

THESIS

LONGITUDINAL ANALYSIS AND CHARACTERIZATION OF ESCHERICHIA COLI
O157:H7 SHEDDING IN DAIRY CATTLE IN NORTHERN COLORADO

Submitted by

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ABSTRACT

LONGITUDINAL ANALYSIS AND CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 SHEDDING IN DAIRY CATTLE IN NORTHERN COLORADO

Escherichia coli O157:H7 (STEC) is an enterohemorrhagic Gram-negative bacteria that is a common source of foodborne illness around the world. Annually, O157 is responsible for approximately 100,000 cases, 3,000 hospitalizations, and 90 deaths in the United States, and has been diagnostically confirmed on every continent except for Antarctica. Dairy cattle serve as asymptomatic carriers of the O157 bacteria, maintaining a continuous cycle of reinfection through their environment, and have been implicated as a potential source of contamination of the food chain. Gathering data on prevalence and shedding cycles of O157 in dairy cattle can provide insight into the scope of the problem and potential mitigation strategies.

The primary objective of this study was to investigate the association between shedding status on a randomly selected day and- shedding on subsequent consecutive days (n=4), daily proportions and patterns of shedding, and how shed status on one day affects shed status on the next day. Two local Northern Colorado dairies were selected for study. Fecal samples were taken from 25 cows from Dairy A and 49 cows from Dairy B and tested for presence of the O157 pathogen. Based on those results, twenty cows from each dairy were randomly chosen for the study, with 10 “shedders” (i.e. cows that tested positive for O157 on Day 1) and 10 “non-shedders” (i.e. cows that tested negative for O157 on Day 1) selected from each dairy for a total of forty study subjects. The cows were then resampled once daily for an additional four days, testing for rfb, stx1, stx2, and eae genes as well as collecting overall health information. Health

information variables were dichotomized based on scoring systems and logistic regression, generalized linear models, and generalized linear mixed models were used for analysis of research questions.

Our study had three main aims and five research questions of interest. Our first aim was to analyze overall shedding events, split into two research questions. First we wanted to know if shedding status on Day 1 was associated with shedding on any subsequent day. We used a logistic regression model with any subsequent shedding as the outcome and Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score as the covariates. Next, we wanted to know what risk factors were associated with cumulative days of shedding. For this question we used a generalized mixed model with a poisson regression. The count of total shedding days was used as the outcome variable and Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score were the covariates.

Additionally, we aimed to analyze day-to-day shedding patterns within the cattle cohort so see if shedding status on one day was associated with shedding status on the next day. First we used a generalized linear mixed model to compare paired days, specifying Day 1 vs Day 2, Day 2 vs Day 3, Day 3 vs Day 4, and Day 4 vs Day 5. The outcome variable was daily shedding status and the primary risk factor was shedding status on the stated previous day, with additional variables including Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score were the covariates. We then used a generalized linear model with a logit link to assess the overall association between day-to-day shedding patterns averaged over the five-day study period, with the outcome variable as daily shedding status. The primary risk factor was shedding status on the previous day, with additional variables

including Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score were the covariates.

Our last research question aimed to assess the associations between our risk factors of interest and daily shedding status, as well as daily shedding patterns. We used a generalized linear model with a logit link to model risk factor associations, with the outcome variable being daily shedding status and the risk factor variables including Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score. We then used proportion testing to assess the differences in proportions of gene and shedding prevalence between Day 1 Shedders and Day 1 Non-Shedders.

Initial shedders had a higher proportion of daily shedding than non-shedders during every sample day, 60% vs 35% on Day 2, 60% vs 45% on Day 3, 50% vs 30% on Day 4, and 45% vs 35% on Day 5, however none of these were statistically significant. Shedders similarly also had a higher overall prevalence of targeted O157 genes than Non-Shedders; 20% vs 10% for Stx1, 35% vs 30% for Stx2, and 30% vs 20% for eae. There were no significant differences in gene prevalence between cows from Dairy A and cows from Dairy B for Stx1 or eae, but there was for Stx2; 15% for both groups for Stx1, 25% vs 40% for Stx2, and 25% for both groups for eae.

Cows in the Shedder cohort were twice as likely to shed O157 on any subsequent sampling day than non-shedders based on logistic regression analysis (OR 2.0, 95% CI: 1.1,3.8). Day 1 shedding status ($p < 0.0001$), fecal score >3 vs 3 ($p 0.02$), and temperature ($p 0.04$) were significantly associated with an increase in cumulative days of shedding. Day 1 shedding status was also a significant predictor of daily shedding status (OR: 1.7, 95% CI: 1.1,2.5). Interestingly, shedding status on one day was not significantly associated with shedding status on the next day, whether looking at specific days (Day 1 vs Day 2- OR: 1.6, 95% CI: 0.4,2.5; Day 2 vs Day 3-

OR: 1.5, 95% CI: 0.2,1.8; Day 3 vs Day 4- OR:1.8, 95% CI: 0.6,4.0; Day 4 vs Day 5- OR: 1.6, 95% CI: 0.3,2.2) or averaged over the 5 day study period (OR: 1.5, 95% CI: 0.9,2.3).

Overall, we found inconsistent and transient shedding patterns among all of our cohorts, which is similar to findings in past literature. Day 1 shedding status was the only variable consistently found to be associated with any subsequent shedding. Although Day 1 Shedders had a higher daily proportion of shedding throughout the entire study period than Day 1 Non-Shedders, these results were not statistically significant. Past literature has said that shedding cycles likely last between two and six days, but we found that shedding status on one day was not associated with shedding status on the next day, whether looking at pair of days or averaged over the five-day period. The inconsistency in our results calls in to question whether shedding patterns are truly transient acts or whether the sampling methods used potentially misclassify Shedders as Non-Shedders.

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headed, and for the many reminders that life outside grad school exists. To my Dad, thank you not only for your financial support but for pushing me to do my best, supporting my dreams, and regaling me with stories from your own grad school experience. And finally to my boyfriend Nick, thank you for putting up with me throughout the last two years of this program. You've listened to me ramble about *E.coli*, cow poop, effect modification, DAGS, SAS, R, exams, homework, projects, models, grant submissions, internship applications, and so many other things, and you've *barely* complained (key word barely). You keep me grounded in reality and excited for the future, and for that I will be eternally grateful.

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CHAPTER 1

INTRODUCTION

Overview

This chapter summarizes the motivation for this study and provides background information which supports the need for the research study. The introduction also includes a statement of purpose, the study aims and hypotheses, as well as the scope of the study.

Background

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC) is a public health problem, resulting in approximately 100,000 cases, 3,000 hospitalizations, and 90 deaths of human infection in the U.S. on an annual basis.^{1,2} O157 was first detected as a food-borne human pathogen in the United States in 1982, and has since been found in more than 50 countries and on every continent except for Antarctica.¹⁶

Dairy cattle have been implicated as a likely source of food chain contamination given their unique involvement in many different food commodities. STEC colonize in the digestive tracts of dairy cattle, who act as asymptomatic carriers of the pathogen, and are then shed in feces where they are dispersed and recolonize in the surrounding environment including manure, soil, water, and air.^{3,4,5,6} *E. coli* infections are maintained in the cows through continuous reintroduction of the bacteria in the fecal-oral route. The human health burden is propagated through exposure of dairy farm workers and food product infection through slaughtered cows, contaminated agricultural land, and dispersal of aerosolized dust on farms.^{6,7}

Currently, there is limited understanding of shedding cycles for cows. Researching shedding cycles in O157 reservoirs and gathering data on risk factors that escalate the potential

for human health implications can provide insight into the scope of the problem and potential mitigation strategies.

Purpose of the Study

The purpose of this study was to longitudinally analyze and characterize O157 shedding patterns in dairy cattle in Northern Colorado. Our specific research questions aimed to determine the association between shedding status on a randomly selected day and any subsequent shedding, the association between shedding status on one day and shedding status on the next day, daily patterns of shedding, and individual health variables associated with shedding status. The aim of these research questions was to gain a better understanding of daily shedding patterns and factors that influence overall shedding status to acquire foundational knowledge of this specific O157 reservoir. Gaining more accurate information about shedding dynamics in dairy cattle could promote more effective mitigation strategies for downstream human health effects and occupational safety policies for dairy workers.

Research Questions and Methods

Aim 1A: Is shedding status on Day 1 associated with shedding on any subsequent day?

To answer this research question we used a logistic regression model with the outcome variable as any shedding between Day 2 through Day 5, and risk factor variables including Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score.

Aim 1B: What risk factor variables are associated with cumulative days of shedding?

We used a generalized linear model with a Poisson distribution to analyze the counts of cumulative days of shedding in comparison with risk factor variables including Day 1 shedding

status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score.

Aim 2A: Does shedding status on one day influence shedding status on the next day, by specific day?

We used a generalized linear mixed model with the outcome variable as shedding status on one day and the primary risk factor variable as shedding status on the previous day, separating by specific day-pairs. Additional variables in the model included Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score.

Aim 2B: Does shedding status on one day influence shedding status on the next day, averaged over the five-day study period?

We used a generalized linear model with a logit link with the outcome variable as daily shedding status and the primary risk factor variable as shedding status on the previous day. Additional variables included Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score.

Aim 3: What risk factors variables are associated with daily shedding status and daily proportions of shedding?

We used a generalized linear model with a logit link with the outcome variable as daily shedding status and the risk factor variables including Day 1 shedding status, dairy, parity, temperature, days in milk, body condition score, hygiene score, and fecal score. We then used proportion tests to assess statistical significance of shedding and gene proportions between Shedders and Non-Shedders.

Scope of the Study

The data for this study was collected during a larger One Health project that examined animal samples, environmental and occupational health data as well. Previous studies have investigated shedding patterns in young cows and targeted enteropathogenic genes present in cattle carrying O157, but prevalence estimates and shedding patterns have shown to be inconsistent throughout the literature.^{18,20,21} Two Northern Colorado dairies were chosen as the study site and samples were collected over a five-day sampling period. Our study targeted fresh cows, defined as cows that had given birth within the last 21 days before the study began, to sample because this group has been shown to have a higher susceptibility for pathogens, including O157.⁶²⁻⁶⁶ Fecal samples were collected once daily for five days and individual health information such as body condition score, temperature, fecal score, and days in milk were collected for each cow. We then used various regression analyses and proportion testing to analyze the association and trends.

CHAPTER 2

LITERATURE REVIEW

Overview

This chapter gives an in-depth overview of the literature related to the various components of this study. This includes background information on *Escherichia coli* O157, targeted O157 genes, the human health concern related to O157, history and evolving trend of O157 in the agricultural industry, and material about dairy cattle and their role in the problem.

Escherichia coli O157:H7

Escherichia coli O157:H7 is an enterohemorrhagic Gram-negative bacteria that has been associated with a wide spectrum of human pathogenic manifestations such as non-bloody diarrhea, hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura.²⁶ In addition to these more severe clinical expressions, O157 is also one of the foremost causes of bacterial diarrhea in the world. Annually, O157 is responsible for 100,000 cases, 3,000 hospitalizations, and 90 deaths in the United States, and has been diagnostically confirmed in 50 countries and on every continent except for Antarctica.¹⁶ *E. coli* O157 was first detected as a pathogen affecting humans in 1982 after a hemorrhagic colitis outbreak in the United States, and has since been a consistent source of diarrheal illness. In 1993, a multistate outbreak of O157 linked to undercooked beef patties from a fast-food chain exposed O157 as an important source of foodborne illness and initiated more thorough public health action plans such as recalls and food safety information.^{33,36}

O157 is most commonly transmitted through direct or indirect contact with infected feces, through contaminated or undercooked food, close proximity to an infected person, water,

animal contact, or less frequently through laboratory exposure.^{32,33} Uncooked meat such as ground beef, unwashed vegetables including lettuce or slaw, and raw dairy products comprised an estimated 50-60% of outbreaks in the United States, with waterborne cases accounting for around 15% of outbreaks, and direct animal contact accounting for 5% .^{33,34,36}

O157 Genes and Serotypes

Escherichia coli O157:H7 is an enteric pathogenic strain of *E. coli* that produces verocytotoxin (VTEC) and shiga toxin (STEC), which are highly pathogenic organisms capable of human illness.¹⁶ VTEC is a newer pathogen commonly reserved in the intestinal tracts of animals or in the food of animals that manifests as diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome in people.³⁹ VTECs cause damage to endothelial cells in the small intestinal, kidney, and brain small vessels and cause thrombotic microangiopathy, which is the appearance of thrombosis in capillaries and arterioles. The bacterial adhesins recruit colonization and begin attachment, which results in the translocation of effectors that alter the structure and function of host cells.^{40,41} STECs are a similar serotype of O157 to VTECs in that they can cause diarrhea, gastroenteritis, hemorrhagic colitis, and hemolytic uremic syndrome in humans.^{42,43} STEC and VTEC differ in that STECs produce shiga toxins, and VTECs are functionally active shiga toxins that are detected using Vero cell toxicity tests.⁷⁷

Within strains of O157 are also several genes that have varying clinical and human health significance. Shiga toxins 1 and 2, also called stx₁ and stx₂, are primary virulence gene factors indicative of STEC located in lambdoid bacteriophages that are then integrated into the host's bacterial genome.⁵² These genes are implicated as the cause for illnesses relating to O157 including hemolytic uremic syndrome (HUS) and hemorrhagic colitis. The O antigen (rfb gene) cluster is an identifier of O157 strains and has been used by past research to classify positivity in

tests. The O side chain is often what starts the bactericidal humoral immunity in infected hosts, but previous research has suggested that this antigen can be under strong selective pressure to avoid the host immune response.⁵³ Another gene of interest in our study was the *eae* gene, which is responsible for producing the 94-kDa outer membrane protein, intimin, to produce the attaching and effacing lesion to epithelial cells.^{55,56}

Detection

After the 1982 identification of O157 as a human pathogen in the United States, scientists began pursuing methods for quick and reliable detection in laboratory tests. Distinct from other *E. coli* strains, O157:H7 does not ferment sorbitol, and the overall frequency of non-sorbitol-fermenting (NSF) species in feces is low.^{35,37} A 1986 study found that use of MacConkey agar containing sorbitol (SMAC medium) for detection of O157 in feces produced poorly defined clear cultures whereas traditional MacConkey agar cultures produced distinguishable results.³⁵ During the 1993 outbreak of O157 in beef patties, researchers used pulsed-field gel electrophoresis to identify the O157 strain and match it between cases to establish commonality.³⁶ A 1998 study from researchers in Australia developed two multiplex polymerase chain reactions (PCR) assays for the detection and genetic characterization of O157 in bacterial cultures from feces. The first assay utilized four PCR primer pairs to identify existence of *stx*₁, *stx*₂, *eae*, and enterohemorrhagic *E. coli hly*_A, as well as producing amplification products of 180, 255, 384, and 534 bp. The second assay used two primer pairs explicit for portions of the *rfb* regions of O157 and O111, creating PCR products of 259 and 406 bp.²⁹

In more recent literature and in our study, the gold standard for O157 detection has been enriching samples in buffered peptone water, then selective plating onto cefixime tellurite sorbitol MacConkey agar plates and incubating them at 37°C for 24 hours.³⁵ Colonies are then

confirmed by latex agglutination and PCR targeting the O-antigen (*rfb* gene) and shiga toxin genes, *stx*₁ and *stx*₂, as well as the *eae* gene.^{18,19,20}

Antimicrobial Resistance

Another emerging public health threat in direct relation to *Escherichia coli* O157:H7 is the increasing incidence of antimicrobial resistance (AMR). Although public health officials are more concerned with AMR in pathogenic bacteria such as O157, there is also concern for resistance in commensal bacteria in non-pathogenic bacteria like *E. coli*.³⁸ According to the CDC, more than two million people in the United States get sick every year due to antibiotic resistant infections and at least 23,000 die from them.⁵⁹ The emergence of so-called “superbugs” is an increasing trend and now outbreaks of bacteria that are resistant to last-resort antibiotics, such as Colistin, have begun appearing.⁶⁰ The development of antibiotics in the 20th century has had a major impact on quality of life, life expectancy, and reduced burden on the healthcare system, however, this progress is beginning to slip to the wayside. The most at-risk populations for antimicrobial resistant complications include the immunocompromised, the elderly, and children, however everyone is susceptible due to the ubiquitous nature of antibiotics in our society.

