Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Available online at www.sciencedirect.com
ScienceDirect
Preventive Veterinary Medicine 82 (2007) 12–28
PREVENTIVE VETERINARY MEDICINE
www.elsevier.com/locate/prevetmed

Calf-level risk factors for neonatal diarrhea and shedding of *Cryptosporidium parvum* in Ontario dairy calves

Lise A. Trotz-Williams [a,*], S. Wayne Martin [a], Kenneth E. Leslie [a], Todd Duffield [a], Daryl V. Nydam [b], Andrew S. Peregrine [c]

[a] *Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ont. N1G 2W1, Canada*
[b] *Department of Population Medicine, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[c] *Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ont. N1G 2W1, Canada*

Received 20 January 2006; received in revised form 7 May 2007; accepted 8 May 2007

Abstract

This work was conducted to investigate calf-level factors that influence the risk of neonatal diarrhea and shedding of *Cryptosporidium parvum* oocysts in calves, on dairy farms in Ontario with histories of calf diarrhea or cryptosporidiosis. Fecal samples were collected weekly for 4 weeks from each of 1045 calves under 30 days of age on 11 dairy farms in south-western Ontario during the summer of 2003 and the winter of 2004. A questionnaire designed to gather information on calf-level management factors was administered on farm for each calf in the study. Samples were examined for *C. parvum* oocysts by microscopy, and a subset of specimens was also tested for enterotoxigenic *Escherichia coli*, *Salmonella*, bovine rotavirus and bovine coronavirus. The consistency of each sample was scored and recorded at the time of collection in order to assess the presence or absence of diarrhea. In addition, a blood sample was taken from each calf upon enrolment in the study, for assessment of maternal antibody transfer and for polymerase chain reaction testing for persistent bovine viral diarrhea virus infection. Using the GLLAMM function in Stata 9.0, multilevel regression techniques were employed to investigate associations between management practices and the risk of *C. parvum* shedding or diarrhea.

C. parvum oocysts were detected in the feces of 78% of the 919 calves from which all four fecal samples had been collected. Furthermore, 73% of the 846 calves for which all four fecal consistency scores had been recorded were diarrheic at the time of collection of at least one sample. Significant predictors of the calf-level risk of *C. parvum* shedding included the use of calf diarrhea prophylaxis in pregnant cows, and the type of maternity facilities in which the calves were born. Factors associated with an increased risk of diarrhea were leaving the calf with the dam for more than an hour after birth, and the birth of a calf in the summer as

* Corresponding author. Tel.: +1 519 824 4120x58951; fax: +1 519 763 8621.
E-mail address: ltrotzwi@uoguelph.ca (L.A. Trotz-Williams).

0167-5877/$ – see front matter
doi:10.1016/j.prevetmed.2007.05.003

opposed to winter. Calves shedding *C. parvum* oocysts had 5.3 (95% CI 4.4, 6.4) times the odds of diarrhea than non-shedding calves, controlling for other factors included in the final multivariable model. Furthermore, infected calves shedding more than 2.2×10^5 oocysts per gram of feces were more likely to scour than infected calves shedding lower numbers of oocysts (OR = 6.1, 95% CI 4.8, 7.8). The odds of diarrhea in calves shedding oocysts that had been allowed to remain with their dams for more than an hour were higher than the odds of diarrhea in shedding calves that had been separated from their dams within an hour after birth.

Keywords: *Cryptosporidium parvum*; Diarrhea; Dairy calves; Risk factors; Ontario

1. Introduction

Cryptosporidium parvum is a ubiquitous intestinal protozoan parasite that commonly infects dairy calves in the first month of life. Mortality may occur in severely affected animals, but, more often, a self-limiting secretory diarrhea results in morbidity and increased labour and veterinary costs (de Graaf et al., 1999). Infected animals usually shed large numbers of infective oocysts in the feces (Nydam et al., 2001). Detection of these oocysts, or of *C. parvum* antigen in feces, is the usual means of diagnosis. In dairy calves, the prevalence of shedding has been reported to be highest among animals 7–21 days of age, with a pre-patent period of 3–6 days (de Graaf et al., 1999). Studies in Canada have reported the prevalence of shedding among dairy calves 0–24 weeks of age to be 15–59% (Olson et al., 1997a,b). In another study, O'Handley et al. (1999) reported a period prevalence of shedding of 100% among dairy calves from birth to 4 months old on a farm in Alberta, Canada. In 2002, 40.6% of 500 calves aged 7–21 days on 51 dairy farms in south-western Ontario were found to be shedding oocysts (Trotz-Williams et al., 2005a).

