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Original Article

Pulsed-Field Gel Electrophoresis Subtypes of Shiga Toxin–Producing *Escherichia coli* O157 Isolated from Ground Beef and Humans, United States, 2001–2006

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Abstract

Pulsed-field gel electrophoresis *Xba*I patterns of Shiga toxin–producing *Escherichia coli* O157 (STEC O157) isolates ($n = 156$) found in ground beef sampled from U.S. processing plants and retail stores during 2001 to 2006 were summarized and compared with *Xba*I patterns from human STEC O157 isolates ($n = 14{,}591$) in the national PulseNet *E. coli* database. Four ground beef samples contained more than one pulsed-field gel electrophoresis subtype of STEC O157. Of the 117 unique patterns found in ground beef, 100 (85%) appeared only once, and 17 (15%) were found in more than one isolate. The six patterns that appeared most frequently in human isolates were also found among the eight most common ground beef patterns. The yearly proportion of human isolates with the two most common patterns changed inversely, such that these patterns traded dominance over the study period. Human isolates with patterns that were first detected in both ground beef and humans contemporaneously were clustered in a 6-month window around the time of the respective ground beef sample. Of the 156 ground beef isolates, 82 (53%) were indistinguishable from at least one human isolate in this 6-month window. The yearly proportions of human STEC O157 isolates that were indistinguishable from ground beef isolates decreased significantly from 2002 to 2003 (12.3–0.8%), and then increased significantly from 2003 to 2006 (overall 0.8–12.6%). This increase in the numbers of human isolates that matched a ground beef isolate occurred during a period of relatively consistent rates of ground beef contamination with STEC O157. Pattern similarity of STEC O157 isolates derived from ground beef and clinical cases may serve as a good predictor of human incidence trends.

Introduction

Shiga toxin–producing *Escherichia coli* O157 (STEC O157) continues to play a substantial role in foodborne illness. Mead *et al.* (1999) estimated that STEC O157 causes over 70,000 illnesses and over 600 deaths each year in the United States. The bovine gastrointestinal tract is the main reservoir for this pathogen (Karch *et al.*, 1999). Contaminated undercooked ground beef is recognized as a major source of human STEC O157 infection (CDC, 1993, 2002; Rangel *et al.*, 2005). Alternate routes of infection exist, including ingestion of other contaminated foods such as vegetables and unpasteurized cider (FDA, 2006, 2007; CDC, 1996, 1997), direct contact with animals (CDC, 2001, 2005; Kassenborg *et al.*, 2004; Voetsch *et al.*, 2006), and secondary transmission from ill humans (Belongia *et al.*, 1993).

The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) began testing ground beef for STEC O157 in 1994 (USDA-FSIS, 2008). As part of ongoing, risk-based verification testing of raw ground beef, inspectors at federal ground beef processing establishments randomly collected one pound of ground beef for STEC

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O157 testing in FSIS laboratories. For each year from 2001 to 2006, FSIS laboratories tested between 6,000 and 12,000 ground beef samples for STEC O157.

The Centers for Disease Control and Prevention (CDC) has used the Foodborne Diseases Active Surveillance Network (FoodNet) to estimate the incidence of human infections with STEC O157 in the United States since 1996 (CDC, 2007a). Figure 1 summarizes yearly human incidence and the percent of contaminated ground beef samples during the study period (CDC, 2007a; USDA-FSIS, 2008). Both the percent-positive rates of STEC O157 in ground beef and the incidence of human STEC O157 infections decreased during 2002 through 2004. In 2005 and 2006, however, the incidence of infections in humans increased above the goal set for the Healthy People 2010 initiative (1 case per 100,000 persons) (USDHHS, 2007), while ground beef percent-positive rates remained relatively unchanged. Possible explanations for this divergence in trends include the increasing roles of routes of STEC O157 transmission other than ground beef (CDC, 2007b) and the apparent recent emergence of strains with enhanced virulence (Manning *et al.*, 2008).

Molecular subtyping may give insights into recent surveillance data. Pulsed-field gel electrophoresis (PFGE) has been used in epidemiologic investigations of infectious disease since the mid-1990s (Bender *et al.*, 1997). In 1996, the CDC created PulseNet—the national subtyping network for foodborne disease surveillance, with databases of PFGE patterns from isolates of STEC O157 and other pathogens. FSIS began contributing PFGE patterns to PulseNet from STEC O157–positive ground beef samples in 1998.

Ground beef and human STEC O157 PFGE patterns have been compared on a case-by-case basis during outbreak investigations, but a systematic multi-year comparison has not been completed. This report summarizes and compares the PFGE patterns of STEC O157 isolated from ground beef and humans in the United States during the period from 2001 to 2006.

