escherichia coli* associated with hemorrhagic colitis. *J. Clin. Microbiol.* 25:1486–1489.
8. Centers for Disease Control and Prevention. 2000. FoodNet surveillance report for 2000: final report. Available at: http://www.cdc.gov/foodnet.annuals.htm. Accessed 29 January 2004.
9. Donohue-Rolfe, A., I. Kondova, S. Oswald, D. Hutto, and S. Tzipori. 2000. *Escherichia coli* O157:H7 strains that express Shiga toxin (Stx) 2 alone are more neurotropic for gnotobiotic piglets than are isotypes producing only Stx1 or both Stx1 and Stx2. *J. Infect. Dis.* 181:1825–1829.
10. Dorn, C. R., S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe. 1989. Properties of Vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7. *Epidemiol. Infect.* 103:83–95.
11. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
12. Fegan, N., and P. Desmarchelier. 2002. Comparison between human and animal isolates of Shiga toxin–producing *Escherichia coli* O157 from Australia. *Epidemiol. Infect.* 128:357–362.
13. Feng, P., M. Dey, A. Abe, and T. Takeda. 2001. Isogenic strain of *Escherichia coli* O157:H7 that has lost both Shiga toxin 1 and 2 genes. *Clin. Diagn. Lab. Immunol.* 8:711–717.
14. Feng, P., P. I. Fields, B. Swaminathan, and T. S. Whittam. 1996. Characterization of nonmotile variants of *Escherichia coli* O157 and other serotypes by using an antiflagellin monoclonal antibody. *J. Clin. Microbiol.* 34:2856–2859.
15. Feng, P., R. C. Sandlin, C. H. Park, R. A. Wilson, and M. Nishibuchi. 1998. Identification of a rough strain of *Escherichia coli* O157:H7 that produces no detectable O157 antigen. *J. Clin. Microbiol.* 36: 2339–2341.
16. Fields, P. I., K. Blom, H. J. Hughes, L. O. Helsel, P. Feng, and B. Swaminathan. 1997. Molecular characterization of the gene encoding H antigen in *Escherichia coli* and development of a PCR–restriction fragment length polymorphism test for identification of *E. coli* O157:H7 and O157:NM. *J. Clin. Microbiol.* 35:1066–1070.
17. Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli,* and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13:60–98.
18. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
19. Janka, A., M. Bielaszewska, U. Dobrindt, L. Greune, M. A. Schmidt,

and H. Karch. 2003. Cytolethal distending toxin gene cluster in enterohemorrhagic *Escherichia coli* O157:H$^-$ and O157:H7: characterization and evolutionary considerations. *Infect. Immun.* 71:3634–3638.

20. Lerman, Y., D. Cohen, A. Gluck, E. Ohad, and I. Sechter. 1992. A cluster of cases of *Escherichia coli* O157 in a day-care center in a communal settlement (kibbutz) in Israel. *J. Clin. Microbiol.* 30:520–521.
21. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11:142–201.
22. Ostroff, S. M., P. L. Tarr, M. A. Neill, J. H. Lewis, N. Hargrett-Bean, and J. M. Kobayashi. 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J. Infect. Dis.* 160:994–998.
23. Paros, M., P. I. Tarr, H. Kim, T. E. Besser, and D. D. Hancock. 1993. A comparison of human and bovine *Escherichia coli* O157:H7 isolates by toxin genotype, plasmid profile, and bacteriophage λ–restriction fragment length polymorphism profile. *J. Infect. Dis.* 168: 1300–1303.
24. Paton, A. W., and J. C. Paton. 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for $stx_1$, $stx_2$, *eaeA,* enterohemorrhagic *E. coli hlyA,* $rfb_{0111}$, and $rfb_{O157}$. *J. Clin. Microbiol.* 36:598–602.
25. Renter, D. G., J. M. Sargeant, R. D. Oberst, and M. Samadpour. 2003. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. *Appl. Environ. Microbiol.* 69: 542–547.
26. Rogerie, F., A. Marecat, S. Gambade, F. Dupond, P. Beaubois, and M. Lange. 2001. Characterization of Shiga toxin producing *E. coli* and O157 serotype *E. coli* isolated in France from healthy domestic cattle. *Int. J. Food Microbiol.* 63:217–223.
27. Schmidt, H., H. Rüssman, and H. Karch. 1993. Virulence determinants in nontoxigenic *Escherichia coli* O157 strains that cause infantile diarrhea. *Infect. Immun.* 61:4894–4898.
28. Schmidt, H., J. Scheef, H. I. Huppertz, M. Frosch, and H. Karch. 1999. *Escherichia coli* O157:H7 and O157:H$^-$ strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. *J. Clin. Microbiol.* 37:3491–3496.
29. Sowers, E., J. G. Wells, and N. A. Strockbine. 1996. Evaluation of commercial latex reagents for identification of O157 and H7 antigens of *Escherichia coli. J. Clin. Microbiol.* 34:1286–1289.
30. Tesh, V. L., J. A. Burris, J. W. Owens, V. M. Gordon, E. A. Wadolkowski, A. D. O'Brien, and J. E. Samuel. 1993. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. *Infect. Immun.* 61:3392–3402.
31. Vernozy-Rozand, D., P. Feng, M.-P. Montet, S. Ray-Gueniot, L. Villard, C. Bavai, A. Meyrand, C. Mazuy, and V. Atrache. 2000. Detection of *Escherichia coli* O157:H7 in heifers' faecal samples using an automated immunoconcentration system. *Lett. Appl. Microbiol.* 30:217–222.
32. Vila, J., M. Vargas, J. Ruiz, F. Gallardo, M. T. Jimenez de Anta, and J. Gascón. 1997. Isolation of verotoxin-producing *Escherichia coli* O-rough:K1:H7 from two patients with traveler's diarrhea. *J. Clin. Microbiol.* 35:2279–2282.