Several management factors have been found to be associated with an increased risk of *C. parvum* shedding in dairy calves. A longitudinal study conducted in the United States (USA) found that infected calves were more likely to be found on farms using multi-cow maternity facilities than on those using single-cow facilities (Garber et al., 1994). Frequent bedding changes in the calf housing area, and increased contact between calves, have also been identified as factors associated with an increased risk of *C. parvum* shedding in dairy calves in the USA (Sischo et al., 2000). Using a cross-sectional study design, Mohammed et al. (1999) found that feeding milk replacer, use of antibiotics and use of fan or exhaust ventilation in calf rearing areas were all associated with a decreased risk of shedding on dairy farms in New York State, USA. In a cross-sectional study conducted in Mexico, use of hay bedding in the maternity area, sweeping the maternity area and feeding starter grain to dairy calves were identified as practices associated with increased shedding of *C. parvum* (Maldonado-Camargo et al., 1998).

Although shedding of *C. parvum*, and cryptosporidiosis, are known to be common in dairy calves in Canada (Trotz-Williams et al., 2005a), little has been published on potential risk factors for infection and neonatal diarrhea (including *C. parvum*-related diarrhea) in dairy calves in Canada. Moreover, it is unknown whether risk factors for *C. parvum* shedding and calf diarrhea identified by previous work done in Ontario or elsewhere, are associated with the risk of infection and diarrhea on Ontario dairy farms. This study was carried out with the objective of identifying potential calf-level risk factors for neonatal diarrhea and *C. parvum* shedding among pre-weaned dairy calves on purposively selected farms with histories of calf diarrhea or cryptosporidiosis in south-western Ontario.

2. Materials and methods

2.1. Sampling frame and sample size estimation

The implied sampling frame consisted of all dairy herds with a history of neonatal calf diarrhea and/or cryptosporidiosis in south-western Ontario, within 250 km of the Ontario Veterinary College, University of Guelph.

Statistical computer software (Stata® 8.0, College Station, TX) was used to estimate the number of study units (calves) required for the comparison of two proportions reflecting exposure to risk factors. These proportions were set at 0.6 for infected calves and 0.25 in non-infected calves, respectively. The estimated sample size (36 calves per group) was adjusted for a cluster size of 30 with an intra-herd correlation of 0.3, and for 10 covariates with an estimated average correlation with infection of 0.2. This gave an estimated sample size of 990 calves (495 per group).

Estimates of the two proportions, the ratio of controls to cases, and the intra-herd correlation coefficient were based on data collected in a previous prevalence study conducted in southern Ontario in 2002 (Trotz-Williams et al., 2005a).

2.2. Animals

Calves from 0 to 30 days of age on 11 dairy farms in south-western Ontario were sampled. Farms were recruited using purposive sampling, with the assistance of dairy practitioners in the area. Clients' farms with histories of calf diarrhea and or cryptosporidiosis within the previous 2 years, milking at least 60 cows and situated within 250 km of the Ontario Veterinary College (OVC), were encouraged to participate. Attempts were made to enrol all calves born on the farms during the sampling periods, up to a weekly maximum of 50 calves per farm.