Materials and Methods

STEC O157 isolates were identified from the 50 states of the United States from 2001 through 2006 using FSIS sampling records, laboratory data (USDA-FSIS, unpublished data, 2007), and the PulseNet *E. coli* database. Isolates were included in the study if the *Xba*I pattern had sufficient visual clarity to assign it a unique identifier, hereafter referred to as a "name." PulseNet PFGE pattern names were used, and isolates were said to match if their *Xba*I patterns were indistinguishable.

STEC O157 isolates ($n = 156$) were identified from regulatory program samples of ground beef that were routinely collected (not associated with outbreak investigation or follow-up sampling) at federally inspected beef processing establishments ($n = 137$) and retail stores ($n = 19$). The sample collection date was used as the reference date for ground beef isolates. Human STEC O157 isolates from 2001 to 2006 ($n = 14{,}591$) were identified. Human isolates from the first 3 months of 2007 ($n = 162$) were also used for comparison to FSIS ground beef isolates found in late 2006. The date on which the isolate was confirmed by culture was used as the reference date for human isolates. In its absence, the following formulas (generally used by PulseNet staff) were used to estimate the culture-confirmed date: the date the isolate was received at the laboratory for PFGE analysis minus 7 days, or (in the absence of the received date) the date the isolate was uploaded to the PulseNet database minus 14 days.

The most frequently appearing patterns in the ground beef and human subsets were compared by pattern name. Changes in the yearly percentage of isolates with the two most common patterns in both the ground beef and human subsets (EXHX01.0047 and EXHX01.0074) were also analyzed for statistical significance using the χ^2-test for trend in the EpiInfo (v. 6) Statcalc program (CDC), with p-values <0.05 considered significant.

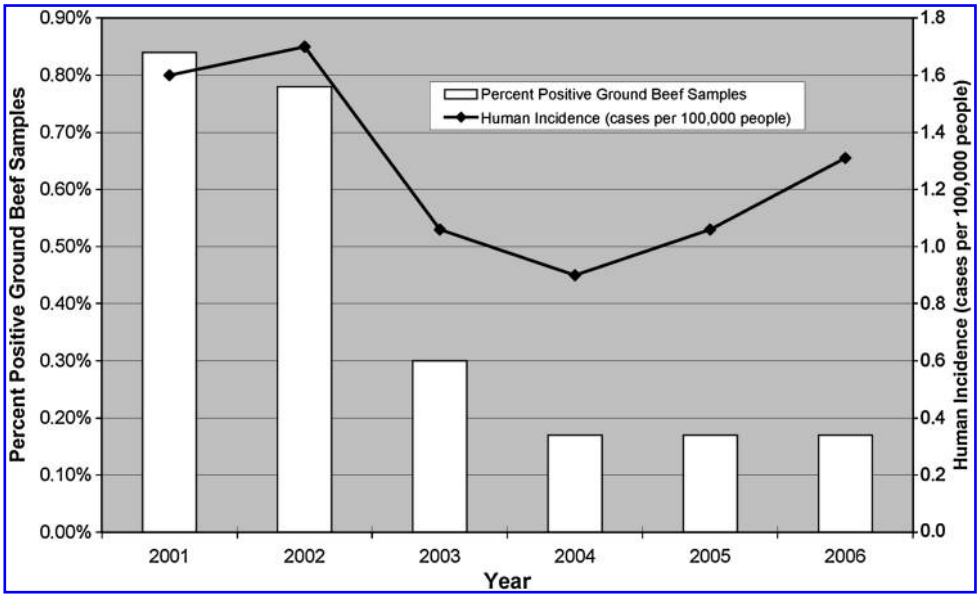

FIG. 1. Yearly percentage of Shiga toxin–producing *Escherichia coli* O157 (STEC O157)–positive ground beef samples and the Foodborne Diseases Active Surveillance Network–estimated incidence of human infection with STEC O157 in the United States, 2001–2006.

