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Survivability of Bovine Derived *Escherichia coli* Subjected to Temperatures Typical of Summer in Texas

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ABSTRACT

Dairy manure and wastewater is often stored in lagoons and applied to fields during summer months. The objective of this study was to determine the effect of moderate durations of typical Texas summer temperatures on the survivability of *E. coli* in dairy manure. Manure samples were collected from two dairy operations and assigned to one of three temperature treatments, 73 °F, 95 °F, or 111 °F. Manure samples were analyzed for *E. coli* on d 0, d 10, and d 21. By d 10, *E. coli* levels in manure samples were reduced ($P < 0.01$) from 5.34 log CFU/g at d 0 to 4.55 and 0.25 log CFU/g in manure samples stored at 95 °F and 111 °F, respectively. All samples showed significant ($P < 0.001$) reductions in *E. coli* populations after 21 d. On d 21, *E. coli* bacteria in manure stored at 111 °F were undetectable, and *E. coli* concentrations averaged 0.32 and 3.69 log CFU/g for the 95 °F and 73 °F treatments, respectively. Survivability of bovine derived *E. coli* was reduced by temperatures that are typical of summer in Texas. Application of manure during periods of high environmental temperatures may facilitate a quicker decline in *E. coli* concentrations as compared to application during cool ambient temperatures.

KEY WORDS: *Escherichia coli*, dairy, manure, temperature

INTRODUCTION

Escherichia coli are a type of bacteria well adapted for survival in natural environments. These bacteria are naturally widely distributed in soils and bodies of water throughout the world, and they are commonly found in the intestines of animals and humans. There are concerns that pathogenic strains of *E. coli* from livestock manure could move into surface or groundwater and pose risks to human health.

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Reports have shown that temperatures above 100 °F decrease the concentrations of *E. coli* in manure (Hess et al., 2004; Michel et al., 2005). Hess et al. (2004) reported in a composting study, where manure and straw were mixed in a 20:1 C:N ratio at 70% moisture, that temperatures as low as 104 ºF reduced the numbers of *E. coli* 0157:H7. Similar temperatures have also been shown to eliminate all strains of *E. coli* bacteria during the process of aerobic digestion (Michel et al., 2005).

Escherichia coli populations do not multiply rapidly under cool temperatures. Temperatures of 46.4 ºF have been shown to initially foster the growth of certain *E. coli* strains, but caused them to decline in subsequent days (Francis and O'Beirne, 2001). Temperatures of 39.2 ºF have been shown to reduce, but not eliminate, the growth of *E. coli* (Francis and O'Beirne, 2001). Furthermore, *E. coli* has the ability to reproduce exponentially as an initial response to exposure to temperatures between 73 ºF and 77 ºF (Fujioka and Byappanahlli, 1998).

The time of year of manure application has been shown to have an effect on bacterial populations (Miller et al., 2003; Sharples et al., 2004). A Canadian study concluded land application of manure in spring or fall was preferable to applications in summer due to the fact that *E. coli* and total coliform populations were found to be significantly greater as summer air temperatures increased to 81 ºF (Miller et al., 2003). A study by Sharples et al. (2004) found that manure applied in June resulted in greater survival rates of *E. coli* as opposed to manure applied in August, and Fukushima et al. (1999) noted decreased persistence of *E. coli* in manure at temperatures of 77 ºF.

In Texas, dairy waste is often stored in lagoons and applied to fields during the summer months. During the summer, air temperatures in Texas can easily exceed 100 ºF and soil temperatures typically reach 86 ºF (ARS, 2009). As a result of high temperatures that are common during the summer, *E. coli* concentrations of manure may decline more rapidly than would be expected in a more moderate climate. The objective of this study was to determine the effect of moderate durations of typical Texas summer temperatures on the survivability of *E. coli* in dairy manure.

MATERIALS AND METHODS

Manure samples for this study were collected from 2 different dairy operations, P and F. The dairy operations were located approximately 20 miles apart and were both in Cooke County, TX. The dairy operations used different management styles and feeding practices. Dairy P was a 150 cow dry-lot dairy that utilized a total mixed ration with a feeding lane, and dairy F was a 90 cow grazing-based dairy that fed supplemental grain inside the milking parlor and fed some supplemental silage. Manure was collected at each dairy from the holding pen outside of the milking parlor.

Sample Collection and Treatments. The manure for the study was collected from the milking parlor holding pens at each dairy within 2 hours of completion of morning milking. Manure was manually collected and compiled from the holding pen with a scraper. After collection, manure was thoroughly mixed. Approximately 210 qt of manure were collected from each dairy and used to fill nine separate 24 qt plastic containers with lids. Each container was filled with ~23 qt of manure. The manure was then immediately transported to temperature controlled storage; reaching storage within 3 hours after collection.

After arrival at the storage location, six manure samples, three composite samples from each dairy, were collected and submitted for analysis to determine *E. coli* levels at the initiation of the trial period. The initial samples were used as the baseline *E. coli* concentrations (d 0).

