Preweaned heifer management on US dairy operations: Part III. Factors associated with *Cryptosporidium* and *Giardia* in preweaned dairy heifer calves

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ABSTRACT

The objective of this study was to evaluate management practices and environmental factors associated with cryptosporidiosis and giardiasis in preweaned heifer calves on US dairy operations. This study was conducted as part of the calf component of the National Animal Health Monitoring System's Dairy 2014 study. The calf component included 104 dairy operations in 13 states and was an 18-mo longitudinal study focused on dairy heifer calves from birth to weaning. Fecal samples were collected from 2,249 calves: 839 calves in the West region (California, Colorado, and Washington) and 1,410 calves in the East region (Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin). Fecal samples were collected only once from calves during the preweaning period. Samples were collected from calves 3 to 66 d of age, with a mean of 22 d. Overall, *Cryptosporidium* and *Giardia* were detected in 43.1 and 30.5% of fecal samples, respectively. Backward elimination logistic model selection was used after univariate screening to determine which management practices and environmental factors significantly affected the presence of *Cryptosporidium* or *Giardia*. The final *Cryptosporidium* model included herd size, days of age at fecal collection, and average temperature-humidity index for the month of fecal collection (fTHI). *Cryptosporidium* was found on a higher percentage of large operations (≥ 500 cows) than small operations (30 to 99 cows). Younger calves were more likely to have a fecal sample positive for *Cryptosporidium* than samples from older calves.

Fecal samples from calves during the warmer parts of the year (fTHI >70) were more likely to be positive for *Cryptosporidium* than samples collected in colder months (fTHI <20). The final *Giardia* model included herd size, days of age at fecal collection, average fTHI, failure of passive transfer status, and average daily gain (kg/d) during the preweaning period. *Giardia* was isolated more frequently from calves on small operations than on large operations and from calves that were older compared with younger calves. *Giardia* was more frequently isolated in warmer months. Samples from calves with failure of passive transfer were more likely to have *Giardia* than calves with adequate passive transfer (>10 g/L IgG). Average daily gain during the preweaning period was lower in calves from which *Giardia* was isolated. These results highlight the factors associated with the presence of *Cryptosporidium* and *Giardia* in preweaned dairy heifer calves.

Key words: dairy heifer calves, *Cryptosporidium*, *Giardia*

INTRODUCTION

Cryptosporidium parvum and *Giardia duodenalis* are intestinal protozoa that are commonly found in calves and have the potential to cause diarrhea (Huetink et al., 2001). *Cryptosporidium* causes diarrhea via destruction of the small intestinal epithelium. Epithelial destruction results in villus atrophy, crypt hyperplasia, and cell death, which lead to impaired nutrient digestion and transport (Di Genova and Tonelli, 2016). *Cryptosporidium* in endemic herds has morbidity rates that may reach 100%; however, mortality is infrequently observed (de Graaf et al., 1999). *Giardia* causes diarrhea via diffuse shortening of the brush border microvilli and decreased activity of the small intestinal brush border enzymes, specifically lipase. The microvillus shortening leads to a decrease in absorptive capacity of the

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small intestine. The combined effects of decreased re-sorption and brush border enzyme deficiencies results in malabsorptive diarrhea and decreased weight gain (Olson, 2004). Currently, there are no licensed drugs in the United States available to treat *Cryptosporidium* or *Giardia* in ruminants. Supportive therapy, including fluids and electrolytes, is recommended for calves with *Cryptosporidium*- or *Giardia*-induced diarrhea (Thompson et al., 2008). Additionally, because infection of both protozoans occurs via the fecal-oral route (directly via the ingestion of feces or indirectly via ingestion of contaminated food or water), good husbandry, including the removal of feces from calf environments, will help minimize re-infection and transmission of *Cryptosporidium* and *Giardia* (Thompson et al., 2008).

Infections with *Cryptosporidium* and *Giardia* are of public health concern because some of their species and assemblages are zoonotic. For *Cryptosporidium*, molecular studies of cryptosporidiosis in cattle have shown that 4 species are mainly responsible for most cattle infections: *C. parvum*, *C. bovis*, *C. ryanae*, and *C. andersoni* (Santín, 2013). The host age-related susceptibility to these species has been described previously; virtually all infections in calves 8 wk of age and younger are caused by *C. parvum* (Santín et al., 2004; Fayer et al., 2006, 2007; Langkjær et al., 2007; Thompson et al., 2007). *Cryptosporidium parvum* is known to infect humans worldwide and is recognized as the major zoonotic *Cryptosporidium* species.

Molecular characterization of *G. duodenalis* has revealed 8 major assemblages with different host ranges, with only assemblages A and B identified in humans (Feng and Xiao, 2011), although both assemblages A and B have been identified in a wide range of other mammalian hosts (Monis et al., 2003; Thompson and Monis, 2004). In cattle, 3 assemblages have been commonly detected: A, B, and E, with E being most frequently reported, followed by A (Trout et al., 2004; Langkjær et al., 2007; Geurden et al., 2008; Santín et al., 2008). Thus, calves should be considered a potential source of human infections.

The objectives of this prospective, longitudinal study were to examine management and environmental associations for *Cryptosporidium* and *Giardia* detection in preweaned dairy heifers.

MATERIALS AND METHODS

Study Design

The USDA's National Animal Health Monitoring System (NAHMS) conducts national surveys to collect information on the health, management, and productivity of domestic livestock species (USDA, 2016).