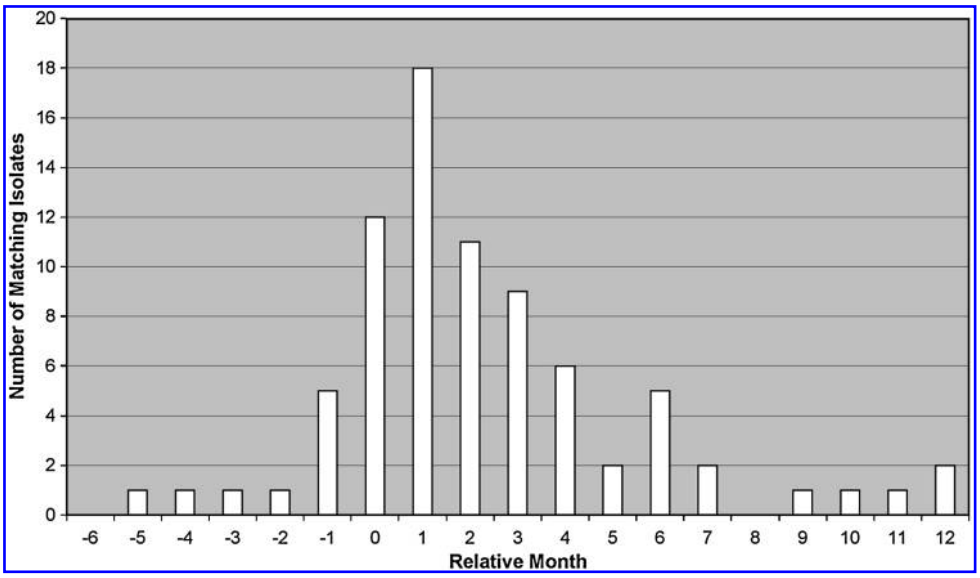

FIG. 2. STEC O157 isolates from humans with pulsed-field gel electrophoresis (PFGE) *Xba*I patterns that first appeared in both humans and ground beef within five calendar months of each other, United States, 2001–2006. Human isolates totaled by month relative to the date of the respective matching ground beef sample. Chart shows human isolate totals from six months before through twelve months after the matching ground beef sampling date.

Ground beef and human isolate patterns were also compared by pattern image. Pattern similarity was assessed by cluster analysis (Dice; UPGMA) and fast band-matching applications of Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium), and confirmed by visual comparison.

Matching human and ground beef isolates were considered to be temporally associated if the human isolate was identified within six calendar months of the date of the ground beef sample. To define this period of temporal association, patterns were identified from first-appearing human isolates that matched a nonrepeating ground beef isolate within five calendar months before or after the month that the ground beef sample was collected. Human STEC O157 isolates with these patterns were totaled by month with reference to the collection month of the matching ground beef sample. From these totals, a cluster of matching isolates was identified in a 6-month window from one calendar month before the ground beef sample through four calendar months after (Fig. 2).

Yearly proportions of ground beef isolates that matched at least one human isolate within the 6-month window were calculated. Similarly, yearly proportions of human isolates that matched a ground beef isolate within the 6-month window were also calculated. The statistical significance of year-to-year differences in proportion was assessed using the two-tailed, Yates-corrected χ^2-test (CDC), with p-values <0.05 considered significant. The Yates' correction for continuity was used to provide a more conservative estimate of significance due to the low yearly numbers of ground beef isolates. The statistical significance of trends in proportion over multiple years was assessed using the χ^2-test for trend (CDC), with p-values <0.05 considered significant.

Results

A summary of ground beef and human STEC O157 isolates in the study, their *Xba*I PFGE patterns, and their matching

Table 1. Summary of Ground Beef and Human Shiga Toxin–Producing *Escherichia coli* O157 Study Isolates and Their Pulsed-Field Gel Electrophoresis (PFGE) *Xba*I Patterns, United States, 2001–2006, Showing the Total Number of Isolates and Distinct Patterns, Distinct Pattern:Isolate (DP:I) Ratios, the Number and Percentage of Isolates with Patterns That Appeared Only Once During the Study Period, and the Number and Percent Age of Ground Beef Isolates That Matched a Human Isolate (and Vice Versa) by PFGE *Xba*I Pattern During the Entire Study Period and Within a 6-Month Window[a]

				Isolates with patterns appearing once		*Isolates matching 2001–2006*		*Isolates matching in 6-month window*[a]	
Isolate source	*Total isolates*	*Distinct patterns*	*DP:I ratio*	#	*% of total*	#	*% of total*	#	*% of total*
Ground beef	156	117	0.75	100	64	110	71	82	53
Human	14,591	3018	0.21	1762	12	5448	37	1177	8

[a]The 6-month window: from one calendar month before the ground beef sample date through four calendar months after, the calendar month of the sample counting as one month.

Table 2. The 17 Repeated PFGE *Xba*I Patterns from Shiga Toxin–Producing *E. coli* O157 Isolates Found in Ground Beef Samples, United States, 2001–2006, by Pattern Name, Count of Isolates, Frequency Rank,[a] and Corresponding Rank[a] Among Human Isolates

Pattern name	*Ground beef isolate count*	*Ground beef rank*[a]	*Human rank*[a]
EXHX01.0074	9	1	2
EXHX01.0047	8	2	1
EXHX01.0008	5	3	11
EXHX01.0224	4	4	3
EXHX01.0800	3	5	No human isolates[b]
EXHX01.0124	3	5	4
EXHX01.1343	3	5	5
EXHX01.0011	3	5	6
EXHX01.1058	2	6	No human isolates[b]
EXHX01.2178	2	6	No human isolates[b]
EXHX01.0200	2	6	9
EXHX01.0097	2	6	12
EXHX01.0238	2	6	26
EXHX01.0272	2	6	28
EXHX01.1401	2	6	120
EXHX01.1068	2	6	3 human isolates[b]
EXHX01.1354	2	6	1 human isolate[b]

[a]Ranked according to how frequently the pattern was found during the study period, with the rank of 1 denoting the most frequently appearing pattern.

