Escherichia coli O157:H7 Prevalence in Fecal Samples of Cattle from a Southeastern Beef Cow-Calf Herd

D. G. RILEY,1* J. T. GRAY,2 G. H. LONERAGAN,3 K. S. BARLING,4 AND C. C. CHASE, JR.1

1U.S. Department of Agriculture, Agricultural Research Service, Subtropical Agricultural Research Station, 22271 Chinsesagut Hill Road, Brooksville, Florida 34601; 2U.S. Department of Agriculture, Agricultural Research Service, Antimicrobial Resistance Research Unit, 950 College Station Road, Athens, Georgia 30605; 3Division of Agriculture, College of Agriculture, Nursing, and Natural Sciences, West Texas A&M University, Canyon, Texas 79016; and 4Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA

MS 03-59: Received 14 February 2003/Accepted 17 May 2003

ABSTRACT

The proportion of fecal samples culture-positive for Escherichia coli O157:H7 was determined for samples collected from 296 beef cows on pasture in a single Florida herd in October, November, and December 2001. The overall proportion of samples that cultured positive was 0.03. The proportion of cows that were culture-positive on at least one occasion was 0.091. No effect of pregnancy status or nutritional regimen on the proportion of culture-positive samples for E. coli O157:H7 was detected. We detected a breed effect on the shedding of E. coli O157, with Romosinuano cows having a lower (P < 0.01) proportion of samples culture-positive than Angus or Brahman cows. This difference might have resulted from the presence of confounding variables; however, it also might represent evidence of breed-to-breed genetic variation in E. coli O157 shedding. Further research is warranted to evaluate breed as a possible risk factor for shedding of this important foodborne pathogen. Further substantiated findings could indicate that breed is a cow-calf-level critical control point of E. coli O157:H7.

Escherichia coli O157:H7 continues to result in substantial human illness in the United States. The Centers for Disease Control and Prevention estimates that each year, approximately 64,000 people are sickened by this bacterium (15). Critical control points in the beef production cycle for this pathogen have largely been elucidated at the harvest level. However, critical control points likely exist at all aspects of the beef production and consumption continuum. Several studies have focused on the feedlot phase of production because it immediately precedes harvest (2, 12). Limited studies of the cow-calf phase include those in the northwest (11) and midwest/Great Plains regions (14, 21) of the United States and in western Canada (8). Beef calves appear to be exposed to E. coli O157:H7 before weaning (8, 14), which suggests that initial exposure occurs early in life, possibly via maternal transmission. Cow status, therefore, could be a subsequent determinant of calf status. No study of the beef cow-calf segment in the southern United States has been conducted; yet, more than one third of the beef produced in the United States is harvested from cattle bred, born, and weaned in the states along the Gulf Coast. An observational study was performed to characterize and longitudinally evaluate E. coli O157 prevalence in a Florida herd.

MATERIALS AND METHODS

Cattie. Cows (females of reproduction age; n = 296) sampled for this study are part of the research herd at the Subtropical Agricultural Research Station in Brooksville, Fla. Three purebreds were represented in the cow herd and included Angus (n = 67), Brahman (n = 109), and Romosinuano (n = 120). (The Romosinuano breed was developed from descendents of Spanish Bos taurus cattle that were isolated in the Sinu Valley region of Colombia from the 15th and 16th centuries. They were first imported into the United States as embryos in the 1990s.) Cows were pastured at two locations (Main Station and Turnley units; hereafter herds 1 and 2, respectively) approximately 10 km apart. During the early months of 2001, cows were randomly reassigned to locations and breeding herds within each location on the basis of age, pregnancy status, and breed. Cows were pastured on bahiagrass (Paspalum notatum Flügge) or a combination of bahiagrass and perennial peanut (Arachis glabrata Benth.) pastures.

Cows ranged in age from 2 to 15 years, and most had calved in January through mid-April. At the time of sampling, most (n = 228) were 5 to 7 months pregnant, as determined by rectal palpation performed by a licensed veterinarian, but some were not pregnant (n = 68). Most (n = 213) had lactated and raised a calf to weaning (September 2001), but some had not (n = 83), either because they did not calve or their calves died.

Young cows (2-year-olds) that had weaned their first calf (n = 48) were pastured as a separate group at the Turnley unit, initially on pearl millet [Pennisetum americanum (L.) Leeke] and later on bahiagrass or perennial peanut pastures (herd 3). These management procedures were designed to help young cows recover from the stress of first lactation and provide additional nutrition. This group consisted solely of Angus and Romosinuano females.

