Research Note

Seasonal Prevalence of Escherichia coli O157:H7 in Beef Cattle Feces†

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ABSTRACT

Cattle are an asymptomatic reservoir of Escherichia coli O157:H7, but the bacterial colonization and shedding patterns are poorly understood. The prevalence and shedding of this human pathogen have been reported to be seasonal with rates typically increasing during warm months. The objectives of this study were (i) to assess the prevalence of E. coli O157:H7 in feces of feedlot cattle in Kansas during summer, fall, and winter months, and (ii) to characterize E. coli O157:H7 by screening for virulence factors. Of 891 fecal samples collected, 82 (9.2%) were positive for E. coli O157:H7. No significant differences in prevalence were detected among summer, fall, and winter months. The highest monthly prevalence (18.1%) was detected in February. All tested isolates were positive for stx2 (Shiga toxin 2) and eaeA (intimin) genes; 14 isolates (12.8%) also carried stx1. Our results indicate the prevalence of E. coli O157:H7 in beef cattle feces is not necessarily season dependent.

Escherichia coli O157:H7 is an important human foodborne pathogen that can cause syndromes such as bloody diarrhea, vomiting, hemolytic uremic syndrome, and in some cases, death (14). E. coli O157:H7 strains commonly carry Shiga toxins (encoded by stx1 and stx2 genes) and factors for attachment to the host mucosa, including intimin (encoded by the eaeA gene) (15). The infectious dose of E. coli O157:H7 can be as low as 10 cells (2). In the United States alone, E. coli O157:H7 cause over 73,000 cases of human infections every year (15). Persons can become infected through the consumption of contaminated food, particularly inadequately cooked ground beef and milk (non-pasteurized or contaminated after pasteurization). Other sources of infection include contaminated unpasteurized apple cider, water (drinking and swimming), vegetables, mayonnaise, cured salami, and direct contact (animal to person or person to person) (2, 3). The number of human infections usually peaks during the summer months (2, 22).

Cattle are considered to be an important asymptomatic reservoir of this pathogen, although the gut colonization and shedding patterns are not well understood. Individual cattle are transiently colonized and shed E. coli O157:H7 in their feces for a short period of time (usually in weeks) (15). The concentration of E. coli O157:H7 in cattle feces ranges from 10^2 to 10^7 CFU/g (2). The prevalence and shedding of E. coli O157:H7 has been reported to be season dependent in the United States and Europe, with increased rates during the summer months (4, 9, 10, 13, 21). Previously, only two studies from Europe (Scotland) indicated increased prevalence and shedding of E. coli O157 in beef cattle feces during winter months (16, 20).

The objectives of this study were (i) to assess the prevalence of E. coli O157:H7 in feces of feedlot cattle in Kansas during summer, fall, and winter months, and (ii) to characterize E. coli O157:H7 isolates by screening for the virulence factors genes (stx1, stx2, and eae) by PCR.

MATERIALS AND METHODS

Between 40 and 50 samples of fresh cattle feces (immediately after defecation) from different animals were collected on a weekly basis from four pens (approximately 200 cattle) on a feedlot in northeastern Kansas from the middle of August 2004 until the end of February 2005 (with exception of December due to technical difficulties). Samples (several grams) were aseptically collected to sterile plastic bags (Whirl-Pak, Nasco International, Fort Atkinson, Wis.) and immediately transported to the laboratory for processing. One gram of feces was enriched in 9 ml of GN broth (Becton Dickinson, Sparks, Md.) supplemented with cefixime (0.05 mg/liter), cefsulodin (10 mg/liter), and vancomycin (8 mg/liter) and incubated at 37°C overnight. After enrichment, 1.0-ml aliquots were processed by immunomagnetic separation technique using Dynabeads anti-E. coli O157 (Dynal Biotech, Oslo, Norway) according to the manufacturer’s instructions. Fifty microliters of immunomagnetic separation-concentrated samples was spread plated onto MacConkey sorbitol agar (Becton Dickinson) with cefixime (25 μg/liter) and tellurite (1.25 mg/liter). Plates were incubated overnight at 37°C, and sorbitol-negative colonies with a morphology characteristic of E. coli O157 were tested for the O157 antigen by the latex agglutination assay (Oxoid, Basingstoke, Hants, UK). One to two colonies per sample positive for the O157 serogroup were subcultured on Trypticase soy agar (Becton Dickinson) and identified by the API Rapid 20E test (bioMerieux, Durham, N.C.). E. coli O157 isolates were tested for the flagellar H7 gene (fliC) by PCR and for the virulence

