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Heather Bayko
hbayko@utk.edu

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Determining the Effect of Seasonal Factors on the Shedding Patterns in Calves of two Strains of Food Borne Pathogenic *E. coli*

Heather Bayko, Spring 2011 Graduate

CASNR, Animal Science

University of Tennessee Knoxville, Chancellor's Honors Program

Faculty Mentor: Dr. Alan Mathew

Introduction:

Shiga toxin-producing *Escherichia coli* (STEC) is an emerging infectious food-borne pathogen that has been associated with significant and sporadic human disease outbreaks worldwide. A STEC infection can be present either asymptomatically or symptomatically - causing diarrhea, hemorrhagic colitis (HC) or hemolytic-uremic syndrome (HUS) which in serious cases can lead to death (Padola et al., 2002). Cattle are generally asymptomatic carriers, shedding the pathogen through their feces; this in turn serves as a reservoir to spread further the pathogen among cattle herds and the environment as well as slaughtering and processing facilities amounting to an important human health risk (Jaeger, 2000). STEC related illnesses have been traced to O157 and non-O157 STEC strains (Hussein, 2005). Although *E. coli* O157:H7 serotype has been commonly associated with HUS cases in the United States, more than 375 other shiga toxin-producing serotypes have been isolated worldwide (Padola et al., 2004). For instance, serotype O91:H21 has previously been isolated from foods and cattle in Argentina and studies have suggested *E. coli* O91:H21 to be more resistant to stress conditions than *E. coli* O157:H7 (Padola et al., 2004). One of the factors proposed to explain this phenomenon is climate condition; several international studies have shown O157:H7 serotype shedding to vary according to season with low shedding in winter, increased shedding in spring, peak levels during the summer months, then decreased shedding through late fall to eventually very low winter month levels. Human outbreaks of *E. coli* O157:H7 bacteria similarly mirror the seasonal shedding patterns in cattle. It is not known however, if similar patterns are seen for other STEC serotypes. The aim of this work is therefore to compare the shedding of *E. coli* O91:H21 and *E. coli* O157:H7 in experimentally inoculated calves and to determine how the temperature influences shedding patterns of these serotypes of bacterium in a controlled environment.

Objective:

To determine the impact of husbandry conditions such as temperature and humidity on the shedding patterns of *E. coli* O157:H7 and *E. coli* O91:H21.

Materials and Methods:

Escherichia coli organisms used in the trial were isolated, from commercially raised cattle in Argentina, by Dr. Nora Lia Padola and collaborators, and sent to Dr. Alan Mathew at the University of Tennessee for preparation as challenge organisms for the study. *Escherichia coli* strains, O157:H7 and O91:H21, were both STEC strains and were cultured to be resistant to nalidixic acid. This resistance was used to grow selectively the two strains when culturing the samples.

A total of eight approximately eight-week old dairy steers were obtained from The University of Tennessee AgResearch Dairy Herd in Spring Hill, TN and moved to JARTU Research facility in Knoxville, TN and maintained under simulated production husbandry conditions and dietary regimens in four identical rooms. Approximately 10 days following acclimation of animals to the JARTU facility, fecal samples were obtained via rectal swabs for bacterial analysis of any pre-existing resistance to nalidixic acid (this was noted as day zero of the trial). Experimental room conditions were set in each room. Two rooms provided a temperature of 65°F for 12 hours and 59°F for 12 hours (cool rooms), and two rooms provided a temperature of 90°F for 12 hours and 80°F for 12 hours (warm rooms). Cool rooms were hosed down with water twice daily throughout the trial to simulate damp conditions. Room light was provided during the 12 hour warmer phase for each treatment room. The steers were randomly assigned to the four rooms with each room receiving two calves and one combination of temperature range and one challenge strain.

Next, animals were orally inoculated with 10^{10} CFU of *E. coli* via nasoesophagial tube, delivered in a total of 200 mL of Luria Bertani broth (this was noted as day one of the trial). Thus, four animals were inoculated with O157:H7 (two in a cool room and two in a warm room) and four animals were inoculated with O91:H21 (two in a cool room and two in a warm room).

Beginning on day two, fecal samples from the calves housed under warm and cool conditions were obtained once daily on alternate days through the remainder of the study - 14 days. Approximately two grams of each fecal sample were diluted 1:4 in 1x phosphate buffered saline (PBS) and vortexed. Mixtures were maintained at room temperature for 30 minutes for precipitation of solid matter. The

supernatant was then serially diluted, and 100ul of each dilution was spread on Sorbitol MacConkey plates containing 50ug/mL nalidixic acid. Plates were incubated at 37°C overnight. Based on the ability to ferment sorbitol O157:H7 grew as white colonies on the plates and O91:H21 as red colonies – therefore enumeration data was recorded for each strain from the respective room.