Although O157 is often connected to human health diseases such as diarrhea and HUS, in recent literature, a link between AMR O157 and inflammation related to respiratory health has been demonstrated.⁵⁸ This new research is beginning to show that AMR has larger health impacts than medicinal resistance and so-called superbugs, but has the ability to cause health effects not previously seen before.

Human Health Concern

Diarrheal illness related to *E. coli* has been a human health problem for many years and has only recently been more thoroughly understood by researchers. In the late 1960's and early 1970's American soldiers stationed in Vietnam were frequently experiencing diarrheal illness, and it was eventually reported to be caused by the *E. coli* bacteria instead of Cholera as originally thought.⁴⁷ In a 1987 study conducted among children in Mexico plagued by acute diarrheal illness, enteropathogenic *E. coli* (EPEC) was identified as the cause of more than 21% of cases.⁴⁸ Nursing homes and day care centers have also shown to be a common location of O157 outbreaks, with bloody diarrhea appearing as the indicator.⁴⁹ Diarrhea becomes clinically significant due to its ability to cause dehydration and demineralization in humans, both of which can lead to severe illness and even death if not treated.

Verocytotoxin-producing *E. coli* is a dangerous and potentially fatal bacterial infection with a wide range of clinical manifestations in people. Illnesses include hemolytic uremic syndrome (HUS), hemorrhagic colitis, diarrhea, and thrombotic thrombocytopenic purpura (TTP).²⁶ O157 is responsible for 100,000 cases, 3,000 hospitalizations, and 90 deaths in the United States on a yearly basis, although the number of cases is estimated to be higher due to non-reporting and underreporting.^{1,2}

Hemolytic uremic syndrome is a kidney disease that causes hemolytic anemia, acute kidney failure, and a low platelet count. HUS is the most common cause of acute kidney injury in children, and includes symptoms such as vomiting, bloody diarrhea, abdominal pain, fever, chills, and headache. More severe symptoms range from edema, albuminuria, hypoalbuminemia, blood in the urine, seizure, stroke, and coma. There are no specific therapies for HUS or vaccines

to prevent it, so treatment is usually involves IV hydration, platelet replacement, mineral replacement, and sometimes dialysis or transplantation.⁴³

Hemorrhagic colitis is a type of gastroenteritis most commonly diagnosed after the onset of bloody diarrhea and abdominal pain. Although more common in young children and the elderly, hemorrhagic colitis can occur in people of all ages.⁴⁵ O157 bacteria adhere to the intestinal mucosa and produce cytotoxins, which cause inflammation of the intestines and eventually bloody diarrhea. Similar to HUS, hemorrhagic colitis has no preventative treatment and is mitigated through supportive care.

Thrombotic Thrombocytopenic Purpura (TTP) is a rare blood disorder where blood clots form throughout the body in small blood vessels. These blood clots can cause blockages to the flow of oxygen-rich blood to vital organs including the heart, brain, and kidneys. O157 has been linked to TTP through the endothelial-cell injury and intravascular platelet aggregation propagated by pathogenic bacterial accumulation. Although TTP is rare disorder and it not a common side effect of O157 infection, it is a fatal and long lasting disease that can result in stroke or death.^{33,46}

Dairy Cattles' Role in the Problem

Dairy cattle have the unique ability to contaminate a multitude of food products and agricultural land as well as transmitting O157 to dairy workers due to their daily proximity. STEC colonize in the digestive tracts of dairy cattle, who act as asymptomatic carriers of the pathogen, and are then shed in cow feces where they are dispersed and recolonize in the surrounding environment including manure, soil, water, and air.^{3,4,5,6} The human health burden is propagated through exposure of dairy farm workers and food product infection through slaughtered cows, contaminated agricultural land, and dispersal of aerosolized dust on farms.^{6,7}

Our preliminary research indicates an O157 prevalence between 19-21% in dairy cattle herds and a 3-5% prevalence of the Stx₁, stx₂, and eae genes.^{17,58} These results demonstrate a likely source of downstream human effects, including those that have the ability to cause potentially life threatening diseases like TTP and HUS. Although non-pathogenic *E. coli* strains that are ubiquitous in agricultural animals such as dairy cattle have been found to be less resistant to antimicrobials, young calves have shown the opposite. Young calves often transmit *E. coli* resistant to numerous antimicrobials, and potentially serve as the origin of resistance genes for nearby bacteria.³⁸ While there has been a decline in the use of antimicrobials in animals in the agricultural industry over the last several years, the past overuse of these medicinal treatments has been implicated as a likely source of human-related AMR cases.

Hypothesized Shedding Risk Factors

During early lactation periods (1-21 days post-partum), female cattle are at an increased susceptibility for both metabolic diseases and infections, which can lead to increased O157 shedding.^{62,63,64} One biological pathway that involved in this association is immune responses to inflammation. During the birthing process and the first several weeks afterwards, female cattle experience cellular tissue damage and enlargement of vascular diameters which correspond to redness, heat, swelling, and pain. These inflammatory responses facilitate movement of leukocytes from the blood to the site of injury, which lessens overall immune responses.^{62,63}

Another reasonable pathway for increased incidence of pathogenic infections in early lactation cattle is the effect of hormone fluctuations on immune responses. During the early lactation period, cows release a significant number of reproductive, regulatory, and stress hormones from the anterior pituitary, which can have effects on other endocrine organs and the immune system. One example of this are glucocorticoids, which are steroid hormones known for

suppressing the immune system, delaying wound healing, and depressing numbers of circulatory lymphocytes.^{63,66}

In previous studies investigating the association between cattle health covariates and O157 shedding, certain risk factors have repeatedly been investigated and contradicting results have been found. Parity is described as the number of times a female cow has had an offspring. Increased parity has been shown to be associated with increased risk of several different pathogenic infections, including mastitis, O157, and *Salmonella*.^{67,68} One mechanism of explanation is that older cows tend to have higher overall numbers of lactations which leads to increased exposure to environmental pathogens. Older cows also tend to show a general increase in presentation of pathogens, likely due to hampered immune systems.⁶⁹ Another variable of interest related to parity was days in milk (DIM), which is the number of days since the birth of the cow's offspring. Past literature suggests that the higher number of days in milk, equivalent to further days past birthing, is considered protective against risk of O157 infection. This aligns with biological associations mentioned previously, such as the stress and injury involved in birthing a calf.^{68,70}

In addition to variables relating to calf birthing and stages of lactation, we also gathered information about several health factor scores; hygiene score, body condition score, and fecal score. Hygiene score is assessed on a four-point scale, measuring visual cleanliness of the head of the tail, upper rear limbs, the ventral abdomen, udders, and the lower rear limbs and averaging the scores into a single grade. Cows shown to be have at least 10% of their body covered in dirt or caked on dirt, hygiene score of three or four, have been revealed to be more likely to be infected with contagious pathogens than cows with less than 10% of their body covered in dirt, hygiene score of one or two.⁷¹ Body condition score (BCS) has also been an important health

variable related to disease prevalence in dairy cattle. BCS is assessed on a five point scale through physical and visual examinations, with a score of three serving as the ideal reference, scores of one or two indicating an underweight cow, and scores of four or five indicating an overweight cow. Underweight and overweight cows face hormone imbalances, metabolic problems, and decreased energy output which can lead to susceptibility to bacterial infection.⁷² The last scored variable indicated as a potential risk factor for O157 infection is fecal score, an evaluation of manure on a scale of one to six. Manure evaluation is comprised of three main parts; the color, the consistency, and the content.⁷³ These defining factors related to manure can often present information about diet and infectious or chronic disease, as well as overall herd health.

One additional variable we collected during sample collection was the rectal temperature of each cow, measured in degrees Celsius. Body temperature is a common measurement used in the assessment of overall health, as it indicates the cow's ability to balance heat gain and heat loss. The normal temperature of an adult cow is 38.5°C, with a temperature equal to or greater than 39.5°C indicating the presence of a fever.⁷⁸ A rise in temperature, congruent with a fever, is often an indication of inflammation or infection related to illness. A decrease in temperature can also be an indication of illness, and can sometimes be a sign in inability to fight off infection.⁷⁹ Temperature is an important variable to consider in relation to O157 shedding because cattle dealing with an illness may be more likely to shed O157 due to increased volume of fecal output, such as diarrhea, or in contrast, decreased O157 shedding due to decreased fecal output from reduced eating.^{78,79}

Summary

E. coli O157 is a pertinent public health problem that has shown to have serious consequences in human populations, especially for the very young and the very old. O157 has several different genotypes that can be present and some of these genes, such as shiga toxins and *eae*, have been implicated as the sources for serious health conditions including diarrhea, HUS, TTP, and hemorrhagic colitis. Dairy cattle have been shown to serve as asymptomatic carriers of the O157 pathogen and can directly infect their surrounding environment, dairy workers, and food products for human consumption. Early lactation cattle have been shown to harbor a higher prevalence of O157 due to dampened immune function and thus serve as a useful target population for study. Based on previous research, certain health factors such as fecal score, hygiene score, days in milk, and more have also been associated with increased risk of O157 infection.

CHAPTER 3

METHODS

Overview

This chapter describes the various methods involved in executing our study and provides rationale for the chosen approaches. The chapter contains field methods, lab methods, and statistical methods, including a sampling scheme diagram for clarity.

Field Methods

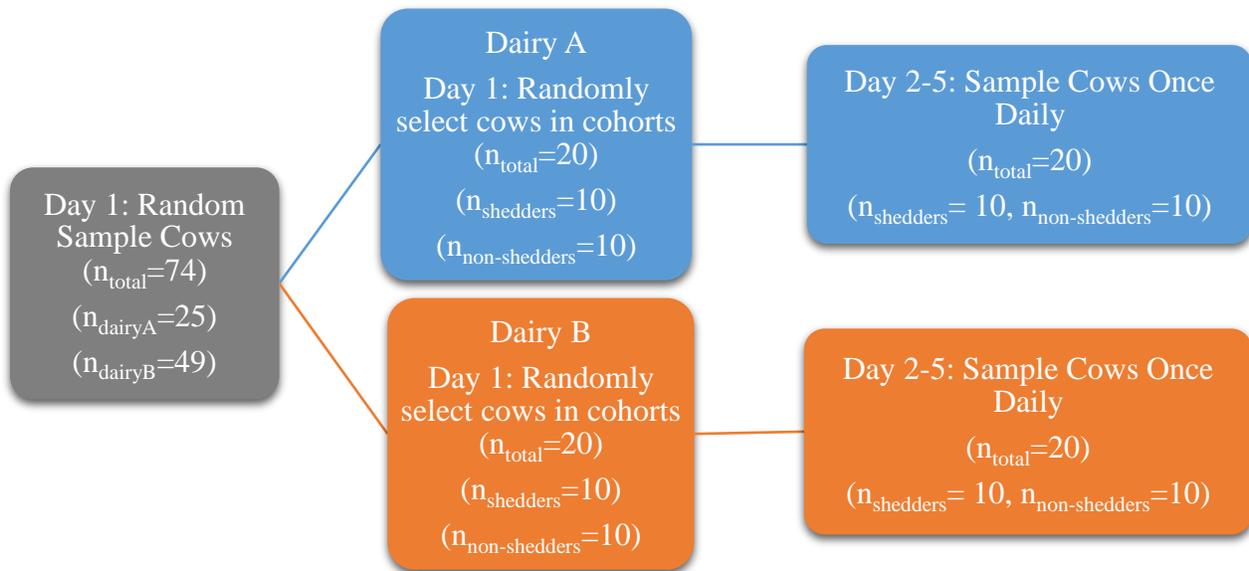


Figure 1 | Field Sampling Scheme

The study sites included two Northern Colorado dairies consisting of approximately 1,400 milking cows each. Early lactation cows ($n=25$ Dairy A, $n=49$ Dairy B) (1-21 days postpartum) were randomly sampled on Day 1 of the study by obtaining approximately 10g feces via rectal palpations or approximately 10g feces via captured defecation. After O157 lab

isolation and identification, (confirmation of shedding status (y/n) 10 shedding and 10 non-shedding cows were randomly selected from each dairy. The 40 cows chosen for the study were then sampled once daily for an additional four consecutive sample days. Fecal, hygiene and body condition scores, temperature, days in milk, and parity information were gathered for each animal on the day of sample collection. Shedders are defined as cows who were shedding O157 on Day 1 and Non-Shedders are defined as cows who were not shedding O157 on Day 1.

Lab Methods

O157 presence was assessed in fecal samples following enrichment in buffered peptone water and selective plating onto cefixime tellurite sorbitol MacConkey agar plates incubated at 37°C for 24 hours.³⁵ Suspect colonies were confirmed by latex agglutination and PCR targeting the O-antigen, shiga toxin genes *stx1* and *2*, and *eae*.

Covariates and Statistical Methods

All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC). In Table 4, the variable coding for the three statistical models is shown. Fecal score was categorized with a score of 3 serving as the reference because it is the ideal score, with comparison groups in those with a score of 2 or below or a score of 4 or higher. Hygiene score was categorized with the reference as a score less than or equal to 2 and the comparison group as those with a score greater than 2. Body condition score was categorized with the ideal being a score of 3 and comparison groups as those with a score of less than 3 or those with a score of greater than 3. Parity was categorized with the reference as 1, and comparison groups as 2 and greater than or equal to 3. Temperature and days in milk were kept continuous, both with a unit of 1 and shedding status and dairy were dichotomized. The variables were categorized according to diagnostically relevant criteria and to be relatable to past research.

I used descriptive statistics to identify daily patterns of shedding and proportion tests between groups of interest; shedders vs non-shedders and Dairy A vs Dairy B. Proc univariate was used to describe mean, median, minimum, maximum, and standard deviation of variables kept continuous including number of days in milk, parity, body condition score, hygiene score, fecal score, and temperature. Frequency tables were used to describe daily proportions of shedding among Shedders, Non-Shedders, cows from Dairy A, and cows from Dairy B.

Aim 1A: Is shedding status on Day 1 associated with shedding status on any subsequent day?

To answer the research question of whether shedding status on day 1 is associated with any subsequent shedding, I used a logistic regression model. To build a best-fit model, I originally ran the full model that included all model covariates, with results being shown in Table 5A. For manual model selection I used a p value cutoff of 0.4 and then excluded any variables with p values higher than that. Afterwards, I individually removed variables with non-significant p values until all variables left in the model were statistically significant. I also used stepwise model selection with an entry cutoff of 0.15 and an exit cutoff of 0.20 to find the final model. In addition, I ran model selection using the best subsets method as an added model selection method. Output generated included an odds ratio estimate, confidence interval, p value, and a Wald diagram for odds ratio. I assessed model fit using the Pearson and Deviance tests, the Hosmer Lemeshow test, and the Osius Rojek test. Given that there were only 2 unique covariate patterns within the 200 observations, the Pearson and Deviance tests were determined as the most appropriate to use to assess model fit.

Aim 1B: What risk factors are associated with cumulative days of shedding?

To assess whether the documented risk factors, dairy, parity, days in milk, fecal score, hygiene score, body condition score, temperature, and day 1 shedding status, were associated with cumulative days of shedding I used a generalized linear model with a Poisson distribution. The outcome variable was a count of the total number of days each cow was shedding O157 and the risk factors were the measured variables included in the list above. The model accounted for repeated observations for each cow by using a repeated statement with cow number as the repeated identifier. From the full model, I used manual model selection with a p value cutoff of 0.3 to exclude non-significant variables. I then individually removed variables from the model until each variable left was significant at the 0.05 level.

Aim 2A: Is shedding status on one day associated with shedding status on the next day, by specific day?

