AGRICULTURE, FOOD & ANALYTICAL BACTERIOLOGY

www.afabjournal.com

Agriculture, Food and Analytical Bacteriology

# BRIEF COMMUNICATION
# Influence of Winter and Summer Hutch Coverings on Fecal Shedding of Pathogenic Bacteria in Dairy Calves

R. L. Farrow[1], T. S. Edrington[1], B. Carter[2], T. H. Friend[2], T. R. Callaway[1], R. C. Anderson[1], and D. J. Nisbet[1]

[1]Food and Feed Safety Research Unit, ARS, USDA, College Station, TX 77845
[2]Texas A&M University, Department of Animal Science, College Station, TX 77843

‡Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

## ABSTRACT

The effects of hutch coverings utilized during the summer and winter months to moderate extreme temperatures were examined on fecal prevalence of *E. coli* O157:H7 and *Salmonella* in newborn dairy calves. In the initial study, the effects of shade using screens in three treatment groups: no shade, partial shade, and full shade were examined. Two additional studies were designed where individual calf hutches were modified with a hutch blanket (treatment) or no hutch blanket (control) in the winter study and a ventilated hutch design added as a third treatment in the summer study. During the summer experiment, prevalence of *E. coli* O157:H7 and *Salmonella* was low; however, *Salmonella* was increased ($P < 0.05$) in the ventilated hutch versus the control treatment. In the winter study, quantifiable results for both *E. coli* O157:H7 and *Salmonella* were largely negative. *Salmonella* positive samples were numerically higher, however no treatment differences were observed. In the shade cloth study all fecal samples were *E. coli* O157:H7 negative. *Salmonella* was cultured from all treatment groups, however no differences were observed between treatments. Summarily, there is no evidence that hutch treatments decreased the period prevalence of fecal shedding of *Salmonella*, *E. coli* O157:H7 or *Enterococcus*.

Keywords: Dairy calves, *Salmonella*, *E. coli*, Hutch covering

*Agric. Food Anal. Bacteriol. 1: 98-104, 2011*

## INTRODUCTION

Dairy producers have long realized the need for individualized care of newborn calves from both management and health perspectives. Dairy calves are born without circulating antibodies that are critical for immune function and current management practices strive to insure that newborns receive adequate amounts of colostrum to develop immunity to a host of potentially pathogenic organisms; however, these calves have an increased susceptibility to disease (Roy, 1970). In 2006 the U. S. Department of Agriculture reported a mortality rate of 11 percent for pre-weaned dairy calves with the majority of those deaths resulting from enteric and respiratory pathogens (USDA, 2006).

Correspondence: T. S. Edrington, tom.edrington@ars.usda.gov
Tel: +1 -979-260-3757 Fax: +1-979-260-9332

Calf hutches have been used as a means to reduce direct contact among newborn calves in an effort to reduce transmission of disease. Dairy calves that were comingled had an increased prevalence of *E. coli* O157:H7 compared to calves that were housed individually (Garber *et al.*, 1995). Poos and Sordillo (1982) reported that calves housed in hutches had improved growth and performance as well as reduced mortality when compared to other methods. In extreme cold weather environments, calves reared in hutches had an overall better performance, ate more starter feed and required fewer medical treatments (McKnight, 1978).

In warm climates, supplemental shade used during the summer months decreased severity of the heat stress experienced by calves when compared to those in hutches alone (Spain and Spears, 1996). Scott *et al.* (1976) reported that calves exposed to chronic heat loads had lower IgG than those that were housed at thermo-neutrality. Given the aforementioned research, it is hypothesized that reducing temperature variations in periods of extreme heat and cold will reduce the level of stress in calves and thereby increase their resistance to pathogenic organisms. The objectives of this research are to determine if the application of shade to dairy hutches decreases fecal prevalence of *E. coli* O157:H7 and *Salmonella* in newborn dairy calves.

