Fecal Shedding of Foodborne Pathogens by Florida-Born Heifers and Steers in U.S. Beef Production Segments†

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ABSTRACT

The objective in this study was to assess breed effects in fecal prevalence of *Escherichia coli* O157:H7 in heifers on a development program in Florida and in their steer half siblings in stocker and feedlot phases in Oklahoma. A secondary objective was to characterize fecal shedding of *Campylobacter* and *Salmonella* in subsets of the same samples. After weaning, heifers (*n* = 501; purebreds and *F*1 crosses of Angus, Brahman, and Romosinuano) were preconditioned and placed in a local development program. Steers (*n* = 481) were transported to Oklahoma, where they grazed wheat for 6 months and then were placed in feedlot pens. Fecal samples were obtained at least every 28 days for 12 months on most animals. None of the 10,982 samples tested positive for *E. coli* O157:H7. Overall fecal prevalences of *Campylobacter* and *Salmonella* in heifers were 1.7 and 0.04%, respectively. Corresponding overall prevalences in steer samples were 27.2 and 0.6%. *Campylobacter* isolates were mostly *C. jejuni* and were tetracycline resistant. Eight *Salmonella* isolates were *Salmonella* Typhimurium that were either quad or penta resistant, most often to ampicillin, chloramphenicol, sulfamethoxazole, and tetracycline. Feedlot steers had greater odds of positive detection of *Campylobacter* (odds ratio, 8.5; confidence interval, 3.7, 19.5) than when grazing winter wheat. No breed effect was detected for fecal prevalence of these pathogens.

The presence of *Escherichia coli* O157:H7 in cattle and the small dose required for human contamination necessitate the attentiveness of all beef industry segments. Detailed critical control points have been proposed for prevention of contamination of fresh beef in the slaughter process (26); beef cattle studies have often focused on the feedlot phase of the industry (e.g., (3, 18)). The competitive characteristics of the beef industry and the potential negative economic impact of foodborne pathogens result in a tendency for downstream industry segments to hold upstream segments responsible for pathogen control (19). Any national animal identification program for livestock in the United States would facilitate inclusion of other production segments in any end product (or near-end product) problems. Recommendations for preharvest intervention have included consideration of procedures on the farm of birth and/or weaning (1, 15).

Genetic control of production traits has been a major focus of beef cattle research since the early 20th century. The historical and still the predominant exploitation of genetics in beef cattle production has been through the use of breeds. One of the easiest, most effective, and well-understood ways to rapidly change a trait or set of traits in cattle is breed selection. Characterization of broad genetic effects on pathogen shedding has been coincidental and minimal (1, 6). Breed and other genetic effects, such as heterosis, have not been assessed for levels of pathogens (such as *E. coli* O157:H7) that may not adversely affect host health. Based on earlier detection of potential breed effects on fecal shedding of *E. coli* O157:H7 in mature cows (25), the objective of the present study was to assess breed and genetic effects on percentage of fecal samples positive for *E. coli* O157:H7 in typical industry management for the production of replacement females in Florida and in their steer half siblings in stocker and feedlot phases in Oklahoma. A secondary objective was to characterize fecal shedding and potential breed effects on shedding of *Campylobacter* and *Salmonella* in a proportion of the samples from the same animals.

MATERIALS AND METHODS

Cattle. Three cattle breeds comprised the Subtropical Agricultural Research Station herd near Brooksville, Fla, and were used in pure and crossbred matings. These breeds represent diverse origins and biological types, and two distinct adaptations to the tropics (*Bos indicus* and Criollo *Bos taurus*). Calves were purebred Brahman, Angus, and Romosinuano, and all reciprocal crosses.
Calves were born in late winter or spring of 2002, 2003, and 2004 (n = 313, 321, and 348 calves, respectively), and were weaned in September of each year at an average of about 7 months of age. Heifers (n = 501) entered a regimen at the research station in Florida for growth and development into replacement females for the station’s cow herd, using typical regional feed resources and management practices. Winter feeding of heifers began at first frost, approximately on 15 November of each year and continued until the last week of May of each year (approximately 200 days) and consisted of 2.2 kg of blackstrap molasses plus 2.2 kg of soybean hulls per heifer per day. Heifers were also fed bahiagrass (Paspalum notatum Flügge) and/or rhizoma perennial peanut (Arachis glabrata Benth.) hay at a rate of about 2% of average body weight (approximately 6.4 kg per day) throughout this period. Summer pastures were bahiagrass and/or a mixture of bahiagrass and rhizoma perennial peanut, and were grazed from approximately the last week of May through September.