In 2014, a nationwide survey was conducted to collect information about the US dairy industry and included an 18-mo longitudinal preweaned heifer calf study, which included a cross-sectional study to evaluate the presence of *Cryptosporidium* and *Giardia*.

The calf component was part of the NAHMS's Dairy 2014 study, and consisted of a convenience sample of 104 dairy operations. These operations were located in 13 states, including California, Colorado, and Washington in the West region, and Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin in the East region. Dairy operations were categorized based on the number of mature cows, as small (30 to 99 cows), medium (100 to 499 cows), and large (≥ 500 cows). Figures describing the study sample and operations can be found in Urie et al. (2018).

Data collection for the calf component of the study occurred from March 2014 through September 2015. Each operation was instructed to enroll 24 heifer calves over a 1-yr period, or an average of 2 calves/mo. Farm personnel selected which calves to enroll in the study; however, a calf must have been alive at 24 h of age to be enrolled. Because fewer operations participated than originally planned, the target number enrolled per operation was increased to 48 calves. Additionally, because enrollment of farms did not occur as quickly as anticipated, the study encompassed 18 mo instead of the 12-mo period that was planned.

Heifer Calf Health Card

Each calf enrolled in the study had a Heifer Calf Health Card ("Calf Card") filled out to record information on events that occurred from birth to weaning (https://www.aphis.usda.gov/animal_health/naahms/dairy/downloads/dairy14ques/CalfHealth.pdf). The Calf Card contained questions in both English and Spanish and was filled out by farm personnel, a veterinary medical officer, extension personnel, veterinarians, or a combination of people involved with calf raising. The Calf Card included birth data (e.g., birth date, weight, and calving ease), colostrum feeding data (including timing, amount, and method of colostrum feeding), preweaning housing and procedures data (e.g., housing, ventilation, bedding, navel disinfection, and dehorning), milk feeding (including type of liquid diet fed, any additives, and method of feeding), milk consumption record (volume and frequency of feedings), preweaning growth record (hip height and heart girth recordings every 2 wk), biologic sampling record (including serum collection and fecal sampling dates), vaccinations, disease incidence and treatment, weaning data (weaning date, primary weaning criteria), and any

additional notes. Starter feed labels and milk replacer labels, if applicable, were also collected.

Producers were provided with the Calf Health Scoring Chart (<https://www.vetmed.wisc.edu/dms/fapm/apps/chs.htm>) to guide the reporting of clinical signs. The goal of the study was to collect recognized clinical signs and causes of death, not to standardize clinical signs and causes of death across operations. All clinical signs and causes of death were reviewed, edited, and categorized, as described in the statistical analysis section.

Due to the variation observed in the quantity and quality of the liquid diet fed per day, kilograms of protein and fat fed per day were calculated for each calf. Information regarding the amount of liquid diet fed per feeding and the frequency of feedings per day were recorded on the Calf Card and used to calculate the total amount of liquid diet fed. For calves fed milk replacer, the percent protein and percent fat were obtained from the producer-reported values or the milk replacer label. For calves fed whole or waste milk, percent protein was set at 3.1% and percent fat as 3.8% (as-fed basis; Chester-Jones and Hoffman, 2003). The total amounts of protein and fat were calculated and then divided by the number of days fed a liquid diet to determine the average kilograms of protein and fat fed per day in the liquid diet.

Additives in the liquid diet were categorized as antibiotics, direct-fed microbials, fly control, acids/preservatives, coccidiostats, vitamins and minerals, or other additives. Information regarding calf starter included the percent protein and the age that starter was first offered; starter intake was not recorded.

Biological Sampling

Before enrollment in the study, calves were screened for persistent infection with bovine viral diarrhea virus (BVDV). V-Cut ear notchers (Nasco, Fort Atkinson, WI) were used to collect ear notch samples from all calves, which were tested on-farm for BVDV using the Idexx SNAP BVDV Antigen Test (Idexx, Westbrook, ME). Calves that tested positive for BVDV were excluded from the study. Colostrum samples (40–50 mL) from the first feeding of colostrum administered to each calf were collected in conical screw-top tubes by farm personnel and frozen until shipping. Blood samples (5 mL) from calves between 1 and 7 d of age were collected in serum separator tubes by veterinary medical officers or animal health technicians, and samples were centrifuged if possible before shipping. Colostrum and blood samples were shipped together on ice to USDA's National Veterinary Services Laboratories (Ames, IA). Blood samples were centrifuged at the National Veteri-

nary Services Laboratories and serum was separated. Serum and colostrum samples were then accumulated and shipped in bulk to the Saskatoon Colostrum Co. (Saskatoon, SK, Canada) for testing. Colostrum samples were tested for IgG concentration using radial immunodiffusion and Brix score using a digital Brix refractometer. Serum samples were tested for IgG concentration using radial immunodiffusion, total protein, and Brix score using a digital Brix refractometer. Blood samples collected within 24 h of birth or after 7 d of age were excluded from this analysis. A complete description of the methods used for Brix scoring and IgG determination can be found in Urie et al. (2018).

For evaluation of enteric parasites, veterinary medical officers or animal health technicians collected approximately 50 g of feces directly from the rectum of each calf between 2 and 4 wk of age. Fecal samples were placed in cylindrical screw-top containers and shipped on ice to the USDA Agricultural Research Service's Environmental Microbial Food Safety Laboratory (Beltsville, MD) as soon as possible after sample collection. Fecal samples were tested by immunofluorescence microscopy for *Cryptosporidium parvum* and *Giardia duodenalis*.