## MATERIALS AND METHODS

This research was conducted on a single, large commercial Holstein dairy (> 2000 head) located in the Texas Panhandle and managed as typical for dairies in Eastern New Mexico and the Texas Panhandle. During the periods at which the observations took place for the summer and winter studies temperatures averaged approximately 24°C (18.1 - 31.4°C) and 4.9°C (-2.9 - 12.7°C), respectively. At the time of these studies this dairy was a closed herd that reared its own replacement females. Three studies were designed to evaluate the effects of hutch modifications on pathogen shedding. The first study (shade cloth) evaluated the effects of a suspended shade cloth over hutches on pathogen shedding. The second (winter) and third (summer) studies evaluated the effects of reflective insulation applied to calf hutches; during the summer study an additional treatment designed to increase hutch ventilation was incorporated. Holstein heifer calves were placed alternately in treatments immediately after birth and managed as typical for dairy calves in this region. Heifers were fed approximately 2.0 L of pasteurized waste milk twice daily until they would drink from a bucket (1 to 3d), after which they were fed approximately 7.5L pasteurized waste milk twice daily. As per protocol of dairies typical of this region calves were provided approximately 7.5L water upon consumption of milk, and in addition calves were gradually introduced to solid feeds between 10 to 14 d of age. All hutches were commercially available polyethylene hutches (Calf-Tel Pro®, Hampel Corp., Germantown, WI) affixed with a 1 x 2 m outdoor pen made of welded wire panels.

### Shade Cloth Study

This study utilized 80% shade cloth (Sunblocker Premium, Farmtek., Dyersville IA) to provide partial (n = 4), full (n = 6) or no shade (n = 14). The cloth was suspended 3 m above the hutches so that a portion of the pen in front of the hutches was also shaded; partially shaded hutches were considered as those that received shade in the morning or evening because they were located near the edge of the shade structure. Heifers were assigned to treatment as described above. However, during the first collection calves had only been placed in half of the hutches, resulting in reduced sample sizes for partial (n = 2), full (n = 2) and no shade (n = 9). Fecal samples were collected via rectal palpation or from freshly voided, uncontaminated fecal pats within the hutch area on 20JUL2006 (day 1), 25AUG2006 (day 37), and 23SEP2006 (day 66).

### Summer Study

As described below, hutch coverings were placed on hutches upon placement of the calf in the hutch, shortly following birth starting June 10, 2007 (day 1),

and were removed on September 1, 2007 (day 84). An additional 10 hutches were modified by creating a 14 x 18 inch hole, 18 inches off the ground, in the back wall of the hutch (ventilation treatment) to increase air flow through the hutch. Heifers were assigned to treatment as described above, calves were fed using this opening on the inside the hutch, versus normal feeding which occurred outside of the hutch in the wire pen, thus allowing them to remain shaded while eating. One calf in this type of hutch died early in the experimental period and was not replaced resulting in 9 hutches of this type at each collection. Fecal samples were collected as above on days 6, 19, 41, 65 and 84 of the experimental period as described above.

### Winter Study

During the winter and summer studies, treated hutches were covered with a 2.2 x 2.5 m sheet of Tempshield™ reflective insulation (Innovative Insulation Inc., Arlington, TX). Grommets were place on the edges of the "hutch blanket" to facilitate attachment to the hutch in three places on each side of the hutches long sides by elastic cord. Treated hutches (n = 20) alternated with control hutches (n = 19) and were all placed in a single row, contained within multiple rows of calf hutches. Heifers were assigned to treatments as above. Hutch blankets were applied on December 18, 2007 (day 1) and removed February 23, 2008 (day 68). Fecal samples were collected as described above on days 29, 42, 56 and 68 of the experimental period. Fecal samples were collected using sterile palpation sleeves, placed on ice and transported to our laboratory in College Station, Texas for bacterial culture described in the following section.