Herds 1 and 2 (n = 248; mature cows plus 2-year-olds that did not wean a calf) were on two separate winter nutritional regimens within each location: (i) heavy blackstrap molasses at 2.0 kg/cow/day beginning at weaning in September, plus, beginning 19 November, ad libitum access to perennial peanut/bahiagrass hay (n = 120); (ii) urea-fortified molasses (16% protein equivalent) at 2.5 kg/cow/day beginning at weaning in September, plus, bahiagrass hay ad libitum beginning 19 November (n = 128).
Molasses was placed in troughs, and hay was fed using large, round bales.

**Sample collection.** Cows were sampled in October, November, and December 2001. Rectal grab samples were obtained using a sterile glove while maintaining aseptic techniques when cows were restrained in a chute. Herd 1 was sampled on 10 October, 7 November, and 5 December. Herds 2 and 3 were sampled on 17 October, 14 November, and 12 December. Samples were shipped overnight to Athens, Ga.

**Selective enrichment and isolation of *E. coli* O157.** Samples were processed as previously described by Gray and others (9, 10) and Smith (23). Feces (10 g) were removed from each sample and transferred to a filter-lined sterile plastic bag (Filter Mixer Bags SFB-410, Spiral Biotech, Norwood, Mass.). Brilliant green bile broth (100 ml; Difco, Sparks, Md.) prewarmed to 37°C was added to each filter stomacher bag containing the fecal sample. Samples were incubated at 37°C for 6 h with shaking (150 rpm). Following enrichment, 1-ml aliquots were removed and added to 20 μl of a solution containing *E. coli* O157 antibody-coated magnetic beads (Dynabeads anti- *E. coli* O157, Dynal, Oslo, Norway). The resulting suspensions were processed by immunomagnetic separation according to the manufacturer’s instructions with some modifications. The suspensions were incubated at room temperature for 30 min with continuous mixing on a Bellco roller drum (model no. 7736-10164; Bellco Glass, Vineland, N.J.) at 6 rpm. Following immunomagnetic separation, the bead-bacteria complexes were washed with phosphate-buffered saline (pH 7.4) containing 0.05% Tween-20 (PBS-Tween) and resuspended in 100 μl of PBS-Tween. The resuspended bead-bacteria complexes (30 μl) were plated onto sorbitol MacConkey (SMAC; Difco) agar, and Rainbow agar O157 (Biolog, Hayward, Calif.) and incubated overnight at 37°C.

**Identification and biochemical confirmation of *E. coli* O157.** Up to 10 isolated colonies from CT-SMAC agar were picked and transferred to additional *E. coli* plate media as follows. Non–sorbitol-fermenting colonies from CT-SMAC agar were tested for the presence of β-glucuronidase and the ability to ferment lactose using EC broth containing 4-methylumbelliferyl-β-glucuronide (MUG) (EC medium with MUG; Difco) and MacConkey broth (Difco), respectively. Lactose-positive isolates and MUG-negative isolates were tested for O157 latex agglutination (RIM *E. coli* O157:H7 latex test; Remel, Lenexa, Kan.). The O157 latex agglutination-positive isolates were then tested for the presence of the H7 antigen using latex agglutination (RIM *E. coli* O157: H7 latex test) according to the manufacturer’s instructions. All isolates that were O157 agglutination-positive were biochemically confirmed as *E. coli* using the API 20E System (bioMérieux Vitek, Hazelwood, Mo.). Up to 10 *E. coli* O157 and H7 latex agglutination-positive isolates were saved per colon fecal sample for further testing.

**Confirmation by PCR.** All biochemically confirmed *E. coli* isolates that were O157 agglutination-positive were tested for the presence of a gene in the *E. coli* O157 antigen gene locus, *rfb*O157:H7, which codes for GDP-erythrose synthetase (*rfb*O157), by PCR analysis using primers previously described (3, 19). Additionally, all biochemically confirmed *E. coli* isolates that were O157 agglutination-positive were tested for the presence of genes encoding for Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), the intimin protein gene (*eaeA*), and the H7 flagellar protein (*fltC*H7) by PCR analysis as previously described (7) with modifications. Multiplex PCR assays were used to test for the presence of the *rfb*O157 and the *fltC*H7 genes and for the presence of the stx1 and the stx2 genes with an annealing temperature of 57°C, whereas the presence of the *eaeA* gene was tested for singly with annealing temperatures of 54°C. An *E. coli* O157:H7 reference strain 933 (7) was used as a positive control, whereas water and a *Salmonella* Typhimurium isolate were used as negative controls. Samples were designated positive when they contained an *E. coli* isolate that was agglutination-positive for O157 and H7 antigens, as well as being positive by PCR for *fltC*H7 and *rfb*O157, stx1, stx2, and *eaeA*.

