Preweaned heifer management on US dairy operations: Part IV. Factors associated with the presence of *Escherichia coli* O157 in preweaned dairy heifers

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ABSTRACT

Dairy calves shed pathogenic *Escherichia coli* O157 (O157) in feces and are a potential route of exposure for human infections. As part of the National Animal Health Monitoring System's (NAHMS) Dairy 2014 study, we evaluated farm, animal, and environmental factors associated with O157 presence in dairy heifer calves. For this O157 study, calves were enrolled from 100 dairy operations in 13 states. Each operation collected data from calves from birth to weaning over an 18-mo period. A single fecal sample was collected from 487 calves in western states and from 871 calves in eastern states ($n = 1,358$ total), and O157 was detected in 2.5% ($n = 34$) of fecal samples. Descriptive statistics and univariable screening were used to determine which farm practices, environmental factors, and calf health measures were associated with O157 detection. Multilevel logistic models, controlling for dairy operation, were created using backward elimination of screened variables. The final O157 main effects model included variables for source of colostrum, temperature-humidity index (THI), and serum IgG concentration. Higher serum IgG was associated with lower odds of O157 shedding, whereas calves fed colostrum from their own dam had higher odds of O157 shedding than calves fed colostrum from pooled sources. Interaction models showed that THI level modified the effect of colostrum source on O157 shedding; calves with a THI indicative of heat stress had a significantly increased presence of O157 when fed colostrum from a first-lactation dam.

The THI level also modified the effects of serum IgG. Calves with thermoneutral or heat stress THI values had increased presence of O157 with poor (<10 g/L) or adequate (10–15 g/L) serum IgG levels compared with those having excellent (≥ 15 g/L) serum IgG levels. These results highlight factors that influence the presence of O157 in preweaned dairy heifer calves and may be used to guide practices that mitigate shedding through improved animal husbandry.

Key words: dairy calf, *Escherichia coli* O157, risk factor, serum IgG, colostrum

INTRODUCTION

Escherichia coli O157:H7 (O157) is a gram-negative, pathogenic bacterium capable of causing bloody diarrhea, abdominal cramps, and hemolytic uremic syndrome in humans (Karmali et al., 2010; Nguyen and Sperandio, 2012; Croxen et al., 2013). The most recent data available estimate that up to 149,000 domestically acquired human O157 infections occur annually in the United States and are associated with ingestion of contaminated bovine products and other food sources (Scallan et al., 2011). Cattle play a central role in contamination of the food chain, as they can shed O157 in their feces. Once shed from bovine reservoirs, the O157 bacterium can survive under a multitude of conditions and travel far from sites of deposition by meteorological factors and processes such as irrigation and manure fertilization (Avery et al., 2008; Chauret, 2011; van Elsland et al., 2011). Attempts to control O157 after it is disseminated in feces (Food Safety and Inspection Service standard operating procedures for slaughter, USDA codes for food preparation, Pasteurized Milk Ordinance; <https://www.fda.gov/downloads/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Milk/UCM612027.pdf>) have had limited success, which is

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evidenced by persistent human outbreaks (Chekabab et al., 2013; Majowicz et al., 2014). To reduce the future burden of human disease, understanding the dynamics of cattle carriage with O157 and developing strategies that reduce shedding at the animal level are necessary.

After infection via the fecal-oral route, dairy cows that shed O157 have the ability to directly contaminate diverse food products, including milk, meat, and manure-fertilized crops (Meyer-Broseta et al., 2001). This is particularly important, because national studies have indicated that O157 is frequently present within the environment of US dairy herds (Wells et al., 1998; USDA, 2003). Previous studies have revealed that dairy cow shedding patterns are intermittent and complex, but can be influenced by environmental, farm management, host health, and lactation factors (Shere et al., 1998; Menrath et al., 2010; Williams et al., 2015; Venegas-Vargas et al., 2016; Stenkamp-Strahm et al., 2017a). Although risk factors in lactating cows have been measured, less is known about O157 shedding in preweaned calves. Calves may play a pivotal role in both maintaining O157 within the farm environment and directly spreading the pathogen to humans. Calves may shed greater numbers of O157 for prolonged periods (Dean-Nystrom et al., 1997b; Cobbold and Desmarchelier, 2000), and both on- and off-farm human contact tend to be greater with calves than with adult cows (Crump et al., 2002; Smith et al., 2004). Shedding of O157 in calves cannot be directly compared with shedding in adult cows because preweaned calves are monogastrics, and their diet, housing, and overall management on a dairy operation are different from that of cows. Further, O157 may cause diarrhea in calves, whereas adult animals do not experience clinical signs of disease during carriage (Cray and Moon, 1995; Quinn et al., 2015).

Previous studies have shown a low prevalence of shedding in calves (Garber et al., 1995; Rugbjerg et al., 2003; Stenkamp-Strahm et al., 2017b), but this finding is not consistent across the literature (Mechie et al., 1997; Cobbold and Desmarchelier, 2000; Irshad et al., 2012; Jaros et al., 2016). When attempting to define factors that influence carriage of O157 by calves, studies have indicated that dietary shifts, the presence of multiple gastrointestinal (GI) pathogens, and administration of anticoccidial agents may play a role (Cray et al., 1998; Smith et al., 2004; Cho et al., 2013). Operation-level factors, including calving strategy, colostrum administration, housing, and amount of contact between adult cows and calves, may also affect preweaning O157 prevalence (USDA, 1996; Rugbjerg et al., 2003; Cernicchiaro et al., 2012). In the United States, dairy operations are located across the country and are diverse in their calf-rearing approaches and

overall methods of farm management (USDA, 2016). Although some factors for calf shedding have been elucidated, results from studies to date have lacked generalizability. This is due to an absence of comprehensive managerial and calf health data and characterization of samples from geographically discrete areas at few seasonal time points. To develop strategies that reduce the prevalence of O157 on-farm, the dynamics of shedding in calves should be analyzed on a national scale that incorporates information for geography, management, and animal health. The objective of the current study was to examine managerial, environmental, and animal health parameters for associations with the presence of O157 in preweaned heifer calves, using USDA's National Animal Health Monitoring System (NAHMS) Dairy 2014 Calf Component study data. Understanding these parameters may then be used to guide the development of management strategies that reduce O157 shedding in calves.

