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IMPACT OF TRANSPORTATION OF FEEDLOT CATTLE TO THE HARVEST FACILITY ON THE PREVALENCE OF *E. COLI* O157:H7, *SALMONELLA* SPP AND TOTAL AEROBIC MICROORGANISMS

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Introduction

The main pathogens of concern found in fresh meat products include *E. coli* O157:H7 and *Salmonella* spp. The prevalence of *E. coli* is used as an indicator of fecal contamination in meat products and its incidence can be highly variable. Because outbreaks of *E. coli* infection have been linked to beef more than any other food product, it is inherent to determine the prevalence levels in the live animal population (Jay 2000). Once determined, measures can be taken to reduce the risk of pathogen contamination in beef products and the need for multiple intervention steps later in processing.

Salmonella spp vary greatly by strain as to how they will affect and survive in an animal and human environment. According to Mulder (1995), the reduction and spread of *Salmonella* in the preslaughter environment can affect the contamination prevalence on swine and poultry carcasses. In addition, Bacon and others (2002) showed that the prevalence of *Salmonella* on the hides of beef animal entering the slaughter facility serves as an indication of contamination that could be transferred to sterile equipment and the environment during the dehiding process, which may not be an indication of the number of animals carrying or shedding *Salmonella*.

Total aerobic microorganisms can be representative of overall cleanliness in the beef processing environment and may be an indicator of spoilage organisms present in the final beef product.

Many factors that affect the microbial prevalence levels in fresh meat and poultry include but are not limited to feeding, transportation, the slaughter process and the use of antimicrobials (Hardin et. al 1995).

The handling and transportation of livestock can cause animals to become stressed, which can increase the shedding of fecal material and pathogens (Fischer 1996, Hails 1978). Research conducted by Williams and Newell (1970) investigated the affect of transport, overcrowding in holding pens, and rough handling before slaughter in swine species. They showed that animals differ in the *Salmonella* excretion patterns, preceding and following the stress of transport. This can result in hidden or masked *Salmonella* infections to become more prevalent. Increased stress and changes in fecal patterns can increase the many points of contamination as animals are transported to slaughter facilities.

Previous research (Barham 2002) indicates an increase in pathogen prevalence on hides of cattle during transportation from the feedyard to the packing plant. The transportation trailer was reported as a possible source of the increased prevalence, but it is not known how cleaning and sanitizing trailers prior to animal transportation will affect contamination on beef hides entering the slaughter plant. A study

conducted by Childers et al. (1977) showed the prevalence levels of bacteria obtained from animal midlines was reduced in those taken from pigs transported and held in sanitized trailers and holding pens, but there was no information collected regarding prevalence levels inside the trailers. In 1998, Rajkowski and others concluded that washing and sanitizing after animal unloading significantly reduced the incidence of *Salmonella* and *E. coli* found in trailers, but corresponding animal data was not collected. Interventions could be expected to decrease the microbial prevalence levels in feedyards, trailers and holding pens; therefore decreasing cross-contamination between farms, animal to animal contact, reduced contamination of the slaughter plant, and decreased microbial loads in fresh meat products. It is also unknown and hypothesized that animals transported on the lower level of the trailer will have increased microbial prevalence levels compared to those transported on the upper level.

Objectives

1. Determine the impact of transportation of beef animals to the harvest facilities on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.
2. Determine the impact of the animal location (upper vs. lower level) during transportation on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.
3. Determine the effectiveness of trailer washing as a means of minimizing hide contamination during transportation of beef animals on the prevalence of *E. coli* O157:H7, *Salmonella* spp. and total aerobic organisms.