2.3. Sampling

From June to October 2003 and from January to April 2004, inclusive, each farm was visited weekly by trained research technicians. At each visit, calves born since the previous visit were enrolled in the study. A 10 mL sample of blood was drawn from each newly enrolled calf by venepuncture: 5 mL into a sterile vacutainer containing ethylene diamine tetra-acetic acid (EDTA) and 5 mL into a vacutainer without anticoagulant (Becton Dickinson, Mississauga, Ont., Canada). Approximately 5 g of fresh feces were collected per rectum in a clean screw-topped vial. Fecal samples were collected weekly on subsequent visits, to a total of four samples per calf. The consistency of each fecal sample was recorded at the time of collection by the research technicians, using the scoring system of 1–4 described by Larson et al. (1977). Scores of 3 and 4 were regarded as being indicative of diarrhea, while scores of 1 and 2 reflected normal fecal consistency. If an attempt to collect feces per rectum was unsuccessful and calves were housed individually, a fresh sample was taken from the ground where available.

2.4. Testing of samples

Blood and fecal samples were transported to the OVC in coolers, and were then refrigerated at 4 °C until processed. Serum was separated from each blood sample within 24 h of collection, and was used to assess passive transfer of maternal immunity by total serum protein refractometry

(Calloway et al., 2002) as well as by using an automated serum analyzer (Hitachi Ltd., Hitachinaka, Japan). One milliliter aliquots of blood from the EDTA vacutainers were frozen at −70 °C and were subsequently tested for persistent bovine viral diarrhea virus (BVDV) infection by polymerase chain reaction (PCR), using the protocol described by Deregt et al. (2002).

Fecal samples were examined for the presence of *C. parvum* oocysts using a standardized sucrose wet mount method. Using polymerase chain reaction-restriction fragment length polymorphism of the *Cryptosporidium* oocyst wall protein as a gold standard, this test showed a sensitivity of 88.6% (95% confidence interval (CI) 80.1%, 94.4%) and a specificity of 93.8% (95% CI 86.0%, 97.9%) when used on 168 calf fecal specimens (Trotz-Williams et al., 2005b). Briefly, 1 g of feces was mixed with 3 mL of sucrose solution of specific gravity 1.32. One drop of the resulting fecal suspension was placed on a slide, covered with a 22 mm × 22 mm coverslip, and examined at ×400 magnification using bright field optical microscopy. *C. parvum* oocysts were identified as pink, refractile, spherical structures 3–6 μm in diameter situated just beneath the coverslip. The average number of oocysts per microscope field was used to estimate the number of oocysts per gram of feces (Trotz-Williams et al., 2005b).

Fecal samples from a specific subset of calves were tested at the University of Guelph Animal Health Laboratory (AHL) for infection with enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* spp., bovine rotavirus (BRV) group A and bovine coronavirus (BCV). Calves were selected for these tests using the two-stage sampling procedure described by Schaubel et al. (1997). Briefly, data from an earlier prevalence study (Trotz-Williams et al., 2005a) were used to assess the relationship between *C. parvum* shedding and diarrhea. This information was used to determine the proportion of exposed (*C. parvum* infected) and diseased (diarrheic) calves that would be selected for bacteriological and virology testing. Random systematic sampling was then used to select fecal samples taken in the first week of life for ETEC and *Salmonella* tests, and specimens from calves 7 to 14 days old for BRV and BCV tests.

Testing for ETEC and *Salmonella* was performed at the AHL by standard culture methods, with bacterial isolates being identified using a semi-automated bacterial identification system (Replianalyzer, Oxoid, Nepean, Ont., Canada).

Testing for bovine rotavirus was carried out at the AHL using a latex agglutination test (Rotascreen®, Microgen Bioproducts, Surrey, UK). An antigen detection ELISA (Syracuse Bioanalytical, Syracuse, New York) was employed for detection of bovine coronavirus.