MATERIALS AND METHODS

Study Design

National surveys of US livestock and poultry industries are conducted annually by NAHMS. In 2014, NAHMS researchers collected information on the health, management, and productivity of the US dairy industry (USDA, 2013). This study also included a longitudinal study that collected data regarding dairy heifer calf health (NAHMS Dairy 2014 Calf Component).

The NAHMS Dairy 2014 Calf Component study was conducted over 18 mo from March 2014 to September 2015. A convenience sample of 104 dairy operations was enrolled from 13 states in 2 regions: California, Colorado, and Washington (western region) and Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin (eastern region). Enrolled operations were categorized based on the number of mature cows: small (30–99 cows), medium (100–499 cows), and large (≥ 500 cows).

At the study outset, each operation was instructed to enroll 24 heifer calves (roughly 2/mo) for a 1-yr period. Due to lower than anticipated participation and a lag in calf enrollment, the target number for participating operations was increased to 48 calves, over an 18-mo period.

Calf Health Card and Sampling of Biologics

Before enrollment, calves were tested for bovine viral diarrhea virus (BVDV) and excluded if positive. For on-farm testing, the Idexx SNAP BVDV Antigen

Test (Idexx, Westbrook, ME) was used, with ear-notch samples collected via V-cut ear notchers. Calves that tested positive for BVDV were excluded so as not to confound the goals of the calf component study; that is, to assess preweaned calf health and growth.

For each enrolled heifer, a Heifer Calf Health Card ("Calf Card"; https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14ques/CalfHealth.pdf) was filled out by the producer, the calf handler, a veterinary medical officer, veterinary extension personnel, or a combination of these individuals. The Calf Card was written in both English and Spanish, and had sections describing birth data (e.g., birth date, birth weight, calving ease of dam), colostrum feeding data (timing, amount, method of feeding), preweaning housing and procedures (e.g., type of housing, ventilation, bedding, dehorning, and navel disinfection), milk feeding (including type of liquid diet, additives, method of feeding) and milk consumption record (volume and frequency of feedings). The Calf Card also contained a growth record (hip height and heart girth recorded every 2 wk), biologic sampling record (serum collection and fecal sampling dates), vaccination history, disease incidence and treatment, weaning data (weaning date, primary weaning criteria), and any additional notes. Starter feed and milk replacer labels were collected if applicable.

For each calf enrolled in the study, colostrum from the initial feeding (40–50 mL) was collected in conical screw-capped tubes and frozen before shipping. Similarly, blood (5 mL) was collected from each calf between 1 and 7 d of age and placed in serum separator tubes, which were centrifuged when possible before shipping. Both colostrum and serum were shipped together on ice to the USDA's National Veterinary Service Laboratories (NVSL; Ames, IA). At the NVSL, serum was separated and serum and colostrum samples accumulated and then shipped to Saskatoon Colostrum Company (Saskatoon, SK, Canada), where they were tested for IgG concentration using radial immunodiffusion.

For radial immunodiffusion, 24-mL agarose plates were punched with 42 wells of 6 μ L volume. Wells 1 to 4 and 39 to 42 were for 2 replicates each of 4 calibrators used to generate a standard curve; wells 5 to 9 and 10 to 14 were for each of the 2 reference standards that were used to qualify the plate; and wells 15 to 38 were used for test samples. Serial 2-fold dilutions (1:4, 1:8, 1:16, 1:32) of the bovine IgG standard (bovine serum calibrator cat. #4055, Midland BioProducts Corp., Boone, IA), reference bovine serum (Center for Veterinary Biologics, Ames, IA, bovine IgG species standard; working stock diluted 1:4), and reference colostrum (diluted 1:15) were prepared in PBS. A 1:4 dilution of test serum and a 1:15 dilution of test colostrum were

prepared using PBS. Two replicates of 4 μ L of each dilution of bovine IgG standard were dispensed for the standard curve. Two replicates of 4 μ L of the diluted reference colostrum and reference serum and 4 μ L of the diluted test samples were also dispensed on the plate. Plates prepared in house using commercially available ingredients and reagents were incubated at 20 to 25°C for 18 to 19 h in a humidified chamber. A plate reader was used to measure and record the ring diameters of precipitin rings surrounding wells (Digital RID reader product code AD400; The Binding Site Inc., San Diego, CA). Using the ring diameters obtained for each of the 2-fold dilutions of bovine IgG standard, a regression line was generated for each plate for the variables R (ring diameter) versus $\log_{10}$ (concentration). The plate was considered acceptable if the coefficient of determination was greater than 0.97 for the standard curve, and the mean values for the reference colostrum and reference serum were the expected values $\pm 10\%$. Concentrations of IgG (g/L) for test samples were determined using the regression line of bovine IgG standards obtained for each plate. To calculate the IgG consumed during the first colostrum feeding for each calf, the concentration of IgG in colostrum samples was multiplied by the volume fed, which was recorded on the Calf Card.

Field personnel were instructed to collect a single fecal sample directly from the rectum of calves between 2 and 4 wk of age. Feces were placed in cylindrical screw-topped containers and shipped on ice to the USDA Agricultural Research Service Environmental Microbial Food Safety Laboratory (EMFSL; Beltsville, MD) for enteric parasite detection. Fecal samples were tested for *Cryptosporidium* and *Giardia* by immunofluorescence microscopy, as described previously (Fayer et al., 2000; Santín et al., 2004). Briefly, 15 g of feces was mixed with 35 mL of distilled water. Each fecal suspension was passed through a sieve with 45- μ m pores. Distilled water was added to the filtrate up to a volume of 50 mL. Samples were then centrifuged for 15 min at $1,800 \times g$. Pellets were resuspended in a solution of 25 mL of distilled water with 25 mL of 1.4 g/L CsCl solution. Samples were then centrifuged at $300 \times g$ for 20 min and the supernatant was aspirated. Each pellet was washed with distilled water, and 2 μ L of pellet suspension was transferred to an 11-mm-diameter well of a glass microscope slide. Two microliters of Merifluor reagent (Meridian Diagnostics, Cincinnati, OH) was added to each well. A Zeiss Axioscop microscope (Jena, Germany) with a 400 $\times$ objective, epifluorescence, and fluorescein isothiocyanate (FITC)-Texas Red dual-wavelength filter, was used to examine each well in its entirety and count oocysts and cysts.