Cryptosporidium oocysts and *Giardia* cysts were concentrated from feces as previously described (Fayer et al., 2000; Santín et al., 2004). Briefly, 15 g of feces from each specimen cup was mixed with 35 mL of distilled water (dH₂O). The suspension was passed through a sieve with a 45- μ m pore size screen. The filtrate volume was adjusted to 50 mL with dH₂O and centrifuged at $1,800 \times g$ for 15 min. The pellet was resuspended in a mixture of 25 mL of dH₂O and 25 mL of CsCl (1.4 g/L) and centrifuged at $300 \times g$ for 20 min. Supernatant (4 mL), aspirated from each suspension, was washed with dH₂O and the final pellet was examined by microscopy as described below.

A 2- μ L suspension of the pellet was transferred to a well (11-mm diameter) of a 3-well glass microscope slide, and 2 μ L of premixed Merifluor reagent (Meridian Diagnostics, Cincinnati, OH) was added. The slide was covered with a 24 mm $\times$ 50 mm coverslip and the entire well area was examined and oocysts/cysts counted by fluorescence microscopy at 400 $\times$ using Zeiss Axioskop fluorescence microscope (Zeiss, Oberkochen, Germany) equipped with epifluorescence and a fluorescein isothiocyanate (FITC)-Texas Red dual wavelength filter.

Growth Measurements

Calves were measured approximately every 2 wk during the preweaning period to measure growth. Height/weight tapes from Coburn (Nasco, Fort Atkinson, WI) were provided for measuring calves. For consistency,

it was recommended that 1 trained veterinary medical officer or animal health technician complete the measurements on all calves enrolled on an operation. Birth weights were estimated using a scale, hoof circumference, or heart girth circumference and reported in pounds or kilograms. The methods that operations used to estimate birth weight were not captured. Hip height and heart girth circumference were measured approximately every 2 wk and recorded in centimeters. Heart girth circumference in centimeters was converted to kilograms using the following equation (Heinrichs et al., 1992):

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

Prewaning weight gain was calculated by subtracting the birth weight from the final weight. Average daily gain was calculated by taking the weight gain during the preweaning period divided by the number of days between birth weight and final weight (approximately the preweaning period). Final weights did not always occur at the same time as weaning. However, only calves with final weight measurements within 14 d of weaning were included in the analysis.

Environmental Factors

Temperature-humidity index (**THI**) data were obtained monthly by county from the National Oceanic and Atmospheric Administration (NOAA, 2016). The THI accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T , °F) and relative humidity (RH). The equation used for this analysis was $\text{THI} = T - [0.55 - (0.55 \times \text{RH}/100)] \times (T - 58)$. Each calf was assigned a THI for the month of fecal collection (**fTHI**).

Statistical Analysis

When each calf was weaned, the Calf Card was mailed to USDA NAHMS (Fort Collins, CO). Initial ongoing validation was performed on every Calf Card as it came in, followed by data entry into SAS (version 9.4; SAS Institute Inc., Cary, NC). Once all Calf Cards were entered, the data were validated again by NAHMS staff and merged with the results from the colostrum, serum, and fecal testing obtained from the laboratories. Descriptive data were analyzed using the FREQUENCY and MEANS procedures for categorical and continuous variables, respectively.

Logistic regression models were constructed to determine the factors associated with *Cryptosporidium* and

Giardia detection. Operation was included in the model as a random variable to account for clustering of calves within operation. Predictor variables were considered for the models based on biological plausibility. The outcome was the presence or absence of protozoa oocysts/cysts, and, to be included in the analysis, calves had to have data for all of the variables initially included in the model. Variables were tested for potential confounding using variance inflation factors. Continuous variables were tested for linearity and if they were not linear, they were categorized and evaluated as categorical variables. For both *Cryptosporidium* and *Giardia* model building, univariate screening was used. Variables with $P < 0.20$ were considered for inclusion in the initial multivariable model. Stepwise backward elimination model selection in PROC GENMOD was used to construct the final model, with $P < 0.05$ considered significant and thus included in the model.

RESULTS

Descriptive Summary

Fecal samples were obtained from 2,249 calves, with the majority (89.2%) of calves being Holstein. Samples were collected from all herd sizes with large operations being over-represented (314, 611, and 1,324; calves on small, medium, and large operations, respectively). The age at fecal sample collection for each calf ranged from 3 to 66 d, with an average age of 22 d. Sample collection occurred throughout the year, starting on April 12, 2014, and ending on July 23, 2015. *Cryptosporidium* was detected in 43.1% of preweaned heifer calves and *Giardia* was detected in 30.5% of preweaned heifer calves. Of all tested calves, 13.3% had a positive result for both *Cryptosporidium* and *Giardia*. Therefore, 39.8% of calves tested were negative for both *Cryptosporidium* and *Giardia* (Figure 1).