2.5. Questionnaire

A pre-tested questionnaire was administered to collect information on the management of each calf immediately after birth and for the first 4 weeks thereafter. Copies of questionnaires were distributed by research technicians and were completed on farm by producers, one following the birth of each calf. At the time of completion of each questionnaire, producers were blind to the status (shedding or non-shedding) of the calf for which information was being recorded. Questions were designed to gather information on management practices reported in the literature as significantly associated with the risk of *C. parvum* shedding, as well as other factors considered by the authors to be potential predictors of the risk of shedding or calf diarrhea. Examples of factors investigated were ease of birth, amount of time for which the calf had remained with its dam after birth, calving area hygiene, quantity and source of colostrum fed, preventive procedures performed on the neonatal calf such as vaccination and navel-dipping, and type of housing in which the calf was kept for the first 4 weeks of life. A copy of the questionnaire used for this study is available from the authors in English.

2.6. Statistical analysis

Data were entered in EpiData 3.02 (EpiData Association, Odense, Denmark) and were exported from EpiData into Stata 9.0 (Stata Corporation, College Station, TX) for screening of variables and analysis. Variables were screened for associations with *C. parvum* shedding and diarrhea by unvariable multilevel generalized linear mixed modelling, using the ''GLLAMM'' function in Stata 9.0 with a liberal *P*-value (0.25). This approach was employed to control for clustering at the herd-level, and repeated observations at the calf-level. During screening, the dependent (outcome) variable used to represent the risk of *C. parvum* shedding was a dichotomous variable representing the positive or negative status of each fecal specimen. Similarly, the dependent variable used to represent the risk of diarrhea was the diarrheic or non-diarrheic status of individual fecal specimens, with specimens having consistency scores of 3 or 4 classified as diarrheic. The intra-class correlation of *C. parvum* shedding was estimated using a one-way analysis of variance approach with the 'loneway' command in Stata 9.0. Collinearity between variables was investigated by examination of covariance matrices, and where necessary by cross-tabulations, comprising groups of variables.

Variables that were significant at the 25% level in univariable screening were used to build multivariable multilevel generalized linear mixed models using the ''GLLAMM'' command in Stata 9.0. As in univariable modelling, *C. parvum* shedding status, and diarrhea status of calves at individual weekly sampling times, were used as response variables in modelling risk of shedding and risk of diarrhea, respectively. Where variables were not mutually exclusive and were highly correlated, only one of the related variables was included in model-building, the decision of which to include being made on the basis of distribution and biological plausibility. Models were built by the manual backwards elimination procedure described by Dohoo et al. (2003) with a significance level of $P > 0.05$ as the criterion for removal of a variable from the model. The statistical significance of continuous and dichotomous variables in each model was assessed using Wald's test, and the likelihood ratio test was used to determine the significance of multilevel categorical predictors. The associations between continuous variables and the outcome were assessed for linearity both graphically, and by the inclusion of polynomial terms in the model; variables were assumed to have an approximately linear relationship with the outcome if the coefficients of the polynomial terms were non-significant at the 5% level. Non-linear associations were modelled by categorizing the variable before inclusion in the models. During the model-building process, whether or not a predictor was a confounder was determined by removing the variable and observing the effect on the coefficients of the remaining significant variables; a change of more than 25% in one or more coefficients was regarded as evidence of confounding (Dohoo et al., 2003). Interaction was assessed by the generation of interaction terms amongst variables in the final first-order model and the inclusion of those terms along with the main effects. A *P*-value of less than 0.05 associated with an interaction term was considered evidence of interaction.

Model diagnostics were performed by generating and examining expected values (probabilities) of the outcome, Pearson residuals, deviance residuals and Cook's distance values.

To explore whether calves born in the summer may have been managed differently from those born in the winter, management variables were regressed on season of calf's birth using logistic modelling, with farm included as a random effect. This showed that differences significant at the 5% level existed between summer and winter born calves with reference to several management practices. To control for these differences, season of birth was forced into the multilevel models previously described.