After testing at EMFSL, leftover feces were stored frozen before being shipped on dry ice to the Colorado

State University Animal Population Health Institute laboratory (APHI; Fort Collins, CO) for detection of enteric bacteria. Once all study samples were received at APHI, they were thawed and diluted 1:10 in buffered peptone water (Oxoid Ltd., Basingstoke, UK). Samples were then incubated for 6 h at 37°C, enrichments were stored at -80°C in 10% sterile glycerol, and DNA was extracted as previously described (Shridhar et al., 2016). Briefly, enrichments were thawed, boiled for 10 min, and centrifuged at $9,300 \times g$ for 5 min. The DNA in culture supernatants was purified using a GeneClean Turbo Kit based on the manufacturer's instructions (MP Biomedicals, Solon, OH).

Each extracted DNA sample was subjected to real-time multiplex PCR reactions (**qPCR**), using a BioRad iQ5 machine (BioRad, Hercules, CA). The multiplex combination described below was previously validated to have bacterial detection sensitivity of <18 cfu/10 g in artificially contaminated ground beef and minimal interference by an internal amplification control (**IAC**; Suo et al., 2010). Reactions targeted the *invA* (*Salmonella enterica*; *Salmonella* spp.) and *rfbE* (*Escherichia coli* O157) genes, and human adenovirus IAC DNA, as previously described (Suo et al., 2010). For the IAC, a 79-bp region of the human adenovirus was used, based on exclusivity of the viral sequence to bacterial genomes. The DNA was originally PCR amplified from a long oligonucleotide (5'-TGGAAGCAATGCCAAATGTGTATGTGGTGGCATTGTCTTCTCCCGTTGTAAC-TATCCACTGAGATGTGTTAGGCGCGCC-3'), using the forward and reverse primers described in Table 1, and purified with a QIAquick gel extraction kit (Qia-gen, Valencia, CA). The IAC DNA was prepared and validated at the USDA Molecular Characterization of Foodborne Pathogens Research Unit (Wyndmoor, PA) and shipped to the APHI Laboratory on ice.

The following amplification procedure was used for all real-time reactions: 95°C × 10 min, and 40 cycles of 95°C × 15 s and 60°C × 1 min. Initially, amplification curves of serially diluted IAC (10^6 to 10^1 copies/μL), *Escherichia coli* O157:H7 ATCC 35150, and *Salmonella enterica* ssp. *enterica* var. Typhimurium DNA were performed. Results showed that IAC concentrations of 1.4×10^3 copies had low impedance of bacterial amplification at both low and high concentrations of bacterial template, similar to that seen by Suo and colleagues (2010). After running dilution curves of individual and multiplexed positive controls, amplification efficiencies for both *rfbE* and *invA* targets were determined to ≥94%.

Forward and reverse primers and TaqMan probe sequences are given in Table 1. All of the primers and probes were obtained from Eurofins Genomics (Louisville, KY). The following was added to each 20-μL

Table 1. Forward and reverse primers and TaqMan probes used in bacterial detection by quantitative PCR

Organism	Gene	Primer/probe	Sequence (5'-3')	Amplicon size (bp)	Probe 5' reporter/ 3' quencher
<i>Escherichia coli</i> O157	<i>rfbE</i>	Forward Reverse Probe	TGTTCCAACACTGACATATATAGCATCA TGCCAAGTTTCATTATCTGAATCAA ATGCTATAAATACACAGGAGCCACCCCA	93	HEX/BHQ 2
<i>Salmonella enterica</i>	<i>invA</i>	Forward Reverse	GTTGAGGATGTTATTCGCAAGG GGAGGTTCCGGGTCAAG	75	6-FAM/BHQ 1
Human adenovirus (control)	IAC	Forward Reverse Probe	CCGTCAGACCTCTGGCAGTACCTTCCTC GGCGCGCTAACACATCT TGGAAGCAATGCCAAATGTGTA TTACAACGGGAGAGACAATGCCACCA	79	Texas Red/BHQ 2

reaction: 1× PerfeCTa qPCR ToughMix (Quanta Biosciences, Beverly, MA), 200 nmol each of forward, reverse, and probe oligonucleotides (3 sets), 1.4×10^3 copies of IAC, and 2 µL of control or test DNA. Each test DNA sample was run in triplicate.

Growth Measurements

Height and weight measures of calves were gathered approximately every 2 wk during the preweaning period. Height/weight tapes from Coburn (Nasco, Fort Atkinson, WI) were used to make measurements. Birth weights (kg) were estimated using a scale, hoof circumference, or heart girth circumference. When measured, hip height and heart girth circumference were recorded in centimeters. Heart girth (cm) was converted to BW in kilograms using the following equation from Heinrichs et al. (1992):

$$\text{BW (kg)} = [(0.02655 \times (\text{cm}^2)) + (-2.876 \times \text{cm}) + 102.71]. \quad [1]$$

Average daily gain was calculated by subtracting the birth weight from the final weight and dividing by the number of days between the 2 measurements (approximately equivalent to the duration of the preweaning period). Final weights did not always occur at the same time as weaning, although only calves with final weight measurements within 14 d (SD = 8.9 d) of weaning were included in analyses.

Meteorological Measurements

County measures of the temperature-humidity index (THI) were derived using data from the National Oceanic and Atmospheric Administration (www.noaa.org). The THI accounts for the effects of temperature and humidity, using dry bulb temperature (T, °F) and relative humidity (RH). The equation for THI used in this analysis was (Buffington et al., 1977; Collier et al., 2011):

$$\text{THI} = T - [0.55 - (0.55 \times \text{RH}/100) \times (T - 58)]. \quad [2]$$

Values from the month of fecal sample collection were obtained, and each calf was assigned a single THI value. The THI values were then categorized based on thermoneutral zones (Scibilia et al., 1987), because external temperature likely influences the shedding of O157 (Williams et al., 2015). The following categories were created: cold stress (THI <50), thermoneutral (THI ≥50 and <70), and heat stress (THI ≥70).