### Bacterial Culture and Isolation

All fecal samples were processed the day following collection for qualitative analysis of *Salmonella* (Edrington *et al.*, 2009) and *E. coli* O157:H7 as described previously (Robinson *et al.*, 2004) and modified (Brichta-Harhay *et al.*, 2007). *Enterococcus* was qualitatively cultured as previously described by Edrington and co-workers (2009). Unless noted otherwise, all reagents and antibiotics were obtained from Sigma Chemical Co. (St. Louis, MO).

Antimicrobial susceptibility was determined on isolates using the Sensititre automated antimicrobial susceptibility system and the National Antibiotic Resistance Monitoring System's (NARMS) testing panels (Trek Diagnostics Systems Inc., Cleveland, OH) for isolates as described previously (Edrington *et al.*, 2009).

### Statistical Analysis

Data were analyzed using SAS Version 9.2 (SAS Inst. Inc., Cary, NC, USA). Quantitative enumeration was infrequent and not sufficient to detect differences among treatments, therefore data is presented as prevalence (% positive) and not actual counts. The incidence of fecal pathogen shedding was subjected to a Chi-square analysis using the PROC FREQ procedure. Additionally, the PROC MIXED procedure was used to examine the main effects of treatment and day, and treatment x day interaction. Differences in means were considered significant at a 5% level of significance.

## RESULTS

### Shade Cloth Study

When combined across sampling dates, partially shaded calves shed *Salmonella* more frequently (70%) when compared to no shade and full shade (54 and 43%, respectively), however no statistical differences between treatments were observed (Table 1). *Salmonella* isolates (n = 29) from the first two collection times were analyzed for antimicrobial susceptibility. Two isolates per positive sample from the first collection period and a single isolate from collection two were subjected to antimicrobial screening. Most isolates (86%) were pan susceptible; resistance to sulphathiazole was observed in two isolates and resistance to tetracycline was observed in a single isolate. One isolate that displayed resistance to

Table 1. Prevalence (number and % positive) of *Salmonella* in fecal samples collected from dairy calves housed in control hutches or hutches with partial shade or full shade. Fecal samples were enriched for qualitative analysis prior to plating (ENR).

| | | Control | | Partial Shade | | Full Shade | |
|---|---|---|---|---|---|---|---|
| Day | Method | no. | % | no. | % | no. | % |
| 1 | ENR | 9/9 | 100 | 2/2 | 100 | 2/2 | 100 |
| 37 | ENR | 8/14 | 57 | 4/4 | 100 | 4/6 | 67 |
| 66 | ENR | 3/14 | 21 | 1/4 | 25 | 0/6 | 0.0 |
| all days | ENR | 20/37 | 54 | 7/10 | 70 | 6/14 | 43 |

No significant differences between treatments were observed

chlortetracycline, oxytetracycline, and tetracycline belonged to serogroup $C_2$. No differences were observed between treatments with respect to antimicrobial resistance. All fecal samples were *E. coli* O157:H7 negative throughout the study.

## Summer Study

Fecal prevalence of *E. coli* O157:H7 was low throughout the study, with 4 of 205 samples positive following enrichment and IMS (Table 2). Three of those positive samples were cultured on d 84 in the hutch covering treatment; however no significant differences were observed between control and ventilated hutch treatments ($P = 0.06$). No samples were culture positive for *E. coli* O157:H7 using quantitative methodology. Similarly, *Salmonella* was cultured infrequently following direct plating with positive samples detected only on d 84 in the control and ventilated treatments (22% positive in each treatment). Following enrichment however, *Salmonella* positive fecal samples were detected on all collection days except d 41. The highest incidence was observed on days 19 and 84, although only on d 19 were significant treatment differences observed. *Salmonella* prevalence was increased ($P < 0.05$) in the ventilated hutch compared to the control treatment, but was similar for control and hutch blanket treatments (17.2, 31.6 and 66.7% for control, hutch blanket and ventilated treatments, respectively). When averaged across collection days, only *E. coli* O157:H7 prevalence (as detected by IMS) was affected by treatment, with a higher ($P < 0.05$) percentage of positive samples in the hutch blanket treatment compared to control and ventilated treatments. There were no treatment x day interactions observed. Five different *Salmonella* serogroups were identified ($C_1$, $C_2$, $E_1$, K and poly A-I, vi) with $C_2$ accounting for 55% of the isolates. Twenty-one *Salmonella* isolates were examined for antimicrobial susceptibility (7, 8 and 6 each from the control, blanket and ventilated hutch treatments, respectively) and all were susceptible to all of the antibiotics examined (data not shown).