Approximately 1 month after weaning, steer calves (n = 481) were shipped by commercial truck to the U.S. Department of Agriculture, Agricultural Research Service (USDA, ARS), Grazinglands Research Laboratory at El Reno, Okla. After a short receiving period, calves were placed on pastures where they grazed winter wheat (Triticum aestivum L.) until the first week of May in each year. At that time, steers were placed in feedlot pens (two sizes of pens in which each calf was allotted either 2.6 or 5 m² of surface area). The feedlot diet contained 12.3% crude protein, 2.06 Mcal of net energy for maintenance and 1.34 Mcal of net energy for growth per kg of dry matter. Calves were fed for 100, 128, or 155 days before slaughter. The entire preharvest production continuum for steers in this project was designed to be representative of that for typical southeastern U.S. beef production.

Sampling procedures. Fecal samples were obtained every 28 days from cattle in both Florida and Oklahoma, beginning at weaning each year and continuing through September of subsequent years, with the exception of the third project year, when sampling was terminated in March. Additional samples were collected from steers immediately prior to shipment, 72 h after arrival, and 2 weeks after arrival in Oklahoma. Rectal grab samples were collected using aseptic procedures from cattle as they were restrained in a chute. Integrity of samples was maintained by using separate sleeves or gloves for each animal and by storing in individually labeled plastic cups or plastic bags. Samples and cold packs were placed in insulated boxes and sent by overnight carrier to the USDA, ARS, Bacterial Epidemiology and Antimicrobial Resistance Research Unit in Athens, Ga.

Beginning in January 2004, on arrival of samples at the Athens laboratory, approximately a quarter of the monthly samples were randomly selected (in order to have equal representation of breed groups and sires within breed groups of the sampled animals) for assay of Salmonella and Campylobacter. This was continued monthly through March of 2005 for assays of both pathogens in heifer samples, and for assay of Salmonella in steer samples; this therefore encompassed animals from both birth years, but not equally across all feeding phases. Steer samples assayed for Campylobacter were from those taken monthly from 2003-born steers in January through March, and July through September of 2004. Samples from 2004-born steers on arrival in Oklahoma in October 2004, were also assayed for Campylobacter.

Bacterial isolation. Samples were assayed for E. coli O157:H7 based on immunomagnetic separation procedures of Gray et al. (14), as detailed previously (25). The bacteriologic culture methods for Salmonella were those described previously (28). Each Salmonella isolate was tested for susceptibility to a panel of 17 antimicrobial drugs (amikacin, amoxicillin-clavulanic acid, ampicillin, apramycin, cefoxitin, cefotiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, naladixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole), using a semiautomated testing system (Sensititre, TREK Diagnostics, Westlake, Ohio) in a broth microdilution format. The MIC for each isolate was determined. Where possible, each isolate was classified as susceptible or resistant according to standards of the Clinical and Laboratory Standards Institute (formerly NCCLS (21, 22)). Otherwise, the breakpoint interpretations as described for the National Antimicrobial Resistance Monitoring System were used (27). Campylobacter isolation from fecal samples was according to those procedures described previously (11, 12). Isolates were tested for antimicrobial susceptibility (amoxicillin-clavulanic acid, azithromycin, cefepime, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, imipenem, nalidixic acid, tetracycline, trimethoprimsulfamethoxazole), using the E-test (AB-Biodisk, Piscataway, N.J.) according to the manufacturer’s directions.

Statistical analyses. The effect of interest was breed group. These consisted of purebred Angus, Brahman, and Romosinuano, and first crosses (F₁) of all three combinations (Brahman-Angus, Brahman-Romosinuano, and Angus-Romosinuano). Data were analyzed separately by animal gender. Heifer data classifications included two levels of feeding phase corresponding to winter (October through May of each year, a time of minimal grass growth in central Florida; heifers were fed as described) and summer (June through September, a time of substantial grass growth and no feeding of heifers). Steer feeding-phase classifications included samples taken immediately on arrival of steers in Oklahoma, samples from steers in the stocker phase (November through April), and samples from steers in the feedlot (May through slaughter dates in August and September). Although they were largely confounded with feeding phase for each sex, months of year were investigated in both heifer and steer data sets. Birth year was investigated as a fixed effect; animals from a given birth year were sampled from October of their birth year through September of the following year. Data were analyzed as binomial response variables (values of 1 and 0 assigned to samples with positive detection of pathogens and no detection, respectively, in separate analyses for each pathogen), when possible using the GENMOD procedures of SAS (SAS Institute, Inc., Cary, N.C.). Main effects from univariable analyses with a probability value of 0.25 or less were investigated in a multivariable model. Terms were removed from multivariable models, using a backward-selection technique if they had a probability value of >0.15. When possible, estimates of odds ratios and 95% confidence intervals were generated. Additionally, class numbers were tested against χ² expectation, using the FREQ procedures of SAS.