Because calves with fecal samples taken toward the beginning of the month may have been assigned THI values that did not truly reflect the time immediately before O157 detection, a sensitivity analysis was performed. Data were categorized based on whether or not calves had a THI value recorded for their birth month. Because these were more likely to reflect calves with THI values that coincided with the time period before shedding, multilevel regression models (see below) were run using both data sets (birth-month matched or non-birth-month matched), in turn, after randomly subsampling to the same number of observations.

Data Management and Statistical Analysis

When each calf was weaned, the Calf Card was mailed to USDA NAHMS (Fort Collins, CO). Each Calf Card was validated by NAHMS personnel when it arrived. Once all Calf Cards were entered, information was again validated by NAHMS employees and merged with results from colostrum and serum testing at Saskatoon Colostrum Company, fecal testing at EMFSL and APHI, and THI data. Variables assessed for association with O157 presence were selected based on their biologic or historically relevant plausibility to affect colonization of the pathogen. Broadly, these variables were related to calf growth parameters, immune status, housing and maintenance, and presence of GI microbes and parasites. These variables are shown in Table 2 and included days of age at fecal sampling, birth weight, weight at weaning, days of age at weaning, ADG, IgG consumed in colostrum, serum IgG, colostrum source, hours until colostrum given, type of liquid diet, milk pasteurization, milk delivery, calf bedding type, size of herd, calf breed, housing ventilation, calving ease, dam lactation, THI, any disease, coccidiostat administration, dehorning, housing type, presence of *Giardia*, presence of *Cryptosporidium*, presence of *Salmonella enterica*, region of farm, navel disinfection, administration of direct-fed agents, and administration of any *E. coli* preventative.

Continuous variables were tested for normality. Hours to colostrum administration was dichotomized based on this variable's nonlinear nature. Because of low values in several categories, the incidence of disease as indicated on Calf Cards (cough, runny nose, runny eyes, breathing difficulty, head tilt, listlessness, droopy ears, off of feed, dull, diarrhea) was collapsed to a binary outcome of "any disease" or "no disease." In addition, the variable for calf bedding initially included options for straw/hay, shavings, sand, manure biosolids, none, or other/combination. This variable was collapsed to reflect the most prominent categories, while still dis-

cerning bedding types shown previously to affect O157 growth: straw/hay, shavings/woodchips, and other/combination (Lejeune and Kauffman, 2005; Ellis-Iversen et al., 2008). Direct fed agents administered to calves in this study, as indicated on calf cards, included Convert Day One Calf Gel (All West SelectSires, Burlington, WA), NuLife ReBOUND (Genex, Shawano, WI), First Arrival (DBC Ag Products, Lancaster, PA), Last Stand Calf Paste (DBC Ag Products), Vita Charge (BioZyme Inc, St. Joseph MO), and probiotics. This variable was dichotomized into “any direct-fed agent” or “no direct-fed agent.” Calves of the current study were also given many vaccines. To reflect vaccinations that might affect colonization or shedding of the O157 pathogen, vaccines indicated on Calf Cards were categorized based on their efficacy against GI microbes. Four vaccines were selected and further investigated for their association with O157 shedding. These vaccines were categorized as “*E. coli* preventatives,” and identified on Calf Cards as Bovi-Sera (Colorado Serum Company, Denver, CO), Piliguard E.coli-1 (Schering-Plough, Kenilworth, NJ), First Defense (Immucell Corp, Portland, ME), and Ecolizer (Novartis Pharmaceuticals, Sacramento, CA). The variable indicating “hours to colostrum feeding” was dichotomized (≤ 4 h or > 4 h) based on industry recommendations. Variables that may temporally be associated with O157 (disease incidence, dehorning, administration of *E. coli* preventative) were classified based on their occurrence before fecal sampling.

Distributions of calf variables by presence of O157 were measured and tested for differences using Fisher's exact test for categorical variables and *t*-tests for continuous variables (Table 2). Because of the small number of calves with O157, variables with $P < 0.20$ were selected and only calves with all variables in Table 2 were included in regression analyses.

Mixed logistic regression models were implemented to determine factors significantly associated with the presence of O157. Screened variables were initially assessed via univariable mixed logistic regression models, with dairy operation as a random variable. Variables with $P < 0.20$ in univariable screening were assessed for collinearity and included in multivariable models in the absence of collinearity. Initial multivariable models were stable. Step-wise backward elimination model selection was then performed, screening for confounding at each step. Variables with $P < 0.05$ were retained in a final model of main effects. An interaction analysis was then performed. Multiplicative interactions between main effects were assessed by introducing a product term for all final model covariate pairs, in turn, in mixed regression models. For interaction variables that met a $P < 0.05$ threshold for significance, stratified odds

ratios (OR) were determined for different combinations of each variable level.

Initial statistical analysis, screening of variables, and interaction assessments were performed in R version 3.3.2 or later (<https://www.r-project.org/>). Mixed logistic regression analysis was performed in SAS (version 9.4; SAS Institute Inc., Cary, NC) using the GLIMMIX procedure.

RESULTS

For the study, fecal samples were obtained from 2,249 calves. Of these samples, 1,358 (60.4%) had sufficient material for laboratory analysis of all parasitic and bacterial pathogens. These calves were from 100 different operations in the western ($n = 487$; CA, CO, WA) and eastern ($n = 968$; IA, MI, MN, MO, NY, OH, PA, VT, VA, WI) regions. For fecal-level measures, 31% ($n = 421$) of calf samples contained *Giardia* and 44% ($n = 593$) of samples contained *Cryptosporidium*. Relatively few samples ($n = 12$, 0.8%) contained *Salmonella* spp. detected via qPCR, and a higher number ($n = 34$, 2.5%) were found to contain O157.