The percentage of samples that tested positive changed based on the age at fecal sample collection. *Cryptosporidium* was more prevalent in young calves, with 63.3% of calves less than 2 wk of age testing positive compared with only 9.1% of calves greater than 6 wk of age testing positive. *Giardia* was more prevalent in older calves, with 36.4% of calves greater than 6 wk of age testing positive and only 16.7% of calves less than 2 wk of age testing positive (Figure 2). Before model building, variables that were thought to affect the presence of *Cryptosporidium* and *Giardia* were analyzed univariately. More *Cryptosporidium*-positive specimens were detected in the West region (48.9%) than in the East region (40.5%). In contrast, more *Giardia*-positive specimens were detected in the East region (33.6%) than in the West region (25.2%; Figure 3). On large

Figure 1. Percentage of preweaned heifer calves testing positive for *Cryptosporidium* and *Giardia*. The total percentage of calves with a positive *Cryptosporidium* result and the total percentage of calves with a positive *Giardia* result are represented by dashed lines. Color version available online.

operations, 47.8% of specimens were *Cryptosporidium*-positive compared with 37.6% on both medium and small operations. Conversely, the prevalence of *Giardia* was highest on small operations (43.3%) compared with medium and large operations (34.0 and 25.8%, respectively; Figure 4). During the month of October, 54.6% of calves excreted *Cryptosporidium*, whereas during March, 35.9% of calves excreted *Cryptosporidium*. *Giardia* followed a similar trend, with the highest

number of positive calves in November (41.7%) and the lowest in February (17.2%; Figure 5).

***Cryptosporidium* Model**

Of the 2,249 calves with submitted fecal samples, 1,824 had complete data and were included in the inferential modeling for *Cryptosporidium*. Initial variables included in the multivariable model after univariable

Figure 2. Percentage of preweaned heifer calves that tested positive for *Cryptosporidium* and *Giardia* by age of calf (in days) at fecal collection. Color version available online.

Figure 3. Percentage of preweaned heifer calves that tested positive for *Cryptosporidium* and *Giardia* by region. West region = California, Colorado, Washington; East region = Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin. Color version available online.

screening for *Cryptosporidium* included operation as a random effect to account for clustering of calves within farm, region, herd size, dam lactation, navel disinfection, failure of passive transfer, housing ventilation, bedding type, days of age at fecal collection, fTHI, and ADG (Tables 1 and 2). The final multivariable model for *Cryptosporidium* in calves included herd size ($P = 0.024$), days of age at fecal collection ($P < 0.001$), and fTHI ($P < 0.001$) (Table 3).

Large operations had a higher percentage of calves with *Cryptosporidium* (46.8% of calves with a positive

fecal result), whereas small and medium-sized operations saw no difference in the number of *Cryptosporidium* positive calves (37.7 and 37.6%, respectively). Thus, when compared with small operations, large operations had an odds ratio (OR) of 1.55. The variance inflation factors with and without region in the model were 1.324 and 1.022, respectively. Given this small difference, region was not forced into the model. After holding other independent variables in the model constant, younger calves at the time of fecal collection had a higher risk of a positive *Cryptosporidium* result than older calves at

Figure 4. Percentage of preweaned heifer calves that tested positive for *Cryptosporidium* and *Giardia* by herd size. Small = 30–99; Medium = 100–499; Large = ≥ 500 . Color version available online.

Figure 5. Percentage of preweaned heifer calves that tested positive for *Cryptosporidium* and *Giardia* by month. Color version available online.

fecal collection (OR = 0.91; Figure 6a). To demonstrate the risk of *Cryptosporidium* with this continuous variable, 2 different ages at fecal collection were selected. A calf at 14 d of age had a predicted *Cryptosporidium* risk of 56.5%, whereas a calf at 35 d of age had a predicted *Cryptosporidium* risk of 16.7%. Similarly, an increase in average THI for the month of fecal collection was associated with an increase of detection of *Cryptosporidium* in the fecal sample (OR = 1.01; Figure 6b). Two extreme fTHI points were selected to demonstrate the predicted risk of this continuous variable. An fTHI of 20, below the reported calf thermoneutral zone, had a predicted *Cryptosporidium* risk of 32.0%, whereas an fTHI of 70, above the reported calf thermoneutral zone, had a predicted *Cryptosporidium* risk of 45.7%.

Giardia Model

Of the 2,249 calves with submitted fecal samples, 1,785 calves had complete data and were included in the inferential modeling for *Giardia*. Initial variables considered for the multivariable model after univariate screening for *Giardia* included region, herd size, calving event attendance, navel disinfection, failure of passive transfer, sex of the primary caretaker, any antibiotics in the liquid diet, any additives in the liquid diet, days of age at fecal collection, fTHI, protein per day (kg/d), and ADG (Tables 1 and 4). The final multivariable model for *Giardia* detection in calves included herd

size ($P = 0.006$), days of age at fecal collection ($P = 0.003$), fTHI ($P < 0.001$), failure of passive transfer status where serum IgG was <10 g/L ($P = 0.037$), and ADG (kg/d) during the preweaning period ($P = 0.021$; Table 5).