Methodology

Sample Collection. A random sample of 40 animals from the same pen (Caprock Feeders, # 6, Lockney, TX) were evaluated and tagged prior to loading on each of 8 days. Spongesicles (SSL 100, International BioProducts, Muncie, IN) hydrated with 10 mL of Butterfields Phosphate Buffer (BPB, Difco Laboratories, Detroit, MI) were used to swab the midline and withers of all animals. Clean trucks were cleaned and sanitized at Excel, Plainview, TX using 4QUAT (K-Klean Chemical Co., INC, Dallas, TX) prior to animal loading. Samples of clean and dirty trailers were collected from the front, right side, left side and floor of each level prior to loading. Ten tagged animals were loaded on the upper and lower compartment of the clean and dirty trailers with the remaining trailer space filled with cattle pen mates. After transportation to the harvest facility, cattle were unloaded and kept in their treatment groups. Trailers swabs were repeated after unloading and animals swabs repeated after exanguination. All swabs were done in duplicate; one used for the *E.coli*/*Salmonella* analysis and one for the total aerobic plate count (TPC) analysis.

Microbiological Analysis. Within 6 hours of collection, samples were returned to Texas Tech University and enriched for further analysis. The spongesicle swab used for *E.coli*/*Salmonella* spp was cut in half and each half was placed in a separate bag for enrichment procedures specific for *E. coli* O157:H7 and *Salmonella* spp. An additional 15 mL of BPB was added to the total aerobic plate count (TPC) samples for dilution.

E. coli O157:H7 swabs were enriched with 45 mLs of GN Broth (Difco Laboratories, Detroit, MI). GN broth specific to organism was made by adding 50 um

Cefixime, 1 mL Vanomycin and 1 mL Cefsulodin to 1 L prepared GN broth. Enriched samples were incubated at 37° C ± 1° C for 6 hours and stored in a refrigerated cooler at 1-4° C. Samples were further enriched in Brain Heart Infusion Broth (Difco Laboratories, Detroit, MI) for 3 hours prior to automated *E. coli* O157:H7 detection. The automated detection of *E. coli* O157:H7 was preformed by Polymerase Chain Reaction assay using the BAX System (Model #1200, Qualicon, Inc., Wilmington, DE).

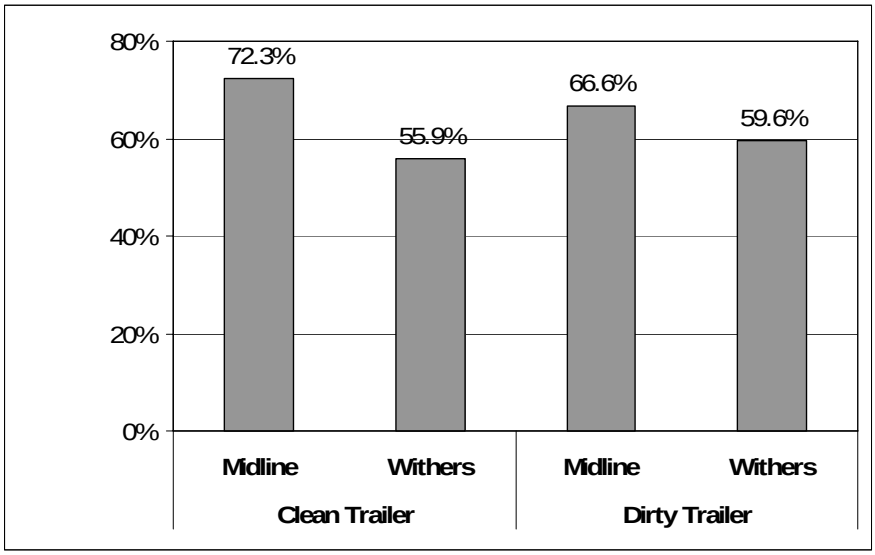
Salmonella spp swabs were enriched using 45 mLs of Tryptic Soy Broth (Difco Laboratories, Detroit, MI) prepared according to manufacturers instructions. Enriched samples were incubated at 42° C ± 1° C for 2 hours, then 37° C ± 1° C for an additional 6 hours and stored in a refrigerated cooler at 1-4° C until automated detection using the BAX System.

Serial dilutions of the TPC samples were made using 1 mL dilutions of BPB and plated on 3M Petrifilm Aerobic Count Plate (3M Corporation, St. Paul, MN) following AOAC Official Methods 998.08. Petrifilm plates were incubated for 48 hours ± 3 hours at 35° C ± 1° C and red colonies were enumerated using a 3M Petrifilim Plate Reader (Model # 6499, 3M Corporation, St. Paul, MN).