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TABLE 1. Prevalence of E. coli O157:H7 in cattle feces during the study period

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of samples tested (2004–2005)</th>
<th>No. (%) positive</th>
<th>% prevalence</th>
<th>Mean temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>76</td>
<td>9</td>
<td>11.8</td>
<td>22.1</td>
</tr>
<tr>
<td>September</td>
<td>160</td>
<td>12</td>
<td>7.5</td>
<td>21.7</td>
</tr>
<tr>
<td>October</td>
<td>160</td>
<td>9</td>
<td>5.6</td>
<td>14.4</td>
</tr>
<tr>
<td>November</td>
<td>190</td>
<td>9</td>
<td>4.7</td>
<td>7.1</td>
</tr>
<tr>
<td>January</td>
<td>134</td>
<td>12</td>
<td>8.9</td>
<td>-2.3</td>
</tr>
<tr>
<td>February</td>
<td>171</td>
<td>31</td>
<td>18.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Total</td>
<td>891</td>
<td>82</td>
<td>9.2</td>
<td>NA*</td>
</tr>
</tbody>
</table>

* Based on a daily average (data are recorded hourly by the K-State Research and Extension Weather Data Station).

b NA, not applicable.

genes (stx1, stx2, and eaeA) by multiplex PCR (5, 7). Briefly, individual isolates were streaked onto Trypticase soy agar and incubated overnight at 37°C. Cells (approximately 10^9 cells per ml) from the overnight culture were transferred to 1.0 ml of sterile deionized water in 1.5-ml microcentrifuge tubes, boiled for 10 min to lyse the cells, and centrifuged for 5 min at 4,000 rpm. One microliter of the supernatant was then used as a template for PCR amplification. PCR was carried in a 25-µl volume containing 10 mM PCR buffer, 3 mM MgCl2, 0.2 mM of each dNTP, 2 mM of each primer, and 2 U of Taq polymerase (all Promega, Madison, Wis.). Temperature cycling consisted of an initial 95°C denaturation for 3 min followed by 36 cycles of 95°C for 20 s, 59°C for 40 s, and 72°C for 90 s. The final extension step was 72°C incubation for 5 min. Amplified DNA was visualized by gel electrophoresis using 3% agarose (3:1) (Amresco, Solon, Ohio) with ethidium bromide.

Amplification. PCR was carried in a 25-µl volume containing 10 mM PCR buffer, 3 mM MgCl2, 0.2 mM of each dNTP, 2 mM of each primer, and 2 U of Taq polymerase (all Promega, Madison, Wis.). Temperature cycling consisted of an initial 95°C denaturation for 3 min followed by 36 cycles of 95°C for 20 s, 59°C for 40 s, and 72°C for 90 s. The final extension step was 72°C incubation for 5 min. Amplified DNA was visualized by gel electrophoresis using 3% agarose (3:1) (Amresco, Solon, Ohio) with ethidium bromide. E. coli O157:H7 (ATCC 43894) was used as a positive control; E. coli DH5α was used as the negative control.

The seasonal prevalence of E. coli O157:H7 (combined summer months [August, September], fall [October, November], and winter [January, February]) was transformed with arcsine square root [arcsin(√(% prevalence/100))] to stabilize error variance (8) and analyzed using analysis of variance (ANOVA). Means were compared using the least-squares means (LSMEANS) procedure (P = 0.05) of general linear model (PROC GLM) (19).

RESULTS AND DISCUSSION

Of 891 fecal samples collected over a 5.5-month sampling period, 82 (9.2%) were positive for E. coli O157:H7. E. coli O157:H7 was isolated every month; the highest monthly shedding frequency (18.1%) was detected in February and the second highest peak (11.8%) was recorded in August (Table 1). Overall, no significant differences in prevalence of E. coli O157:H7 were observed among summer (August, September), fall (October, November), and winter (January, February) months (P = 0.1241). All E. coli O157:H7 isolates were positive for stx2 and eaeA genes and therefore potentially pathogenic to people (Table 2). In addition, 14 isolates (12.8%) carried both Shiga toxins (stx1 and stx2) (Table 2).