A smaller herd size was correlated with a positive *Giardia* result. Calves on small operations had a 43.3% prevalence of *Giardia*; medium operations had a 34.0% prevalence; and large operations had a 25.8% prevalence. Thus, when compared with large operations, medium operations had an OR of 1.50 and small operations had an OR of 2.30. After holding other independent variables in the model constant, calves that were older at the time of fecal collection had a higher risk of a positive *Giardia* result than younger calves at the time of fecal collection (OR = 1.04; Figure 6c). To demonstrate the risk of *Giardia* with this continuous variable, 2 different ages at fecal collection were selected. A calf 14 d of age at fecal collection resulted in a predicted *Giardia* risk of 28.3% and a calf 35 d of age at fecal collection had a predicted *Giardia* risk of 44.7%. Similarly, an increase in fTHI was directly correlated with an increase in detection of *Giardia* in the fecal sample (OR = 1.02; Figure 6d). For example, an fTHI of 20, selected to represent cold months, had a *Giardia* risk of 24.5%, whereas an fTHI of 70, representing hot months, had a *Giardia* risk of 41.1%. Failure of passive transfer status (serum IgG <10 g/L) was directly correlated with a positive *Giardia* fecal sample. A calf

Table 1. Percentage of heifer calves for all categorical variables initially included in *Cryptosporidium* and *Giardia* univariate screening, which included operation as a random effect to account for clustering of calves within farm, by *Cryptosporidium* and *Giardia* status (positive or negative)¹

Variable	Level	Cryptosporidium model				Giardia model					
		Calves (no.)	Calves (%)	Positive (%)	Negative (%)	P-value	Calves (no.)	Calves (%)	Positive (%)	Negative (%)	P-value
All calves	West	1,824	100.0	43.9	56.1	0.079	1,785	100.0	30.6	69.4	0.024
	East	688	37.7	18.9	18.8		659	36.9	9.2	27.7	
Herd size (adult cows)	Small (30–99 cows)	1,136	62.3	25.0	37.3	0.032	1,126	63.1	21.4	41.7	0.002
	Medium (100–499 cows)	219	12.0	4.6	7.4		219	12.3	5.5	6.8	
	Large (≥500 cows)	535	29.3	10.5	18.8		531	29.7	10.1	19.6	
		1,070	58.7	28.8	29.9		1,035	58.0	14.9	43.1	
Breed	Holstein	1,627	89.2	39.1	50.1	0.890	1,590	89.1	27.7	61.4	0.825
	Jersey	69	3.8	1.7	2.1		68	3.8	0.7	3.1	
Dam lactation	Other	126	6.9	3.0	3.9	0.158	125	7.0	2.1	4.9	0.364
	First	674	37.0	17.2	19.7		649	36.5	10.8	25.7	
Calving ease	Second and greater	1,049	57.5	24.2	33.3	0.600	1,039	58.2	18.1	40.1	0.859
	Unknown	101	5.5	2.5	3.1		97	5.4	1.8	3.6	
	Unassisted	1,680	92.1	40.6	51.5		1,646	92.2	28.4	63.8	
	Assisted	102	5.6	2.5	3.1		98	5.5	1.5	4.0	
Number of calves born	Unknown	42	2.3	0.8	1.5	0.778	41	2.3	0.7	1.6	0.156
	Single	1,757	96.3	42.2	54.1		1,719	96.3	29.9	66.4	
Calving event attendance	Twins	64	3.5	1.6	1.9	0.600	63	3.5	0.7	2.8	0.004
	Unknown	3	0.2	0.1	0.1		3	0.2	0.0	0.2	
Navel disinfection	Unattended	842	46.2	18.9	27.3	0.012	817	45.8	16.4	29.4	0.002
	Attended	982	53.8	25.0	28.8		968	54.2	14.1	40.1	
Failure of passive transfer	Yes	1,427	78.2	36.3	41.9	0.134	1,388	77.8	21.7	56.1	0.017
	No	397	21.8	7.5	14.3		397	22.2	8.9	13.3	
Sex of primary caretaker	Yes	225	12.3	4.8	7.5	0.227	220	12.3	5.0	7.3	0.052
	No	1,599	87.7	39.0	48.6		1,565	87.7	25.6	62.1	
Housing type	Male	1,151	63.1	28.3	34.8	0.537	1,116	62.5	20.6	41.9	0.483
	Female	592	32.5	14.1	18.4		590	33.1	8.6	24.5	
Housing ventilation	Male and female	81	4.4	1.4	3.0	0.128	79	4.4	1.6	2.8	0.567
	Individual	1,694	92.9	40.6	52.2		1,655	92.7	28.2	64.5	
Bedding type	Group	130	7.1	3.2	3.9	0.082	130	7.3	2.4	4.9	0.914
	Natural/outside ventilation	1,446	79.3	35.3	44.0		1,411	79.0	24.0	55.0	
Dehorning status	Positive pressure tubes	188	10.3	3.6	6.7	0.235	188	10.5	3.8	6.7	0.553
	Cross ventilation	190	10.4	5.0	5.4		186	10.4	2.8	7.6	
Preventative drugs	Straw/hay	970	53.2	21.2	32.0	0.694	960	53.8	16.8	37.0	0.201
	Shavings	430	23.6	11.3	12.2		430	24.1	7.2	16.9	
Disease status before fecal sample	Sand/none	206	11.3	6.5	4.8	0.759	177	9.9	2.8	7.1	0.345
	Combination/other	218	12.0	4.9	7.1		218	12.2	3.8	8.4	
Digestive signs before fecal sample	Yes with medications	267	14.6	7.0	7.6	0.443	263	14.7	3.3	11.4	0.642
	Yes without medications	704	38.6	17.7	20.9		676	37.9	11.1	26.8	
Liquid diet type	Not dehorned	793	43.5	17.6	25.9	0.443	790	44.3	14.7	29.6	0.856
	Yes	39	2.1	1.0	1.1		39	2.2	0.5	1.7	
Only whole waste milk	No	1,785	97.9	42.8	55.0	0.443	1,746	97.8	30.1	67.7	0.642
	Any disease	428	23.5	9.9	13.5		403	22.6	6.4	16.2	
Any milk replacer	No disease	1,396	76.5	33.9	41.4	0.443	1,382	77.4	23.7	53.7	0.856
	Any digestive signs	248	13.6	6.6	7.0		223	12.5	3.6	8.9	
Any milk replacer	No digestive signs	1,576	86.4	37.2	48.4	0.443	1,562	87.5	30.8	56.7	0.642
	Only whole waste milk	1,119	61.3	25.9	35.4		1,080	60.5	28.4	32.1	
Any milk replacer	Any milk replacer	705	38.7	17.9	20.7	0.443	705	39.5	22.2	17.3	0.642