In order to extrapolate the data on bacterial and viral enteropatogens recorded for a subset of the calves, the method described by Cain and Breslow (1988) was used to adjust the regression coefficient representing the relationship between the overall *C. parvum* shedding status of calves and diarrhea, in a logistic model including all enteropathogens as fixed effects and farm as a random effect. The same method was used to adjust the standard error of that coefficient. Briefly, the unadjusted coefficient was adjusted using the formula:

$$\beta_2^{(\text{adj})} = \beta_2^{(\text{unadj})} + \log\left[\frac{N_{12}N_{01}n_{11}n_{02}}{N_{11}N_{02}n_{12}n_{01}}\right]$$

where N_{01}, N_{11}, N_{02}, N_{12}, n_{01}, n_{11}, n_{02} and n_{12} refer to the numbers of calves in subsets of the first (N) and second (n) stage of the study population, respectively. Subsets were defined by *C. parvum* (i) and diarrhea (j) status.

3. Results

Fecal samples were collected from 1045 calves on the 11 farms. The number of calves per farm enrolled in the study ranged from 4 to 521 (median 32).

For 126 calves, data on *C. parvum* status were missing for one or more of the 4 weeks. Reasons for missing data included difficulties locating calves during a farm visit, removal of calves from farms (sale or transfer) and death of calves. There was no significant difference in the risk of shedding or diarrhea between these calves and the calves for which data was available for all 4 weeks. In addition, examination of the data showed no significant difference in management for the two sets of calves. Of the 919 calves for which no data were missing, 721 (78%) tested positive for *C. parvum* at one or more sampling times. Oocysts were detected in fecal samples of calves from all of the 11 farms. Within-herd prevalence of shedding ranged from 35% to 100%. Calves under 1 week old were least likely to be shedding *C. parvum* oocysts (prevalence = 5%), while calves aged 7–14 days had the highest prevalence (53%) of shedding. Shedding of oocysts was moderately clustered within herds; the intra-class correlation coefficient (rho) for shedding was 0.4.

For 199 calves, data on consistency of one or more fecal sample were missing. Reasons for this were similar to those stated for missing *C. parvum* data. Of the remaining 846 animals, 619 (73%) were diarrheic at the time of collection of at least one sample. Scouring was most prevalent in calves at the time of the second sampling (aged 7–14 days) and was least prevalent in calves less than 1 week old; samples from 47% of calves aged 7–14 days were scored as 3 or 4, while only 15% of calves under 1 week old were diarrheic at the time of sampling.

The prevalence of bacterial and viral enteropathogens is summarized in Table 1.

Table 1
Prevalence of bacterial and viral enteropathogens in dairy calves from south-western Ontario tested in summer 2003 and winter 2004

Enteropathogen	Summer 2003 (n)	Winter 2004 (n)	Overall 2003–2004 (n)
Enterotoxigenic *Escherichia coli* (ETEC)	48% (217)	57% (122)	51% (339)
Salmonella spp.	9% (217)	16% (123)	12% (340)
Bovine rotavirus	22% (222)	31% (126)	26% (348)
Bovine coronavirus	2% (222)	9% (126)	5% (348)
Bovine viral diarrhea virus (persistent infection)	2% (634)	1% (372)	1% (1006)

Table 2a
Results of univariable multilevel generalized linear mixed modelling, showing variables significantly associated ($P < 0.25$) with *Cryptsporidium parvum* shedding in calves 0–30 days old on south-western Ontario dairy farms

Variable	Calf-level frequency[a]	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Age of calf at sampling[a]	25% quartile: 8 days 50% quartile: 15 days 75% quartile: 22 days	(0.883)	(0.806, 0.959)	<0.001
Age squared[a]	N/A	(−0.031)	(−0.033, −0.028)	<0.001
Season of calf's birth				
Winter	389	0		
Summer	656	1.14	0.98, 1.32	0.098
Calf born in multi-cow calving pen				
No	553	0		
Yes	467	0.81	0.69, 0.95	0.009
Positive test result for bovine coronavirus infections				
No	332	0		
Yes	16	0.59	0.30, 1.16	0.129
Quantity of colostrum fed to calf in first 24 h after birth[a]	25% quartile: 4 L 50% quartile: 6 L 75% quartile: 7 L	(0.05)	(0.005, 0.092)	0.030
Failure of passive transfer[b]				
No	913	0		
Yes	90	1.34	1.02, 1.75	0.033
Calf fed colostrum from single cow other than dam				
No	976	0		
Yes	60	0.55	0.35, 0.88	0.012
Calf fed colostrum by bottle				
No	113	0		
Yes	923	0.66	0.49, 0.90	0.008
Calf fed whole waste milk after colostrum				
No	263	0		
Yes	777	0.82	0.61, 1.08	0.160
Calf fed saleable milk after colostrum				
No	828	0		
Yes	212	0.53	0.42, 0.66	<0.001
Calf fed milk replacer after colostrum				
No	882	0		
Yes	158	2.35	1.67, 3.31	<0.001
Calf feed contained coccidiostat				
No	344	0		
Yes	681	0.86	0.74, 1.01	0.068
Dam of calf treated with ScourGuard or Ecostar[c]				
No	104	0		
Yes	903	0.58	0.41, 0.81	0.002