We quantified farm practices, environmental factors, and calf health measures by O157 status (Table 2). Thirteen variables with differences ($P < 0.2$) in O157 presence were retained for evaluation in univariable mixed models (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-14659>). These variables included days of age at fecal collection, days of age at weaning, ADG (kg), serum IgG, calf bedding, size of herd, hours until colostrum feeding, colostrum source, THI, *Giardia*, *Cryptosporidium*, *Salmonella*, and any *E. coli* preventative. Calves with missing values for any of the retained variables were omitted from further analysis. The regression data set included information from 1,106 calves (omitting 252), with 2.5% ($n = 28$) of calf samples containing O157.

Associations with O157 presence were measured for each variable individually, using mixed logistic regression with operation as a random variable (Supplemental Table S1; crude OR). Those with individual $P < 0.2$ significance (all variables except *Giardia* and *Cryptosporidium*) were used in an initial mixed multivariable model, with operation as a random variable (Supplemental Table S1; adjusted OR). After backward step-wise elimination, 3 main effects variables were significantly ($P < 0.05$) associated with O157 presence in calves: colostrum source, THI, and serum IgG (Table 3). Compared with pooled sources, colostrum from the calf's dam (lactation 1, 2, or 3+), was associated with an increased odds of O157 presence (e.g., OR from second-lactation calf dam = 1.045, 95% CI

Table 2. Herd- and calf-level descriptors by *Escherichia coli* O157 presence in preweaned dairy calves

Descriptor	No O157, no. (SD or %) (n ≤ 1,324)	O157, no. (SD or %) (n ≤ 34)	P-value
Days of age at fecal sampling (SD)	21.8 (6.2)	23.6 (7.5)	0.18
Birth weight, ¹ kg (SD)	42.8 (5.5)	43.0 (5.5)	0.88
Weight at weaning, ¹ kg (SD)	88.4 (20.8)	86.5 (20.7)	0.62
Days of age at weaning ¹ (SD)	63.8 (17.4)	69.3 (17.2)	0.08
ADG, ¹ kg (SD)	0.7 (0.2)	0.6 (0.2)	0.05
IgG ¹ (g) consumed in colostrum (SD)	220.9 (115.9)	197.8 (119.9)	0.31
Serum IgG ¹ (g/L) ₂ (SD)	22.2 (10.6)	15.7 (8.9)	<0.01
Colostrum source ²			
First-lactation dam	228 (18)	8 (23)	0.021
Second-lactation dam	171 (13)	8 (23)	
≥Third-lactation dam	236 (18)	10 (30)	
Unknown-lactation dam/not calf's dam	377 (29)	7 (21)	
Pooled	262 (20)	1 (3)	0.76
Commercial replacer	20 (2)	0 (0)	
Type of liquid diet ² (%)			
Milk replacer	454 (34)	10 (29)	
Whole/waste milk	508 (38)	13 (38)	0.80
Both milk replacer and waste milk	362 (27)	11 (32)	
Milk pasteurization ² (%)			
Pasteurized	428 (32)	11 (32)	
Not pasteurized	442 (33)	13 (38)	0.35
Milk replacer only	454 (34)	10 (29)	
Milk delivery ² (%)			
Bottle	348 (26)	8 (24)	0.13
Bucket	230 (17)	2 (6)	
Milk bar	18 (1)	0 (0)	
Robot	17 (1)	0 (0)	
Bottle + bucket	639 (48)	23 (68)	0.15
Other combinations	72 (5)	1 (3)	
Calf bedding ² (%)			
Straw/hay	732 (55)	15 (44)	
Shavings/woodchips	299 (23)	13 (38)	0.68
Other/combo	293 (22)	6 (18)	
Size of herd ² (%)			
Small (30–99 cows)	188 (14)	8 (24)	0.38
Medium (100–499 cows)	353 (27)	11 (35)	
Large (500 or more cows)	783 (59)	15 (44)	
Breed ² (%)			
Holstein	1,182 (89)	31 (91)	1.00
Jersey	57 (4)	2 (6)	
Other	82 (6)	1 (3)	
Housing ventilation ² (%)			
Outside/natural	1,073 (81)	31 (91)	0.98
Positive-pressure tubes	130 (10)	1 (3)	
Other/cross ventilation	121 (9)	2 (6)	
Calving ease ² (%)			
Unassisted	954 (78)	27 (79)	0.23
Easy (1 person help)	205 (17)	6 (18)	
Difficult (≥2 people help)	58 (5)	1 (3)	
Mechanical/surgical extraction	7 (1)	0 (0)	
Dam lactation ² (%)			0.86
1	467 (39)	14 (41)	
2	317 (27)	9 (26)	
≥3	406 (34)	11 (32)	
Temperature-humidity index (THI) ² (%)			<0.01
<50, cold stress	594 (45)	3 (9)	
50–69, thermoneutral	576 (44)	18 (53)	
≥70, heat stress	154 (12)	13 (38)	
Any disease ² (%)			0.86
Yes	335 (25)	5 (15)	
No	989 (75)	29 (85)	
Coccidiostats added to milk ² (%)			
Yes	516 (40)	14 (41)	
No	789 (60)	20 (59)	

Continued

Table 2 (Continued). Herd- and calf-level descriptors by *Escherichia coli* O157 presence in preweaned dairy calves

Descriptor	No O157, no. (SD or %) (n ≤ 1,324)	O157, no. (SD or %) (n ≤ 34)	P-value
Dehorned ² (%)			
Yes	286 (24)	7 (23)	1.00
No	925 (76)	23 (77)	
Calf housing ² (%)			
Pen/hutch	1,173 (89)	31 (91)	1.00
Group	151 (11)	3 (9)	
<i>Giardia</i> isolated ² (%)			
Yes	407 (31)	14 (41)	0.18
No	917 (69)	20 (59)	
<i>Cryptosporidium</i> isolated ² (%)			
Yes	574 (43)	19 (56)	0.16
No	750 (57)	15 (44)	
<i>Salmonella enterica</i> isolated ² (%)			
Yes	9 (1)	3 (9)	<0.01
No	1,315 (99)	31 (91)	
Region ^{2,3} (%)			
Western	475 (36)	12 (35)	1.00
Eastern	849 (64)	22 (65)	
Navel disinfection ² (%)			
Yes	981 (76)	25 (74)	0.84
No	315 (24)	9 (26)	
Any direct-fed agent ² (%)			
Yes	15 (1)	1 (3)	0.34
No	1,309 (99)	33 (97)	
Any <i>E. coli</i> preventative ² (%)			
Yes	166 (13)	0 (0)	0.02
No	1,158 (87)	34 (100)	
Hours until colostrum given ² (%)			
≤ 4	1,016 (82)	20 (61)	<0.01
>4.0	201 (18)	13 (39)	