[b]Denotes the number of human isolates with this *Xba*I pattern found in PulseNet, 2001–2006.

characteristics can be found in Table 1. Roughly two-thirds of ground beef isolates matched a human isolate sometime in the study period, and about one-half matched in the 6-month window. There was more pattern diversity (greater distinct pattern:isolate ratio) among ground beef isolates than among human isolates. Of the 117 distinct patterns found in ground beef, 100 (85%) appeared in only one isolate, and 17 (15%) were represented by two or more ground beef isolates. For each repeated ground beef pattern, none of the representative isolates came from samples collected at the same location.

Details about the 17 patterns repeated in ground beef isolates, as well as comparisons with the patterns most commonly found in human isolates, are given in Table 2. The six most common human patterns were found among the top eight ground beef patterns. Three of the most common ground beef patterns were not found in humans. Similarly, 2 of the top 10 human patterns (8th ranked, EXH01.0087, and 10th ranked, EXH01.0079) were not found in ground beef (data not shown). The two most common patterns, EXH01.0047 and EXH01.0074, were the same in both ground beef and humans, albeit opposite for each subset in order of representative isolates.

The yearly percentage of human isolates with pattern EXH01.0047 significantly increased overall, from 3.5% (95 isolates) in 2001 to 11.6% (334 isolates) in 2006 ($\chi^2 = 150.4$, $p < 0.001$), while the yearly percentage of isolates with pattern EXH01.0074 significantly decreased overall from 6.5% (176 isolates) in 2001 to 3.7% (107 isolates) in 2006 ($\chi^2 = 48.7$, $p < 0.001$) after an initial increase (Fig. 3). Similar trends are suggested from ground beef isolates with these patterns, but are less certain due to the relatively low numbers of ground beef isolates (data not shown).

Four ground beef samples yielded multiple isolates (with different patterns) per sample. Three of these multiple-isolate samples contained two isolates each (pairs), and one contained three (triplet). Each single-sample group of isolates was different in terms of whether the isolates matched a human isolate in the 6-month window, as follows. Both ground beef isolates from one pair and two isolates from the triplet matched at least one human isolate; the other ground beef isolate in the triplet did not match. In another pair, one ground beef isolate matched, and the other did not. Neither of the ground beef isolates from the remaining pair matched.

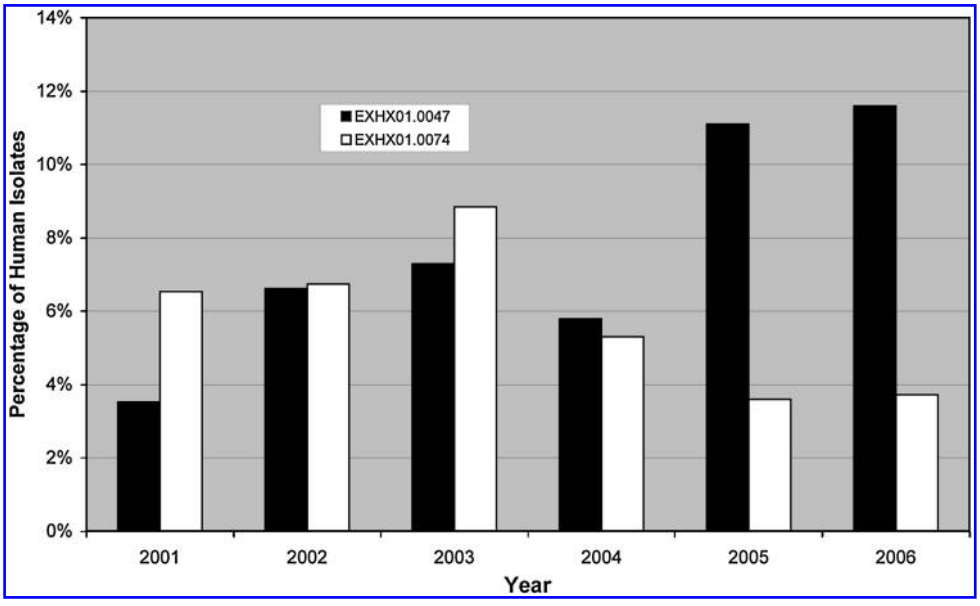

FIG. 3. Yearly percentage of STEC O157 isolates from humans with PFGE *Xba*I patterns EXH01.0047 and EXH01.0074, United States, 2001–2006.