*Enterococcus* isolates (n = 14) cultured from hutch calves on this farm during the shade study were examined for antimicrobial susceptibility. All 14 isolates were resistant to quinupristin/dalfopristin and four of these isolates were additionally resistant to erythromycin, lincomycin, streptomycin, tetracycline, and tylosin. No treatment differences were observed and all *Enterococcus* isolates were resistant to vancomycin (data not shown). Based on this data and similar data generated in the Winter Study, *Enterococcus* isolates were not examined in the summer hutch covering study.

## Winter Study

Results of the quantitative culture of *E. coli* O157:H7 and *Salmonella* over the course of the winter study were largely negative (Table 3). None of the samples contained quantifiable populations of *Salmonella* (0/136) while only two animals had quantifiable concentrations of *E. coli* O157:H7 (d 56 and

Table 2. Prevalence (number and % positive) of *E. coli* O157:H7 (EC) and *Salmonella* (Salm) in fecal samples collected during the summer study, by day of collection (when at least on sample was culture positive) and across all collection days, from dairy calves housed in control hutches or hutches modified with a blanket or ventilated to improve calf comfort. Fecal samples were either plated directly to quantify bacterial populations (DIR) or enriched for qualitative analysis prior to plating (ENR).

| | | | Control | | Hutch blanket | | Ventilated hutch | |
|---|---|---|---|---|---|---|---|---|
| Bacteria | Day | Method | no. | % | no. | % | no. | % |
| EC | 6 | ENR | 0/4 | 0 | 1/4 | 25 | ns[a] | . |
| | 84 | ENR | 0/18 | 0 | 3/15 | 20 | 0/9 | 0 |
| | All days | ENR | 0/100 | 0[b] | 4/73 | 5.48[c] | 0/32 | 0[b] |
| Salm | 6 | ENR | 2/4 | 50 | 2/4 | 50 | ns[a] | . |
| | 19 | ENR | 5/29 | 17.2[b] | 6/19 | 31.6[b] | 6/9 | 66.7[c] |
| | 65 | ENR | 1/22 | 4.55 | 0/16 | 0 | 0/5 | 0 |
| | 84 | DIR | 4/18 | 22.2 | 0/15 | 0 | 2/9 | 22.2 |
| | 84 | ENR | 3/18 | 16.7 | 1/15 | 6.67 | 1/9 | 11.1 |
| | All days | ENR | 11/100 | 11 | 9/73 | 12.3 | 7/32 | 21.9 |

[a] no samples collected.
[bc] Treatment means within a row with different superscripts differ ($P < 0.05$).

68, control treatment). Enrichment of the samples followed by IMS identified the same two positive animals in the control treatment and an additional six positive animals in the hutch blanket treatment (2 on d 42, 4 on d 56). The number of *Salmonella* positive samples increased slightly with enrichment, although no treatment differences were observed. The majority of serogrouped *Salmonella* belonged to group K.

Antimicrobial susceptibility screening was conducted on five *E. coli* O157:H7 isolates (data not shown). All isolates were resistant to sulphisoxazole and two of the five were also resistant to streptomycin. *Enterococcus* isolates from d 29 (n = 32) and 42 (n = 11) collections were also examined (data not shown). Most isolates however were susceptible to all of the antibiotics examined with the exception of quinupristin/dalfopristin, to which all but three were resistant. Isolates (in both treatments) displayed resistance to seven different antibiotics (chloramphenicol, erythromycin, lincomycin, quinupristin/dalfopristin, streptomycin, tetracycline and tylosin) including three isolates in the control treatment and two in the hutch blanket treatment, accounting for most of the observed resistance. Of the 11 isolates examined 13 d later on d 42, only one isolate in the hutch blanket treatment was multi-resistant (6 antibiotics, exhibiting the same pattern as the previous isolates). As discussed above, the majority of the isolates were susceptible to all antibiotics with the exception of quinupristin/dalfopristin (data not shown). All *Enterococcus* isolates were susceptible to vancomycin. Due to the limited number of *Salmonella* positive samples, isolates were not retained for antimicrobial susceptibility screening.

