

# Effect of Transportation on *E. coli* O157:H7 Prevalence and Coliform Concentrations in Feces of Feedlot Cattle

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## Introduction

Foodborne illness from *Escherichia coli* O157:H7 is a major concern for the food industry. Contamination of food products can occur at slaughter by contact with hide or feces. Limiting *E. coli* O157:H7 shedding is important to prevent outbreaks. Previous studies have demonstrated a relationship between stress and levels of pathogens shed in feces. During transport to the slaughterhouse, animals are subjected to large amounts of stress. This stress could increase shedding of *E. coli* O157:H7 prior to slaughter, and in so doing increase the risk of contamination of beef products by contact with hides or feces. Our objective in this study was to evaluate the effects of transportation on fecal shedding of *E. coli* 4 and 24 hours after transport compared with non-transported animals.

## Experimental Procedures

The experiment was repeated three times over 29-hour periods. We used groups of 20 steers for each replication. Control steers remained in their pens at all times throughout the experiment. Transported cattle were loaded onto a trailer and transported for 1 hour to mimic the stress of transport to the abattoir, returned to the feedlot, and placed into concrete-surfaced pens to mimic pre-slaughter lairage. We collected fecal samples from freshly voided fecal pats from each animal at hours 0, 5, and 29 relative to loading onto the trailer. After the 0-hour sampling, the hauled group was loaded onto a trailer, transported for 1 hour, unloaded and placed into pens, and left to rest for 4 hours. At the end of the rest period (hour 5), all animals were sampled again. A final sampling was obtained 24 hours later (hour 29). Fecal samples were placed into plastic bags and kept on ice until they were transported to the Preharvest Food Safety Laboratory. Approximately 1 gram of feces was subsampled from each fecal pat, weighed, and transferred to a tube containing 9 mL Gram Negative Broth (Difco) with 0.05 mg/L cefixime, 10 mg/L cefsulodin, and 8 mg/L vancomycin (GNccv) and to another tube containing PBS. Tubes were vortexed for 1 minute. The PBS tube was serially diluted up to  $10^{-6}$  and subsequently plated onto Petrifilm plates for enumeration of *E. coli* and total coliforms. Petrifilm plates were incubated at 99°F for 24 hours. The GNccv tubes were incubated at 104°F for 6 hours. After incubation, tubes were subjected to immunomagnetic separation using serotype-specific beads for *E. coli* O157:H7. Beads were resuspended in 200  $\mu$ L of phosphate buffer and plated onto two MacConkey Sorbitol plates (CT-SMAC) containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L). Up to 6 non-sorbitol fermenting colonies from the CT-SMAC plate were selected and inoculated into 5 mL TSB. Colonies were grown overnight at 37°C and tested for indole production. Indole-positive colonies were plated on SMAC and further tested for O157 antigen agglutination. Colonies positive for indole production and antigen agglutination were confirmed as *E. coli* O157:H7 by Gram staining and API 20E.

## Results and Discussion

The statistical analysis revealed differences in *E. coli* O157 prevalence in the fecal samples from one replication to the next. This result was to be expected, because shedding of the pathogen is known to be transient. Additionally, we observed an interaction between sampling time and treatment, as well as an effect of treatment (Figure 1). We interpret these observations to suggest that transport may influence the timing of shedding or fecal pathogens. Prevalence of *E. coli* O157 in the transported group was fairly constant across the three sampling times (10, 3.3, and 16.7%, respectively;  $P = 0.43$ ); however, a significant increase in the pathogen prevalence was observed in the control group at hour 5 (33%) compared with hour 0 (17%,  $P = 0.06$ ) and hour 29 (13%,  $P < 0.02$ ). These findings illustrate a change in shedding patterns of transported cattle relative to their non-transported counterparts, which may be the consequence of transport-related stress. The 4-hour lairage period after transport was chosen arbitrarily, and sampling at additional time points could reveal important changes in timing of pathogen shedding by transported and non-transported cattle.