Experimental Design and Statistical Analysis. The experiment was conducted in 8 replications (days) with 10 experimental units per treatment. Data analysis was performed using the PROC MIXED application of SAS Version 8 (SAS Institute, Cary, NC). The design was completely random with model including the main effects of truck, animal location (level), hide swab location and day. Means were separated using the appropriate error terms with a significance level set at $P < 0.05$.

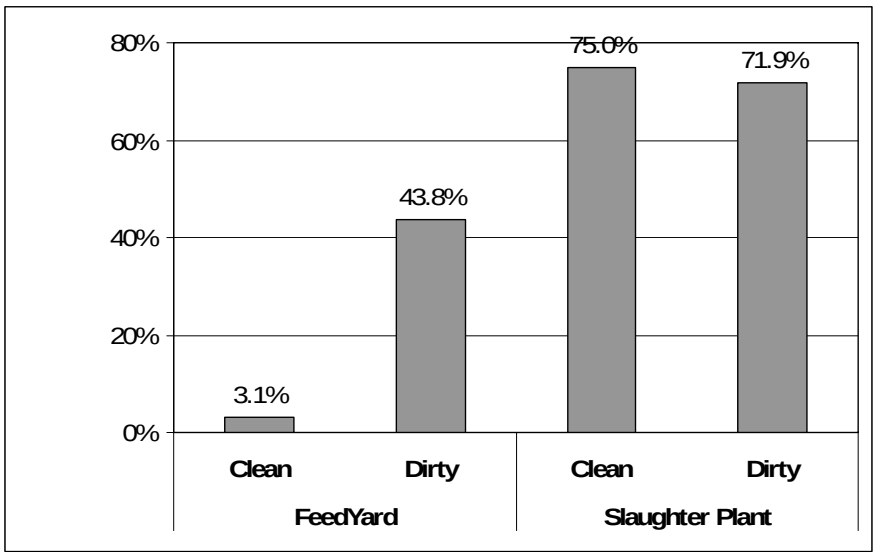
Results & Discussion

***Salmonella* spp.** Animal swabs collected showed a significant interaction for trailer and hide swab. Swab samples collected from the midline had a higher *(P = 0.038)* percentage of *Salmonella* positive samples than those collected from the withers for animals transported on both trailers (Figure 1).



Animal swabs taken at the plant had significantly more positive samples than those taken at the feed yard at both hide swab locations. Trailer swabs collected for *Salmonella* spp analysis showed a significant interaction for location x trailer. At the feedyard, swabs taken from the clean truck (3.1%) had a lower *(P < 0.001)*

percentage of positive samples than those collected from the dirty truck (43.8%). However, at the plant swabs taken from the clean truck (75.0%) had a higher *(P = 0.001)* percentage of positive samples than those collected from the dirty truck (71.9%, Figure 2).



In addition, an interaction for location x level in trailer was observed for trailer swabs collected. At the feedyard, a significant difference was found as the top level reported only 15.6% positive samples while the bottom level had 31.3% positive samples.

Another interaction for the prevalence of *Salmonella* spp in trailer swabs taken was the trailer x level. The clean trailer had a lower percentage of positive samples than those found in the dirty trailer for the bottom level, which could be expected. However, the trailer swabs collected from the top level were similar *(P = 0.524)* for the clean and dirty truck. The main effect of swab location within the trailer also showed significant differences in the prevalence of positive samples. The floor samples collected were significantly higher *(P = 0.001)* than those collected from the right, left and front sides of the clean and dirty trailers.

E. coli 0157:H7. The swab location on the animal hides had a higher *(P =.051)* percentage of positive samples at the plant (1.3%) when compared to the feedyard (0.3%). Trailer had no effect on prevalence as seen with 256 swabs taken from the trailers, less than 2% of samples were positive for *E. coli* 0157:H7.

Total Aerobic Organisms. The location swabs taken at the plant had a higher *(P = 0.003)* CFU log count than those taken at the feedyard. Also, hide swabs showed higher *(P = 0.008)* counts at the midline location compared to the withers (Table 7).