¹Continuous variables (SD in parentheses) assessed with Student's *t*-test.²Categorical/dichotomous variables (%) in parentheses) analyzed with Fisher's exact test.³Western region included California, Colorado, and Washington; Eastern region included Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin.

= 1.011–1.081). When compared with a thermoneutral THI, a heat stress THI showed a significant association with increased O157 presence (OR = 1.057, 95% CI = 1.023–1.089) and a cold stress THI showed significant association with decreased O157 presence (OR = 0.974, 95% CI = 0.955–0.993). Values for serum IgG showed

that each 1-g increase was significantly associated with a reduced odds of O157 presence (OR = 0.998, 95% CI = 0.997–0.999).

With regard to interaction analyses, THI was found to significantly modify the effect of colostrum source and serum IgG (Table 4; *P* < 0.001 for THI × colos-

Table 3. Main effects in multivariable mixed logistic regression model of *Escherichia coli* O157 presence in preweaned dairy calves

Variable	Unit increase	Odds ratio ¹	SE	95% CI	P-value
Serum IgG (g/L)	1	0.998	0.0005	0.997–0.999	<0.001
Temperature-humidity index for stress (THI)					
>70, heat stress		1.057	0.0149	1.023–1.089	<0.001
50–69, thermoneutral		Referent			
<50, cold stress		0.974	0.0100	0.955–0.993	0.009
Colostrum source					
First-lactation dam		1.033	0.0158	1.002–1.066	0.040
Second-lactation dam		1.045	0.0171	1.011–1.081	0.010
≥Third-lactation dam		1.042	0.0157	1.010–1.075	0.009
Unknown-lactation dam/not calf's dam		1.012	0.0145	0.983–1.041	0.420
Colostrum replacer		0.977	0.0388	0.906–1.055	0.555
Pooled colostrum		Referent			

¹Odds of O157 presence, calculated from mixed logistic regression using operation as random variable.

trum source and $P = 0.0007$ for THI $\times$ serum IgG). To measure the relationship between these variables, serum IgG was categorized and samples were stratified based on standard industry cut-offs (<10 g/L, 10.1–14.9 g/L, ≥ 15 g/L; USDA, 2010). Mixed logistic regression modeling was performed using multiplicative interaction terms, and O157 logit values were plotted for each level of serum IgG (Figure 1) and colostrum source (Figure 2) using the lowest risk categories from the main effect model (low THI, high serum IgG, pooled colostrum). The odds of O157 presence increased as THI level increased and serum IgG level decreased (Table 4). Specifically, compared with calves exposed to a cold stress THI with high (≥ 15 g/L) serum IgG values, calves exposed to thermoneutral or heat stress THI, with low or moderate values of serum IgG, were at increased risk of O157 presence (thermoneutral and low IgG OR: 1.076, 95% CI = 1.008–1.149; heat stress and low IgG OR: 1.203, 95% CI = 1.103–1.311). Compared with calves exposed to a cold stress THI and fed pooled colostrum,

calves fed colostrum from their own first-lactation dam and exposed to a heat stress THI were at increased risk of O157 presence (OR: 1.238, 95% CI = 1.128–1.360). Calves fed colostrum from their own second-lactation dam and exposed to a thermoneutral THI were also at increased risk of O157 presence compared with the reference group (OR: 1.076, 95% CI = 1.007–1.150).

We performed a sensitivity analysis using THI values from birth-month-matched or non-birth-month-matched calves. The change in model fit (Akaike's information criterion value) was negligible between data sets (data not shown).

DISCUSSION

In the current study, we detected O157 in 2.5% of tested NAHMS calf fecal samples. The calves studied were raised on diverse operations across the United States. By comparison, during the NAHMS 1991–1992 National Dairy Heifer Evaluation Project (NDHEP),

Table 4. Interaction multivariable mixed logistic regression model of *Escherichia coli* O157 presence in preweaned dairy calves