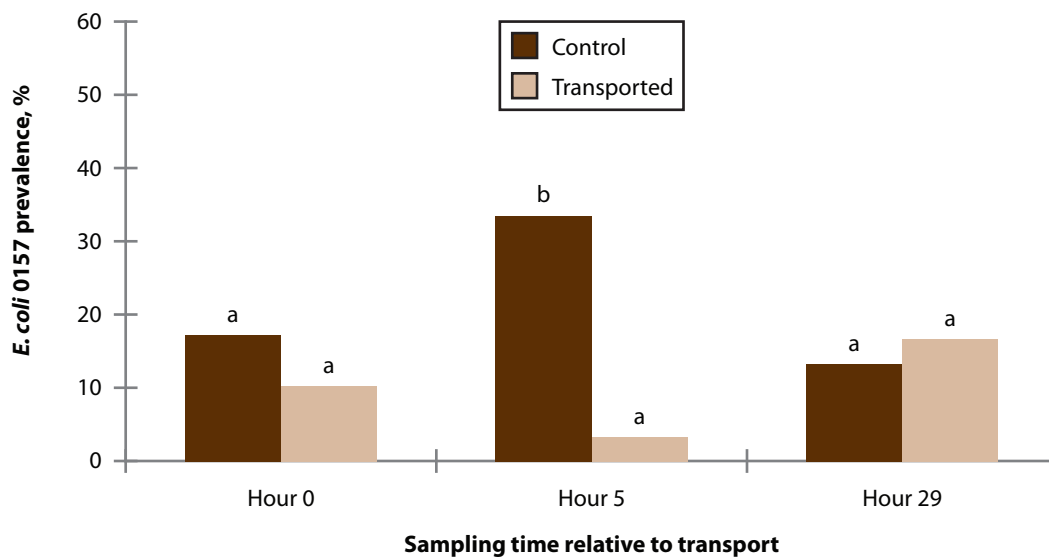
As a secondary objective, we evaluated concentrations of *E. coli* (Figure 2) and other coliforms (Figure 3) in the samples to determine if these populations were related to variations in *E. coli* O157 prevalence. Most *E. coli* bacteria produce glucuronidase and will appear as blue colonies on Petrifilm, whereas the other coliforms will appear red. *Escherichia coli* O157 does not produce beta-glucuronidase, and thus is enumerated along with other coliforms. Numbers of *E. coli* or other coliforms remained fairly constant across replications (replication effect,  $P > 0.1$ ). There were no significant correlations between prevalence of *E. coli* O157 and concentrations of total fecal coliforms. For the control group, coliform concentrations remained relatively stable over the different sampling times (Figure 3). *Escherichia coli* numbers decreased at hour 5 ( $P < 0.05$ ) but rebounded by hour 29. The transported cattle had decreased fecal coliform concentrations at hour 5 (3.2 log CFU/gram;  $P < 0.02$ ), but returned to pre-transport levels of 4.5 log CFU at hour 29. Total enumerable *E. coli* followed the same pattern, decreasing slightly by hour 5, then rebounding ( $P < 0.01$ ) to 6.2 log at hour 29.

These results show a tendency for coliform concentrations to decrease at hour 5 in both of the treatments, suggesting that transport is not the causative factor in this change.

