Evaluation of manila ropes for the on farm food safety monitoring of Escherichia coli O157:H7 in feedlot cattle.

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Introduction

Past attempts at identifying cattle shedding E. coli O157:H7 have focused on collection of fecal samples or rectal swabs from individual animals or from existing fecal pats on the pen floor. This requires monitoring staff to enter the pen, a practice that may disturb the cattle and be unacceptable to feedlot operators. The observation that E. coli O157:H7 is also harbored within the oral cavity of cattle (Keen and Elder 2002) raises the possibility that culturing of E. coli O157:H7 from objects that have been licked or chewed could serve as a method of estimating the prevalence of E. coli O157:H7 in pens of feedlot cattle. As beef cattle are managed in groups/pens and rarely handled as individuals, this study was conducted to compare the utility of a number of methods for monitoring pens of cattle experimentally challenged with E. coli O157:H7 or naturally colonized in commercial feedlots. Additionally, it was our intent to identify some characteristics of cattle grouped in pens, which might further clarify the ecology of E. coli O157:H7 within the feedlot environment and to identify any short coming that might be associated with using the rope technique as an on farm food safety monitoring method.

MATERIALS AND METHODS

Challenge study: Animals and inoculation. Thirty Hereford x Angus yearling steers were housed in 4 outdoor pens. Animals were fed once daily a barley grain (80%)- barley silage diet. Steers were fecal sampled for twelve weeks prior to inoculation to confirm the absence of nalidixic acid (nal) resistant strains of E. coli O157:H7. Steers were inoculated with a four-strain mixture (1010 CFU) of nal-resistant E. coli O157:H7. During the 12-week experimental period, samples were collected on the day of inoculation, and weekly thereafter. Samples (feces; 10 g) were collected rectally and saliva was obtained using swabs. Samples of three fecal pats (20 g) were collected in each pen and pooled. Feed samples (20 g) were collected from 5 locations in the feed bunk of each pen and pooled. A water sample (100 mL) was collected from each pen and swabs were taken from water trough surface. Manila ropes (120 cm, n=2) were tied above the feed bunk of each pen and cattle were allowed oral access for a period of 4 h.

Commercial feedlots: Collection of samples. Over a 1-y period, rope samples and pooled fecal pats were collected monthly from 4 commercial feedlots in southern and central Alberta from 1,160 pens containing a total of 202,878 cattle. For each pen, fecal pats (one for every 20 animals in the pen) and one rope was hung adjacent to the feeding area of each pen for a 4 h period.

Isolation and enumeration of *E. coli* O157:H7. For enumeration of *E. coli* O157:H7 from feces after inoculation, serial dilutions (1:10) of 1.0 g of fecal sample were prepared in PBS. When *E. coli* O157:H7 could no longer be detected by dilution plating, 10 g samples were enriched in 90 mL mTSB for 6 h at 37°C and immunomagnetic separation (IMS). IMS was used for detection of *E. coli* O157:H7 on ropes in water and feed and for all samples from commercial feedlots. Three sorbitol-negative colonies from each plate were tested for the presence of the O157 antigen using the *E. coli* O157 latex kit and the presence of *vt*, *eaeA*, and *flicC (H7)* genes using multiplex PCR assays for final confirmation of *E. coli* O157:H7.

RESULTS AND DISCUSSION

Challenge study: For 2 wk after inoculation, a higher proportion of rectal-derived feces (100%) were positive for E. coli O157:H7 as compared to fecal pats (60%), but the frequency of detection did not differ between these sources after wk 2 (Figure 1). Sampling of fecal pats that were excreted prior to inoculation is likely responsible for lower isolation of E. coli O157:H7 from fecal pats during the first two weeks of the study. Compared to feces and fecal pats, isolation of E. coli O157:H7 from ropes or oral swabs was more sporadic, with higher proportions of positive isolates collected for ropes than

oral swabs in wks 3, 4, 6, and 12. In the first 4 h after placement of a single rope in feedlot pens averaging 196 head, 18.3% of animals chewed the rope, but access rate was influenced by climate and cattle age (Stanford et al. unpublished data). Apparently, even though all animals in the pen do not chew the rope, the greater salivation on the rope may be more likely to detect a pen as being positive for E. coli O157:H7 than mouth swabs from individuals.

In wks 2, 3, 4, and 7, the proportion of pens positive for E. coli O157:H7 was higher for feces than oral swabs, whereas the frequency of detecting isolates on ropes did not differ from feces. After wk 7, the proportion of positive isolates was equal in oral swabs and feces (wks 8, 9, 10, 12) with a greater proportion of positive isolates in oral swabs than feces in wk 11 (Figure 1). In contrast, Keen and Elder (2002) found a higher proportion of positive oral isolates (74.8%) as compared to fecal isolates (60.4%), in naturally-infected feedlot cattle. Re-infection through an oral route during wk 8 may have occurred based on the increase in proportion of positive rope and oral isolates after this time, contrary to the expected decline that is frequently observed in challenge studies (Brown et al. 1997; Cray and Moon 1995). Compared to wk 7, positive isolates increased for rope in wks 10 and 12 and for oral swabs in wks 9 and 11. Immediately prior to the increase in positive ropes and oral swab isolates, the number of cfu/g of E. coli O157:H7 isolated from feces increased in wk 7 as compared to wk 6, the only time during the challenge study when the number of cfu/g feces of E. coli O157:H7 increased over time (data not shown). During the latter weeks of the challenge study, oral monitoring methods (i.e., rope, oral swabs) more readily detected E. coli O157:H7 than did fecal monitoring (i.e., feces or fecal pats). Based on these results and those of Keen and Elder (2002), when the primary source of the organism is the environment, oral monitoring methods may be more effective than fecal samples.

With the exception of 1 water sample on week 11, the organism was not isolated in samples of feed, water or water-bowl interface collected from 5 to 12 wk post inoculation, despite the large number of CFU shed into the environment during this time. Although trough water and water-bowl interface have been proposed as a primary mechanism behind the spread of E. coli O157:H7 in the farm environment (Hancock et al. 1998; Van Donkersgoed et al. 2001), this was not the case in our study. Chlorination of water in the present study also may have controlled the proliferation of E. coli O157:H7 in water, but others have reported that chlorination does not control E. coli O157:H7 (LeJeune et al. (2004). As 20% or more of the fecal pats were positive for E. coli O157:H7 prior to wk 10 and given that cattle routinely lick the pen floor, it seems plausible that oral ingestion of feces is likely the primary route of transmission of E. coli O157:H7. Comparison of the effectiveness of collecting samples of feces and oral swabs from one animal per pen with collecting one fecal pat or placing one rope per pen for detecting pen infection status, the probability of finding a single rope sample positive was higher than that of all other sampling techniques.

Based on the results of our challenge study and from our commercial feedlot study (results to appear shortly in the Journal of Food Protection), ropes show promise as a tool for the on farm monitoring of infection status for E. coli O157:H7 in pens of feedlot cattle, provided animals are acclimated to the pen environment. Further study is required to characterize the period of acclimation required prior to maximal effectiveness of the rope technique. The period shortly after entry to the feedlot is critical for understanding the ecology of E. coli O157:H7 and the development of mitigation strategies at this point is essential as stressed animals seem more likely to shed E. coli O157:H7 and naïve animals may be more susceptible to colonization.

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Figure 1. Four methods (mouth swabs, ORL; fecal samples by rectal palpation, FEC; pooled fecal pats, PAT, manila ropes, ROP) of monitoring infection in pens (n=4) of feedlot cattle (n=30) inoculated with 10^{10} CFU of nalidixic acid-resistant *E. coli* O157:H7.