Prevalence of *Escherichia coli* O157 in Cattle Feeds in Midwestern Feedlots

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Comparisons of enrichment methods (with or without antibiotics and with or without a preenrichment step) using gram-negative (GN) broth or tryptic soy broth (TSB) were conducted with feeds inoculated with *Escherichia coli* O157:H7. TSB was more sensitive than GN broth, and TSB with preenrichment and isolation methods need to be designed specifically for the feed environment. The purposes of this study were (i) to develop more-sensitive isolation techniques with feed samples inoculated with *E. coli* O157 and (ii) to assess the prevalence of *E. coli* O157 in cattle feeds from midwestern feedlots.

**MATERIALS AND METHODS**

Comparison of GN broth and TSB enrichment for detection of *E. coli* O157 in inoculated feeds. (i) Feces and feed inoculation. FRIK 2000, a strain of *E. coli* O157:H7 adapted to nalidixic acid (20 μg/ml; Nař), was used for experimental inoculations. The organism was grown overnight in gram-negative (GN) broth (BD, Franklin Lakes, N.J.) at 37°C and diluted with phosphate-buffered saline to obtain various concentrations of *E. coli* O157:H7. Colony counts of the dilutions were done by plating onto sorbitol MacConkey (SMAC) agar plates supplemented with nalidixic acid (20 μg/ml). Feeces were collected per rectum from healthy, lactating dairy cows from a local dairy. Samples of a total mixed ration composed of corn silage, cracked corn, chopped brome hay, cottonseed, soybean meal, and a vitamin and mineral supplement were collected from feed bins at the same dairy. Fresh feces were inoculated with 10⁸, 10⁹, and 10⁴ CFU of Nař *E. coli* O157/g and stored over...
night at room temperature (20°C). For each inoculation level, 5 g of inoculated feces was mixed with 15 ml of phosphate-buffered saline and vortexed to a uniform mixture. The suspension was added to 500 g of feed and thoroughly mixed. This resulted in feed inoculation concentrations of 102, 103, and 104 CFU/g, respectively. Mixed feeds were stored for 60 to 72 h at room temperature (20°C) before culturing by enrichment broths.

(ii) Experiment 1: GN broth enrichments. Ten-gram samples of inoculated feeds (105, 106, or 107 CFU/g) were placed in sterile plastic bags and mixed with 90 ml of GN broth with no antibiotics added; 90 ml of GN broth supplemented with cefixime (0.05 mg/ml), cefsulodin (10.0 mg/ml), and vancomycin (8.0 mg/ml) (GNccv); or 10 ml of GN broth with no antibiotics added that was held at room temperature (20°C) for 2 h as a preenrichment step, followed by the addition of 80 ml of GNccv broth (GN/GNccv). There were 10 replicates of feed samples at each inoculation level, for a total of 30 replicates for each enrichment method (GN broth only, GNccv, or GN/GNccv).

(iii) Experiment 2: comparison of GN versus TSB. Ten-gram samples of inoculated feeds (105 or 106 CFU/g) were placed in sterile plastic bags and mixed with 90 ml of TSB with no antibiotics added, 90 ml of TSB supplemented with cefixime (0.05 mg/ml) and vancomycin (8.0 mg/ml) (TSBccv), or 10 ml of TSB with no antibiotics added that was held at room temperature (20°C) for 2 h as a preenrichment step, followed by the addition of 80 ml of TSBccv broth (TSB/TSBccv). There were 10 replicates of feed samples at each inoculation level, with a total of 45 replicates for each enrichment method (TSB only, TSBccv, or TSB/TSBccv).

All broths were incubated for 6 h at 37°C, followed by immunomagnetic separation (IMS) (Dynal; Biotech Inc., Lake Success, N.Y.) and spread plated onto SMAC agar supplemented with cefixime (50 mg/ml) and tellurite (2.5 μg/ml) (CT-SMAC). Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies with characteristic E. coli O157 morphology were streaked onto CT-SMAC. Plates were incubated overnight at 37°C followed by IMS and spread plated onto CT-SMAC. Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies with characteristic E. coli O157 morphology were streaked onto SMAC agar plates supplemented with nalidixic acid (20 μg/ml). Nalidixic acid-resistant isolates were tested for O157 antigen by a latex agglutination assay (Oxoid Limited, Basingstoke, Hampshire, England).