Coveralls, gloves, boots, hair nets, face masks, and goggles were worn during collection of samples. Coveralls were changed and boots were disinfected in a boot wash between rooms of the two challenge strains to prevent contamination.

On day 15, animals were moved one at a time to a separate room in JARTU where they were euthanized by lethal injection (Beuthanasia 1mL/ 10 lb BW).

Results:

After analyzing fecal samples collected from the eight animals on day zero, one of the animals in a cool room, inoculated with the O91:H21 strain, on day zero was seen to have preexisting nalidixic acid resistant bacteria which showed similar phenotype (red colony) on Sorbital MacConkey agar containing 50ug/mL nalidixic acid as that of O91:H21; therefore further data from this animal was excluded. Samples collected on day zero from the remaining seven calves showed to be clear of nalidixic acid resistant bacteria and data from these animals were used in the trial.

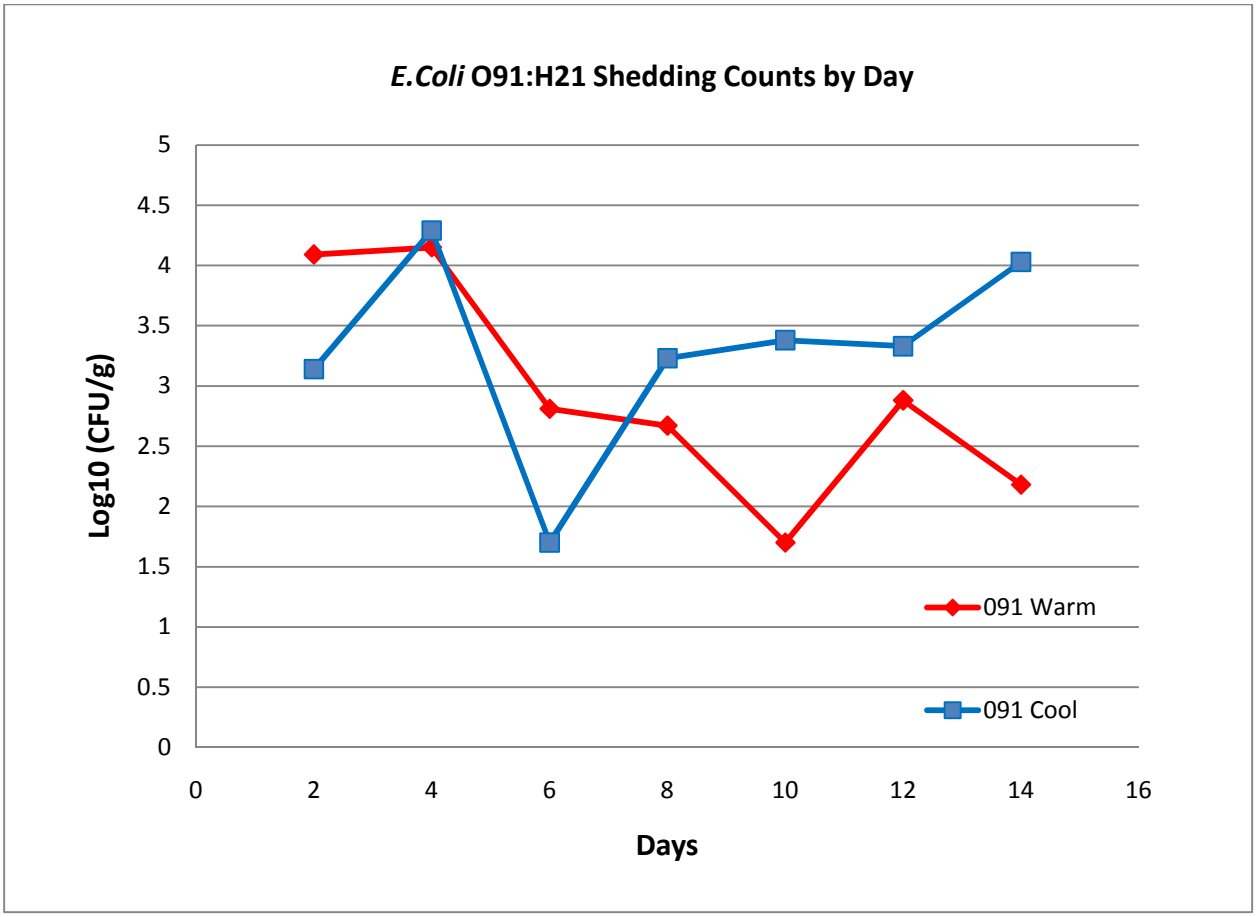
Samples collected from one of the animals in a cool room, inoculated with O157:H7 indicated contamination beginning on day 10 – phenotypes of the bacteria growing on the plates showed to be a mix of white and red/pink colonies. Therefore data from this animal was excluded from day 10 to day 14.