To assess whether there was an association between shedding status on one day and shedding status on the next day, by specific days, I used a generalized linear mixed model. Proc glimmix was used to compare day to day shedding patterns by pair, Day 1 to Day 2, Day 2 to Day 3, Day 3 to Day 4, and Day 4 to Day 5. Dairy was included as a random effect and the model accounted for the documented risk factor variables including fecal score, body condition score, parity, temperature, days in milk, and hygiene score. Output from the GLMM comprised odds ratio estimates, confidence intervals, and p values.

Aim 2B: Is shedding status on one day associated with shedding status on the next day, averaged over the five-day study period?

I then used a generalized linear model with a logit link to assess the overall risk of shedding on one day given shedding status on the previous day, averaged over the 5 days. Risk

factor variables included fecal score, body condition score, parity, temperature, days in milk, and hygiene score. From the full model, I used manual model selection with a p value cutoff of 0.3 to exclude non-significant variables. I then individually removed variables from the model until each variable left was significant at the 0.05 level.

Aim 3: What risk factors variables are associated with daily shedding status and proportions of shedding?

I used a generalized linear model with a logit link and a binary distribution to identify which risk factors were associated with shedding on any sampling day. For this analysis, I used proc genmod for the generalized linear model, adjusting for dairy as a random effect, accounting for repeated measures, and using a binomial distribution. Variables considered in the model included shedding status on day 1, parity, fecal score, hygiene score, body condition score, temperature, and days in milk. Output from the GLM included odds ratios, confidence intervals, and p values. From the full model, I used manual model selection with a p value cutoff of 0.3 to exclude non-significant variables. I then individually removed variables from the model until each variable left was significant at the 0.05 level.

CHAPTER 4

RESULTS

Overview

This chapter presents the results gathered from the statistical analyses for this study. The chapter describes the descriptive statistics, the logistic regression model, the generalized linear models, and the generalized linear mixed model.

Descriptive Statistics

Daily Prevalence

The prevalence of cows shedding O157 and specifically shedding the four serotypes (rfb, stx1, stx2, eae) by day is shown in Table 1B, broken down into different groups. Day 3 had the highest overall prevalence of O157 shedding with 52% and Days 4 and 5 had the lowest prevalence of shedding with 40% of the cows shedding O157, while 47% of the cows were shedding on Day 2. The Shedder cohort consistently had a higher prevalence of O157 shedding on every single sample day than Non-Shedders cohort, 60% vs 35% on Day 2, 60% vs 45% on Day 3, 50% vs 30% on Day 4, and 45% vs 35% on Day 5, although none of these proportion differences were statistically significant (Table 1B). There was a statistically significant difference in the proportion of cows shedding on Day 2 and Day 4 between Dairy A and Dairy B (Day 2 p 0.02, Day 3 p 0.03).

Daily Gene Prevalence

When Non-Shedders were positive for O157 they typically had a higher prevalence of stx2 and eae present than Shedders. Prevalence of stx2 in the positive samples for Non-Shedders ranged from 14% on Day 2 up to 50%, whereas the highest prevalence of stx2 in Shedders was

22% on Day 5. Shedders were also lower in daily prevalence for eae every day except for Day 5; 0% vs 14% on Day 2, 8% vs 11% on Day 3, 10% vs 17% on Day 4, and 22% vs 14% on Day 5.. When comparing Dairy A to Dairy B, there were no statistically significant difference in the prevalence with any of the serotypes (Table 1B).

Overall Prevalence

Table 1A shows the number and percentage of cows in each group that had at least one positive O157 test result during sample days two through five. Approximately 85% of Shedders and 80% of Non-shedders were positive for O157 at least once during sample Days 2 through 5, but these differences were not statistically significant (p 0.21). Dairy A had 95% of their cows shed O157 at least once during sample days two through five whereas Dairy B only had 70%, with this difference being statistically significant (p 0.02).

Cumulative Shedding

Shown in Table 1C are the cumulative days of shedding for each cow by group. Shedders most commonly shed for a total of three days (50%) or four days (25%), with one day (15%) and five days (10%) as the next most common. Non-shedders most commonly shed for one day (35%), two days (30%), or no days (20%), with only 10% shedding for three days and 5% shedding for four days. Cows from Dairy A were more likely to shed for three days (35%), one day (25%), or two days (20%), whereas cows from Dairy B were more likely to shed for three days (25%), one day (25%), four days (15%), or zero days (15%). The only significant difference in shedding patterns was that Day 1 Shedders were more likely to shed for three total days than Day 1 Non-Shedders (p 0.04).

Continuous Variable Summary

The variable descriptives for the risk factor variables as well as the average number of days with a positive O157 results are presented in Table 2. The overall mean number of sample days with a positive O157 test result for all 40 cows was 2.3 days and the median was 2.5 days. Shedders had a statistically significant higher mean number of days with a positive O157 result than the Non-Shedders, with 3.1 days for the Shedders and 1.4 days for the Non-Shedders ($p = 0.03$). There was no significant difference in mean number of days with a positive O157 results between Dairy A and Dairy B, with the mean being 2.3 days and the median being 2.5 days for both ($p = 0.76$). The mean parity for all 40 cows was 2.1 and the median was 2, and all groups had nearly the same mean; 2.2 for Shedders, 2 for Non-Shedders, and 2.1 for both Dairy A and Dairy B. The overall mean number of days in milk for all cows was 9.5, with Non-Shedders having a slighter higher number of days (10) compared to Shedders (8.7). Dairy B also had a higher number of days in milk (11) than Dairy A (7.9). Mean fecal scores and body condition scores remained consistent throughout all groups, staying between 2.8 and 2.9. There was slight variation in hygiene score, with Dairy A having the highest mean (2.4), Dairy B having the lowest mean (2.0), and Shedders (2.1) and Non-Shedders (2.3) falling in the middle. Mean temperature also remained at 39°C across all groups.

Overall Gene Prevalence

Table 3A presents the overall prevalence for the targeted O157 genes we tested for in each group throughout the entire five-day study period. Shedders had a higher prevalence of all three pathogenic genes than Non-Shedders; 20% vs 10% for Stx₁, 35% vs 30% for Stx₂, and 30% vs 20% for eae. Dairy A and Dairy B had the same prevalence for Stx₁ (15%) and eae (25%), however Dairy B had a higher prevalence of Stx₂ (40%) than Dairy A (25%).

Gene Test Proportions

Tables 3B, 3C, 3D, and 3E include test proportions throughout the five-day study period. Since there were 20 cows in each group and 5 days of sampling, there were a total of 100 tests total for each group. Cows could either be not shedding, shedding without Stx₁/Stx₂/eae, or shedding with Stx₁/Stx₂/eae. Our study used rfb positivity as the criteria for positive O157 shedding status, so 100% of cows positive for O157 were positive for rfb. Results indicate that 63% of the tests for Sheddors were positive and 37% were negative, whereas only 29% were positive and 71% were negative for Non-Sheddors. Dairy A and Dairy B had the same proportions with 54% of tests being negative and 46% of tests being positive. There was a statistically significant difference in the proportions of shedding rfb between Day 1 Sheddors and Day 1 Non-Sheddors (p 0.03).

Table 3C shows the test proportions for the Stx₁ gene. For Sheddors, 37% of the tests were negative, 58% were positive for O157 but negative for Stx₁, and 5% were positive for both rfb and Stx₁. Non-Sheddors tested negative for Stx₁ 71% of the time, positive for rfb but negative for Stx₁ 27% of the time, and positive for both rfb and Stx₁ 2% of the time. Dairy A and Dairy B were the same, with both having 54% of their tests negative for Stx₁. However, Dairy B had 4% of their tests positive for both rfb and Stx₁ compared to 3% for Dairy A. There was a statistically significant difference in the proportions of shedding Stx₁ between Day 1 Sheddors and Day 1 Non-Sheddors (p 0.04).

Table 3D includes the test proportions for the Stx₂ gene. For Sheddors, 37% of tests were negative, 55% were positive for rfb but negative for Stx₂, and 8% were positive for both rfb and Stx₂. For Non-Sheddors, 71% of tests were negative, 19% were positive for rfb but negative for Stx₂, and 10% were positive for both rfb and Stx₂. Dairy A and Dairy B had the same test

proportions, with 54% of the tests negative for Stx₂, 41% positive for rfb but negative for Stx₂, and 5% positive for both rfb and Stx₂. There was a statistically significant difference in the proportions of shedding Stx₂ between Day 1 Shedders and Day 1 Non-Shedders (p 0.02).

Table 3E shows the test proportions for the eae gene. For Shedders, 37% of the tests were negative, 57% were positive for rfb but negative for eae, and 6% were positive for both rfb and eae. For Non-Shedders, 71% of tests were negative, 25% were positive for rfb but negative for eae, and 4% were positive for both rfb and eae. Dairy A and Dairy B had the same test proportions, with 54% of the tests negative for eae, 41% positive for rfb but negative for eae, and 5% positive for both rfb and eae. There was a statistically significant difference in the proportions of shedding eae between Day 1 Shedders and Day 1 Non-Shedders (0.04).

Research Aim I A and B

Is shedding status on Day 1 associated with shedding on any subsequent day?

A logistic regression model was used to analyze whether shedding status on Day 1 was associated with any shedding on any subsequent sampling day. In the original model shown in Table 5A that contained all covariates of interest, I found that Day 1 Shedding status was the only significant predictor of any shedding, with an odds ratio of 4.0 (95% CI: 2.1,7.5). After using manual model selection and automated model selection to get the final model, I again found that Day 1 shedding status was the only significant predictor of any subsequent shedding. The model showed that Day 1 shedders were 2.0 (95% CI: 1.1, 3.8) times as likely to shed O157 on any subsequent sample day than Day 1 non-shedders, shown in Table 5B. Pearson and Deviance goodness of fit tests, however, showed that this model was poorly fit.

Are any risk factors associated with cumulative days of shedding?

I used a generalized linear model (GLM) with a Poisson distribution to assess whether any of our risk factors were associated with cumulative days of shedding for each cow. The full model containing all covariates of interest shown in Table 8A displays that Day 1 shedding status ($p < 0.0001$), fecal score <3 vs 3 ($p 0.02$), fecal score >3 vs 3 ($p 0.01$), and body condition score >3 vs 3 ($p 0.04$) were all associated with increased cumulative days of shedding while temperature ($p 0.03$) was associated with decreased cumulative days of shedding. I then used manual model selection to condense the model and found that Day 1 shedding status ($p < 0.0001$), fecal score >3 vs 3 ($p 0.02$), and temperature ($p 0.04$) were all significant predictors of increased cumulative days of shedding; results are shown in Table 8B.

Research Aim II A and B

Does shedding status on one day influence shedding status on the next day, by specific day?

To identify the association between shedding status on one day and shedding status on the next day, by specific day, I implemented a generalized linear mixed model. In the model I accounted for all risk factors variables and then ran significance tests between each combination of paired day. Cows who were shedding on day 1 were 60% more likely to shed on Day 2 than cows who did not shed on day 1 (OR: 1.6, 95% CI: 0.4,2.5). Cows who were shedding on day 2 were 50% more likely to shed on day 3 than cows who did not shed on day 2 (OR: 1.5, 95% CI: 0.2,1.8). Cows who were shedding on Day 3 were 80% more likely to shed on Day 4 than cows who were not shedding on Day 3 (OR: 1.8, 95% CI: 0.6,4.0). Finally, cows shedding on Day 4 were 60% more likely to shed on Day 5 than cows who were not shedding on Day 4 (OR: 1.6, 95% CI: 0.3,2.2). None of these comparison tests were statistically significant and results are shown in Table 7A.

Does shedding status on one day influence shedding status on the next day, averaged over 5 days?

I used a generalized linear model with a logit link to assess the overall relationship between shedding on one day and shedding on the subsequent day, averaged over the 5 day study period. I accounted for all risk factor variables in the model and found that cows were 50% more likely to shed on one day if they shed on the previous day (OR: 1.5; 95% CI: 0.9,2.3). Results are shown in Table 7B.

Research Aim III

Are documented risk factors influence shedding status and proportions of shedding on each day?

I used a generalized linear model with a logit link in order to assess daily health factor variables and risk of shedding O157 on a day to day basis. In the initial full model, I included all covariates of interest and found that cows shedding on Day 1 had 30% greater odds to shed on any subsequent sample day than cows who were not shedding on Day 1 (OR: 1.3, 95% CI: 1.1,1.6) and cows who have had two calves were two times as likely to shed than cows who have had one calf (OR: 2.0, 95% CI: 1.1,4.0). In contrast, cows with a body condition score of less than 3 had 50% less odds to shed on any subsequent sample day than cows with the reference body condition score of 3 (OR: 0.5, 95% CI 0.3,0.8). Results are shown in Table 6A. I then used manual model selection and found that day 1 shedding status was the only significant predictor of subsequent shedding status (OR 1.7, 95% CI: CI 1.1,2.5).

CHAPTER FIVE

DISCUSSION

Overview

This chapter discusses limitations and strengths of the study, conclusions generated from analyses, and future research projects related to this topic.

Conclusions

O157 has shown to be an important public health consideration and dairy cows serve as a unique transporter of the bacteria. With more than 100,000 cases yearly being attributed to the O157 pathogen in the United States, it is pertinent that risk factors are identified and mitigation strategies proposed. Our research has shown that even in cows chosen in the Non-Shedder cohort (Day 1 non-shedders) based on Day 1 sampling, the prevalence of O157 shedding throughout the remainder of the study period was upwards of 80% with a 10-30% prevalence specifically of pathogenic genes *stx*₁, *stx*₂, and *eae*.

Among our multiple analyses, we found several interesting results that add more questions to past conclusions in this field of research. We found that Day 1 Shedders were statistically more likely to shed on subsequent sample days than Day 1 Non-Shedders, but there were no statistically significant differences in the daily proportions of shedding. We also observed that when Day 1 Non-Shedders did shed O157 during sample Days 2 through Day 5, they were significantly more likely than Day 1 Shedders to shed the *stx*₂ gene, which is the more precarious gene in relation to human health.

Aligning with past research, we found inconsistent shedding patterns within the study cows and it is unclear whether these irregularities are indicative of true transience or due to

sampling errors.^{13,19,25} Since once daily sampling has been the standard field method for prevalence estimation of O157 in dairy herds and we observed these inconsistencies, we will be conducting a follow-up study the summer of 2016 that employs hourly sampling for four study days. These methods would allow us to not only analyze between day shedding patterns but within day shedding patterns and within sample accuracies. The multitude of research delving into this field of study will give us better prevalence estimates, information about shedding cycles within dairy cows, and best practices for gathering this data.

Limitations

Sampling Inconsistencies

Through the collaboration for this project we have identified a limiting factor in the transmission dynamics of O157 to be the inconsistent exposure assessment and sample analysis of the fecal samples. Our work supports published literature with evidence of inconsistent shedding patterns within the dairy cows, with shedding status changing transiently from day to day. There is still question about if the shedding cycle of O157 is short-lived itself or if there are sampling method errors resulting in false negatives.

Sample Size

This study only used 40 cows throughout the five sampling days, for a total of 200 samples. With a relatively small sample size as this, it is difficult to find statistical significance and to check for effect modification. I cannot be sure if the variables in my model are truly non-significant or if there isn't enough statistical power to detect significant associations.

Single point sampling

Our field methods involved taking one 10g fecal sample from each cow once a day, which is a fraction of the amount of manure each cow excretes each day. This lends uncertainty

in the accuracy of the tests deemed negative, because we cannot be sure if the cow is truly negative that day or if it is just chance in the sample. This could lead to an underestimation of positive results, though I do not think there would be a difference between in this area in comparing first day shedders and non-shedders.

Strengths

Longitudinal Design

Our study design included sampling our cohort of cows over the span of five days, as opposed to only one or two days like many previous studies. This repeated measure design allowed us to analyze shedding patterns over a longer period of time and also added more samples for increased statistical power.

Generalizability

As shown in literature, gut health and fecal shedding can vary among cows based on factors including diet, overall health, age, stage of lactation, and more. Different dairies feed varying diets and have cows ranging in health factors, so our study was conducted at two different dairies. This made our results more generalizable across dairy cattle and also allowed us to assess whether dairy was associated with O157 shedding.