Variable	O157 (+)/O157 (–) (%)	Odds ratio ¹	SE	95% CI	P-value
Categorized serum IgG (g/L)					
<10	10/117 (7.9)	0.992	0.025	0.944–1.04	0.732
10–14.9	6/150 (3.8)	0.993	0.019	0.957–1.03	0.704
≥ 15	12/811 (1.5)	Referent			
Temperature-humidity index for stress (THI)					
≥ 70 , heat stress	12/131 (8.4)	0.988	0.031	0.930–1.050	0.697
50–69, thermoneutral	14/457 (3.0)	0.9970	0.022	0.956–1.041	0.905
< 50 , cold stress	2/490 (0.4)	Referent			
Colostrum source					
First-lactation dam	6/190 (3.0)	1.001	0.022	0.959–1.046	0.956
Second-lactation dam	7/138 (4.8)	1.003	0.025	0.955–1.053	0.920
$\geq$ Third-lactation dam	9/191 (4.5)	1.025	0.023	0.980–1.072	0.274
Unknown-lactation dam/not calf's dam	5/300 (1.6)	1.000	0.021	0.960–1.042	0.986
Colostrum replacer	1/17 (5.6)	1.001	0.055	0.899–1.115	0.984
Pooled colostrum	1/262 (0.4)	Referent			
Categorized serum IgG $\times$ THI					
<10 g/L, heat stress	5/19 (20.8)	1.203	0.044	1.103–1.311	< 0.001
10–14.9 g/L, heat stress	3/17 (15.0)	1.108	0.042	1.02–1.202	0.014
<10 g/L, thermoneutral	5/53 (8.6)	1.076	0.033	1.008–1.149	0.028
10–14.9 g/L, thermoneutral	3/54 (5.3)	1.047	0.029	0.989–1.107	0.114
Any serum IgG, cold stress	2/490 (0.4)	Referent			
≥ 15 g/L, any THI	12/811 (1.5)	Referent			
Colostrum source $\times$ THI					
First-lactation dam, thermoneutral	1/79 (1.4)	1.004	0.031	0.944–1.067	0.908
Second-lactation dam, thermoneutral	6/60 (9.0)	1.076	0.034	1.007–1.150	0.031
$\geq$ Third-lactation dam, thermoneutral	5/83 (5.7)	1.020	0.031	0.959–1.084	0.532
Unknown lactation/other dam, thermoneutral	1/127 (0.8)	0.999	0.028	0.945–1.057	0.983
Colostrum replacer, thermoneutral	0/9 (0.0)	0.949	0.079	0.813–1.107	0.506
First-lactation dam, heat stress	5/16 (24.0)	1.238	0.048	1.128–1.360	< 0.001
Second-lactation dam, heat stress	1/16 (5.9)	1.000	0.053	0.902–1.110	0.996
$\geq$ Third-lactation dam, heat stress	2/23 (7.7)	1.020	0.046	0.932–1.117	0.662
Unknown lactation/other dam, heat stress	4/42 (8.7)	1.038	0.041	0.957–1.125	0.370
Colostrum replacer, heat stress	1/0 (100)	0.999	0.162	0.727–1.374	0.998
Any THI and pooled colostrum	1/262 (0.4)	Referent			
Cold THI and any colostrum source	2/490 (0.4)	Referent			

¹Odds of O157 presence, calculated from mixed logistic regression using operation as random variable.

approximately 7,000 calf samples from over 1,000 US operations were tested for O157, and the prevalence was found to be 0.36% (USDA, 1996). Calves positive for O157 from both the current study and NDHEP showed no regional clustering across the United States. Another report that included case and control herds from the original NDHEP showed calf O157 prevalence to be 1.9% (Zhao et al., 1995). These results support a systematically low prevalence of O157 in preweaned calves, with little variation across the country.

In these analyses, calves given colostrum from their own dam, compared with pooled sources and colostrum replacer, had a higher risk of O157 presence. This finding is in contrast to the view that pathogen transmission may be increased by administration of pooled colostrum, because pooled aliquots can be contaminated by multiple dam sources. Although NAHMS did not capture the amount of time that calves spent with their dam, the average time to colostrum feeding was significantly greater for calves fed colostrum from their own dam compared with other sources (data not shown). We speculate that calves fed colostrum from their own dams likely spent more time with their dam after birth than did calves fed colostrum from another dam or a

pooled source. Because the specific route of colostrum administration (suckling versus hand feeding) and amount of time spent in the calving pen was not included in current analyses, future studies would need to corroborate this hypothesis. Previous studies have shown that calves left unsupervised to nurse colostrum from their own dams may take longer to ingest colostrum, ingest less colostrum overall, and ultimately experience poor passive transfer (USDA, 2010). Although total colostral IgG given was not associated with O157 presence, our interaction model showed that calves fed colostrum from their own first-lactation dam were at increased risk of shedding O157 when also experiencing a heat stress THI. Previous studies have shown that first-lactation dams experience a higher prevalence of O157 shedding, which tends to increase earlier in their lactation cycle and during warm months of the year (Menrath et al., 2010; Venegas-Vargas et al., 2016; Stenkamp-Strahm et al., 2017a). Coliforms have also been shown to exist in high quantities in raw colostrum (Schneider et al., 2008). Very few operations enrolled in the NAHMS Dairy 2014 study pasteurized their individual cow colostrum (2.6%), and we were unable to correlate pasteurization with a potential reduction in

Figure 1. Interaction of categorized serum IgG and temperature-humidity index for stress on *Escherichia coli* O157 shedding status of calf fecal samples. Logit values were obtained via mixed logistic regression using calf operation as a random variable. Color version available online.

O157 presence in the current study. Further research is needed to determine the frequency by which O157 may be transmitted to calves via colostrum, exposure to the dam, or exposure to the calving pen environment.

Calves that experienced high THI representative of heat stress were more likely to have O157-positive fecal samples. Previous studies have shown that O157 prevalence in dairy cows fluctuates during the year. A study by Williams et al. (2015) showed that higher temperature and humidity were positively associated with increased shedding in dairy heifers on pasture. Other studies that looked at annual dairy farm prevalence of O157 showed levels to be highest during the warm late summer and early autumn months (USDA, 2003; Menrath et al., 2010; Stenkamp-Strahm et al., 2017a).

Sheng and colleagues (2015) reported that a change in seasonal exposure, rather than intrinsic animal change, caused warm-month increases in cattle shedding. Although O157 is capable of surviving in a multitude of environments, survival in manure is lower when temperatures are fluctuating (Semenov et al., 2007). It is known that very high temperatures can stress O157 microbes, but protection within manure and lack of temperature variation during the warm months may

equate to enhanced O157 survival. This may increase oral exposure in calves and lead to the increased presence during periods of heat stress. Our results also showed a slight reduction of O157 presence in calves experiencing cold stress, as only 2 individuals had O157 at THI values <50. Although O157 can survive in cold or frozen manure, exposure of O157 to calves may be reduced because of decreased environmental movement by moisture (Kudva et al., 1998).