Continued

Table 1 (Continued). Percentage of heifer calves for all categorical variables initially included in *Cryptosporidium* and *Giardia* univariate screening, which included operation as a random effect to account for clustering of calves within farm, by *Cryptosporidium* and *Giardia* status (positive or negative)¹

Variable	Level	Cryptosporidium model				P-value	Giardia model				P-value
		Calves (no.)	Calves (%)	Positive (%)	Negative (%)		Calves (no.)	Calves (%)	Positive (%)	Negative (%)	
Coccidiostats in liquid diet	Yes	743	40.7	17.1	23.6	0.638	737	41.3	22.8	18.5	0.915
	No	1,046	57.3	26.8	32.5		1,017	57.0	31.3	25.7	
Direct fed microbials in liquid diet	Yes	306	16.8	6.9	9.9	0.560	306	17.1	5.0	12.2	0.824
	No	1,518	83.2	37.0	46.2		1,479	82.9	25.6	57.3	
Any antibiotics in liquid diet	Yes	224	12.3	5.0	7.2	0.735	220	12.3	2.7	9.6	0.006
	No	1,600	87.7	38.8	48.9		1,565	87.7	27.8	59.8	
Any additives in liquid diet	Yes	996	54.6	24.4	30.2	0.572	990	55.5	15.5	39.9	0.053
	No	828	45.4	19.5	25.9		795	44.5	15.1	29.5	
Pasteurization of liquid diet	Yes	528	28.9	13.4	15.6	0.558	493	27.6	7.8	19.8	0.271
	No	649	35.6	16.0	19.6		645	36.1	12.1	24.0	
Bacterial counts of milk	Not applicable	647	35.5	14.5	21.0	0.467	647	36.2	10.7	25.5	0.467
	Yes	181	9.9	4.1	5.9		181	10.1	2.6	7.5	
Milk delivery system	Not applicable	996	54.6	25.3	29.3	0.393	957	53.6	17.3	36.4	0.406
	Bottle	416	35.5	14.5	21.0		647	36.2	10.7	25.5	
	Bucket/pail	287	15.7	7.8	7.9		383	21.5	7.6	13.9	
	Milk bar	54	3.0	1.0	1.9		287	16.1	4.5	11.5	
	Automated feeder	44	2.4	0.7	1.8		54	3.0	0.6	2.5	
	Bottle and bucket	895	49.1	21.5	27.5		44	2.5	1.0	1.5	
Other combinations		128	7.0	3.2	3.8		889	49.8	14.9	34.9	
							128	7.2	2.1	5.1	

¹Bolded variables were included in the initial models (P -value < 0.20).²West region: California, Colorado, Washington; East region: Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin.

Table 2. Continuous variables initially evaluated in univariate screening, which included operation as a random effect to account for clustering of calves within farm, for *Cryptosporidium* prevalence of heifer calves¹

Variable	<i>Cryptosporidium</i> status									
	Percentile					Positive		Negative		P-value
	Mean	SE	5th	25th	50th	75th	95th	Mean	SE	
Days of age at fecal collection	21.54	0.14	15.00	17.00	20.00	25.00	32.00	22.80	0.18	0.20
THI for the month of fecal collection (fTHI) ²	49.93	0.42	17.20	35.50	51.20	66.10	72.40	48.13	0.59	0.009
Birth weight (kg)	42.71	0.13	34.09	39.09	42.73	45.91	52.27	42.84	0.19	0.17
Protein intake per day (kg/d)	0.20	0.00	0.10	0.15	0.17	0.23	0.35	0.20	0.00	0.829
Fat intake per day (kg/d)	0.21	0.00	0.10	0.15	0.20	0.24	0.34	0.21	0.00	0.986
ADG (kg/d)	0.73	0.01	0.36	0.57	0.71	0.87	1.16	0.74	0.02	0.048

¹Bolded variables were included in the initial *Cryptosporidium* model (*P*-value < 0.20).

²Temperature-humidity index (THI) accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T, °F) and the relative humidity (RH). The equation used for this analysis was $THI = T - [0.55 - (0.55 \times RH/100)] \times (T - 58)$.

Table 3. Continuous variables initially evaluated in univariate screening, which included operation as a random effect to account for clustering of calves within farm, for *Giardia* prevalence of heifer calves¹

Variable	<i>Giardia</i> status									
	Percentile					Positive		Negative		P-value
	Mean	SE	5th	25th	50th	75th	95th	Mean	SE	
Days of age at fecal collection	21.60	0.14	15.00	21.00	32.00	25.00	32.00	21.22	0.17	0.0056
THI for the month of fecal collection (fTHI) ²	49.80	0.43	17.20	51.00	72.40	66.30	72.40	48.35	0.71	0.0003
Birth weight (kg)	42.71	0.13	34.09	39.09	42.73	45.91	52.27	42.51	0.21	0.2811
Protein intake per day (kg/d)	0.20	0.00	0.10	0.17	0.35	0.23	0.35	0.21	0.00	0.1436
Fat intake per day (kg/d)	0.21	0.00	0.10	0.20	0.34	0.24	0.34	0.21	0.00	0.2152
ADG (kg/d)	0.73	0.01	0.36	0.57	0.71	0.87	1.16	0.75	0.02	0.0028

¹Bolded variables were included in the initial *Giardia* model (*P*-value < 0.20).