Study Subject Selection

Fresh cows, defined as those cows who had given birth in the last 21 days, have shown to have higher prevalence of O157 shedding than non-fresh cows. Our study targeted fresh cows for our study subjects, which allowed us to see higher rates of shedding even when using a relatively small sample size. This also allowed our research to be comparable to past research looking at the same populations and risk factors.

Future Research

CRC Sampling Grant

Due to the sampling inconsistencies we saw during the analysis of this project and the need for continued research in this area of study, we are beginning a continuation project in July 2016 titled “Implementation of Systematic Sampling Methods for Prevalence Estimation of *E. coli* O157 Shedding in Dairy Cattle”. This study aims to implement systematic repeated measures sampling to test within and between fecal samples for presence of O157 pathogen. We hypothesize that extensive sampling methods will provide more accurate quantification methods for analyzing O157 shedding patterns and sampling error margins. My role for this project is to serve as the primary student investigator, lead field work and sample collection, and manage and analyze the data for one of my dissertation publications.

Ionophore Experiment

The shedding cycle of *O157* in dairy cattle intestinal tracts is not well known, and the impact of supplemental medications and feed additives has become a question in agricultural science. Ionophores are antimicrobial feed additives traditionally used in beef cattle to maximize body weight gain and feed efficiency by modulating rumen fermentation and flora patterns.⁸ Ionophores act on the metal sequestration pores in bacteria and tip populations of bacteria towards those that create metabolic components better used by cows to create proprionate and drive milk production. Hence, the use of ionophores is becoming more popular in dairy farm operations as well.⁹ Previous research has suggested that as a gram-negative bacterium, ionophores could potentially increase the incidence of *E. coli* in cattle by inhibiting competitive gram-positive species.^{10,11,12} In contrast, survey data and experimentation in cattle has shown the opposite; a decrease in *E. coli* colonization.^{13,14,15} Further research into the effect of ionophores

in dairy cattle is needed to better understand how the modulation of rumen flora specifically impacts *E. coli* and our research team hopes to propose a study looking into this phenomenon. The ionophore study would aim to characterize the prevalence of *E. coli* O157 in dairy cattle fed a diet enriched with ionophores compared to cows fed a normal diet.

R01 Grant

Antimicrobial resistance has been a growing global problem, and the misuse and overuse of antibiotics in the agricultural industry is one of the potential sources of the issue. Research into the association between agricultural occupational exposure to antimicrobial resistant bacteria, including *E. coli* O157, and respiratory disease has been lacking. A research grant has been submitted by the High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) to investigate the role of workplace exposure on human-hosted bacterial communities, antibiotic resistance genes, and precursors, and health status in livestock workers. We hypothesize that consistent exposure to antimicrobial resistant bacteria is associated with a significant increase in inflammation and decreases overall lung function.

TABLES

Table 1A | Proportion of Cows Shedding O157 at Least Once During Sample Days 2-5

<i>Classification</i>	<i>Number</i>	<i>Prevalence</i>	<i>P value</i>
<i>Shedders</i>	17	85%	0.21
<i>Non-Shedders</i>	16	80%	
<i>Dairy A</i>	19	<u>95%</u>	<u>0.02</u>
<i>Dairy B</i>	14	<u>70%</u>	
<i>Overall</i>	33	83%	

Results that are bolded and underlined were significantly different using proportion testing. There was a significant difference in the overall proportion of cows shedding O157 between Dairy A and Dairy B.

Table 1B | Prevalence of Shedding Events and Genes by Day

<i>Classification</i>	<i>Event</i>	<i>Day 2</i> <i>% (n)</i>	<i>Day 3</i> <i>% (n)</i>	<i>Day 4</i> <i>% (n)</i>	<i>Day 5</i> <i>% (n)</i>
<i>Overall</i>	Shedding	19 (47%)	21 (52%)	16 (40%)	16 (40%)
	Not Shedding	21 (52%)	19 (47%)	24 (60%)	24 (60%)
	rfb	19 (100%)	21 (100%)	16 (100%)	16 (100%)
	Stx₁	0 (0%)	4 (19%)	0 (0%)	1 (6%)
	Stx₂	2 (11%)	4 (19%)	4 (25%)	5 (31%)
	eAe	1 (5.3%)	2 (9.5%)	2 (12%)	3 (19%)
<i>Shedders</i>	Shedding	12 (60%)	12 (60%)	10 (50%)	9 (45%)
	Not Shedding	8 (40%)	8 (40%)	10 (50%)	11 (55%)
	rfb	12 (100%)	12 (100%)	10 (100%)	9 (100%)
	Stx₁	0 (0%)	2 (17%)	0 (0%)	1 (11%)
	Stx₂	1 (8%)	1 (8.3%)	1 (10%)	2 (22%)
	eAe	0 (9%)	1 (8.3%)	1 (10%)	2 (22%)
<i>Non Shedders</i>	Shedding	7 (35%)	9 (45%)	6 (30%)	7 (35%)
	Not Shedding	13 (65%)	11 (55%)	14 (70%)	13 (65%)
	rfb	7 (100%)	9 (100%)	6 (100%)	7 (100%)
	Stx₁	0 (0%)	2 (22%)	0 (0%)	0 (0%)
	Stx₂	1 (14%)	3 (33%)	3 (50%)	3 (43%)
	eAe	1 (14%)	1 (11%)	1 (17%)	1 (14%)
<i>Dairy B</i>	Shedding	<u>5 (25%)</u>	12 (60%)	<u>12 (60%)</u>	7 (35%)
	Not Shedding	15 (75%)	8 (40%)	8 (40%)	13 (65%)
	rfb	5 (100%)	12 (100%)	12 (100%)	7 (100%)
	Stx₁	0 (0%)	2 (17%)	0 (0%)	0 (0%)
	Stx₂	2 (40%)	2 (17%)	4 (33%)	3 (43%)
	eAe	0 (0%)	0 (0%)	2 (17%)	1 (14%)
<i>Dairy A</i>	Shedding	<u>14 (70%)</u>	9 (45%)	<u>4 (20%)</u>	9 (45%)
	Not Shedding	6 (30%)	11 (55%)	16 (80%)	11 (55%)
	rfb	14 (100%)	9 (100%)	4 (100%)	9 (100%)
	Stx₁	0 (0%)	2 (22%)	0 (0%)	1 (11%)
	Stx₂	0 (0%)	2 (22%)	0 (0%)	2 (22%)
	eAe	1 (7%)	2 (22%)	0 (0%)	2 (22%)

*Shedders= Cows who shed on day 1, Non-Shedders= cows who did not shed on day 1

*Shedding= Shed O157 on that day, Not shedding= not shedding O157 that day

Results that are bolded and underlined were significantly different using proportion testing. There was a significant difference in the proportion of cows shedding between Dairy A and Dairy B on Day 2 and Day 4.

Table 1C | Shedding Patterns for Cows by Group

<i>Classification</i>	<i>None % (n)</i>	<i>One Day % (n)</i>	<i>Two Days % (n)</i>	<i>Three Days % (n)</i>	<i>Four Days % (n)</i>	<i>Five Days % (n)</i>
<i>Shedders</i>	0% (0)	15% (3)	0% (0)	<u>50% (10)</u>	25% (5)	10% (2)
<i>Non-Shedders</i>	20% (4)	35% (7)	30% (6)	<u>10% (2)</u>	5% (1)	0% (0)
<i>P value</i>	0.22	0.36	0.13	<u>0.04</u>	0.19	0.43
<i>Dairy A</i>	5% (1)	25% (5)	20% (4)	35% (7)	15% (3)	0% (0)
<i>Dairy B</i>	15% (3)	25% (5)	10% (2)	25% (5)	15% (3)	10% (2)
<i>P value</i>	0.44	0.83	0.36	0.38	0.80	0.51
<i>Overall</i>	10% (4)	25% (10)	15% (6)	30% (12)	15% (6)	5% (2)

Results that are bolded and underlined were significantly different using proportion testing. There was a significant difference in the proportion of cows shedding for three total days between shedders and non-shedders.

Table 2 | Variable Descriptives for Continuous Variables

<i>Variable</i>	<i>Classification</i>	<i>Mean</i>	<i>Median</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Standard Deviation</i>	<i>P value</i>
<i>Positive O157 results</i>	Shedders	<u>3.1</u>	3	1	5	1.1	<u>0.03</u>
	Non Shedders	<u>1.4</u>	1	0	4	1.0	
	Dairy A	2.3	2.5	0	4	1.1	0.76
	Dairy B	2.3	2.5	0	5	1.6	
	Overall	2.3	2.5	0	5	1.4	
<i>Parity</i>	Shedders	2.2	2	1	6	1.5	0.25
	Non Shedders	2.0	2	1	6	1.3	
	Dairy A	2.1	1	1	6	1.6	0.68
	Dairy B	2.1	2	1	5	1.1	
	Overall	2.1	2	1	6	1.4	
<i>Days in Milk</i>	Shedders	8.7	8	0	18	4.3	0.12
	Non Shedders	10	10	1	21	4.8	
	Dairy A	7.9	7	0	18	4.2	0.08
	Dairy B	11	11	2	21	4.4	
	Overall	9.5	9	0	21	4.6	
<i>Fecal Score</i>	Shedders	2.9	3	1	4	0.40	0.81
	Non Shedders	2.9	3	1	4	0.36	
	Dairy A	2.8	3	1	4	0.42	0.87
	Dairy B	2.9	3	1	4	0.31	
	Overall	2.9	3	1	4	0.38	
<i>Hygiene Score</i>	Shedders	2.1	2	1	3	0.5	0.38
	Non Shedders	2.3	2	2	4	0.65	
	Dairy A	2.4	2	2	4	0.67	0.54
	Dairy B	2.0	2	1	3	0.44	
	Overall	2.2	2	1	4	0.60	
<i>Body Condition Score</i>	Shedders	2.9	3	2	5	0.74	0.66
	Non Shedders	2.8	3	1	4	0.68	
	Dairy A	2.9	3	2	5	0.74	0.81
	Dairy B	2.8	3	1	4	0.68	
	Overall	2.8	3	1	5	0.71	
<i>Temperature (in °F)</i>	Shedders	39	39	38	40	0.34	0.89
	Non Shedders	39	39	38	41	0.43	
	Dairy A	39	39	38	41	0.41	0.91
	Dairy B	39	39	38	40	0.36	
	Overall	39	39	38	41	0.38	

Results that are bolded and underlined were significantly different using proportion testing. There was a statistical difference between the mean cumulative days of shedding between shedders and non-shedders.

Table 3A- Overall Prevalence of Select O157 Genes

<i>Group</i>	<i>Rfb</i>	<i>P value</i>	<i>Stx₁</i>	<i>P value</i>	<i>Stx₂</i>	<i>P value</i>	<i>eae</i>	<i>P value</i>
<i>Shedders</i>	20 (100%)	0.99	4 (20%)	0.45	7 (35%)	0.68	6 (30%)	0.45
<i>Non-Shedders</i>	20 (100%)		2 (10%)		6 (30%)		4 (20%)	
<i>Dairy A</i>	20 (100%)	0.99	3 (15%)	0.99	5 (25%)	0.28	5 (25%)	0.99
<i>Dairy B</i>	20 (100%)		3 (15%)		8 (40%)		5 (25%)	
<i>Overall</i>	40 (100%)		6 (15%)		13 (33%)		10 (25%)	

There were no statistically significant differences in proportions between shedders and non-shedders or Dairy A and Dairy B.

Table 3B | Rfb Test Proportions

<i>Classification</i>	<i>Daily Shedding Status</i>	<i>Negative % (n)</i>	<i>Positive % (n)</i>	<i>Total</i>	<i>P value</i>
<i>Shedders</i>	Not Shedding	<u>37 (37%)</u>	0 (0%)	37	<u>0.03</u>
	Shedding	0 (0%)	63 (63%)	63	
<i>Non-Shedders</i>	Not Shedding	<u>71 (71%)</u>	0 (0%)	71	
	Shedding	0 (0%)	29 (29%)	29	
<i>Dairy A</i>	Not Shedding	54 (54%)	0 (0%)	54	0.99
	Shedding	0 (0%)	46 (46%)	46	
<i>Dairy B</i>	Not Shedding	54 (54%)	0 (0%)	54	
	Shedding	0 (0%)	46 (46%)	46	

Results that are bolded and underlined were significantly different using proportion testing. The proportion of tests positive for rfb is significantly different between shedders and non-shedders.

Table 3C | Stx₁ Test Proportions

<i>Classification</i>	<i>Daily Shedding Status</i>	<i>Negative % (n)</i>	<i>Positive % (n)</i>	<i>Total</i>	<i>P value</i>
<i>Shedders</i>	Not Shedding	<u>37 (37%)</u>	0 (0%)	37	<u>0.04</u>
	Shedding	<u>58 (58%)</u>	5 (5%)	63	
<i>Non-Shedders</i>	Not Shedding	<u>71 (71%)</u>	0 (0%)	71	
	Shedding	<u>27 (27%)</u>	2 (2%)	29	
<i>Dairy A</i>	Not Shedding	54 (54%)	0 (0%)	54	0.84
	Shedding	43 (43%)	3 (3%)	46	
<i>Dairy B</i>	Not Shedding	54 (54%)	0 (0%)	54	
	Shedding	42 (42%)	4 (4%)	46	

Results that are bolded and underlined were significantly different using proportion testing. The proportion of positive O157 tests negative for Stx₁ is statistically different between shedders and non-shedders.

Table 3D | Stx₂ Test Proportions

<i>Classification</i>	<i>Daily Shedding Status</i>	<i>Negative % (n)</i>	<i>Positive % (n)</i>	<i>Total</i>	<i>P value</i>
<i>Shedders</i>	Not Shedding	<u>37 (37%)</u>	0 (0%)	37	<u>.02</u>
	Shedding	<u>55 (55%)</u>	8 (8%)	63	
<i>Non-Shedders</i>	Not Shedding	<u>71 (71%)</u>	0 (0%)	71	
	Shedding	<u>19 (19%)</u>	10 (10%)	29	
<i>Dairy A</i>	Not Shedding	54 (54%)	0 (0%)	54	0.77
	Shedding	41 (41%)	5 (5%)	46	
<i>Dairy B</i>	Not Shedding	54 (54%)	0 (0%)	54	
	Shedding	41 (41%)	5 (5%)	46	

Results that are bolded and underlined were significantly different using proportion testing. The proportion of positive O157 tests negative for Stx₂ is statistically different between shedders and non-shedders.

Table 3E | eae Test Proportions

<i>Classification</i>	<i>Daily Shedding Status</i>	<i>Negative % (n)</i>	<i>Positive % (n)</i>	<i>Total</i>	<i>P value</i>
<i>Shedders</i>	Not Shedding	<u>37 (37%)</u>	0 (0%)	37	<u>0.04</u>
	Shedding	<u>57 (57%)</u>	6 (6%)	63	
<i>Non-Shedders</i>	Not Shedding	<u>71 (71%)</u>	0 (0%)	71	
	Shedding	<u>25 (25%)</u>	4 (4%)	29	
<i>Dairy A</i>	Not Shedding	54 (54%)	0 (0%)	54	0.79
	Shedding	41 (41%)	5 (5%)	46	
<i>Dairy B</i>	Not Shedding	54 (54%)	0 (0%)	54	
	Shedding	41 (41%)	5 (5%)	46	

Results that are bolded and underlined were significantly different using proportion testing. The proportion of positive O157 tests negative for eae is statistically different between shedders and non-shedders.

Table 4 | Variable Coding

<i>Variable name</i>	<i>Type</i>	<i>Levels</i>
<i>Dairy farm</i>	Categorical	Dairy A, Dairy B
<i>Fecal score</i>	Categorical	<=2, 3 (ref), >= 4
<i>Hygiene score</i>	Categorical	<=2 (ref), >2
<i>Body condition score</i>	Categorical	<=2, 3 (ref), >=3
<i>Parity</i>	Categorical	1 (ref), 2, >=3
<i>Temperature</i>	Continuous	Unit= 1 degree
<i>Days in milk</i>	Continuous	Unit= 1 Day

Table 5A | Original Logistic Regression Model Output; Is shedding status on day 1 associated with shedding status on any subsequent day?