[a] For continuous variables, regression coefficients are reported instead of odds ratios, and quartiles are provided to reflect the distribution of each variable.

[b] Failure of passive transfer was defined as total serum protein ≥5.2 g/dL as measured by serum refractometry.

[c] Ecostar (Novartis, Mississauga, Ontario) or ScourGuard (Pfizer, Orangeville, Ontario).

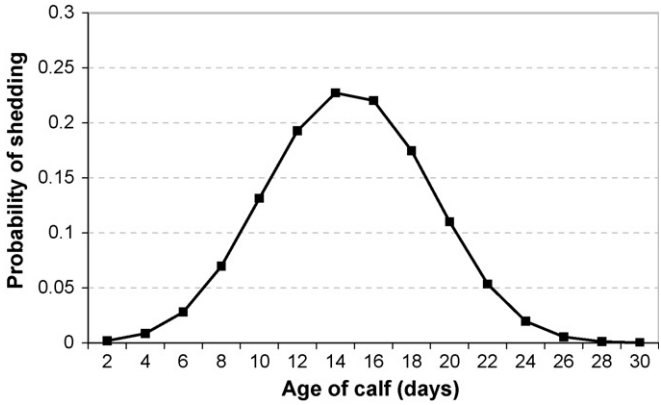

Fig. 1. Probability of shedding *Cryptosporidium parvum* oocysts in Ontario dairy calves by age of calf, as represented by the model shown in Table 3. The relationship illustrated pertains to calves for which all covariates listed in Table 3 were at their referent levels (see Table 2a).

3.1 Predictor variables associated with C. parvum shedding

Table 2a shows the results of unconditional screening of predictor variables for association with *C. parvum* shedding.

During multivariable model-building, the variables representing the quantity of colostrum fed to calves in the first 24 h following birth, and whether calves had been born in a multi-cow calving area, were identified as confounders. These variables were therefore forced into the model. Because several management variables had been found to be statistically associated with season, season of calf's birth was also forced into the model. The final model is shown in Table 3. In addition, the quantity of colostrum fed to calves in the first 24 h after birth, and whether calves had been born in a multi-cow calving pen, appeared to be confounders and were therefore forced into the model. There was no statistical evidence of interaction between variables in this model. Predicted probabilities, Pearson and deviance residuals and Cook's distance values indicated that the model fit the data moderately well.

Fig. 1 illustrates the relationship between the probability of calves shedding *C. parvum* oocysts and age of the calves, as represented by the model in Table 3. This relationship reflects the previously mentioned finding that shedding of oocysts was most prevalent in the second and third weeks of life, with the risk of shedding being lowest in calves under 1 week of age.

3.1. Predictor variables associated with diarrhea

Variables significantly associated with diarrhea at the 25% level in unconditional analysis are shown in Table 2b.

The multivariable model constructed with diarrhea as the response variable is summarized in Table 4. As for the model described in Section 3.1, season of birth was forced into the model. No other confounders were identified, and there was no statistical evidence of interaction between variables in this model. Predicted probabilities, Pearson and deviance residuals and Cook's distance values indicated that this model fit the data moderately well.