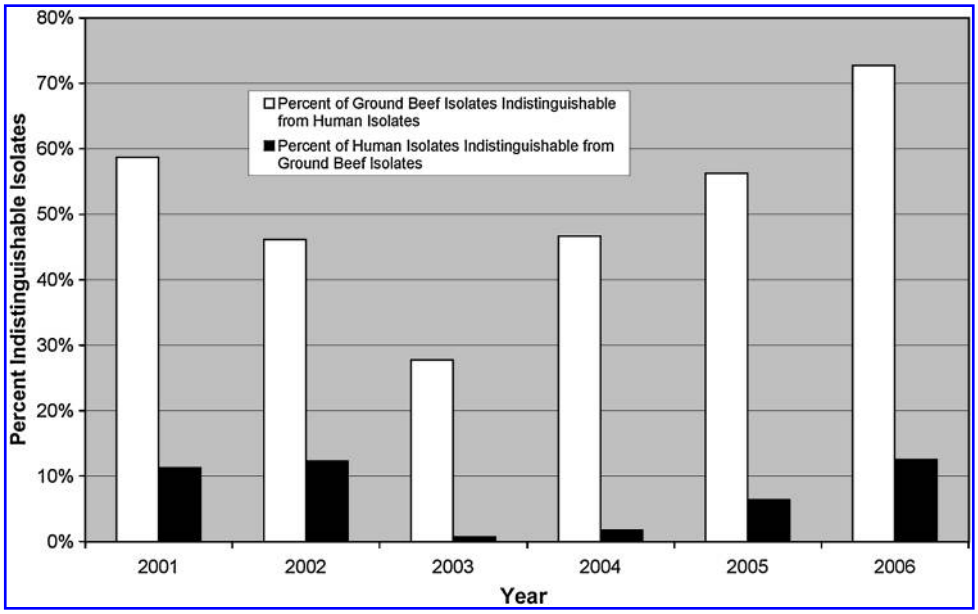

FIG. 4. Yearly percentage of STEC O157 isolates from ground beef indistinguishable from human isolates by PFGE *Xba*I pattern, United States, 2001–2006. Similarly, yearly percentage of STEC O157 isolates from humans indistinguishable from ground beef isolates by PFGE *Xba*I pattern. Matching analyzed in a period from one calendar month before through four calendar months after ground beef sample collection (6-month time window).

Figure 4 shows both the yearly percentage of ground beef isolates that matched a human isolate in the 6-month window, and the yearly percentage of human isolates that matched a ground beef isolate in the 6-month window. For ground beef isolates, year-to-year matching percentage differences were not statistically significant. However, the decreasing trend from 58.7% in 2001 to 27.8% in 2003 was significant ($\chi^2 = 4.97$, $p = 0.026$), as was the increasing trend from 27.8% in 2003 to 72.7% in 2006 ($\chi^2 = 8.10$, $p = 0.004$). For human isolates, the decrease in matching percentage from 12.3% in 2002 to 0.8% in 2003 was statistically significant ($\chi^2 = 206.8$, $p < 0.001$), as was the increasing trend from 0.8% in 2003 to 12.6% in 2006 ($\chi^2 = 351.8$, $p < 0.001$).

Discussion

This study compared subsets of ground beef and human isolates of STEC O157 found in the United States during 2001 to 2006 by PFGE *Xba*I patterns. The year 2001 was chosen as the first year of the study because complete FSIS sampling data were not available before this year, and since 2001 was the first year in which all 50 states submitted PFGE patterns of STEC O157 isolates to PulseNet (Gerner-Smidt *et al.*, 2005).

PFGE patterns made by digestion enzymes other than *Xba*I (*Bln*I/*Avr*II and *Spe*I) were not analyzed because they were not available for all isolates in the study. Patterns from additional enzymes can strengthen the case for genetic relatedness (Singer *et al.*, 2004) and can be particularly helpful in providing more evidence of an association during an outbreak investigation. For the purposes of this multi-year study, however, consistent comparison by one enzyme was considered adequate to reveal trends in similarity.

The genetic diversity observed among isolates from ground beef is consistent with surveys of STEC O157 isolated from cattle on dairy farms (Faith *et al.*, 1996; Wetzel and Lejeune, 2006), feedlots (Lejeune *et al.*, 2004), and at slaughter (Arthur *et al.*, 2007). Also, the resemblance between the lists of frequently appearing human and ground beef patterns is in harmony with the findings of a recent USDA's Agricultural Research Service study that found substantial similarity between the most common human *Xba*I patterns in PulseNet and patterns isolated from cattle at slaughter (Arthur *et al.*, 2007). Further, many of the pattern names in the Agricultural Research Service report were found among the patterns from the ground beef isolates in the present study. The similarities between cattle and ground beef isolates suggest that PFGE patterns from ground beef isolates may be good indicators of STEC O157 PFGE subtypes emerging from the cattle reservoir. The likeness between common human and ground beef patterns also lends support to the idea that contaminated ground beef continues to be a major vehicle for transmission of STEC O157 to people.