<i>Variable</i>	<i>Level</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<u>Day 1 Shed Status</u>	Shedding vs Not Shedding	<u>4.0</u>	2.1	7.5
	Dairy Parity	A vs B	1.3	0.6
Days in Milk Fecal Score	2 vs 1	0.4	0.1	1.1
	>=3 vs 1	0.5	0.2	1.1
Hygiene Score Body Condition Score	Unit= 1 day	0.9	0.8	1.0
	<3 vs 3	1.2	0.4	3.3
Temperature	>3 vs 3	0.2	0.02	2.6
	>=3 vs 0-2	0.8	0.3	1.8
	<3 vs 3	1.5	0.6	3.2
	>3 vs 3	2.0	0.7	5.4
	Unit= 1°C	0.7	0.2	1.6

Table 5B | Final Logistic Regression Model Output; Is shedding status on day 1 associated with shedding status on any subsequent day?

<i>Variable</i>	<i>Level</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<u><i>Day 1 Shed Status</i></u>	Shedding vs Not Shedding	<u>2.0</u>	1.1	3.8

Table 6A | Initial Generalized Linear Model; What risk factor variables are associated with shedding on any day?

<i>Variable</i>	<i>Level</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<u>Day 1 Shed status</u>	Shedding vs Not Shedding	<u>1.3</u>	1.1	1.6
	<i>Dairy</i> Dairy B vs Dairy A	0.7	0.4	1.3
<i>Fecal Score</i>	<3 vs 3	5.2	0.4	61
	>3 vs 3	6.8	0.4	107
<u>Body Condition Score</u>	<3 vs 3	<u>0.5</u>	0.3	0.8
	>3 vs 3	0.7	0.4	1.3
<i>Hygiene Score</i>	>=3 vs 0-2	1.2	0.7	2.1
<u>Parity</u>	2 vs 1	<u>2.0</u>	1.0	4.0
	>=3 vs 1	0.9	0.3	2.6
<i>Temperature</i> <i>Days in Milk</i>	Unit= 1°C	0.5	0.2	1.9
	Unit= 1 Day	0.9	0.8	1.0

Table 6B | Final Generalized Linear Model; What risk factor variables are associated with shedding on any day?

<i>Variable</i>	<i>Level</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<u><i>Day 1 Shed status</i></u>	Shedding vs Not Shedding	<u>1.7</u>	1.1	2.5

Table 7A | Generalized Linear Mixed Model; For each day, what is the risk of shedding on that day given shedding status on the previous day?

<i>Variable</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<i>Day 1 vs Day 2</i>	1.6	0.4	2.5
<i>Day 2 vs Day 3</i>	1.5	0.2	1.8
<i>Day 3 vs Day 4</i>	1.8	0.6	4.0
<i>Day 4 vs Day 5</i>	1.6	0.3	2.2

Table 7B | Generalized Linear Model; What is the overall risk of shedding on one day given shedding on the previous day?

<i>Variable</i>	<i>Odds Ratio</i>	<i>LL</i>	<i>UL</i>
<i>Previous Shedding</i>	1.5	0.9	2.3

Table 8A | Initial Generalized Linear Model Output with a Poisson Distribution; Are documented risk factors associated with the cumulative days of shedding for each cow?

<i>Variable</i>	<i>Level</i>	<i>Estimate</i>	<i>P value</i>
<u>Day 1 Shed status</u>	Shedding vs Not Shedding	<u>2.1</u>	<0.0001
	<i>Dairy</i> Dairy B vs Dairy A	1.0	0.7
<u>Fecal Score</u>	<3 vs 3	<u>1.0</u>	0.02
	>3 vs 3	<u>0.9</u>	0.01
<u>Body Condition Score</u>	<3 vs 3	1.1	0.2
	>3 vs 3	<u>1.3</u>	0.04
<i>Hygiene Score</i>	>=3 vs 0-2	0.9	0.4
<i>Parity</i>	2 vs 1	0.8	0.3
	>=3 vs 1	0.9	0.1
<u>Temperature</u> <i>Days in Milk</i>	Unit= 1°C	<u>0.9</u>	0.03
	Unit= 1 Day	0.9	0.8

Table 8B | Final Generalized Linear Model Output with a Poisson Distribution; Are documented risk factors associated with the cumulative days of shedding for each cow?

<i>Variable</i>	<i>Level</i>	<i>Estimate</i>	<i>P value</i>
<u><i>Day 1 Shed status</i></u>	Shedding vs Not Shedding	<u>2.1</u>	<0.0001
	<u><i>Fecal Score</i></u>	<3 vs 3	<u>1.0</u>
	>3 vs 3	<u>1.0</u>	0.02
<u><i>Temperature</i></u>	Unit= 1°C	<u>1.0</u>	0.04

FIGURES

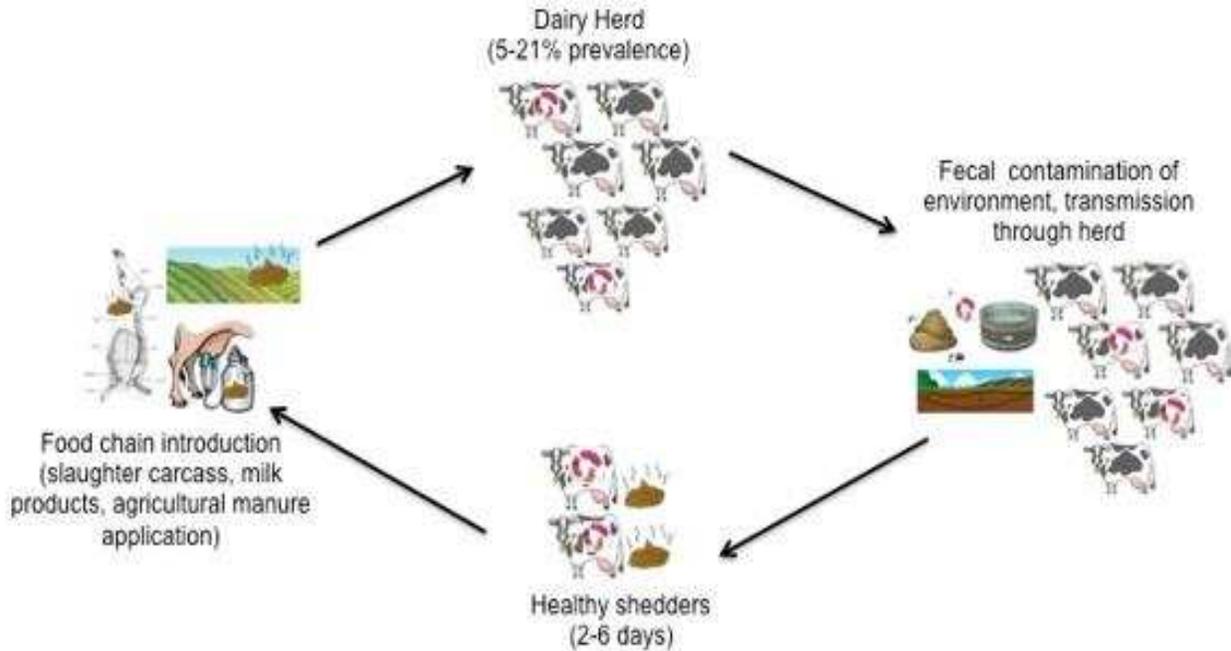


Figure 2 | Spread and Transmission of Dairy-Produced *E. coli* O157

Our preliminary data show O157:H7 shedding prevalence to be between 19-21% in early lactation cattle on farms in northern Colorado.¹⁷ Dairy cattle asymptotically harbor O157 bacteria in their lower intestinal tracts and shed the pathogen in their feces. Due to their multifaceted use in the food industry, dairy cattle are capable of exposing many different food products to O157 including milk, meat, agricultural manure.²

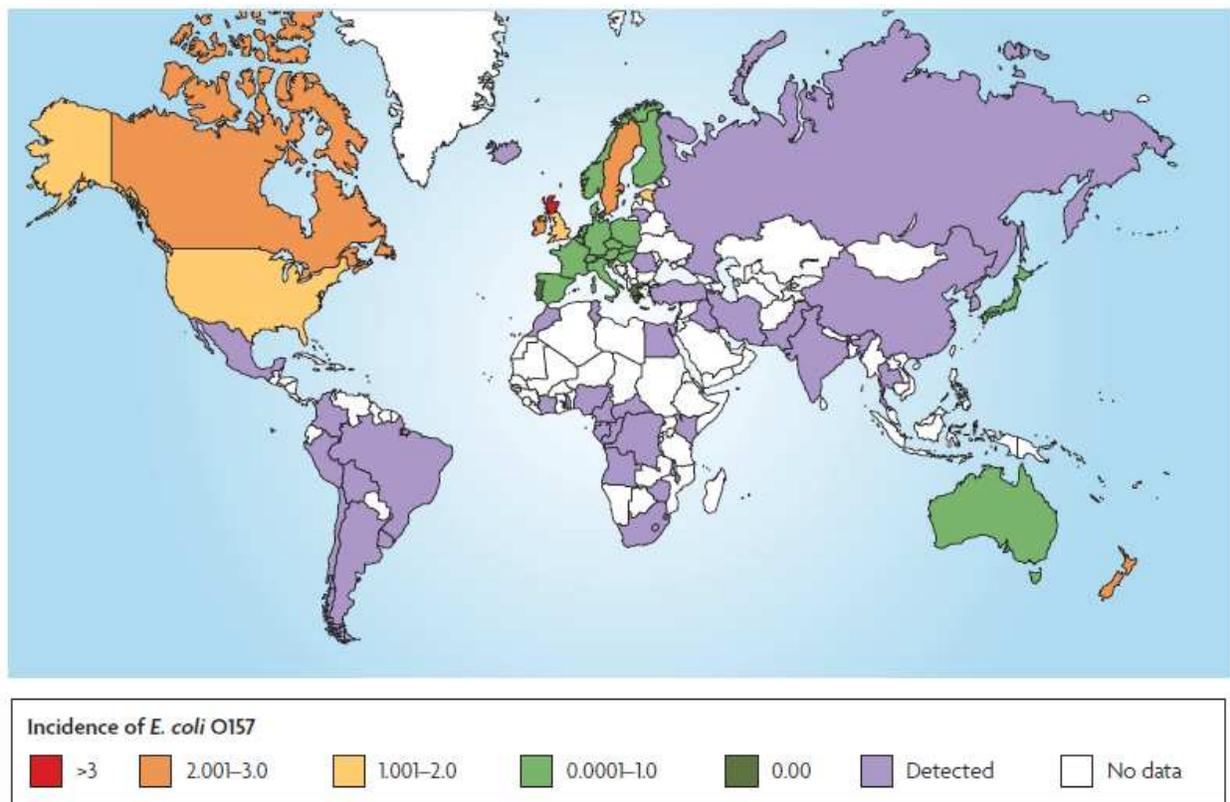


Figure 3 | The Worldwide Burden of *E. coli* O157

Figure 3 shows a map of the 2005 worldwide relative burden of O157 in human populations, per 100,000 individuals. The purple color is shown for countries where there has been detection of O157, but where no incidence rate is available. The white color is shown for countries where no information is available. The rates reported for all other colors are based on individual surveillance systems.¹⁶

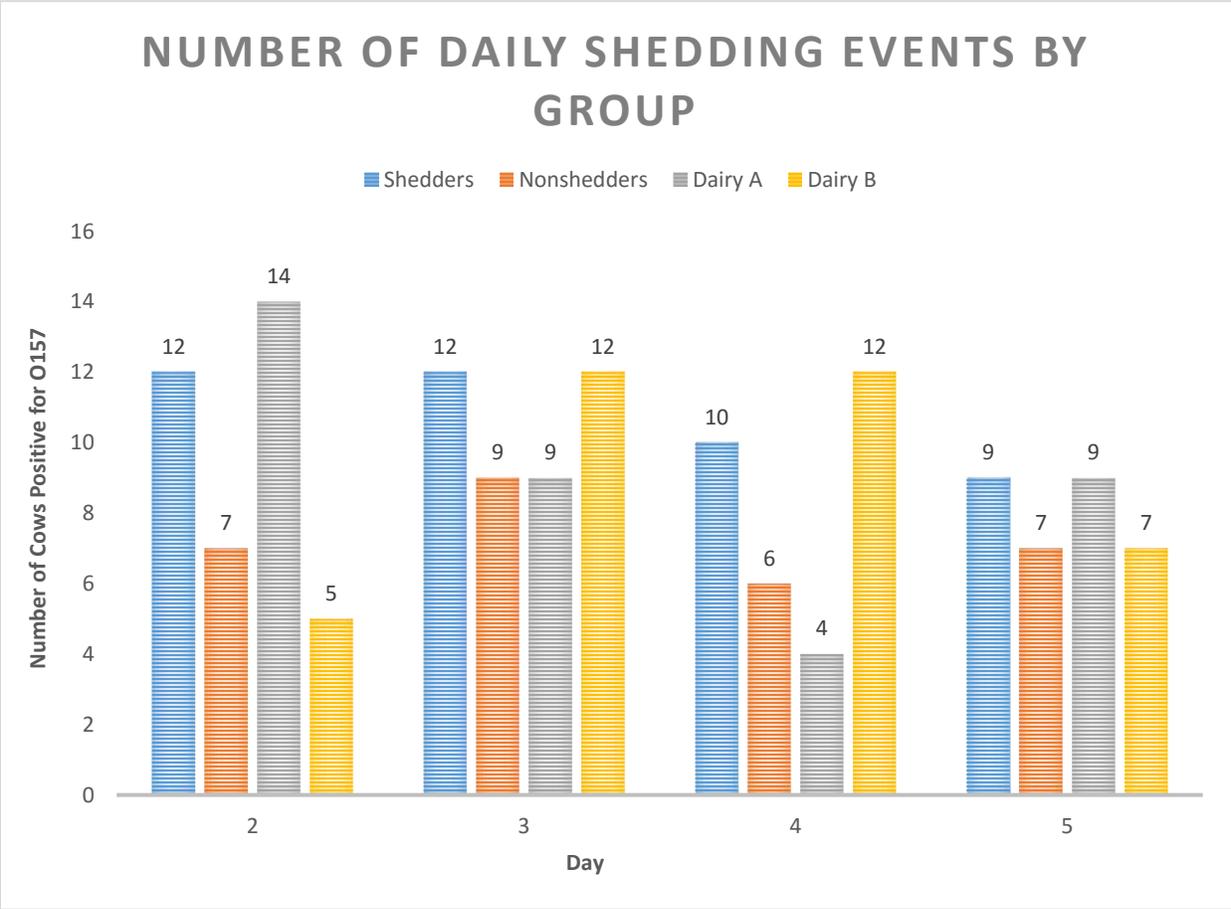


Figure 4 | Number of Daily Shedding Events by Group

Figure 4 shows the number of positive O157 shedding events by day and by classification group. Only sample days 2-5 are included because shedding status on day 1 was used to determine cohorts. Shedders consistently had a higher number of cows test positive for O157, with an average of 11 positive cows per day compared to an average 7 positive cows per day for the Non-Shedders. There is no clear difference between Dairy A and Dairy B shown here, with Dairy A being higher on days 2 and 5 and Dairy B being higher on days 3 and 4.