The relationship between the probability of diarrhea in *C. parvum* negative calves and age of the calves as represented by the model in Table 4 is shown in Fig. 2.

Table 2b
Results of univariable multilevel generalized linear mixed modelling, showing variables significantly associated ($P < 0.25$) with the risk of diarrhea in calves 0–30 days old on south-western Ontario dairy farms

Variable	Calf-level frequency[a]	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Age of calf at sampling[a]	25% quartile: 8 days 50% quartile: 15 days 75% quartile: 22 days	(0.31)	(0.267, 0.356)	<0.001
Age squared[a]	N/A	(−0.01)	(−0.012, −0.009)	<0.001
Season of calf's birth				
Winter	389	0		
Summer	656	1.84	1.58, 2.15	<0.001
Shedding of *Cryptosporidium parvum* oocysts at sampling				
No	198	0		
Yes	721	6.75	5.61, 8.14	<0.001
Number of oocysts per gram of feces				
Low	3302 samples	0		
High[b]	601 samples[c]	9.24	7.41, 11.53	<0.001
Calf allowed to remain with dam for more than 1 hour following birth				
No	526	0		
Yes	513	1.59	1.35, 1.85	<0.001
Calf born in multi-cow calving pen				
No	553	0		
Yes	467	1.19	1.02, 1.39	0.024
Calving pen cleaned before birth of calf				
No	564	0		
Yes	109	0.77	0.60, 0.98	0.031
Positive test result for bovine rotavirus infection				
No	259	0		
Yes	89	0.82	0.62, 1.10	0.186
Quantity of colostrum fed to calf in first 24 h after birth[a]	25% quartile: 4 L 50% quartile: 6 L 75% quartile: 7 L	(−0.10)	(−0.139, −0.055)	<0.001
Calf fed pooled colostrum				
No	577	0		
Yes	459	0.70	0.61, 0.81	<0.001
Calf fed fresh colostrum				
No	234	0		
Yes	707	0.75	0.63, 0.89	0.001
Calf fed colostrum by bottle				
No	113	0		
Yes	923	0.77	0.57, 1.04	0.094
Total protein refractometry reading in first week of life[a]	25% quartile: 5.8 g/dL 50% quartile: 6.2 g/dL 75% quartile: 6.8 g/dL	(0.006)	(−0.003, 0.015)	0.023

Table 2b (*Continued*)

Variable	Calf-level frequency[a]	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Calf fed whole waste milk after colostrum				
No	263	0		
Yes	777	1.25	0.92, 1.70	0.152
Calf fed saleable milk after colostrum				
No	828	0		
Yes	212	0.79	0.60, 1.03	0.085
Calf feed contained coccidiostat				
No	344	0		
Yes	681	1.58	1.32, 1.89	<0.001
Calf received vaccinations				
No	769	0		
Yes	215	0.54	0.45, 0.65	<0.001
Navel of calf dipped				
No	722	0		
Yes	273	0.61	0.51, 0.72	<0.001

[a] For continuous variables, regression coefficients are reported instead of odds ratios, and quartiles are provided to reflect the distribution of each variable.

[b] High = More than 2.2×10^5 oocysts per gram of feces. Categorization based on grouping of number of oocysts using the 'lintrend' option in Stata 9.0, with number of oocysts grouped into 6 groups of similar sample size.

[c] Sample-level frequencies provided for levels of oocyst shedding.

3.2. Predictor variables associated with diarrhea in calves shedding *C. parvum*

Table 2c lists variables significantly associated ($P < 0.25$) with diarrhea in univariable analysis for calves shedding *C. parvum* oocysts. Multivariable modelling of these variables, with presence or absence of diarrhea as the response variable, yielded the final model summarized in Table 5. As in the models described in Sections 3.1 and 3.2, season of birth was forced into the model. No other confounders were identified, and there was no statistical evidence of interaction