There were also some intriguing differences between the most common PFGE patterns in STEC O157 isolates from humans and ground beef. Two of the 10 most common human patterns from 2001 to 2006 did not appear in ground beef over the same period. These could simply have been missed in ground beef because the amount of ground beef sampled in the routine regulatory program is relatively small compared to the amount produced nationally. It may also be that these human patterns represent transmission routes other than ground beef.

Another difference between the two pattern subsets is that three repeated ground beef patterns did not appear in a human isolate in the database, nor did many other once-appearing ground beef isolates. Possible reasons that these patterns did not appear among humans isolates include limitations in surveillance, the proper cooking of meat by consumers, and the fact that STEC O157–positive product detected by FSIS usually does not reach commerce. Another possible explanation,

however, is that these patterns were associated with isolates that tended to make people sick less often, or with milder symptoms. This possibility is supported by recent research showing that certain STEC O157 strains are specific to the bovine reservoir (Besser *et al.*, 2007; Bono *et al.*, 2007).

The increasing and decreasing trends in the two most common patterns show that certain PFGE subtypes can become more or less prominent over time. The shifting dominance of strains may also help explain trends in incidence of human infections, since certain STEC O157 strains, such as those with different Shiga toxin subtypes, are more pathogenic and/or virulent than others (Roldgaard *et al.*, 2004; Kawano *et al.*, 2008).

The four instances of multiple PFGE patterns isolated from the same ground beef sample are not surprising given that meat from multiple animals is often combined in ground beef processing, and that different cattle on the same farm have been shown to harbor distinct STEC O157 strains (Faith *et al.*, 1996; Wetzel and Lejeune, 2006). Moreover, one animal can carry multiple STEC O157 strains (Faith *et al.*, 1996), so it is possible that ground beef contaminated with STEC O157 from only a single animal might contain more than one strain. Most STEC O157 outbreaks are thought to involve cases that all exhibit the same PFGE pattern (Tauxe, 2006). However, the fact that two ground beef samples each contained two distinct subtypes that matched contemporaneous human case isolates suggests that one contaminated batch of ground beef could cause human STEC O157 cases with different strains. Outbreaks of this nature have been reported (Proctor *et al.*, 2002; CDC, 2007c). Public health officials should be alert for such multi-strain outbreaks.

It has been suggested that the decrease in human incidence of STEC O157 infections from 2002 to 2004 was largely the result of decreased contamination of the ground beef supply (CDC, 2006; Naugle *et al.*, 2005, 2006; USDA-FSIS, 2006). The fact that ground beef positive rates remained at record lows while FoodNet incidence rates climbed in 2005 and 2006 indicates that factors other than the level of ground beef contamination also have an influence on human incidence. The increasing role of illness from contaminated leafy greens, animal contact, and other non-ground beef sources may partly explain recent trends (CDC, 2007b). Ecological shifts such as the possible emergence of increasingly virulent STEC O157 strains could also help explain the data (Manning *et al.*, 2008).

Comparison of the human and ground beef isolate subsets showed proportions of matching similar to FoodNet human incidence data. This similarity suggests that contaminated ground beef is still a major contributor to the burden of human STEC O157 illness. These findings also suggest that the proportion of human isolates that match ground beef may be a more accurate predictor of human incidence than ground beef contamination rates alone.

Adding STEC O157 isolates from sources other than ground beef to the comparison described in this study could reveal clues about different routes of disease transmission. FSIS has recently broadened its sampling program to include beef trim and other raw beef products (USDA-FSIS, 2007a, 2007b). Animal and environmental testing on cattle farms and feedlots should be considered to better understand the strain characteristics at the ultimate source of most STEC O157 infections. Indeed, the USDA's Animal and Plant Health Inspection Service already collects routine fecal samples of cattle on farms as part of ongoing studies (USDA-APHIS, 2006, 2007). Another method of surveillance is to test cattle at slaughter plants (Arthur *et al.*, 2007). Thinking beyond beef, outbreaks from leafy greens, raw milk, and contact with cattle and other animals over the past few years have highlighted the need for a reevaluation of STEC O157 attribution. Comparing molecular subtypes of farm, food, and human isolates may help us better understand and prevent pathways of STEC O157 transmission.

Enhanced surveillance and molecular comparison will require increased data-sharing between FSIS, CDC, FDA, and other current and future PulseNet partners. The collaboration required for the present study has strengthened the bond between FSIS and CDC, and has shown the benefits of interagency cooperation. The recent increases in human STEC O157 incidence should provide renewed motivation for public health agencies to cooperate toward decreasing transmission. Given the complex nature of STEC O157 ecology, transmission, and regulation, increased surveillance and cooperation are essential for our nation to reach the STEC O157 Healthy People goals of 2010 and beyond.

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Disclosure Statement

No competing financial interests exist.

References

Arthur TM, Bosilevac JM, Nou X, Shackelford SD, Wheeler TL, and Koohmaraie M. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 from the hides of beef cattle in different regions of North America. J Food Prot 2007;70:1622–1626.