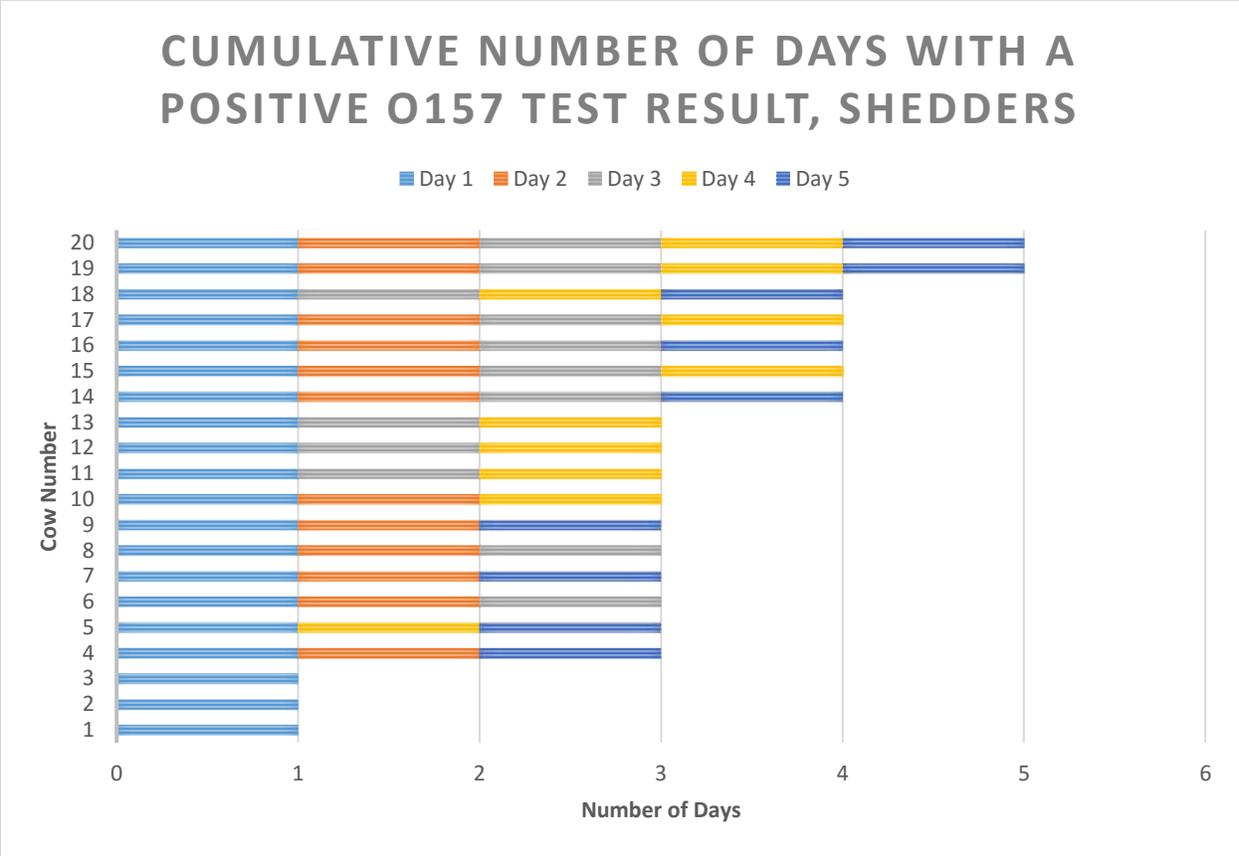


Figure 5A | Cumulative Number of Days With a Positive O157 Result for Shedders

Figure 5A shows the cumulative number of days with a positive O157 result for the Shedder cohort, by cow. Three cows only shed for 1 day, ten cows shed for 3 days, five cows shed for 4 days, and two cows shed for all 5 days.

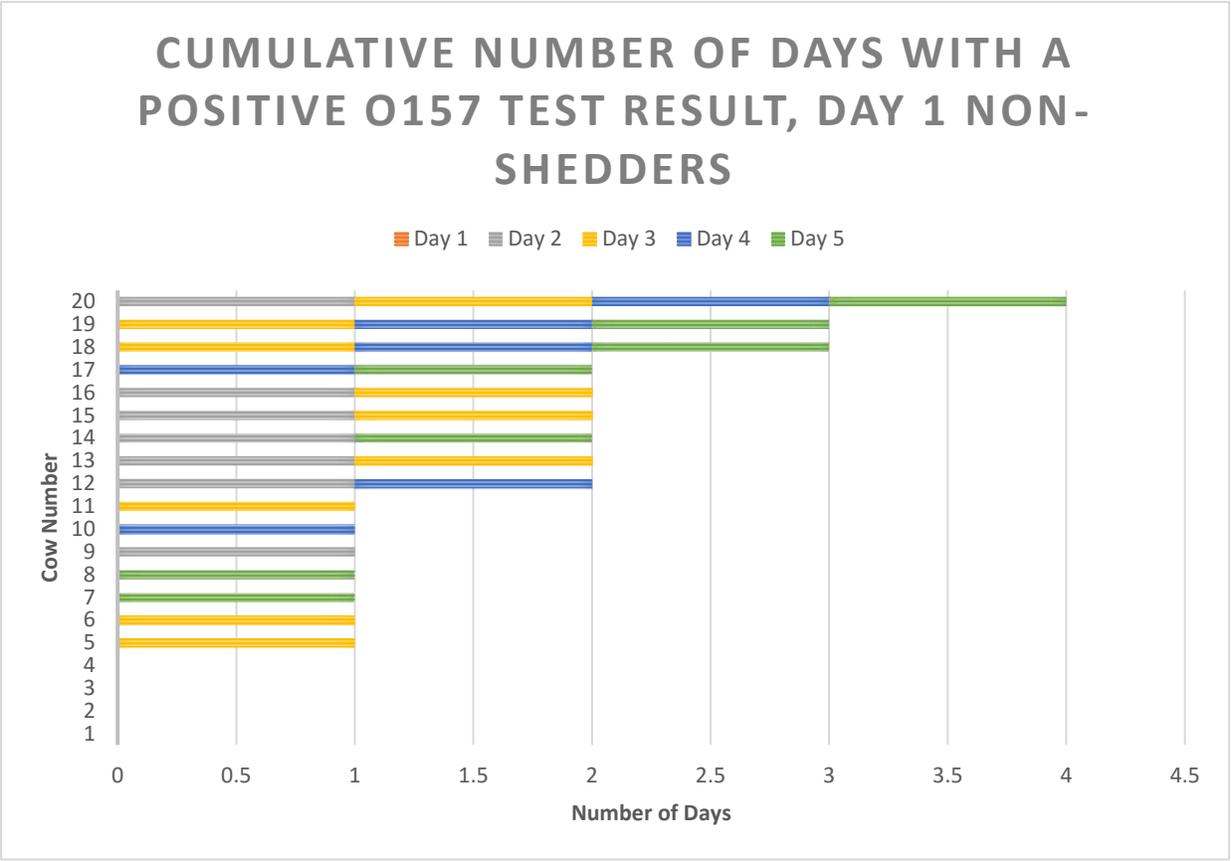


Figure 5B | Cumulative Number of Days With a Positive O157 Result for Non-Shedders

Figure 5B shows the cumulative number of days with a positive O157 result for the Non-Shedder cohort, by cow. Four cows shed for 0 days, seven cows shed for 1 day, two cows shed for 3 days, and one cow shed for 4 days.

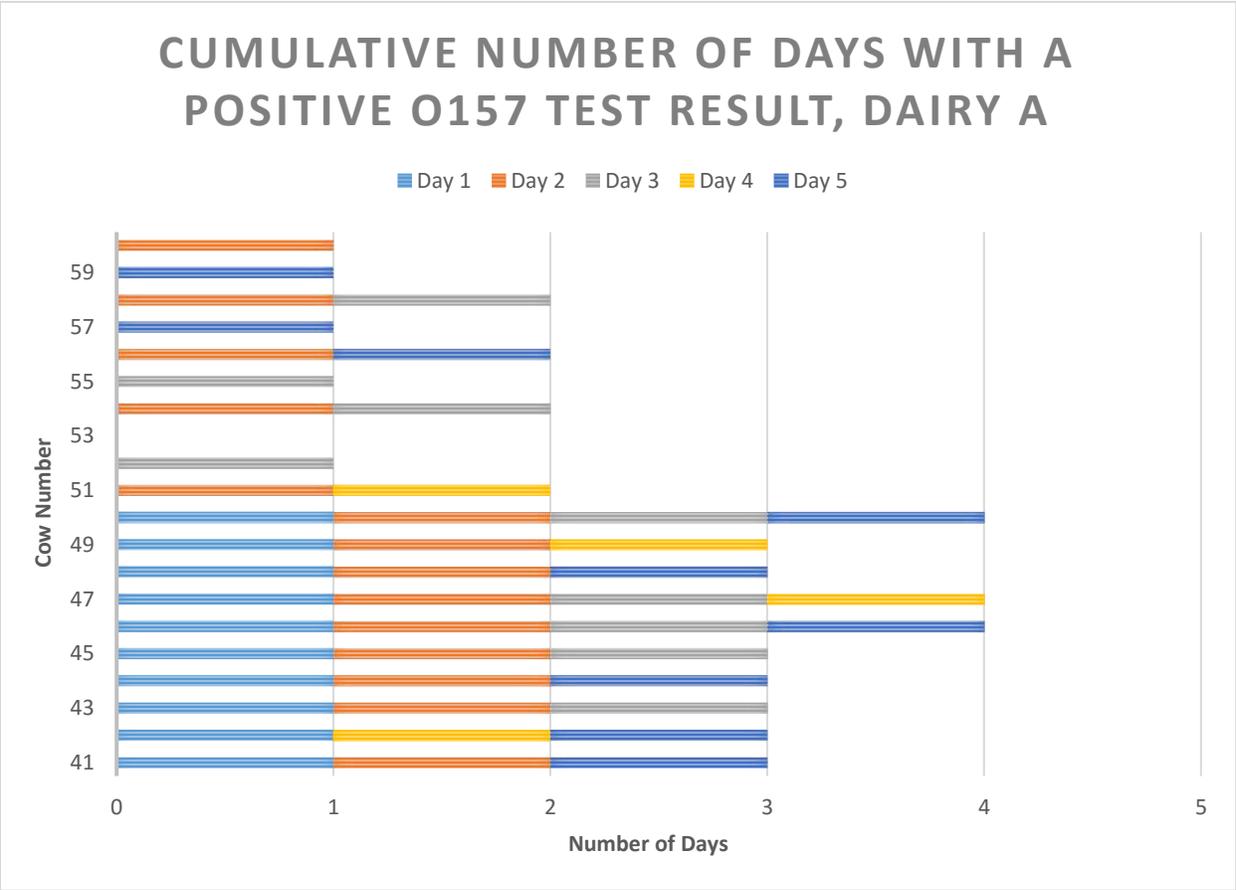


Figure 5C | Cumulative Number of Days With a Positive O157 Result for Dairy A

Figure 5C shows the cumulative number of days with a positive O157 result for Dairy A, by cow. One cow shed zero day, five cows shed for 1 day, four cows shed for 2 days, seven cows shed for 3 days, and three cows shed for 4 days.

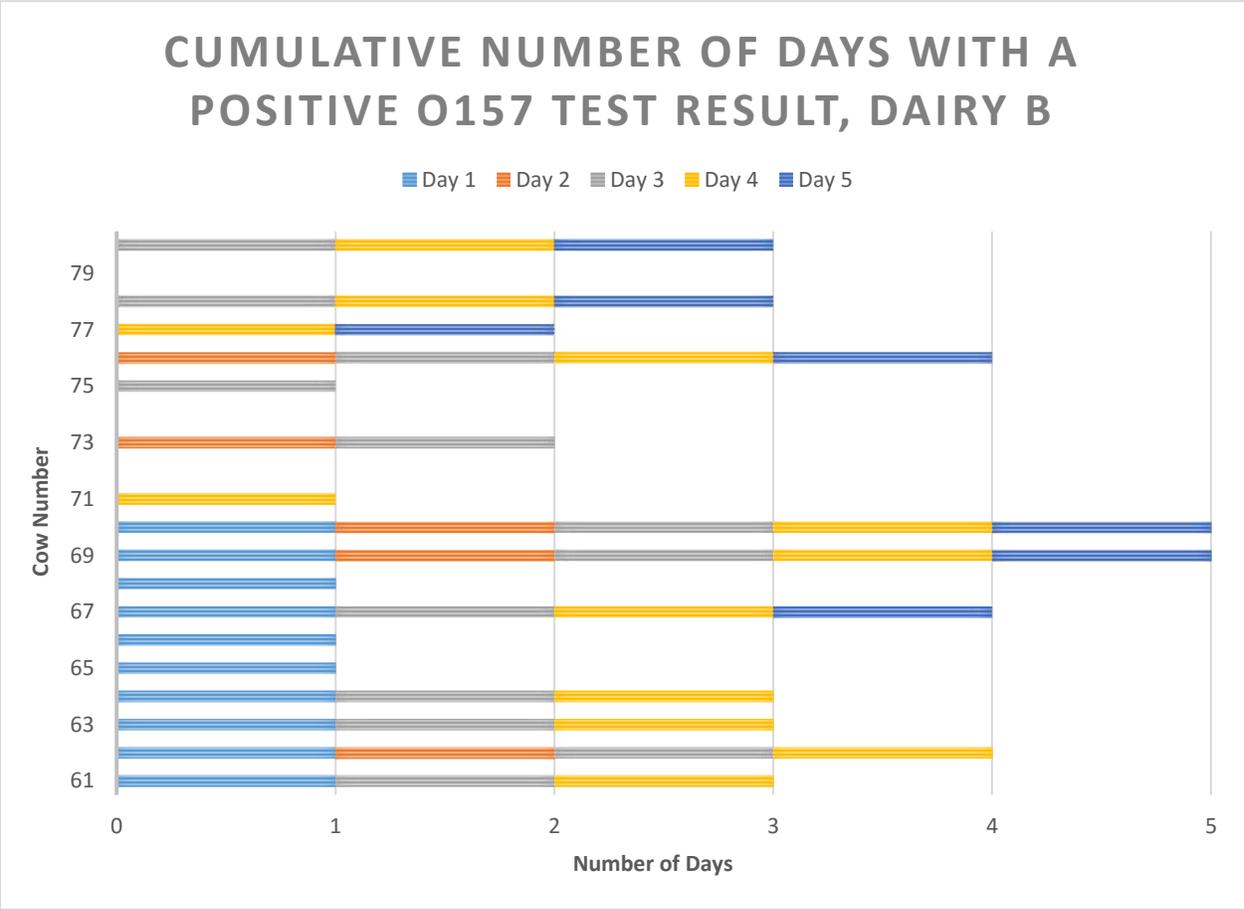


Figure 5D | Cumulative Number of Days With a Positive O157 Result for Dairy B

Figure 5D shows the cumulative number of days with a positive O157 result for Dairy B by cow. Three cows shed for zero days, five cows shed for 1 day, two cows shed for 2 days, five cows shed for 3 days, three cows shed for 4 days, and two cows shed for 5 days.