Prevalence of E. coli O157 in feedlot feed samples. (i) Feed sample collection. Feed samples were collected as a part of a concurrent E. coli O157 prevalence study in cattle feedlots in Nebraska, Kansas, Oklahoma, and Texas during June, July, and August 2001 (J. M. Sargeant, D. D. Griffin, R. A. Smith, M. W. Sanderson, and X. Shi, Abstr. 82nd Conf. Res. Workers Anim. Dis., abstr. 94, 2001). Feed samples were collected from two to four feedlots each week. At each feedlot, approximately 1.0-kg samples of total mixed feed, consisting of 10% corn, corn silage or hay, and a protein supplement such as soybean meal or urea. Mixed feeds were stored for 60 to 72 h at room temperature (20°C) followed by IMS and spread plated onto CT-SMAC. Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies with characteristic E. coli O157 morphology were streaked onto blood agar plates with sterile toothpicks and incubated overnight at 37°C. Growth on blood agar plates was tested for indole production. Indole-positive colonies were tested for nalidixic acid resistance by determining their growth on SMAC agar plates supplemented with nalidixic acid (20 μg/ml). Nalidixic acid-resistant isolates were tested for the O157 antigen by a latex agglutination assay (Oxoid Limited, Basingstoke, Hampshire, England).

(ii) Experiment 1: GN broth enrichments. Ten-gram samples of inoculated feeds (105, 106, or 107 CFU/g) were placed in sterile plastic bags and mixed with 90 ml of TSB with no antibiotics added, 90 ml of TSB supplemented with cefixime (0.05 mg/ml) and vancomycin (8.0 mg/ml) (TSBccv), or 10 ml of TSB with no antibiotics added that was held at room temperature (20°C) for 2 h as a preenrichment step, followed by the addition of 80 ml of TSBccv broth (TSB/TSBccv). There were 10 replicates of feed samples at each inoculation level, with a total of 45 replicates for each enrichment method (TSB only, TSBccv, or TSB/TSBccv).

(iv) Standard coliform counts. Standard coliform counts were performed on each feed sample collected from feedlots. Ten grams of feed was placed in a sterile plastic bag containing 90 ml of distilled water. The sample was mixed for 1 min and serially diluted (10−1, 10−2, 10−3, 10−4, and 10−5), and 100 μl from each dilution was spread plated onto MacConkey agar and incubated at 37°C for 18 h.

Statistical analysis. Analyses were performed with a commercially available software package (Stata statistical software, release 7.0; StataCorp). Experimental isolation methods were compared by the exact McNemar’s chi-square test, controlling for multiple-comparison error rate by the Bonferroni method. The agreement of isolation methods used for prevalence data was determined by using the kappa test of agreement.

RESULTS

Comparison of GN broth and TSB enrichment for detection of E. coli O157 in inoculated feeds. There were no significant differences (P > 0.05) in the sensitivity of detection of E. coli O157 in inoculated feeds among GN broth, GNccv, and GN/GNccv enrichment methods (Table 1, experiment 1). The 105 concentration in experiment 1 was excluded in experiments 2 and 3, as it allowed overgrowth of E. coli O157. Comparison of the two enrichment broths indicated that TSB with no antibiotics added was more sensitive for detection (P < 0.05) than GN broth with no antibiotics (Table 1). In experiment 3, the TSB/TSBccv enrichment method was more sensitive for detection (P < 0.05) than the TSB enrichment method. This was the only significant difference between the three TSB methods (Table 1).

Prevalence of E. coli O157 in feedlot feed samples. The numbers of feedlots sampled in Nebraska, Kansas, Oklahoma, and Texas were 23, 12, 12, and 7, respectively. The average numbers of animals in each pen, square feet of pen space per animal, and available feed bunk length were 124.8, 313 ft2, and

| Table 1. Comparison of enrichment methods with GN broth or TSB to detect E. coli O157 in inoculated cattle feeds |
|-----------------|-----------------|---------------|-----------------|
| Expt            | Enrichment procedure | Total no. of samples | No. of positive samples | Sensitivity* (%) |
|-----------------|-----------------|---------------|-----------------|-----------------|
| 1               | GN broth with no antibiotics added | 30 | 21 | 70.0* |
|                 | GNccv          | 30 | 26 | 86.7* |
|                 | GN/GNccv       | 30 | 24 | 80.0* |
| 2               | GN broth with no antibiotics added | 40 | 10 | 25.0* |
|                 | TSB with no antibiotics added | 40 | 20 | 50.0† |
| 3               | TSB with no antibiotics added | 45 | 16 | 35.6* |
|                 | TSBccv         | 5 | 20 | 44.4*† |
|                 | TSB/TSBccv     | 45 | 24 | 53.3† |