A significant 3-way interaction for location x trailer x trailer swab was determined for TPC from truck samples (Table 8). The dirty trailers had higher counts of aerobic organisms at all trailer locations (floor, front, right and left) at both the feedyard and the slaughter plant. In addition, trailer swabs collected from the top deck (7.50 log CFU) had less *(P = 0.008)* aerobes compared to the bottom deck (7.86 log CFU).

Other research has shown that the number of *Salmonella* spp positive animals increased from entry into feedyard when compared after 30 days in the feedyard (Corrier et. al 1990, Cray et. al 1998). This suggests that animal to animal contact may be a contributing factor to microbial transmission. Current data agrees with this suggestion as the *Salmonella* prevalence increased at the slaughter facility compared to the feedyard. However, there are not any current time and space parameters known for the transmission of *Salmonella* spp from animal to animal.

Even though the prevalence of *Salmonella* spp and total aerobic organisms in the trailer showed some significant interactions, there does not appear to be a direct relationship between the cleanliness of trailer, the level in which the cattle were transported and the actual microbial prevalence found on the hide of the cattle. Barham and others (2002) showed that the prevalence of *Salmonella* spp increased due to transportation and the current data agrees with these previous findings. However, current data shows that the trailer itself is not the source of the increased contamination. In addition to animal contact, other possible sources of contamination include the dirt/dust present in the loading area at the feedyard, holding areas at the feedyard just prior to loading, holding areas at the plant prior to slaughter and equipment/personnel found inside the plant during stunning and exanguination.

Conclusions

Even with increased levels of *Salmonella* spp and aerobic organisms at the harvest facility, positive samples for microorganisms found in trailers did not relate to the contamination found on animal hides. Neither the cleanliness of trailers nor level in which the animals were transported affected the contamination present on the animal at the harvest facility. Increased levels found on animal hides agrees with previous research, but is not caused by the trailer itself. Other sources of contamination include animal to animal contact, dirt/dust present in the loading area at the feedyard, holding areas at the feedyard just prior to loading, holding areas at the plant prior to slaughter and equipment/personnel found inside the plant during stunning and exsanguinations.

References

Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and antibiotic susceptibility of Salmonella isolated from beef animal hides and carcasses. J. Food Prot. 65:284–290.

Barham, A. R., B. L. Braham, A. K. Johnson, D. M. Allen, J. R. Blanton, JR., and M. F. Miller. 2002. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of Escherichia coli O157 and Salmonella spp. J. Food Prot. 65:280–283.

Corrier, D. E., C. W. Purdy, and J. R. DeLoach. 1990. Effects of marketing stress on fecal excretion of Salmonella spp. in feeder calves. Am. J. Vet. Res. 51:866–869.

Cray, W. C., T. A. Casey, B. T. Bosworth, and M. A. Rasmussen. 1998. Effects of dietary stress on fecal shedding of Escherichia coli O157:H7 in calves. Appl. Environ. Microbiol. 64:1975–1979.

Fischer, K. 1996. Transport of slaughter animals. Fleischwirtschaft 76:521–526.

Hails, M. R. 1978. Transport stress in animals: a review. Animal Reg. Studies 1:289–343.

Jay, J. M. 2000. Modern Food Microbiology. Aspen Publishers, Inc., Gaithersburg, Maryland.

Mulder, R. W. A. W. 1995. Impact of transport and related stresses on the incidence and extent of human pathogens in pigmeat and poultry. J. Food Safety 15:239–246.

Rajkowski, K.T., S. Eblen, and C. Laubauch. 1998. Efficacy of washing and sanitizing trailers used for swine transport in reduction of Salmonella and Escherichia coli. J. Food Prot. 61:31–35.

SAS Institute. 1999. User's Guide: Statistics, Version 8. SAS Institute Inc., Cary, N.C.

Williams, L. P. Jr. and K. W. Newell. 1970. Salmonella excretion in joy-riding pigs. Am. J. Pub. Health. 60:926–929.