²Temperature-humidity index (THI) accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T, °F) and the relative humidity (RH). The equation used for this analysis was $THI = T - [0.55 - (0.55 \times RH/100)] \times (T - 58)$.

that had failure of passive transfer was 1.34 times more likely to have a positive *Giardia* fecal sample than a calf with adequate passive transfer. Additionally, ADG (kg/d) was inversely correlated with the detection of *Giardia* (OR = 0.71; Figure 6e). Two points, to represent low and adequate ADG, were selected for this continuous variable to illustrate the risk of *Giardia*. A calf with an ADG of 0.45 kg/d resulted in a predicted *Giardia* risk of 38.7%, whereas a calf with an ADG of 1.1 kg/d resulted in a predicted *Giardia* risk of 27.2%.

DISCUSSION

Cryptosporidium oocysts and *Giardia* cysts were detected in 43.1 and 30.5% of the 2,249 fecal specimens examined by immunofluorescence assay. The prevalence found in this study for *Cryptosporidium* and *Giardia* is similar to that found in previous studies in preweaned calves in the United States using the same detection method (Santín et al., 2004, 2008, 2009; Trout et al., 2004). In 2 studies in preweaned dairy calves from 7

Figure 6. Model of *Cryptosporidium* and *Giardia* risk by days of age at fecal collection (a, c), average temperature-humidity index (THI) during the month of fecal collection (b, d), and ADG during the preweaning period (e). The THI accounts for the effects of temperature and relative humidity (RH) and the equation uses the dry bulb temperature (T, °F). The equation used for this analysis was $THI = T - [0.55 - (0.55 \times RH/100)] \times (T - 58)$. Color version available online.

Table 4. Results of multivariable modeling, which included operation as a random effect to account for clustering of calves within farm, of factors associated with *Cryptosporidium* in heifer calves (n = 1,824)

Final model variable	Model results			Example model outcomes	
	Variable level	β-Estimate (SE)	Odds ratio (SE)	Factor P-value	Predicted <i>Crypto</i> risk (SE)
Herd size (adult cows)	Small (30–99 cows) Medium (100–499 cows) Large (≥500 cows)	Referent 0.014 (0.013) 0.440 (0.221)	Referent 1.01 (0.164) 1.55 (0.440)	0.024	0.372 (0.315) 0.476 (0.427) 0.3690 (0.285)
Days of age at fecal collection		–0.089 (0.013)	0.91 (0.012)	<0.001	0.565 (0.488)
THI for the month of fecal collection (fTHI) ¹				35	0.167 (0.116)
				20.0	0.320 (0.254)
		0.012 (0.003)	1.01 (0.003)	<0.001	0.457 (0.397)

¹Temperature-humidity index (THI) accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T, °F) and the relative humidity (RH). The equation used for this analysis was $THI = T - [0.55 - (0.55 \times RH/100)] \times (T - 58)$.

Table 5. Results of multivariable modeling, which included operation as a random effect to account for clustering of calves within farm, of factors associated with *Giardia* in heifer calves (n = 1,785)

Final model variable	Model results			Example model outcomes	
	Variable level	β-Estimate (SE)	Odds ratio (SE)	Factor P-value	Predicted <i>Giardia</i> risk (SE)
Herd size (adult cows)	Small (30–99 cows) Medium (100–499 cows) Large (≥500 cows)	0.831 (0.219) 0.4040 (0.187) Referent	2.30 (0.504) 1.50 (0.280) Referent	0.006	0.485 (0.198) 0.381 (0.181) 0.291 (0.147)
Days of age at fecal collection		0.034 (0.011)	1.04 (0.011)	0.003	0.283 (0.143)
THI for the month of fecal collection (fTHI) ¹				35	0.447 (0.202)
				20.0	0.245 (0.176)
		0.015 (0.004)	1.02 (0.004)	<0.001	0.411 (0.137)
Failure of passive transfer	Yes No	0.2925 (0.129) Referent	1.34 (0.097) Referent	0.037	0.298 (0.159)
ADG (kg/d)		–0.347 (0.118)	0.71 (0.083)	0.021	0.241 (0.097)
				0.45	0.387 (0.132)
				1.1	0.272 (0.176)

¹Temperature-humidity index (THI) accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T, °F) and the relative humidity (RH). The equation used for this analysis was $THI = T - [0.55 - (0.55 \times RH/100)] \times (T - 58)$.

eastern US states, prevalences of 50.3 and 44% were reported for *Cryptosporidium* and *Giardia*, respectively (Santín et al., 2004; Trout et al., 2004). In studies that examined preweaned dairy calves from a farm in Maryland, a prevalence of 35.8% for *Cryptosporidium* and a prevalence of 60.8% for *Giardia* were reported (Santín et al., 2008, 2009). In a similar study in Ohio, using also immunofluorescence assay, a prevalence of similar prevalence was observed in calves 1 to 8 wk of age for *Cryptosporidium* (43.3%), and a higher prevalence was observed for *Giardia* (70%; Xiao and Herd, 1994). However, the prevalence of infection in cross-sectional studies likely underestimates true prevalence because only one fecal specimen was collected per calf. If that specimen were identified as negative during a period when the calf was experiencing intermittent oocyst/cyst excretion, the calf would be considered negative. A cumulative prevalence of 100% has been reported for *Cryptosporidium* and *Giardia* dairy calves in longitudinal studies (Xiao and Herd, 1994; O'Handley et al., 1999; Santín et al., 2008, 2009).