Changes in ambient temperature and humidity can affect not just environmental but also calf physiological factors. Our results showed an interaction between THI and serum IgG value. Calves with a high IgG had lower odds of shedding during thermoneutral and heat stress THI than calves with moderate or low IgG in the same groups. This suggests that the immune health of the calf is important to reduce the presence of the O157 pathogen, particularly when calves may be physiologically predisposed to shedding due to high seasonal exposure. Because of this, strategies that mitigate the effect of seasonal increases in temperature (e.g., modifying ventilation, providing reflective roofing, placing hutch in shaded areas) may be advantageous to reduce shedding in calves with poor passive transfer. It

Figure 2. Interaction of colostrum source and temperature-humidity index for stress on *Escherichia coli* O157 shedding status of calf fecal samples. Logit values were obtained via mixed logistic regression using calf operation as a random variable. Color version available online.

is also possible that heat stress of calves in utero or of dams during dry-off impairs IgG absorption in the calf, resulting in lower levels of serum IgG (Tao et al., 2012; Dahl et al., 2016). To avoid poor passive transfer in calves, the timely administration of sufficient amounts of high-quality colostrum, especially during times of heat stress, may also be an advantageous strategy (Shivley et al., 2018). More work is required to better understand whether the association between THI value and O157 presence is due to environmental influence, animal health factors, or both.

During this study, serum IgG values were inversely associated with the presence of O157 in calves. Immune status may be important for O157 presence in calves because of calves' potential to have a clinical response to colonization. In contrast to adults, calves colonized with O157 can develop diarrhea and attaching and effacing lesions along the GI villous border (Dean-Nystrom et al., 1997a,b). However, we detected no association between history of any disease (including diarrheal disease) and O157 presence in the current study. Compared with infections of O157 alone, the literature suggests that loose stools are more commonly a result of other calf pathogens or other pathogens in concert with O157 (Kang et al., 2004; Bartels et al., 2010; Quinn et al., 2015). As incubation periods may influence when clinical disease is detected, a sensitivity analysis was performed to look at any disease within 4 d of fecal sample collection (data not shown). We found no significant association between disease and the presence of O157.

It is difficult to tie serum IgG levels directly to O157 colonization in the absence of disease because dams are not likely passing anti-O157 antibodies to calves in colostrum. This is evidenced by previous studies. In one study that looked at 1-wk-old calves at slaughter, serum IgG values were not associated with O157 carriage (Irshad et al., 2012). Other results have shown that colostrum-fed experimentally infected calves did not have greatly different clinical outcomes than colostrum-deprived experimentally infected calves (Dean-Nystrom et al., 1997a). In another study, newborn calves were fed hyperimmune colostrum from dams immunized with the O157 antigens γ -intimin C280 and EspB. These specific antigens were selected because they allow O157 to bind eukaryotic host cells (γ -intimin) and create pores in these cells post-binding (EspB), and adult cattle also respond serologically to these antigens during experimental infection (Bretschneider et al., 2007). Although calves showed increased mucosal antibodies to both of these antigens at sites of O157 colonization (the recto-anal junction and ileum), fecal shedding of O157 was not reduced and enterocyte erosion still occurred after

infection challenge at 10 wk of age (Rabinovitz et al., 2014). This body of evidence indicates that cattle do not pass anti-O157 antibodies in colostrum, and colostrum quantity and quality may not affect clinical or shedding outcomes in O157-infected calves. Interpreting changes in serum IgG alone should be done cautiously, because a strong and significant interaction was found with this variable and THI in the current models. However, it is plausible that the detected association between O157 presence and serum IgG was due to broadly enhanced health parameters related to improved immunity. This correlates with previous research showing that O157 is associated with the presence of other GI microbes (Smith et al., 2004). The immunity and GI health of calves is undoubtedly influenced by their GI microbiota. Work has shown that the presence of other gram-negative pathogens, reductions in microbial diversity, and the absence of key taxa may influence whether O157 is able to colonize cows post-ingestion (Zhao et al., 2013; Chopyk et al., 2016; Stenkamp-Strahm et al., 2018). Pathogen variables analyzed in the current study (i.e., *Salmonella* spp., *Cryptosporidium*, *Giardia*) were associated with O157 presence in initial screening but did not remain in the final multivariable model. This supports the idea that immunity and overall health of the calf GI tract are important factors that may influence the fecal presence of O157. Although the importance of GI tract health cannot be understated, serum IgG values also represent systemic health parameters of calves. In the NAHMS Dairy 2014 Calf Component study, an increase in serum IgG was associated with both decreased calf morbidity and mortality (Urie et al., 2018). Taken together, these outcomes suggest that adequate passive transfer is important to reduce the overall prevalence of disease and death in US dairy calves but it also may reduce the fecal presence of O157.

The current study had several limitations. The low prevalence of shedding in calves left little power to detect associations between O157 and several variables (e.g., housing ventilation, calving ease, milk delivery method, direct-fed agents, disease history, and calf breed). Although consistency of weight measurements was a goal, the variable for ADG (initially associated with O157 in crude univariable analyses) may have been confounded by individual calves that had final weight measurements taken in days before or after the pre-weaning period. Further, studies to date have not assessed whether O157 colonization in calves may occur during a specific time after birth, similar to what is seen for K99 enterotoxigenic *E. coli* and other GI pathogens. The collection of only a single fecal sample from these calves between 2 and 4 wk of age, therefore, may have limited the overall detection of O157 shedding. Finally,

significant odds ratios found in the current work had values very close to 1. Augmentation of management factors found to influence O157 in this study may only modestly reduce the overall environmental contamination with the pathogen.

CONCLUSIONS

Certain environmental, management, and calf health factors may affect the occurrence of preweaned calf O157 shedding. Feeding colostrum from the calf's dam, compared with pooled sources, increased the likelihood of the presence of O157 in the calf. This was affected by the level of THI—calves that drank colostrum from their own first-lactation dam during heat stress THI had an even greater risk of shedding. Additionally, a decrease in serum IgG concentration increased the likelihood of O157 presence at different THI levels. Specifically, calves with poor or moderate serum IgG had increased odds of shedding during thermoneutral or heat stress THI compared with calves with excellent serum IgG. These outcomes highlight factors that influence the odds of O157 presence in preweaned calves and may be used to guide future management strategies aimed at reducing O157 on dairy operations across the United States. On-farm management strategies that mitigate shedding in calves may only modestly reduce environmental contamination with the pathogen because the prevalence of preweaned calves shedding O157 at any given time is low. Regardless, the potential to limit the number of human infections due to direct animal and manure contact makes pursuing these strategies valuable.

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