* Sensitivity values within each experiment with different symbols are significantly different (P < 0.05).
† Ceftaxime, cefsulodin, and vancomycin were included in the medium at 0.05, 10.0, and 8.0 mg/liter, respectively.
| E. coli O157 prevalence study in cattle feedlots in Nebraska, Kansas, Oklahoma, and Texas during June, July, and August 2001 (J. M. Sargeant, D. D. Griffin, R. A. Smith, M. W. Sanderson, and X. Shi, Abstr. 82nd Conf. Res. Workers Anim. Dis., abstr. 94, 2001). Feed samples were collected from two to four feedlots each week. At each feedlot, approximately 1.0-kg samples of total mixed feed, consisting of 10% corn, corn silage or hay, and a protein supplement such as soybean meal or urea. Feed samples were shipped overnight to the Food Animal Health and Management Center laboratory at Kansas State University. A total of 504 feed bunk samples from 54 feedlots were sampled. Data on the number of animals in each pen, square feet of pen space per animal, and available feed bunk length were collected on the day of visit. (ii) Culture methods. Based on the results obtained with inoculated feeds, the most sensitive method (TSB/TSBccv) was selected and applied in parallel with a standard method (GN broth) to feed samples. Ten grams of feed samples, collected from feedlots, was placed in a sterile plastic bag containing 90 ml of GN broth with no antibiotics added or 10 ml of TSB with no antibiotics added and held at room temperature (20°C) for 2 h as a preenrichment step, followed by the addition of 80 ml of TSBccv broth (TSB/TSBccv). There were 10 replicates of feed samples at each inoculation level, for a total of 30 replicates for each enrichment method (GN broth only, GNccv, or GN/GNccv).
TABLE 2. Comparison of generic coliform concentrations and prevalences of E. coli O157 in cattle feeds

| Generic coliform conc (CFU/g) | Total no. of feed samples | No. of positive samples | % Positive |
|-------------------------------|--------------------------|-------------------------|------------|
| 10^1                          | 131                      | 25                      | 19.1       |
| 10^2                          | 76                       | 17                      | 22.4       |
| 10^3                          | 117                      | 16                      | 13.7       |
| 10^4                          | 117                      | 9                       | 7.7        |
| 10^5                          | 53                       | 6                       | 11.3       |
| 10^6                          | 8                        | 1                       | 12.5       |
| 10^7                          | 2                        | 1                       | 50.0       |

136.3 ft, respectively. Animals in all feedlots sampled were provided feed ad lib.

E. coli O157 was detected by one or both culture methods in 75 of 504 (14.9%) of feed bunk samples from 54 feedlots. At least one positive feed bunk sample was found in 44.4%, or 24 of 54, of the feedlots. There was no significant difference in the prevalence of E. coli O157 detected by the two methods (P > 0.05), but there was only moderate agreement between the two, with a kappa statistic of 0.41. The GN broth enrichment method detected E. coli O157 in 52 feed bunk samples (10.3%), and the TSB/TSBcv method detected E. coli O157 in 46 feed bunk samples (9.1%). There were 29 pens for which only the GN broth method detected E. coli O157. There were 23 pens for which only the TSB/TSBcv method detected E. coli O157. Both methods detected E. coli O157 in 23 out of 75 (30.7%) positive samples.

PCR analysis revealed that 75 out of 85 (88.2%) latex agglutination-positive isolates possessed the eaeA gene. Of the 75 eaeA-positive isolates, 65 isolates (86.7%) possessed both stx1 and stx2 genes, 8 isolates (10.7%) possessed only the stx1 gene, and 2 isolates (2.6%) possessed only the stx2 gene.

Generic coliform counts in feed samples ranged from 0 to 1.2 × 10^7 CFU/g, with a mean count of 1.6 × 10^6 CFU/g and a median count of 5.9 × 10^5 CFU/g. There was no correlation between E. coli O157 prevalence and generic coliform counts in feed samples (P > 0.05; Table 2).