## DISCUSSION

Results of the current research highlight the sporadic nature of pathogen shedding in naturally-colonized animals. Prevalence of *E. coli* O157:H7 and *Salmonella* was relatively low in this study compared to previous research conducted by our laboratory examining pathogen prevalence in dairy cattle during the summer months (Edrington *et al.*, 2004). Even so,

Table 3. Prevalence (number and % positive) of *E. coli* O157:H7 (EC) and *Salmonella* (Salm) in fecal samples collected during the winter study, by day of collection (when at least one sample was culture positive) and across all collection days, from dairy calves housed in control hutches or hutches modified with a reflective blanket to improve calf comfort. Fecal samples were either plated directly to quantify bacterial populations (DIR) or enriched for qualitative analysis prior to plating (ENR).

| | | | Control | | Hutch blanket | |
|---|---|---|---|---|---|---|
| Bacteria | Day | Method | no. | % | no. | % |
| EC | 42 | ENR | 0/17 | 0 | 2/17 | 11.8 |
| | 56 | DIR | 1/18 | 5.6 | 2/15 | 13.3 |
| | 56 | ENR | 1/18 | 5.6 | 4/15 | 26.7 |
| | 68 | DIR | 1/18 | 5.6 | 0/16 | 0 |
| | 68 | ENR | 1/18 | 5.6 | 0/16 | 0 |
| | All days | DIR | 2/71 | 2.8 | 2/65 | 3.1 |
| | All days | ENR | 2/71 | 2.8 | 6/65 | 9.2 |
| Salm | 29 | ENR | 1/18 | 5.6 | 0/17 | 0 |
| | 42 | ENR | 0/17 | 0 | 1/17 | 5.9 |
| | 56 | ENR | 2/18 | 11.1 | 1/15 | 6.7 |
| | All days | ENR | 3/71 | 4.2 | 2/65 | 3.1 |

some significant treatment differences and trends were noted in fecal shedding of *E. coli* O157:H7 and *Salmonella* on various collection days, suggesting hutch coverings increased pathogen prevalence. Various scenarios including environmental changes within the hutch due to hutch coverings may have contributed to this outcome, however identifying a single cause is not possible. It is unclear to the authors what contributed to a greater proportion of *Salmonella* positive calves to have originated from the partially shaded treatment relative to both no shade and full shade within the shade study. It should be noted that for a study of this type to achieve an $\alpha = 0.95$ and a $ß = 0.20$ with 20% prevalence expected in the control versus 10% in the treated groups it would require a sample of 219 animals per treatment group. Given the resources available to the researchers sample sizes of this magnitude were unobtainable. However, due to the low number of sampling units used in this study readers are cautioned not to over interpret these data.

Serogrouping of the *Salmonella* isolates identified the majority as belonging to groups $C_2$ (summer study), K (winter study) and $C_1$ and $C_2$ (shade cloth study). This is not surprising as we have reported seasonal differences in serogroup prevalence in dairy cattle previously (Edrington *et al.*, 2004). *Salmonella* Newport, a serotype frequently Multidrug-Rresistant (MDR), belongs to serogroup $C_2$. However, antimicrobial susceptibility testing revealed no MDR isolates; therefore it is likely that these isolates belonged to another common dairy serotype within the $C_2$ group, likely Kentucky. Serotyped isolates from previous dairy research belonging to serogroup K, were frequently identified as Cerro (Edrington *et al.*, 2004).