Enumeration data was recorded for each serial dilution for each animal on each day sampled and was then averaged. After averaging the data for the two animals in each room, the average CFU/g of either *E. coli* O91:H21 or *E. coli* O157:H7 for each room on each day was then found. This data is shown in table 1 below. Graphical representation of data in Table 1 can be seen in graph 1 and graph 2 below.

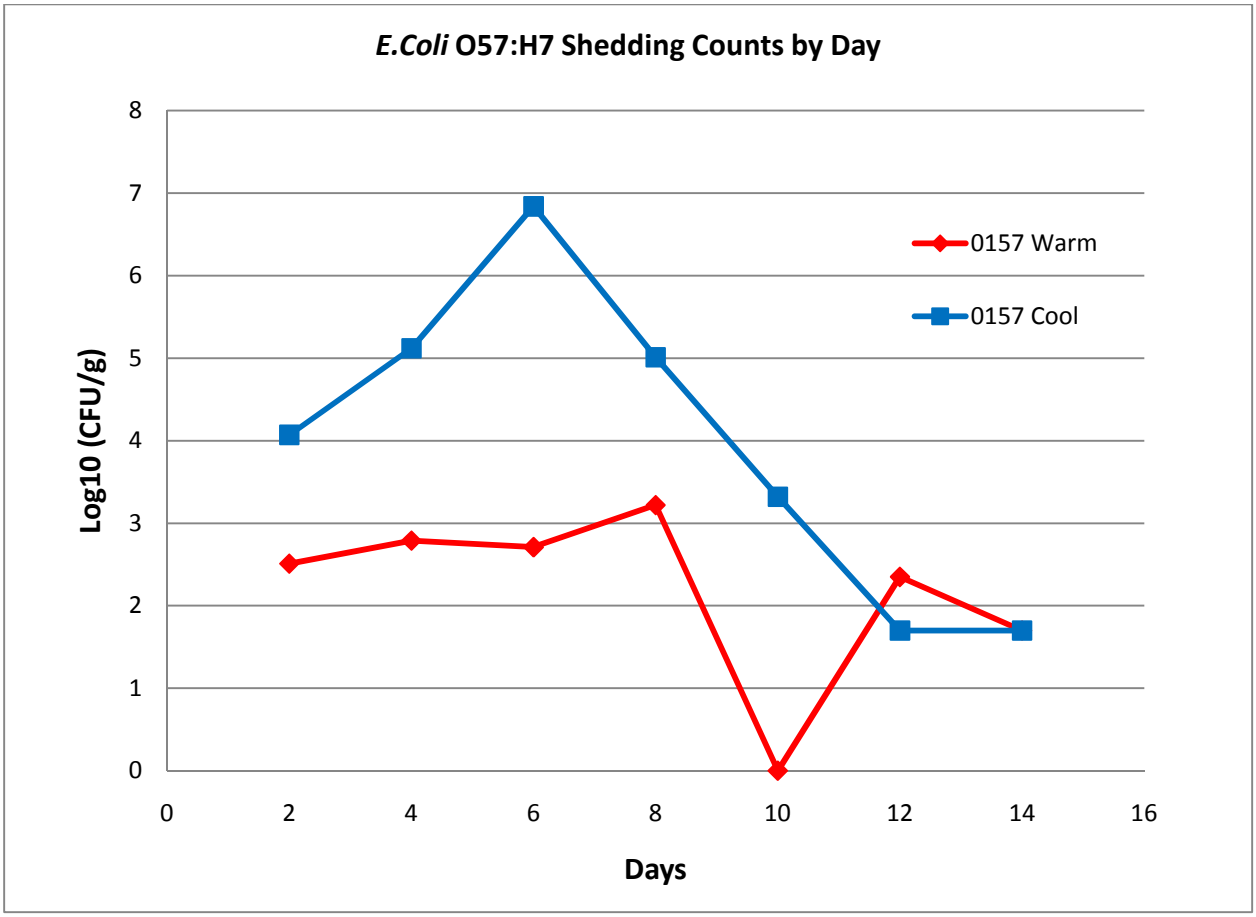
Table 1

	O91:H21				O57:H7			
	Warm Room		Cool Room		Warm Room		Cool Room	
	Average CFU/g	Log10	Average CFU/g	Log10	Average CFU/g	Log10	Average CFU/g	Log10
Day 2	$1.23x10^4$	4.09	$1.38x10^3$	3.14	$3.25x10^2$	2.51	$1.17x10^4$	4.07
Day 4	$1.41x10^4$	4.15	$1.93x10^4$	4.29	$6.13x10^2$	2.79	$1.33x10^5$	5.12
Day 6	$6.5x10^2$	2.81	$5.0x10^1$	1.7	$5.13x10^2$	2.71	$7.07x10^6$	6.84
Day 8	$4.63x10^2$	2.67	$5.0x10^1$	3.23	$1.68x10^3$	3.22	$1.03x10^5$	5.01
Day 10	$5.0x10^1$	1.7	$2.43x10^3$	3.38	0.0	0	$2.10x10^3$	3.32
Day 12	$7.5x10^2$	2.88	$2.15x10^3$	3.33	$2.25x10^2$	2.35	$5.0x10^1$	1.7
Day 14	$1.5x10^2$	2.18	$1.08x10^4$	4.03	$5.0x10^1$	1.7	$5.0x10^1$	1.7

Graph 1


E.Coli O91:H21 Shedding Counts by Day
Log10 (CFU/g)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
16
Days
091 Warm
091 Cool

Graph 2


E.Coli O57:H7 Shedding Counts by Day
Log10 (CFU/g)
8
7
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
Days
0157 Warm
0157 Cool

Discussion:

After comparison of shedding levels of both strains between the two temperature conditions, no clear difference can be observed for the O91:H21 strain. Looking at graph 1, from approximately day seven to day 14, higher numbers of CFU/g were seen for the animals in the cool room; however, seeing that data from day two to day seven varied so greatly, no strong conclusions can be drawn regarding the seasonal effects on the shedding pattern of *E. coli* O91:H21.

Regarding the O157:H7 strain, from graph 2 it can be seen that animals in the cold room shed two to three log units of bacteria higher than those animals in the warm room during the first 10 days of the trial. Individual variation of the animals in the study however can vary greatly, thus, with only eight animals used in just one trial if just one individual varies more than slightly from the others, data can be skewed incorrectly. For instance, the test strain was only detected in two sampling days near the beginning of the trial in one of the calves that was inoculated with *E. coli* O157:H7 in a warm room. Thus, enumeration numbers were zero for the days that the strain was not seem in the sample. That animal was observed to have watery diarrhea and coughing throughout the majority of the trial, which may have contributed to the undetectable shedding since bacteria is more diluted in higher contents of liquid. This most likely lowered the average number of CFU/g for that room for the days the challenge strain was not detected, ultimately skewing the data to have it appear that a larger difference of shedding of challenge bacteria was present between the two temperature ranges for the O157:H7 strain.