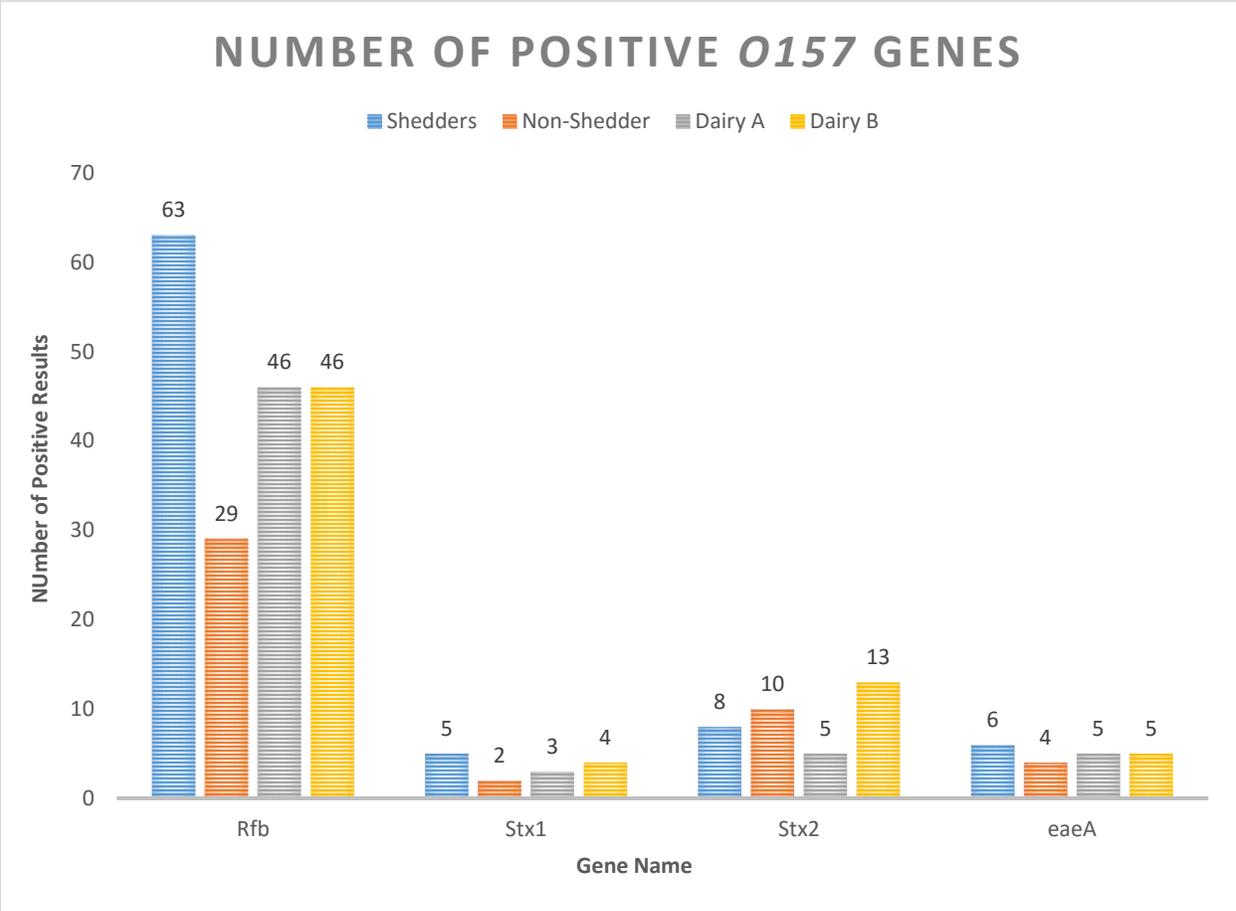


Figure 6 | Number of Positive *O157* Genes by Group

Figure 6 shows the total number of tests that were positive for *O157* targeted genes during the 5 sample days. *O157* positivity was based on the *rfb* gene, so all of the samples positive for *O157* were positive for *rfb*. *Stx*₂ was the next most common gene present, followed by *eae*, and lastly *Stx*₁.

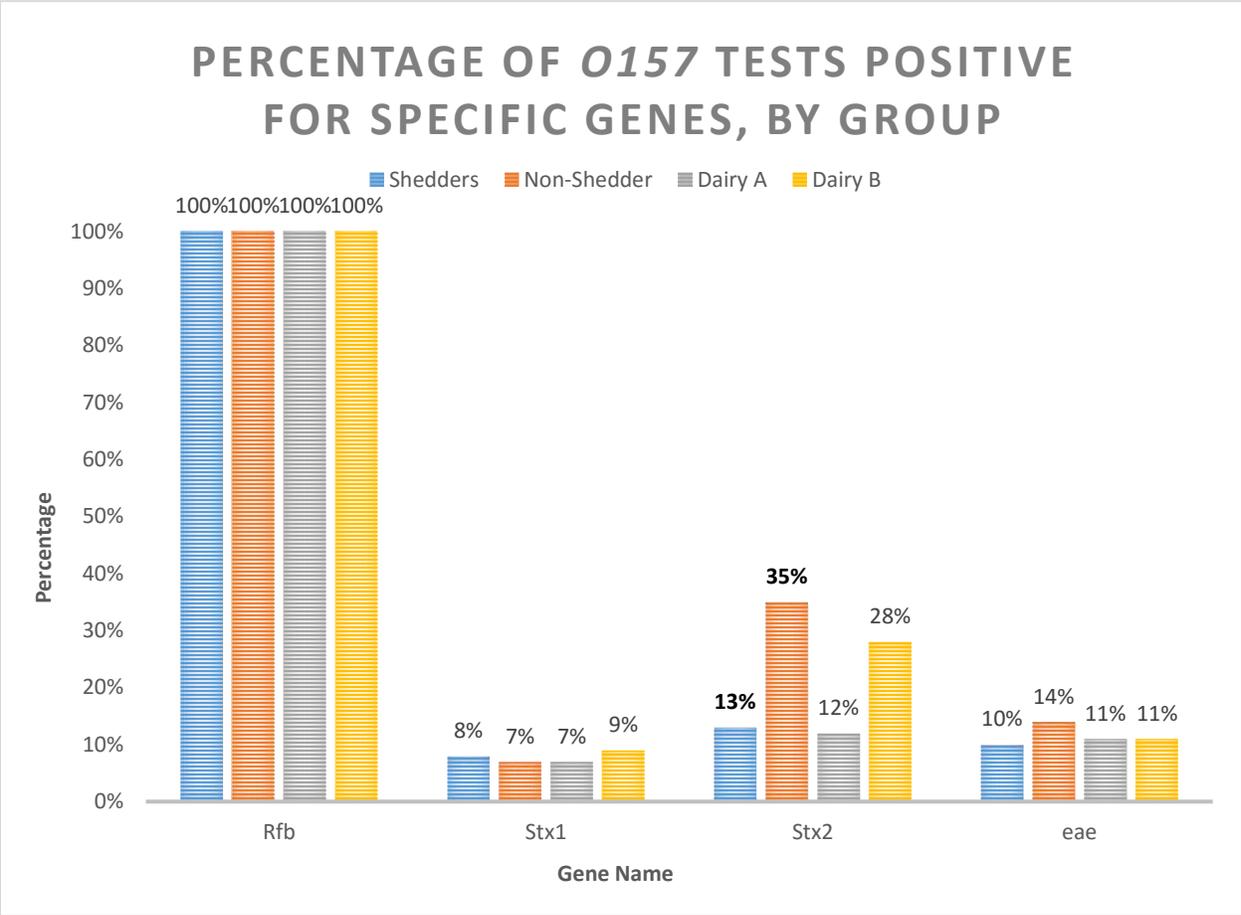


Figure 7 | Percentage of O157 Tests Positive for Targeted Genes by Group

Figure 7 shows the percentage of tests that were positive for O157 that were also positive for targeted genes during the 5 sample days. O157 positivity was based on the rfb gene, so 100% of samples positive for O157 were positive for rfb. The percentage for the Stx₁ and eae remains relatively similar among the groups, with Stx₁ hovering between 7-9% and 10-14% for eae. More than one third (35%) of the tests positive for O157 were positive for Stx₂ in Non-Shedders, compared to 28% for Dairy B. There was a statistically significant difference for the prevalence of Stx₂ genes when comparing shedders and non-shedders.

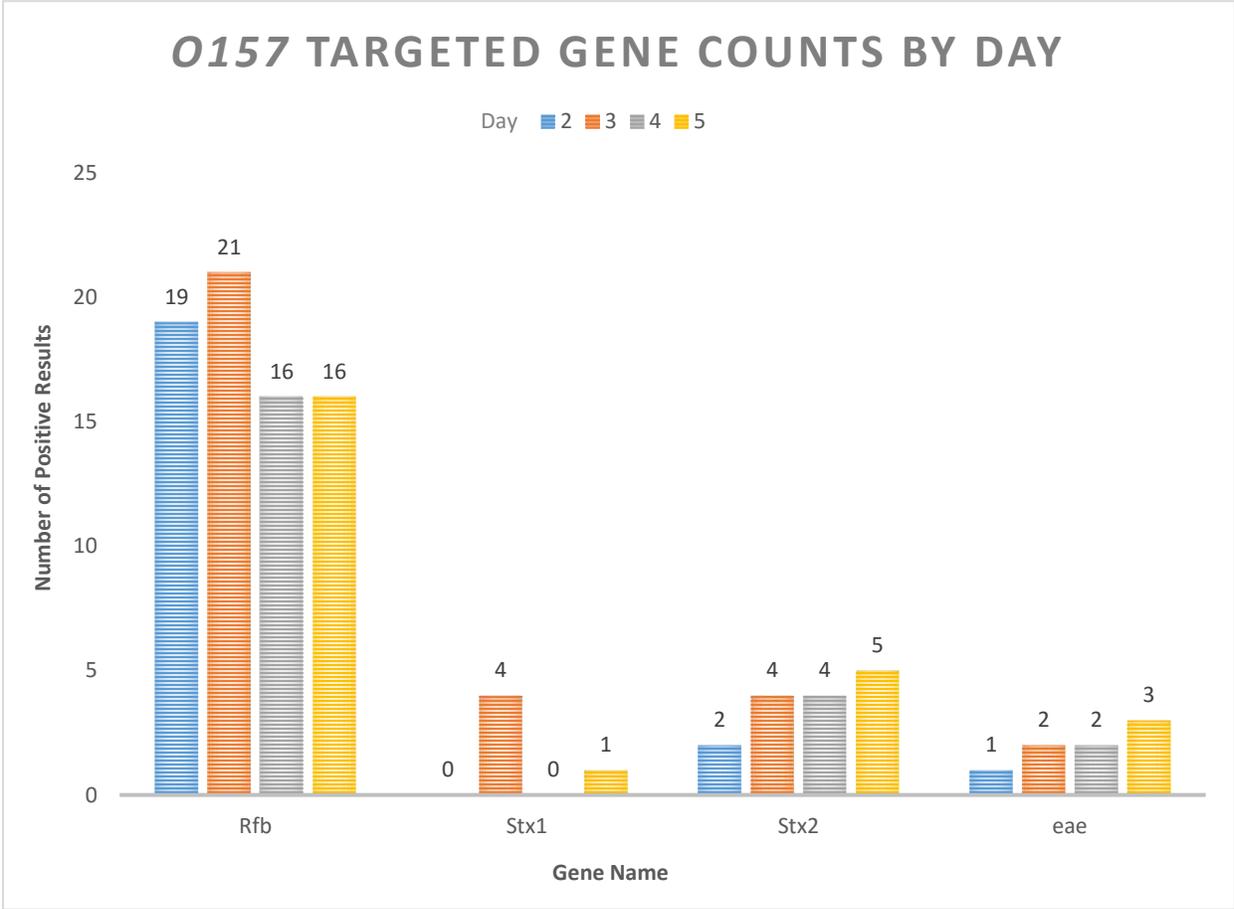


Figure 8 | Number of Positive O157 Genes by Day

Figure 8 presents the number of cows positive for specific O157 genes by day. Day 1 is excluded because cohorts were chosen based on that day. Although Day 5 had the least number of cows shedding O157 (16), the highest number of cows shedding that day were positive for the Stx₂ (5) and eae (3) genes. Day 2 had the lowest number overall of pathogenic gene counts; 0 for Stx₁, 2 for Stx₂, and 1 for eae.

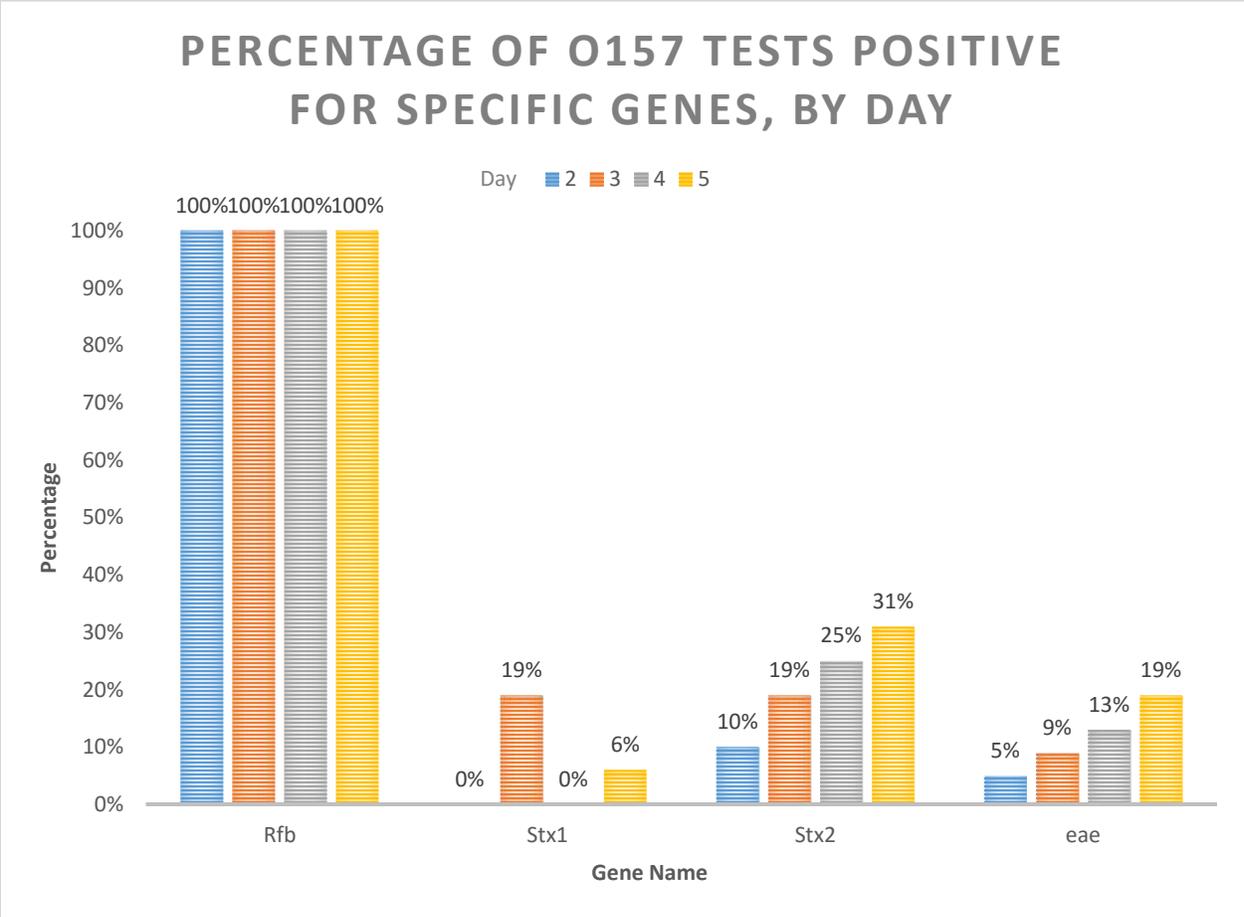


Figure 9 | Percentage of Positive O157 Tests Positive for Specific Genes by Day

Figure 9 shows the percentage of cows that were positive for targeted O157 genes among all cows shedding that day. There is an increasing proportion of cows shedding the Stx₂ and eae genes for each subsequent study day, whereas the trend for Stx₁ is more sporadic. There were no statistically significant differences for these proportions between days.