**Statistical methods.** Effects of interest included herd, nutritional regimen, lactation status (in the summer of 2001), pregnancy status, cow age, and breed of cow. Lactation status, pregnancy status, and nutritional regimen were categorized as two-level effects, i.e., whether or not the cow was lactating until weaning, whether or not the cow was palpated pregnant, and one of the two nutritional regimens. Initial evaluation of cow age data indicated an uneven distribution and some strata with sparse data. Therefore, strata were collapsed into three levels: (i) 2- and 3-year-old cows (*n* = 49); (ii) 4- through 9-year-old cows (*n* = 156); and (iii) 10-year-old cows and older (*n* = 43). These levels are similar to recommended groupings by cow age for the purposes of analyzing cow and calf performance traits (1).

Data were analyzed as binomial response variables using the GENMOD procedure of SAS (22). Univariable analyses of main effects were performed, and those variables with a probability value of 0.25 or less were included in a multivariable model. Multivariable logistic regression analysis (with repeated measures), using generalized estimating equations with a compound symmetry correlation matrix, was used to determine the final model. Generalized estimating equations were used to account for clustering of culture-positive animals within cow age group factor levels. Terms were removed from the multivariable model using a backward selection technique if they had a probability value of >0.15. Estimates of odds ratios and 95% confidence intervals (CI) were generated. Adjusted means were back-transformed to the observed scale and converted to proportions for presentation.

**RESULTS**

The overall proportion of samples that were culture-positive for *E. coli* O157:H7 was 0.03 (27 of 888 samples). The proportion of cows (*n* = 296) that were culture-positive on at least one occasion was 0.091 (*n* = 27). Unadjusted proportions and total numbers of samples evaluated are shown for each level of investigated factors in Table 1. Unadjusted proportions and total numbers of cows that had a sample culture-positive at any time within factor levels are shown in Table 2. There was a single sample that cultured positive for *E. coli* O157:H7 among those of herd 3. Consequently, no analyses were possible for data from herd 3.

Results of statistical analyses of the samples from herds 1 and 2 (*n* = 774) are shown in Table 3. Lactation status, herd, and breed effects were significant (i.e., univariable *P*-value of 0.25 or less) for inclusion in the multivariable model in the model-building process and were subsequently included together in the final model. A lower proportion of positive samples were detected for cows that did not wean a calf than for cows that did wean a calf (*P* = 0.05). The proportion of positive samples detected in herd 2 was great-
TABLE 1. Proportion (count) of samples (n = 888) cultured positive for E. coli O157:H7

<table>
<thead>
<tr>
<th>Factor</th>
<th>Level</th>
<th>Proportion (count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd(^a)</td>
<td>1</td>
<td>0.028 (360)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.042 (384)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.007 (144)</td>
</tr>
<tr>
<td>Nutritional regimen(^b,f)</td>
<td>1</td>
<td>0.039 (360)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.034 (384)</td>
</tr>
<tr>
<td>Lactation status(^c)</td>
<td>0</td>
<td>0.024 (249)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.033 (639)</td>
</tr>
<tr>
<td>Pregnancy status(^d)</td>
<td>Open</td>
<td>0.034 (204)</td>
</tr>
<tr>
<td></td>
<td>Pregnant</td>
<td>0.031 (684)</td>
</tr>
<tr>
<td>Cow age group(^e)</td>
<td>1</td>
<td>0.027 (147)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.045 (468)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.008 (129)</td>
</tr>
<tr>
<td>Breed(^f)</td>
<td>Angus</td>
<td>0.058 (156)</td>
</tr>
<tr>
<td></td>
<td>Brahman</td>
<td>0.034 (327)</td>
</tr>
<tr>
<td></td>
<td>Romosinuano</td>
<td>0.023 (261)</td>
</tr>
</tbody>
</table>

\(^a\) Herds 1 and 2 consisted of 3-year-old and older cows managed at separate locations and weaned on separate days. Herd 3 consisted of 2-year-old Angus and Romosinuano cows that had just completed their first lactation and were managed separately.