RESULTS

Numbers of samples, numbers of animals sampled, and numbers and percentages of positive samples for each pathogen are presented in Table 1. E. coli O157:H7 was not recovered from any sample (n = 10,982) from the 982 animals in this study. Campylobacter was detected in 7 of 412 samples from 2003-born heifers (n = 111) and 4 of 246 samples from 2004-born heifers (n = 57); however, neither birth year nor any other effect met the requirements for inclusion in the logistic regression model for Campylobacter in heifer fecal samples. Two of the Campylobacter isolates were C. coli
TABLE 1. Incidence of pathogens in fecal samples from Florida heifers on pasture and from Florida steers through the stocker and feeder phases

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Heifers</th>
<th>Heifer samples</th>
<th>Steers</th>
<th>Steer samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli O157:H7</td>
<td>501</td>
<td>5,294</td>
<td>481</td>
<td>5,688</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>168</td>
<td>658</td>
<td>171</td>
<td>206</td>
</tr>
<tr>
<td>C. coli</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>9</td>
<td>9</td>
<td>44</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>11</td>
<td>45</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>(6.6)</td>
<td>(1.7)</td>
<td>(26.3)</td>
<td>(27.2)</td>
</tr>
<tr>
<td>Salmonella Newport</td>
<td>331</td>
<td>2,753</td>
<td>339</td>
<td>1,782</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV40:g, Z51</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(0.3)</td>
<td>(0.04)</td>
<td>(3.0)</td>
<td>(0.6)</td>
</tr>
</tbody>
</table>

*a Heifers and steers were born and raised to weaning in central Florida. Heifers remained on local pastures and steers were transported to north-central Oklahoma.

were susceptible in all resistance tests. A single sample of the 2,753 fecal samples from heifers (n = 331) tested positive for Salmonella Newport serogroup C1, and this isolate was susceptible to all evaluated antimicrobials.

DISCUSSION

The failure to detect even one positive sample for E. coli O157:H7 was unexpected after previously detecting it in fecal samples from mature cows at this location in 2001 (25). It is possible—due to the sporadic nature of this organism—that animals in the current study did shed the pathogen in between the 28-day sampling periods and were not detected, either through intermittent shedding or lack of culture sensitivity (15). Although the mean duration of shedding has been reported to be approximately 1 month, the minimum duration of shedding may be as short as a week (7), and even relatively aggressive sampling may be inadequate to detect sporadic shedders. Additionally, since this study, culture techniques have been refined to provide a greater level of sensitivity (4, 23). Pasturing heifers may have contributed to nondetection of this pathogen (1); however, others have associated forage-based diets with increased shedding (16). Still others have reported no association of diet with shedding (6). No heifers from outside of the research station were added to the evaluated group of heifers throughout this project. It is possible that introduction of bacteria may have been arrested by keeping the herd closed.

Southeastern U.S. steers that are shipped to the southern Great Plains undergo an extreme environmental and nutritive change. The stress of changing from forage to high-concentrate feeding has been associated with higher prevalence (26). Transportation and the associated limited access to feed and water are preharvest stressors that may increase shedding (1); however, other work appeared to show that the influence of transportation on prevalence was minimal and indicated that incoming cattle were apparently the source of environmental contamination with regard to this pathogen (20). Prevalence in stocker calves originating in the Great Plains was 2.1% (24), and feedlot prevalence has been as high as 20% and appears to increase with time on feed (17, 18). Confinement of cattle to barns or small pens within barns may be associated with increased shedding rates (13). All of the animals in the present study were preconditioned, which may have a beneficial effect on subsequent prevalence (1, 10). Commingling cattle from different sources has been reported to be associated with higher prevalence of E. coli O157:H7 (10); there was no commingling of steers in the present study with steers from other locations. Our results may diminish the importance of the farm of origin on beef safety events at or immediately prior to consumer level, consistent with recent feedlot results in North Dakota (17). Others have reported very low E. coli O157:H7 prevalence in hot, tropical environments (8); some aspect of such environments may be inhibitory on shedding or detection of shedding.

Results for Campylobacter prevalence in steers were consistent with high prevalence previously reported in feedlot cattle (2) and much higher than their heifer siblings on
pasture, which appears to be consistent with reports of high feedlot prevalence relative to cattle in other phases of production (2, 5). The different percentages of positive samples in steers immediately after transportation and later in the stocker phase were not consistent with the reported lack of fecal prevalence differences for steers before and after transportation (5), but that study did report a reduction in hide prevalence after transportation.

The percentages of fecal samples positive for Salmonella in this study were lower than those reported (2 to 20%) for U.S. feedlot or slaughter phases of beef production (3, 5) and in on-farm prevalence in dairy production (9).

Breed selection as a method of pathogen control is an attractive notion. A Canadian study (1) reported a breed effect (Charolais greater than Angus) on total (log) CFU per gram of feces for E. coli O157:H7 in one treatment of the study. However, researchers in Nebraska did not detect a breed effect on E. coli O157:H7 fecal shedding (6). Results from the present study failed to support any breed effect on fecal prevalence of any of the studied bacteria.

REFERENCES