Given that the majority of small and medium-sized operations in the data set were located in the East region, we suspected that confounding between herd size and region was possible; however, this was ruled out by evaluation of variance inflation factors. Therefore, the association between herd size and prevalence of *Cryptosporidium* and *Giardia* is likely due to management factors that vary based on operation size. Although no publications speculate regarding the association between protozoa and herd size, this association was also reported in the 1992 USDA National Dairy Heifer Evaluation Project (USDA, 1992). One possible hypothesis to explain why *Cryptosporidium* was more prevalent on large operations is that large operations have a greater number of calves throughout the year. Therefore, these large operations may have more calves shedding *Cryptosporidium* at approximately 2 wk of age, while simultaneously having more calves susceptible to *Cryptosporidium*. *Giardia* was more common on small and medium-sized operations. This may be due to smaller operations housing preweaned calves in proximity to older weaned calves, or differences in cleaning and disinfecting protocols on small and medium operations than on large operations. Older asymptomatic calves, which are more likely to have been exposed to *Giardia*, may be an infection source for preweaned calves on small and medium operations.

Similarly, age at fecal collection was associated with both *Cryptosporidium* and *Giardia* detection. Younger calves were at a higher risk of having a *Cryptosporidium*-positive fecal result. A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 yr of age found similar results, with the highest prevalence of

infection at 2 wk of age (Santín et al., 2008). Given the lifecycle of *Cryptosporidium*, it is likely that calves become infected via direct contamination near the time of birth, and, therefore, the peak shedding of oocysts in feces is seen at approximately 2 wk of age (Olson et al., 2004). On the other hand, older calves were at a higher risk of having a *Giardia*-positive fecal result. Similar results were observed in a longitudinal study of giardiasis in dairy cattle in which the number of *Giardia*-infected animals peaked at 4 and 5 wk of age (Santín et al., 2008). Other studies also showed that calves, both dairy and beef, greater than 30 d of age have a higher prevalence of shedding *Giardia* (Xiao, 1994; O'Handley et al., 1999; Ralston et al., 2003).

Additionally, an increase in THI for the month of fecal collection was positively correlated with both *Cryptosporidium*- and *Giardia*-positive fecal results. Several studies have illustrated that extreme temperatures, both hot and cold, can inactivate protozoal oocysts and cysts (Fayer, 1994; Fayer and Nerad, 1996; Jenkins et al., 1997, 2002; Olson et al., 1999; Herk et al., 2004). Given that very few locations in this study had extreme high temperatures, fewer *Cryptosporidium*- and *Giardia*-positive calves detected in cold temperatures are consistent with these previous studies. However, it is important to note that the amount of water in the environment may change the number of oocysts inactivated at extreme temperatures (Jenkins et al., 1997, 2002). Therefore, extreme temperatures at which oocysts are no longer active may be regionally dependent.

A calf that had failure of passive transfer (serum IgG <10 g/L) was more likely to have a positive *Giardia* fecal result compared with calves with adequate passive transfer status. O'Handley et al. (2003) showed that antibody titers to *Giardia* were significantly higher in colostrum than in milk, and that antibody titers in colostrum reacted strongly with many *Giardia* antigens. Consequently, we might conclude that calves fed higher quality colostrum received a higher amount of *Giardia* antibodies and were less likely to have a positive fecal result. Although passive transfer was not significant in the *Cryptosporidium* model, previous studies have shown that adequate passive transfer may help minimize or eliminate *Cryptosporidium* infection (Lopez et al., 1988; Fayer et al., 1989; Petersen et al., 1992). Therefore, feeding high-quality colostrum and ensuring passive transfer is an important management practice to minimize protozoal infections.

Lower ADG was associated with a positive *Giardia* fecal result. Previous studies have shown that various livestock species, including sheep and cattle, have a decreased ADG when infected with *Giardia* (Ralston et al., 2003; Olson et al., 2004). As initially discussed, *Giardia* is known to cause chronic infection, resulting

in prolonged diarrhea, compared with *Cryptosporidium*. Given this prolonged infection, it may explain why a decrease in ADG was seen in the *Giardia* model but not in the *Cryptosporidium* model.

CONCLUSIONS

Various factors play a role in the prevalence of *Cryptosporidium* and *Giardia* in preweaned dairy heifer calves. The *Cryptosporidium* prevalence of 43.1% and the *Giardia* prevalence of 30.5% were similar to those of previously reported studies, illustrating the persistence of these 2 pathogens in the dairy industry. The size of the dairy operation and the THI when calves are in the preweaning period may change the likelihood of positive *Cryptosporidium* or *Giardia* fecal results. However, ensuring adequate passive transfer will decrease the likelihood of protozoal infection. Additionally, decreasing the likelihood of protozoal infection may result in calves with a higher ADG throughout the preweaning period.

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