DISCUSSION

Previous studies have addressed the growth and survivability of E. coli O157 in cattle feeds (7, 25). Detection methods with various media have generally balanced selectivity with enrichment of E. coli O157 (4). E. coli O157 organisms can be injured or stressed and can enter a viable-but-nonculturable state in certain environments (4, 26). Lynn et al. (25) found that moistening feeds with sterile, distilled water increased recovery of generic E. coli from cattle feeds. Additional studies have found that use of a nonselective preenrichment step increased the recovery of potentially stressed E. coli O157 (4, 16, 38). Isolation of E. coli O157 in water was also improved by using a nonselective preenrichment step (24). In our experiments, we chose to vary the selectivity of the enrichment broths while maintaining the rest of the isolation procedures (IMS, CT-SMAC, indole, latex agglutination, API, PCR) constant. We compared TSB and GN broth because they have been previously used to detect E. coli O157 in feces, feed, and water (17, 24, 25). GN broth is a selective medium that supports coliform growth while inhibiting the growth of most competitive flora; TSB is not a selective medium. After determining that TSB was significantly more sensitive in detecting E. coli O157 in inoculated feeds than GN broth, we compared three enrichment procedures with TSB. We found that the TSB/TSBcv enrichment method was more sensitive than the TSB enrichment method in inoculated feeds. Because this was the only significant difference among the three TSB methods, we chose to use TSB/TSBcv in our feed prevalence study.

This study indicates that the prevalence of E. coli O157 (14.9%) in cattle feed may be significantly higher than previously reported (11, 14, 25, 36). The prevalence may reflect the use of more-sensitive detection methods (29, 34, 41). This study provides evidence that feed may be a vehicle for dissemination and colonization, yet the source of E. coli O157 contamination in cattle feed remains unidentified. The cattle in the pens that we sampled had access to the feed bunks prior to sampling; therefore, it is plausible that the feed could have been contaminated by E. coli O157 from cattle saliva (17). Another obvious source is shedding cattle that defecate in the feed. Other possible sources include fecal contamination by other livestock species (19) or by wildlife (8, 31), including birds (14, 39, 40), rodents (14), and insects (14, 29). Another possible source is contaminated feed components that are mixed into the feed (11).

If E. coli O157 fecal shedding is clustered temporally (1, 11, 13), we would expect that our collection of samples at a single point in time would underestimate the feed bunk prevalence (12, 25, 35), assuming that there is a direct relationship between these two factors. We sampled during the summer, which could increase our chance of detecting E. coli O157 if there is a seasonal effect or unknown risk factors associated with season (27). Lack of correlation between feed generic coliform counts and the feed bunk prevalence of E. coli O157 was surprising. If coliform counts are a measure of fecal contamination, feed E. coli O157 prevalence should generally parallel coliform counts (25); alternately, a high number of coliforms could competitively inhibit E. coli O157 recovery. We found no association between feed coliform count and E. coli O157 presence. Ten feed bunk samples that were positive for E. coli O157 had coliform counts that were less than 10. This suggests that fecal contamination may not be the source of E. coli O157 in the feed in all cases.

The lack of agreement between the two methods that we used to detect prevalence suggests that there were interactions that allowed one method to detect when the other didn’t within the same sample. This lack of agreement could be due to sampling variance from within each composite feed sample, or the enrichment methods we used could have interacted with the feed additives or components present in the feed, environmental conditions at the feed bunk, or other factors unknown to us at this time. Additionally, while the TSB/TSBcv method was the most sensitive in the inoculation study, it did not detect more positives in the field samples. The reasons for this are not clear but may include feed interactions with the method as well. The inoculation study used a total mixed ration for lactating cows from a local dairy that was different from the typical feedlot ration and that did not include ionophores. It is plausible that the TSB/TSBcv method may have been more sensitive for this ration but not for general feedlot rations. Clarification of these possible interactions will require more
investigations of isolation methods with various feed types and additives. A majority (88.2%) of our isolates possessed a portion of eaeA, which has been identified as highly specific for E. coli O157:H7 (28), and at least one stx gene. The lack of virulence genes in some latex agglutination-positive isolates indicates that the prevalence of E. coli O157 could be overreported if PCR was not used. The presence of both Shiga toxin genes in a majority (86.7%) of the confirmed isolates was similar to findings from other studies of E. coli O157 isolates from cattle (2, 22, 32).

Further studies are needed to determine sources and associated risk factors for feed contamination before we can establish appropriate safety programs for cattle feed. Possible risk factors for feed contamination are numerous, including components of the feed (5, 6, 20, 29), E. coli O157 shedding in calves before they enter the feedlot (9, 21), and manure handling practices (12). To assess this potential risk of colonization in cattle, we need to know the role of feed in the colonization of cattle and persistence of E. coli O157 within the feedlot environment. The use of typing methods and geographical mapping of specific E. coli O157 isolates (2, 10, 28, 32) will also aid in determining feed contamination sources and links. Further investigations of E. coli O157 detection methods and feed-related factors are needed so that we can learn more of the on-farm ecology and develop strategies to prevent food-borne diseases in humans.

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