The containers from both dairy F and dairy P were separated into three groups of three replicates each. Each group of replicates from the two dairies was assigned to one of three temperature treatments. The control treatment was set at a "room temperature" of 73 °F. The second treatment was stored at 95 °F, and the third treatment was subjected to a greater temperature of 111 °F. Manure samples were stored at their respective assigned treatments within 3 hours of collection, where they were held for 21 days.

Containers for each replicate were 24 qt plastic storage boxes, with loose fitting lids to allow for venting of gases. Room temperature replicates were stored in an air conditioned laboratory, and medium and high temperature replicates were stored in large, electric temperature-controlled incubators. Individual containers were stored at the same temperature throughout the study.

On d 10 and d 21, each manure container was opened and thoroughly mixed. After mixing, 8 oz of manure from each container was collected and submitted for analysis.

Sample Analysis. All analysis of manure was done by Midwest Labs in Omaha, Nebraska. After collection, manure samples were immediately cooled to 39 °F. The 8 oz manure samples were packed in temperature controlled containers and sent next-day delivery to insure that samples were available for analysis within 24 h of collection.

Manure samples were analyzed for *E. coli* with 3M™ Petrifilm™ E. coli/Coliform count plates (AOAC Official Method 991.14; 3M Microbiology Products, St. Paul, MN) using 25 g of manure. A composite sample from each dairy was analyzed at the end of the study to determine the nutrient content of the manure.

Samples collected during the initial manure collection were also used to test for the presence of *E. coli* O157: H7. Manure samples (25 g) were analyzed using the RapidCheck® *E. coli* O157 Test Kit (Part Number 7000160 and Part Number 7000162; Strategic Diagnotics, Inc., Newark, DE) to determine the presence of *E. coli* O157:H7.

Statistical Analysis. The study was arranged in 2 x 3 factorial design with repeated measures. Numbers of *E. coli* were measured in colony forming units (CFU)/g. These populations were transformed using logarithms to achieve normal distributions. The data were analyzed using the Proc Mixed procedure of SAS (SAS Institute, 2004). The simple covariance structure resulted in the lowest Bayesian Information Criterion and was used as the covariance structure. The repeated statement was included to analyze the differences over time. Differences were declared significant when $P < 0.05$, as determined by the least squares means test. Statistical analysis of bacteria populations is reported in logarithmic form.

RESULTS

Initial physical characteristics of the manure from the two dairy operations appeared to differ at the time of collection. Manure from dairy F appeared to be drier, more fibrous, and had visible signs of whole grain sorghum in the manure. Manure from dairy P appeared to have a greater moisture content, possibly due to the water sprinkler system that was in use in the cattle holding pen for cow cooling at dairy P. Moisture and nutrient analysis of the manure samples at the end of the study are shown in Table 1. Average concentrations of *E. coli* at the start of the study were 5.53 log CFU/g for dairy F and 5.14 CFU/g for dairy P.

The initial manure samples from dairies F and P were analyzed for the presence of *E. coli* O157:H7. Analysis for *E. coli* O157:H7 in the manure samples did not return any positive results, with *E. coli* O157:H7 concentrations below the detection limit of 0.04 cells/g for the manure samples from each of the dairies.

Table 1. Moisture and nutrient composition of manure samples from dairy P and dairy F.

Parameter	Dairy P	Dairy F
Total Solids, %	21.0	22.1
	---------------DM Basis------------	
Ash, %	23.05	18.55
Total Nitrogen, %	3.48	4.34
Ammonium Nitrogen, %	1.00	0.77
Organic Nitrogen, %	2.48	3.57
Phosphorus, %	1.33	1.13
Potassium, %	3.43	4.52
pH	7.6	6.5

The differences between the treatments at each sampling date for dairy F and dairy P are shown in Tables 2 and 3, respectively. On d 10, *E. coli* concentrations in the manure from dairy F stored at 95 ºF were significantly less than *E. coli* concentrations in the manure stored at 73 °F. However, *E. coli* concentrations in the manure stored at 73 °F from dairy F actually increased as compared to concentrations at the start of the study, whereas there was a slight decrease in *E. coli* concentrations for the 73 °F treatment on d 10 for dairy P.

Table 2. Concentrations of *E. coli* (log CFU/gram) of manure samples at dairy F.

	Treatment			
Day[*]	73 ºF	95 ºF	111 ºF	SE
0	5.53	5.53	5.53	0.28
10	6.28[a]	4.89[b]	ND[c]	0.28
21	3.87[a]	0.63[b]	ND[b]	0.28

[*]Differing superscripts within row indicate significant differences ($P < 0.05$).
ND – *E. coli* were below detectable levels (< 0.3 log CFU/g).

Table 3. Concentrations of *E. coli* (log CFU/gram) of manure samples at dairy P.

	Treatment			
Day[*]	73 ºF	95 ºF	111 ºF	SE
0	5.14	5.14	5.14	0.28
10	4.61[a]	4.21[a]	0.51[b]	0.28
21	3.51[a]	ND[b]	ND[b]	0.28

[*]Differing superscripts within row indicate significant differences ($P < 0.05$).
ND – *E. coli* were below detectable levels (< 0.3 log CFU/g).