\(^b\) Nutritional regimen consisted of two levels: (1) heavy blackstrap molasses at 2.0 kg/cow/day beginning at weaning and perennial peanut/bahiagrass hay ad libitum beginning 19 November; (2) urea-fortified molasses (16% protein equivalent) at 2.5 kg/cow/day beginning at weaning and bahiagrass hay beginning 19 November.

\(^c\) Lactation status in the most recent lactation: 0, did not wean a calf; 1, did wean a calf in 2001.

\(^d\) Pregnancy status was determined by rectal palpation in September.

\(^e\) Cow age group: 1, 2- and 3-year-old cows; 2, 4- through 9-year-old cows; 3, 10-year-old cows and older, limited to herds 1 and 2.

\(^f\) Frequencies for nutritional regimens, cow age group, and breed (n = 744) exclude herd 3 (first-calf heifers managed separately) records (n = 144).

er (P < 0.01) than in herd 1. The proportion of culture-positive samples from Romosinuano cows was less (P < 0.01) than in either Angus or Brahman cows.

DISCUSSION

Results suggest that E. coli O157 prevalence in southern U.S. beef herds is similar to those in other regions of the country. The proportion of animals testing positive for E. coli O157:H7 herein was generally greater than those reported for beef cows in Washington (0.71%; \(^{11}\)) and in Kansas (1.9% \(^{21}\)), but these earlier studies did not use the more sensitive culture methodology used in our study, and as such, the estimates reported may have underestimated prevalence \(^{20}\). Although northern states or regions have greater reported frequency of human disease associated with this pathogen \(^{12,16}\), a study of dairy calves in 28 states (including some southern states) did not detect a regional difference in prevalence \(^{25}\). There could be some differences in the E. coli O157 prevalence of cattle housed in feedlots of the southern and northern Great Plains \(^{26}\), but this is not conclusive \(^{12}\).

Intermittent shedding of E. coli O157:H7 has been in-
E. coli O157:H7 in Southeastern Beef Cows

An effect of lactation was significant in the present study. Lactation (and probably its cessation) is a stressor for beef cows. All cattle were at least 1 month postlactation at the first sampling date, which might have allowed a sufficient time to recover from lactation stresses prior to sampling. Nonlactating dairy cows were shown to have a slightly higher, but not significant, prevalence than lactating cows (11), but the lactation events of dairy and beef operations are markedly different and represent a different kind and extent of stress for the two groups. In the present study, an effect of lactation might have been confounded with pregnancy because many open (and nonlactating) cows were culled as a part of normal management procedures prior to the beginning of this project and consequently were not sampled.

Multivariable regression with generalized estimating equations was used to account for the within–age group effects. An age effect associated with the prevalence of E. coli O157:H7 has been observed, with a higher prevalence in calves than cows (4, 11) and a higher prevalence (at slaughter) in yearlings than cows (27). In the present study, the higher proportion of positive samples among cows in their prime production years (4 to 9 years of age) than in older cows appears to suggest the presence of an age–dependent variation in prevalence. However, results are potentially confounded with pregnancy or lactation status—in this study, older cows that failed to wean a calf or become pregnant were the first cows to be culled. First-calf young cows experience the most stress in any beef production system, but they did not have the greatest E. coli O157 prevalence, which is not consistent with the higher shedding rates of cows <5 years of age compared with older cows (8). This might be due to the management procedures that were implemented to increase weight and allow these animals to recover from lactation.

Breed differences observed might have resulted from a true breed effect on the prevalence of E. coli O157:H7. To our knowledge, breed differences have not been detected previously. The prevalence reported for beef and dairy (at best, a broad generalization of breed) cattle appeared similar in a study performed in Washington (11). Furthermore, prevalence in Japanese Black and Holstein cattle did not appear to differ (18). In each of the previous studies (11, 18), however, breed differences would be heavily confounded with management system. Although not explicitly stated as a likely effect on prevalence of E. coli O157:H7, breed has been mentioned as a possible confounding factor with geographic region (16). Breed might be a critical control point for cow-calf producers. Before any recommendations can be developed, however, further research is required to evaluate the effects of breed on E. coli O157 shedding.

ACKNOWLEDGMENT

This research was supported by the Florida Agricultural Experiment Station and approved for publication as journal series no. R-09311.

REFERENCES

Animal and Dairy Science Complex, University of Georgia, Athens, Ga. [E-mail: rsilcox@uga.edu].