Furthermore, for the limited number of days a difference in shedding levels of bacteria for each strain between the hot and cold rooms can be seen on the graphs, the data showed a pattern different than originally expected with the number of CFU/g being higher in the cold rooms when compared to the warm rooms. From previously established experimental data, it was expected to see higher CFU/g number from animals in the warm rooms than the cold rooms in this experimental trial.

Analysis of the data overall showed great variation among both strains of *E. coli* in both temperature conditions and thus the determination of the effect of seasonal factors on the shedding patterns of *E. coli* O91:H21 and 057:H7 cannot concretely be determined from this experiment.

Conducting only one trial, animal numbers were very limited and results can be greatly affected by individual variations. Furthermore, since only one trial was conducted, statistical analysis was not conducted as it would have shown little meaning to the results. Combined with experimental error that may have been present in the sampling or enumeration of data, it is possible that using a different antibiotic resistant marker may have proven more successful since other intestinal bacteria can exhibit similar resistance features and phenotypes to those of the experimental challenges. It is certain that more data from additional trials – three or more – would provide a better picture of the impact seasonal factors have on the shedding of these two strains of *E. coli*.

Acknowledgements and Remarks:

Many thanks to the department of Animal Science at the University of Tennessee, Knoxville, for allowing me, to help with the larger research project being conducted under the direction of Dr. Alan Mathew and his graduate student and to develop a senior honors thesis from the work being done. I was able to use microbiology techniques learned in lab and apply them to real life research and develop a protocol to evaluate an objective of the study that interested me.

The *E. coli* strains used in the study were not pathogenic to cattle, but are pathogenic to humans if consumed in significant quantities on contaminated meat for instance that has not been properly cooked. I saw this experimental trial as an attempt to evaluate a factor (seasonal conditions) that if understood fully could greatly benefit human health and play a large role in controlling infectious food borne disease outbreaks worldwide.

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