In this study, *E. coli* concentrations of manure stored at 111 °F declined significantly more quickly than manure stored at either 73 °F or 95 °F. By d 10, *E. coli* was only detectable in one replicate from dairy P, and the concentration in that sample was 1.52 CFU/g. At d 21, *E. coli* was not detectable in any of the samples stored at 111 °F. In contrast, *E. coli* was still present in all of the manure samples stored at 73 °F. However, even the samples stored at 73 °F showed a significant decline in the concentrations of *E. coli* over the 21 d period (Figure 1).

DISCUSSION

The lower concentrations of *E. coli* in manure at dairy P were partially accounted for by the difference in moisture concentration between the two farms. The differences in the manure nutrient, moisture, and bacteria concentrations were most likely a result of the different management styles of the two dairy operations, as dietary feed ingredients, housing, and animal management varied between the two dairy operations. Overall, the *E. coli* concentrations in the fresh manure samples were similar to previously reported values as Meals and Braun (2006) reported *E. coli* concentrations averaging 5.7 CFU of *E. coli*/g of wet dairy manure for samples collected in June in Vermont. The varying moisture or nutrient content of the manure from the two dairies may have played a roll in the greater sustained growth of *E. coli* in the manure from dairy F stored at 73 °F.

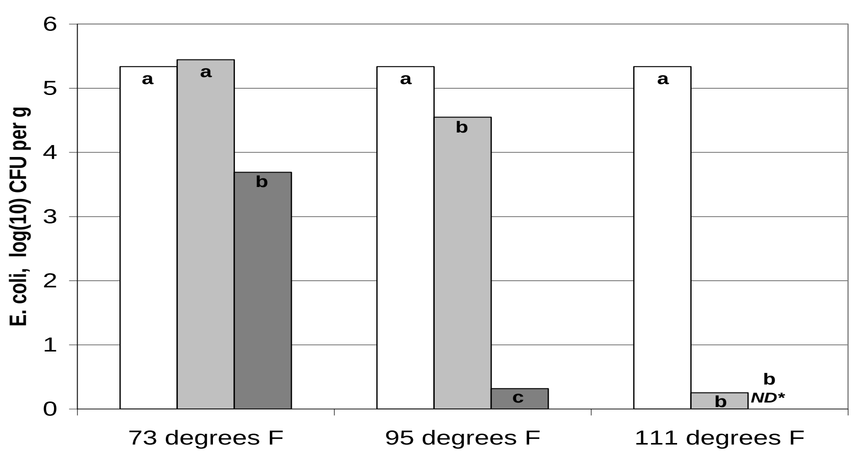

Figure 1. Average concentrations of *E. coli* in dairy manure from two dairy operations on day 0, day 10, and day 21 when held at various temperatures. Day of Sampling: Day 0 (□), Day 10 ( ), and Day 21 ( ).
*ND - *E. coli* were below detectable levels (< 0.3 log CFU/g) at day 21.
abcBars with different letters at a given temperature indicate significance at $P < 0.05$.

With increased time and/or increased heat, the survivability of *E. coli* decreased significantly in this study. When the temperature of the manure was held at 111 ºF, *E. coli* concentrations in manure declined quickly and were not detectable in most of the manure samples within 10 d. The decline in *E. coli* at 73 ºF occurred without exposure to solar radiation or drying effects that would be expected in a natural environment. When the storage temperature of manure increased, the mortality rate of *E. coli* increased significantly.

Escherichia coli O157:H7 is the strain implicated in most human health concerns. In this study, *E. coli* O157:H7 was below the detection limit of the assay and was not detected in the manure samples. According to Vinten et al. (2002), *E. coli* O157 appears to be equal to or more susceptible to environmental stress than generic *E. coli* and less likely to be present in runoff from land where agriculture slurry may be applied.

The Environmental Protection Agency uses generic *E. coli* bacteria as the criteria to determine when the levels of fecal contamination in surface waters need to be addressed, regardless of the strain of *E. coli* present. Animal derived *E. coli* survivability is dependent on the environment in which it must reside. This study concludes that temperatures of 95 °F and 111 °F will create conditions that increase the mortality rate of *E. coli* bacteria found in dairy manure. Since air temperatures in summer months in Texas commonly exceed 100 °F (ARS, 2009), summer air temperatures would assist in reducing concentrations of *E. coli* in dairy manure.

Recommendations for manure management practices concerning the timing of the application of dairy manure to cropland or pasture in Texas may need to be revisited. In Texas or other areas with hot climates where *E. coli* is a concern, application of manure when ambient temperatures are at the greatest may be a management practice that facilitates reductions in *E. coli* concentrations. Recommendations for manure application during high temperatures are in contrast to past studies (Miller et al., 2003) that imply cooler temperatures will reduce the rate of *E. coli* multiplication. Elevated air temperatures, along with solar radiation (LeJeune et al., 2001; Berney et al., 2006), may reduce *E. coli* populations in manure, thereby reducing the amount of viable *E. coli* that could be transported to nearby water sources. The results of this study indicate recommendations for manure application should be based on the climate of the application location.

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