Belongia EA, Osterholm, MT, Soler JT, Ammend DA, Braun JE, and MacDonald KL. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA 1993; 269:883–888.

Bender JB, Hedberg CW, Besser JM, Boxrud DJ, Macdonald KL, and Osterholm MT. Surveillance for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. N Engl J Med 1997;337:388–394.

Besser TE, Shaikh N, Holt NJ, Tarr PI, Konkel ME, Malik-Kale P, Walsh CW, Whittam TS, and Bono JL. Greater diversity of shiga-toxin encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. Appl Environ Microbiol 2007;73:671–679.

Bono JL, Keen JE, Clawson ML, Durso LM, Heaton MP, and Laegreid WW. Association of *Escherichia coli* O157:H7 *tir*

polymorphisms with human infection. BMC Infect Dis 2007;7:98.

[CDC] Centers for Disease Control and Prevention. Update: multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers—Western United States, 1992–1993. MMWR Morb Mortal Wkly Rep 1993;42:258–263.

[CDC] Centers for Disease Control and Prevention. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. MMWR Morb Mortal Wkly Rep 1996;45:975.

[CDC] Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts—Michigan and Virginia, June–July 1997. Morb Mortal Wkly Rep 1997;46:741–744.

[CDC] Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits—Pennsylvania and Washington, 2000. MMWR Morb Mortal Wkly Rep 2001;50:292–297.

[CDC] Centers for Disease Control and Prevention. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef—United States, June–July 2002. MMWR Morb Mortal Wkly Rep 2002;51:637–639.

[CDC] Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 associated with petting zoos—North Carolina, Florida, and Arizona, 2004 and 2005. MMWR Morb Mortal Wkly Rep 2005;54:1277–1280.

[CDC] Centers for Disease Control and Prevention. FoodNet surveillance report for 2004 (final report), 2006. Available at: http://www.cdc.gov/foodnet/annual/2004/Report.pdf. (Online.)

[CDC] Centers for Disease Control and Prevention. FoodNet reports [1996–2006]. 2007a. Available at http://www.cdc.gov/foodnet/reports.htm. (Online.)

[CDC] Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2006. MMWR Morb Mortal Wkly Rep 2007b;56:336–339.

[CDC] Centers for Disease Control and Prevention. Multistate outbreak of *E. coli* O157 infections linked to Topp's brand ground beef patties. 2007c. Available at http://www.cdc.gov/ecoli/2007/october/100207.html. (Online.)

Faith NG, Shere JA, Brosch R, Arnold, KW, Ansay SE, Lee MS, Luchansky JB, and Kaspar CW. Prevalence and clonal nature of *Escherichia coli*: O157:H7 on dairy farms in Wisconsin. Appl Environ Microbiol 1996;62:1519–1525.

[FDA] Food and Drug Administration. Questions & Answers: Taco Bell *E. Coli* O157:H7 lettuce outbreak. 2006. Available at http://www.cfsan.fda.gov/~dms/tacobqa.html. (Online.)

[FDA] Food and Drug Administration. Final report: investigation of an *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach. 2007. Available at http://www.fda.gov/bbs/topics/NEWS/2007/NEW01593.html. (Online.)

Gerner-Smidt P, Kincaid J, Kubota K, Hise K, Hunter SB, Fair M, Norton D, Woo-Ming A, Kurzynski T, Sotir MJ, Head M, Holt K, and Swaminathan B. Molecular surveillance of shigatoxigenic *Escherichia coli* O157 by PulseNet USA. J Food Prot 2005;68:1926–1931.

Karch H, Bielaszewska M, Bitzan M, and Schmidt H. Epidemiology and diagnosis of shiga toxin-producing *Escherichia coli* infections. Diagn Microbiol Infect Dis 1999;34:229–243.

Kassenborg HD, Hedberg CW, Hoekstra M, Evans MC, Chin AE, Marcus R, Vugia DJ, Smith K, Desai Ahuja S, Slutsker L, and Griffin PM. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 FoodNet sites. Clin Infect Dis 2004;38:S271–S278.

Kawano K, Okada M, Haga T, Maeda K, and Goto Y. Relationship between pathogenicity for humans and stx genotype in Shiga toxin-producing *Escherichia coli* serotype O157. Eur J Clin Microbiol Infect Dis 2008;27:227–232.

Lejeune JT, Besser TE, Rice DH, Berg JL, Stilborn RP, and Hancock DD. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. Appl Environ Microbiol 2004;70:377–384.

Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D, and Whittam TS. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proc Natl Acad Sci USA 2008;105:4868–4873.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, and Tauxe RV. Food-related illness and death in the United States. Emerg Infect Dis 1999;5:607–625.