## Implications

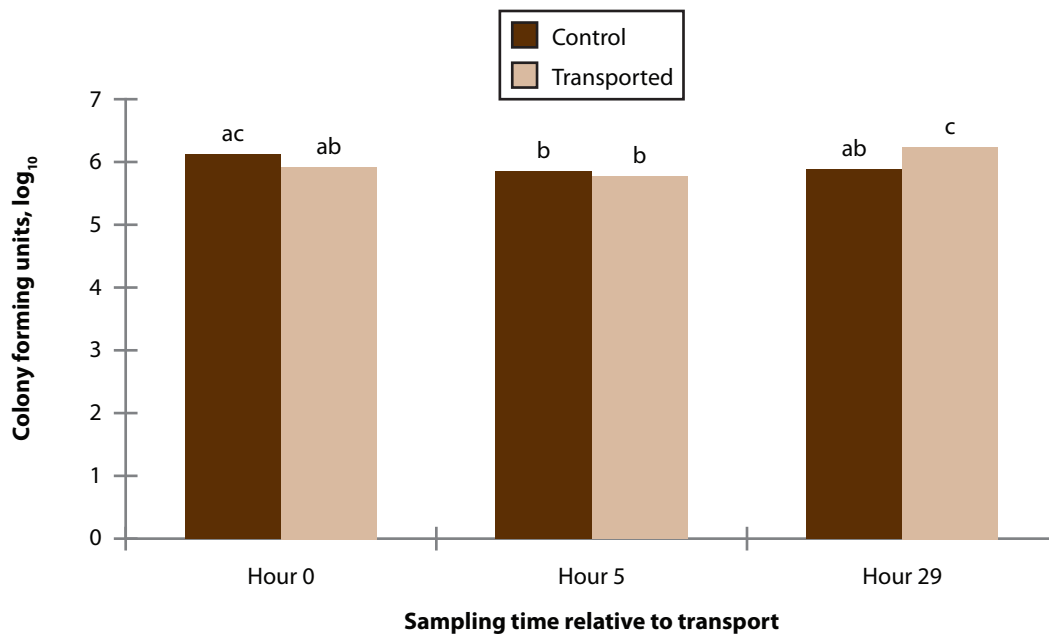
Our hypothesis was that stress from transport would alter the timing for fecal shedding of *E. coli* O157. Results suggest that shedding patterns for pathogens can indeed be influenced by transportation, which has potentially important ramifications for beef safety. Moreover, the data reveal the highly transient behavior of pathogen shedding within a period of only 29 hours, suggesting that pathogen populations can amplify and decay relatively quickly. In future experiments, investigating additional post-transit sampling times may be useful to determine more precisely the pathogen shedding patterns associated with cattle transportation and lairage.

MANAGEMENT



<sup>ab</sup> Columns with different letters differ at  $P < 0.05$ .

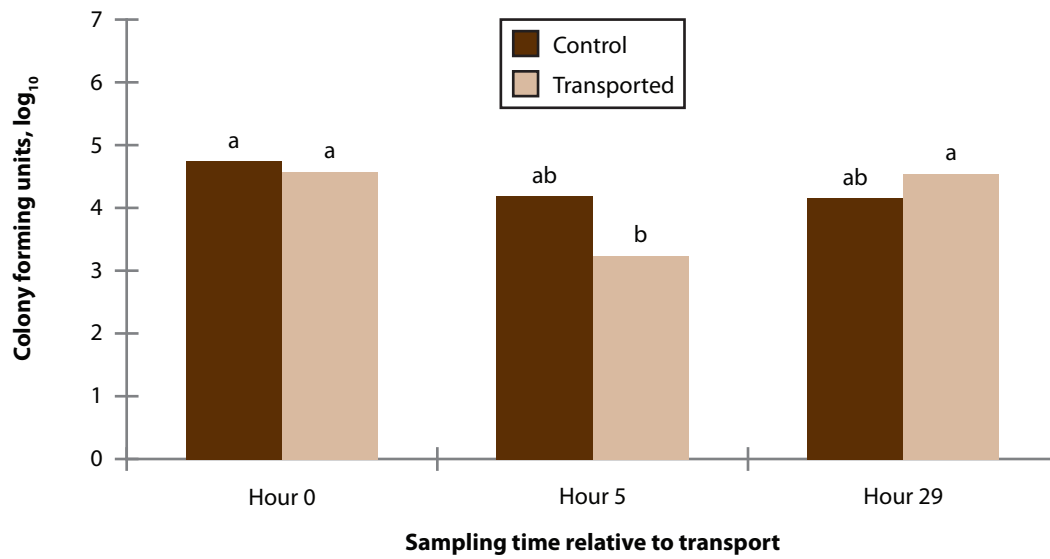
Figure 1. Prevalence of *E. coli* O157 in feces of feedlot cattle following transport and lairage.



<sup>abc</sup> Columns with different letters differ at  $P < 0.05$ .

Figure 2. Fecal concentrations of generic *E. coli* in feedlot cattle following transport and lairage.

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<sup>ab</sup> Columns with different letters differ at  $P < 0.05$ .

**Figure 3. Fecal concentrations of coliforms in feedlot cattle following transport and lairage.**