The mean temporal prevalence of E. coli O157:H7 found in our study (9.2%) is comparable to that reported in other studies using the similar culturing and isolation techniques, including the immunomagnetic separation step (2, 12, 18). All isolates tested in our study carried the stx2 and eaeA genes and 12.8% of the isolates also carried stx1. Although both toxins are important virulence factors, isolates with stx2 are typically more virulent and more likely to cause hemolytic uremic syndrome in people than those with stx1 only (6). Because all isolates in our study carried stx2, they represented potentially highly pathogenic strains.

The majority of human infections caused by E. coli O157:H7 occur in warm summer months (17); however, this may be due to a number of factors other than the actual shedding of this pathogen by cattle. These factors likely include greater outdoor activities of people such as more frequent consumption of ground beef in the form of homemade barbecued hamburgers, other potentially undercooked meat, and unwashed vegetables, as well as more frequent contacts with domestic animals (e.g., petting zoo). In addition, insects, especially houseflies which commonly develop in animal manure and that are attracted to human food, might contribute to the contamination of food and drinks by fecal bacteria during the summer months. It has been shown previously that houseflies and blow flies can carry relatively high concentrations of potentially virulent E. coli O157:H7 (1, 11).

Our study indicates that prevalence and shedding of E. coli O157:H7 in beef cattle is not season dependent; the highest monthly shedding frequency (18.1%) was observed in February when temperatures in Kansas typically drop below freezing point (Table 1). On the other hand, the second highest peak (11.8%) was recorded in August when daylight temperatures in Kansas commonly rise above 30°C. Nevertheless, it is also important to point out that we have used an enrichment technique for E. coli O157:H7 detection and isolation. Therefore, the concentration of this potential pathogen in cattle feces was not assessed. It is possible that high temperatures in the summer months in-

TABLE 2. Virulence characteristics of E. coli O157:H7 isolated from cattle feces during the study period

<table>
<thead>
<tr>
<th>Period of isolation (2004–2005)</th>
<th>No. of isolates tested (no. of samples)</th>
<th>eaeA</th>
<th>stx1 only</th>
<th>stx2 only</th>
<th>stx1 + stx2</th>
<th>fliC</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>16 (9)</td>
<td>16</td>
<td>0</td>
<td>13 (81.3)</td>
<td>3 (18.7)</td>
<td>16</td>
</tr>
<tr>
<td>September</td>
<td>15 (8)</td>
<td>15</td>
<td>0</td>
<td>15 (100)</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>October</td>
<td>8 (7)</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8 (100)</td>
<td>8</td>
</tr>
<tr>
<td>November</td>
<td>14 (8)</td>
<td>14</td>
<td>0</td>
<td>11 (78.6)</td>
<td>3 (21.4)</td>
<td>14</td>
</tr>
<tr>
<td>January</td>
<td>19 (11)</td>
<td>19</td>
<td>0</td>
<td>19 (100)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>February</td>
<td>37 (29)</td>
<td>37</td>
<td>0</td>
<td>37 (100)</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>109 (72)</td>
<td>109</td>
<td>0 (0)</td>
<td>95 (87.2)</td>
<td>14 (12.8)</td>
<td>109</td>
</tr>
</tbody>
</table>
fluence the quantity of E. coli O157:H7 in the manure and consequently affect the potential for contamination of the environment. Evidently, the temporal shedding of E. coli O157:H7 by cattle greatly fluctuates, suggesting that the factors influencing the gut colonization and prevalence of E. coli O157:H7 in feces are complex and require further investigations. Clearly, our limited study shows that the shedding of E. coli O157:H7 can be relatively high during cold winter months and suggests that seasonality does not necessarily play a major role in this process. Therefore, the proper steps in controlling and minimizing the spread of E. coli O157:H7 from cattle to food products and the environment is critical throughout the year regardless of the season.

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REFERENCES