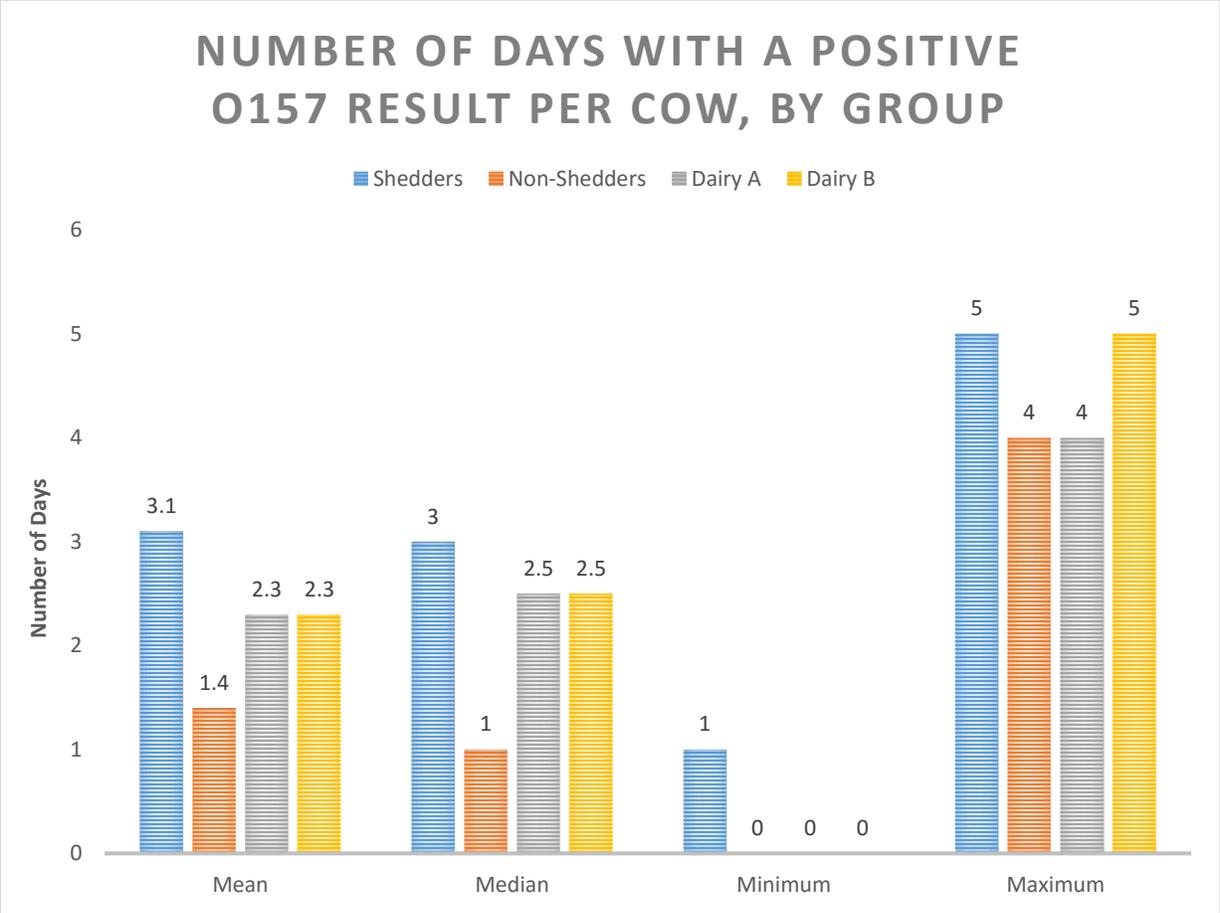


Figure 10 | Number of Days with a Positive O157 Result by Group

Figure 9 shows descriptive information about the number of days each cow was shedding during the study period. Cows in the Shedder cohort had a mean of 3 days during the study that they were shedding compared to between 1 and 2 days for Non-Shedders. There was no difference in the mean between Dairy A and Dairy B.

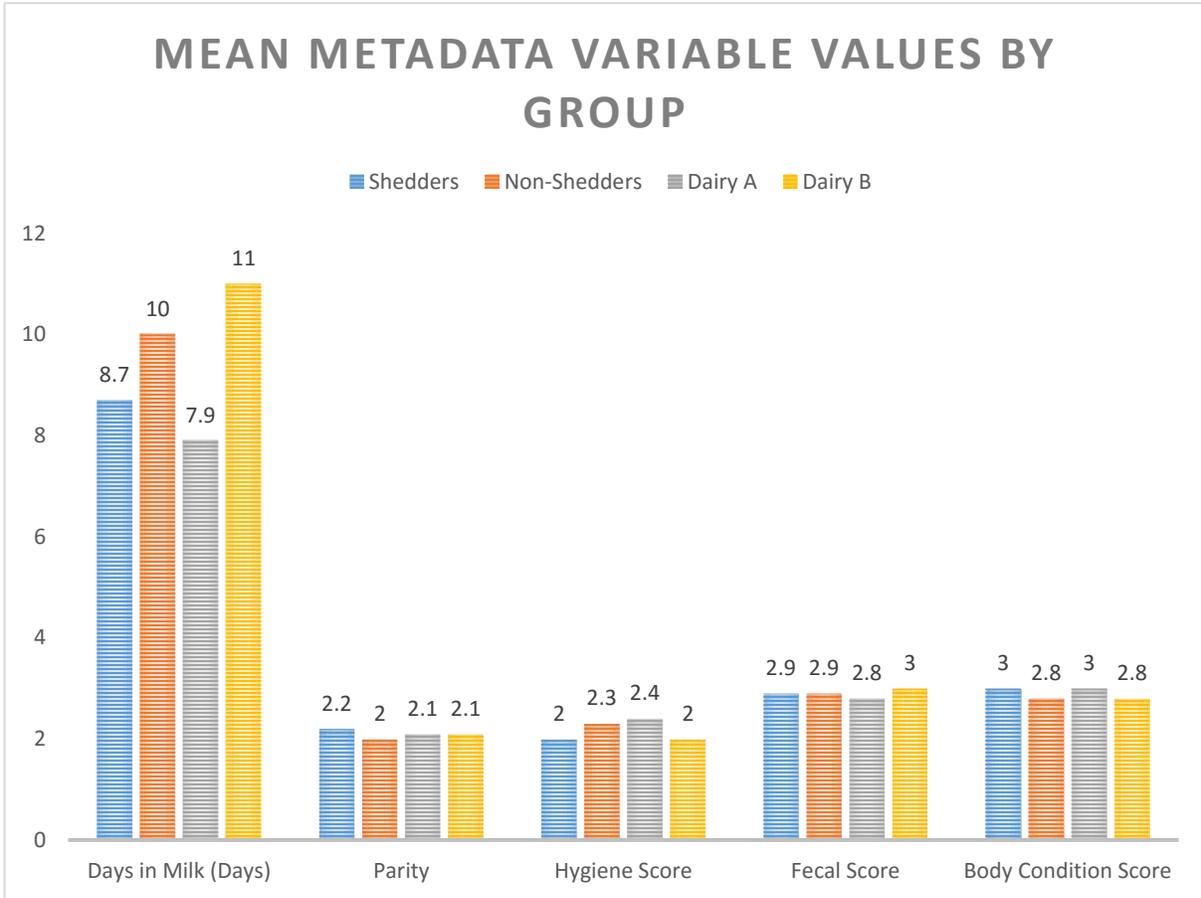


Figure 11 | Mean Metadata Variable Values by Group

Figure 11 shows the mean values for the health information variables collected for each cow. Shedders had a lower average number of days in milk (9 days) compared to Non-Shedders (10 days), and Dairy B had a higher average number of days in milk (11) compared to Dairy A (8 days).

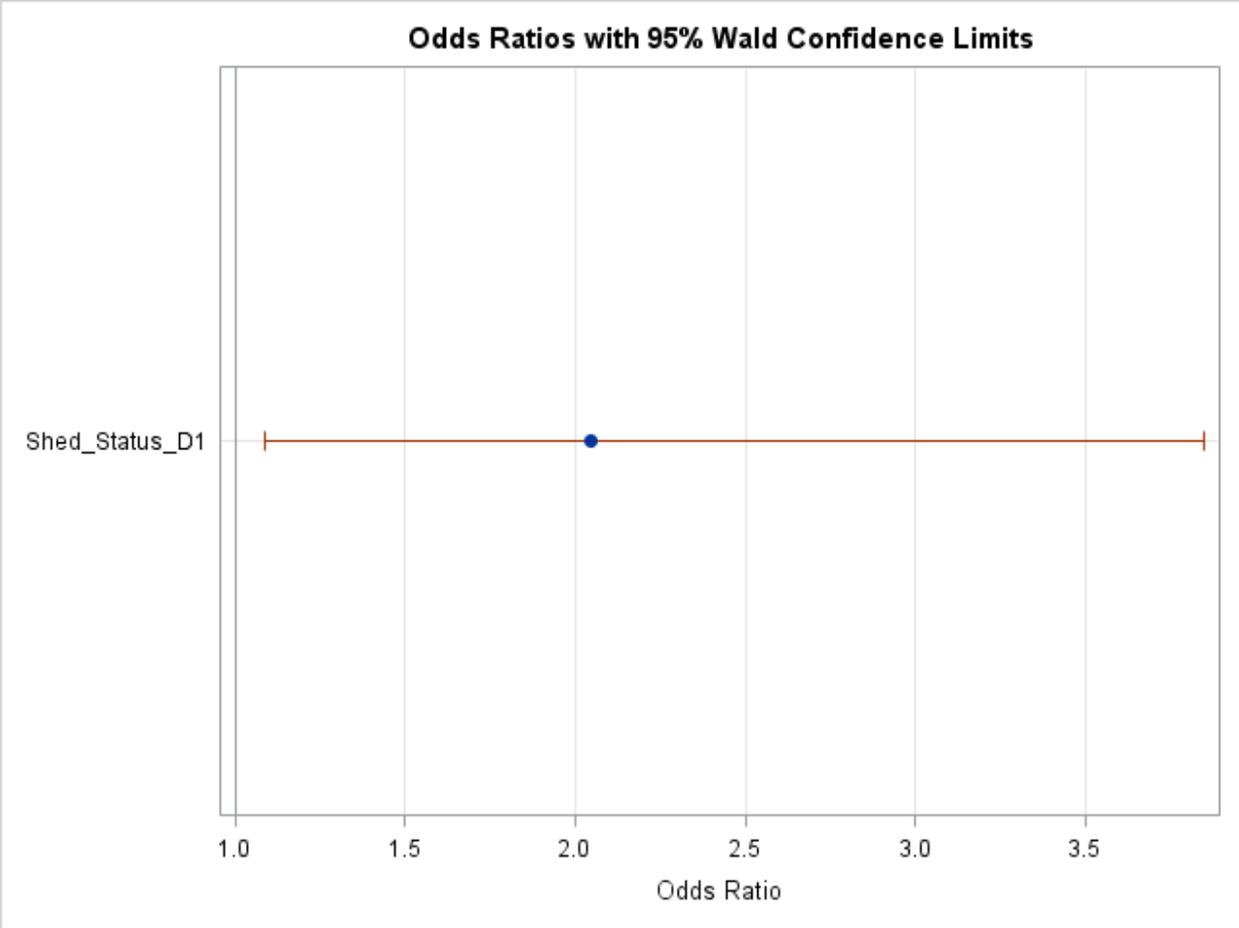
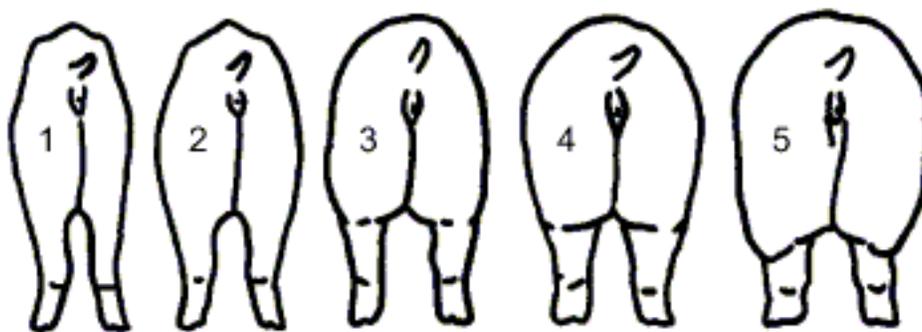


Figure 12 | Odds Ratio for the Logistic Regression Model

Figure 12 shows the Wald output for the shedding status on Day 1 variable in the Logistic Regression Model. Cows who were shedding O157 on Day 1 were about 2 times as likely to shed on any subsequent shedding day than cows who were not shedding on Day 1.



Score	Condition	Detection of ribs, backbone, "H" bones, and "pin" bones
1	Emaciated	Obvious
2	Thin	Easily detected with pressure
3	Ideal	Barely felt with firm pressure
4	Fat	None
5	Overly fat	None

Figure 13 | Body Condition Scoring Criteria

Figure 13 shows the scoring criteria for body condition score. This scoring variable identifies and scores distribution of fat around the cow's body. A score of 1 or 2 indicates underweight, a score of 4 or 5 indicates overweight, and a score of 3 is considered ideal.⁷⁴

Upper Leg & Flank

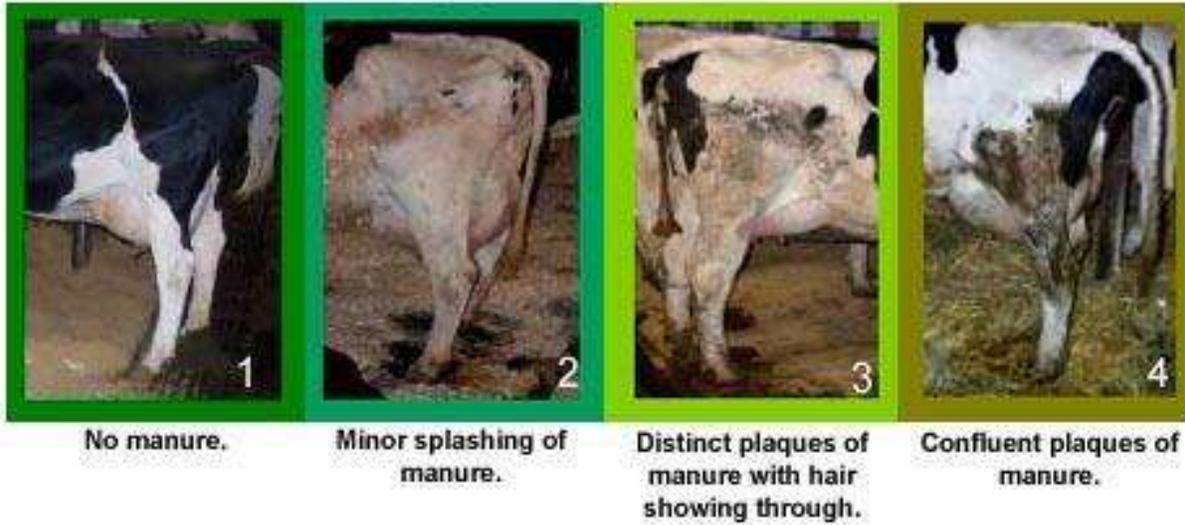


Figure 14 | Hygiene Score Criteria

Figure 14 shows the scoring criteria for Hygiene Score. Hygiene score identifies the percentage of a cow's body that is covered in dirt or manure and then ranks it. A score of 1 indicates very little coverage up to a score of 4 which indicates a high percentage of coverage.⁷⁵

MANURE SCORING



SCORE 1.
This manure is very liquid with the consistency of pea soup. The manure may actually "arc" for the rump of the cow. Excess protein or starch, too much mineral, or lack of fiber can lead to this score. Excess urea in the hind gut can create an osmotic gradient drawing water in the manure. Cow with diarrhea will be in this category.



SCORE 2.
This manure will appear runny and does not form a distinct pile. It will measure less than an inch in height and splatters when it hits the ground or concrete. Cows on lush pasture will commonly have this type of manure. Low fiber or a lack of functional fiber can also lead to this manure score.



SCORE 3.
This is the optimal score! The manure has a porridge-like appearance, will stack up 1 1/2 to 2 inches, have several concentric rings, a small depression or dimple in the middle, make a plopping sound with it hits concrete floors, and it will stick to the toe of your shoe.



SCORE 4.
The manure is thicker, will stick to your shoe, and stacks up over 2 inches. Dry cows and older heifers may have this type of manure (this may reflect that low quality forages are fed and/or a shortage of protein). Adding more grain or protein can lower this manure score.



SCORE 5.
This manure appears as firm fecal balls. Feed a straw based diet or dehydration would contribute to this score. Cows with a digestive blockage may exhibit this score.

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Figure 15 | Fecal Scoring Criteria

Figure 15 shows the scoring criteria for Fecal Score. Fecal score is characterized by the content, color, and consistency of a cow's manure. A score of 1 or 2 indicates liquid or runny diarrhea consistent with diarrhea, a score of 4 or 5 indicates thicker or grainy manure, and a score of 3 is considered ideal.⁷⁶

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