Naugle AL, Holt KG, Levine P, and Eckel R. Food Safety and Inspection Service regulatory testing program for *Escherichia coli* O157:H7 in raw ground beef. J Food Prot 2005;68:462–468.

Naugle AL, Holt KG, Levine P, and Eckel R. Revised letter to the editor: sustained decrease in the rate of *Escherichia coli* O157:H7-positive raw ground beef samples tested by the Food Safety and Inspection Service. J Food Prot 2006;69:480–481.

Proctor ME, Kurzynski T, Koschmann C, Archer JR, and Davis JP. Four strains of *Escherichia coli* O157:H7 isolated from patients during an outbreak of disease associated with ground beef: importance of evaluating multiple colonies from an outbreak-associated product. J Clin Microbiol 2002;40:1530–1533.

Rangel JM, Sparling PH, Crowe C, Griffin PM, and Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 2005;11:603–609.

Roldgaard BB, Scheutz F, Boel J, Aabo S, Schultz AC, Cheasty T, Nielsen EM, Olsen KEP, and Christensen BB. VTEC O157 subtypes associated with the most severe clinical symptoms in humans constitute a minor part of VTEC O157 isolates from Danish cattle. Int J Med Microbiol 2004;294:255–259.

Singer RS, Sischo WM, and Carpenter TE. Exploration of biases that affect the interpretation of restriction fragment patterns produced by pulsed-field gel electrophoresis. J Clin Microbiol 2004;42:5502–5511.

Tauxe RV. Molecular subtyping and the transformation of public health. Foodborne Pathog Dis 2006;3:4–8.

[USDA-APHIS] United States Department of Agriculture, Animal and Plant Health Inspection Service. National animal health monitoring system, dairy 2007. 2006. Available at http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/dairy07/dairy07brochure.pdf. (Online.)

[USDA-APHIS] United States Department of Agriculture, Animal and Plant Health Inspection Service. Animal health monitoring and surveillance. 2007. Available at http://nahms.aphis.usda.gov/about.htm. (Online.)

[USDA-FSIS] United States Department of Agriculture, Food Safety and Inspection Service. Letter to FSIS employees. 2006.

[USDA-FSIS] United States Department of Agriculture, Food Safety and Inspection Service. FSIS notice 18-07: routine sampling of beef manufacturing trimmings intended for use in raw ground beef. 2007a. Available at http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/18-07.pdf. (Online.)

[USDA-FSIS] United States Department of Agriculture, Food Safety and Inspection Service. FSIS notice 68-07: routine

sampling and testing of raw ground beef components other than trim and imported raw ground beef components for *Escherichia coli (E. coli)* O157:H7. 2007b. Available at http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/68-07.pdf. (Online.)

[USDA-FSIS] United States Department of Agriculture, Food Safety and Inspection Service. Microbiological results of raw ground beef products analyzed for *Escherichia coli* O157:H7. 2008. Available at http://www.fsis.usda.gov/Science/Ground_Beef_E.coli_Testing_Results/index.asp. (Online.)

[USDHHS] United States Department of Health and Human Services. Healthy people 2010 objective 10-1: reduce infections caused by key foodborne pathogens. 2007. Available at http://www.healthypeople.gov/document/html/objectives/10-01.htm. (Online.)

Voetsch AC, Kennedy MH, Keene WE, Smith KE, Rabatsky-Her T, Zansky S, Thomas SM, Mohle-Boetani J, Sparling PH, McGavern MB, and Mead PS. Risk factors for sporadic Shiga toxin-producing *Escherichia coli* O157 infections in FoodNet sites, 1999–2000. Epidemiol Infect 2007;135:993–1000.

Wetzel AN and Lejeune JT. Clonal dissemination of *Escherichia coli* O157:H7 subtypes among dairy farms in northeast Ohio. Appl Environ Microbiol 2006;72:2621–2626.

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2. H. C. So, D. L. Pearl, T. von Königslöw, M. Louie, L. Chui, L. W. Svenson. 2013. Spatio-Temporal Scan Statistics for the Detection of Outbreaks Involving Common Molecular Subtypes: Using Human Cases of Escherichia coli O157:H7 Provincial PFGE Pattern 8 (National Designation ECXAI.0001) in Alberta as an Example. *Zoonoses and Public Health* **60**:10.1111/zph.2013.60.issue-5, 341-348. [CrossRef]
3. Deirdre M. Prendergast, Lynsey Lendrum, Rachel Pearce, Caroline Ball, Joanne McLernon, Don O'Grady, Lourda Scott, Seamus Fanning, John Egan, Montserrat Gutierrez. 2011. Verocytotoxigenic Escherichia coli O157 in beef and sheep abattoirs in Ireland and characterisation of isolates by Pulsed-Field Gel Electrophoresis and Multi-Locus Variable Number of Tandem Repeat Analysis. *International Journal of Food Microbiology* **144**, 519-527. [CrossRef]