Antimicrobial susceptibility screening of the various isolates yielded few MDR isolates (mostly *Enterococcus*) and these were resistant to antimicrobials frequently utilized in veterinary medicine and susceptible to antibiotics used in human medicine.

In summary, further research is necessary to provide additional evidence to support the use of hutch coverings to moderate temperatures and consequently increase calf comfort. These data indicate that in an effort to increase animal level comfort

dairy producers may inadvertently increase pathogen shedding. Additional studies should be focused on locating herds that have high enough levels of pathogen shedding and sufficient quantity of subjects to discern measures of effect should they exist.

## CONCLUSION

Efforts to mitigate extreme temperatures within calf hutches via hutch coverings have been shown to increase the pathogen burden of dairy calves within this study. However, due to the small number of observations further work is warranted to better understand the effects of temperature modification within hutches with various forms of shades and reflective material. Therefore, the authors recommend that each dairy assess their needs and situation individually to best determine how increase calf comfort while ensuring that an increased pathogen burden has not been imposed.

## ACKNOWLEDGEMENT

The authors wish to thank the Food Animal Concerns Trust for partial funding of this research and Shannon Garey for assistance during sample collection.

## REFERENCES

Brichta-Harhay D. M., T. M. Arthur, J. M. Bolsilevac, M. N. Guerini, N. Kalchayanand, M. Koohmaraie. 2007. Enumeration if *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. J. Appl. Microbiol. 103:1657-1668.

Edrington, T. S., B. H. Carter, T. H. Friend, G. R. Hagevoort, T. L. Poole, T. R. Callaway, R. C. Anderson, D. J. Nisbet. 2009. Influence of sprinklers, used to alleviate heat stress, on fecal shedding of *E. coli* O157:H7 and *Salmonella* and antimicrobial susceptibility of *Salmonella* and *Enterococcus* in lactating dairy cattle. Lett. Appl. Microbiol. 48:738-743.

Edrington, T. S , M. E. Hume, M. L. Looper, C. L. Schultz, A. C. Fitzgerald, T. R. Callaway, K. J. Genovese, K. M. Bischoff, J. L. McReynolds, R. C. Anderson, D. J. Nisbet. 2004. Variation in the faecal shedding of *Salmonella* and *E. coli* O157:H7 in lactating dairy cattle and examination of *Salmonella* genotypes using pulsed-field gel electrophoresis. Lett. Appl. Microbiol. 38:366-372.

Garber, L. P., S. J. Wells, D. D. Hancock, M. P. Doyle, J. Tuttle, J. A. Shere, T. H. Zhao. 1995. Risk factors for shedding *Escherichia coli* O157:H7 in dairy calves. J. Am. Vet. Med. Assoc. 207:46-49.

McKnight, D. R. 1978. Performance of newborn dairy calves in hutch housing. Can. J. Anim. Sci. 58:517-520.

Poos, M. I. and L. Sordillo. 1982. The effect of type of housing and supplementation on performance of dairy calves from birth to weaning. J. Dairy Sci. 65:121.

Robinson, S. E, E. J. Wright, N. J. Williams, C. A. Hart, and N. P. French. 2004. Development and application of a spiral plating method for the enumeration of *Escherichia coli* O157:H7 in bovine feces. J. Appl. Microbiol. 97:581-589.

Roy, J. H. B. 1970. The calf: management and feeding. Butterworths, London, UK. 224 p.

Scott, G. H., F. Wiersma, B. E. Menefee, F. R. Radwanski. 1976. Influence of environment on passive immunity in calves. J. Dairy Sci. 59:1306-1311.

Spain, J. N. and D. E. Spears. 1996. Effects of supplemental shade on thermoregulatory response of calves to heat challenge in a hutch environment. J. Dairy Sci. 79:639-646.

USDA. 2006. Characterization and enhancement of immune responses of calves. Annual Report FY 2006. United States Department of Agriculture, Agricultural Research Services, National Animal Disease Center, Ames, IA. p 1-5.