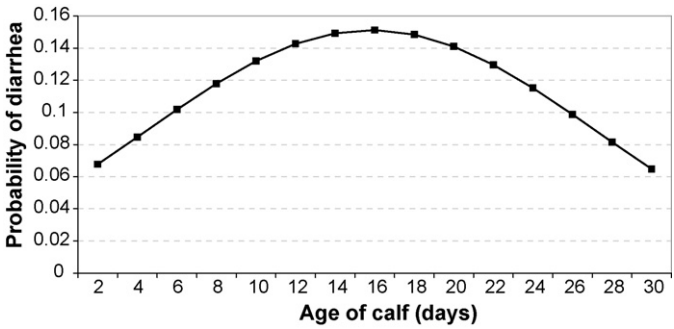

Fig. 2. Probability of diarrhea in Ontario dairy calves 0 to 30 days old, by age of calf, as represented by model shown in Table 4. The relationship illustrated pertains to calves for which all covariates listed in Table 4 were at their referent levels (see Table 2b).

Table 2c
Results of univariable multilevel generalized linear mixed modelling, showing variables significantly associated ($P < 0.25$) with the risk of diarrhea in calves 0–30 days old shedding *Cryptosporidium parvum* on south-western Ontario dairy farms

Variable	Calf-level frequency[a]	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Age of calf at sampling[a]	25% quartile: 8 days 50% quartile: 15 days 75% quartile: 22 days	(0.343)	(0.292, 0.395)	<0.001
Age squared[a]	N/A	(−0.012)	(−0.013, −0.010)	<0.001
Season of calf's birth				
Winter	241	0		
Summer	480	1.79	1.49, 2.14	<0.001
Number of oocysts per gram of feces				
Low	2275 samples	0		
High[b]	609 samples[c]	8.67	6.85, 10.97	<0.001
Calf allowed to remain with dam for more than 1 h following birth				
No	327	0		
Yes	392	1.58	1.34, 1.86	<0.001
Calf born in multi-cow calving pen				
No	428	0		
Yes	279	1.22	1.02, 1.45	0.027
Calving pen cleaned before birth of calf				
No	458	0		
Yes	78	0.82	0.63, 1.08	0.158
Positive test result for bovine rotavirus infection				
No	191	0		
Yes	58	0.76	0.54, 1.06	0.108
Positive test result for *Salmonella* infection				
No	223	0		
Yes	19	1.40	0.86, 2.28	0.169
Quantity of colostrum fed to calf in first 24 h after birth[a]	25% quartile: 2 L 50% quartile: 6 L 75% quartile: 6 L	(−0.114)	(−0.166, −0.061)	<0.001
Calf fed pooled colostrum				
No	414	0		
Yes	302	0.69	0.58, 0.82	<0.001
Calf fed frozen colostrum				
No	260	0		
Yes	384	0.86	0.73, 1.02	0.091
Calf fed whole waste milk after colostrum				
No	126	0		
Yes	594	1.24	1.00, 1.54	0.052
Calf feed contained coccidiostat				
No	257	0		
Yes	452	1.51	1.24, 1.83	<0.001

Table 2c (*Continued*)

Variable	Calf-level frequency[a]	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Calf received vaccinations				
No	605	0		
Yes	76	0.73	0.55, 0.96	0.024
Navel of calf dipped				
No	575	0		
Yes	112	0.84	0.67, 1.06	0.141

[a] For continuous variables, regression coefficients are reported instead of odds ratios, and quartiles are provided to reflect the distribution of each variable.

[b] High = More than 2.2×10^5 oocysts per gram of feces. Categorization based on grouping of number of oocysts using the 'lintrend' option in Stata 9.0, with number of oocysts grouped into 6 groups of similar sample size.

[c] Sample-level frequencies provided for levels of oocyst shedding.

between variables in this model. Examination of the predicted probabilities, Pearson and deviance residuals, and Cook's distance values associated with this model indicated that this model fit the data well.

3.3. Two-stage adjustment of logistic coefficients

In multivariable generalized mixed modelling, none of the enteropathogens for which the calves were tested were significantly associated with diarrhea at the 5% level. This may have been partly due to low power, as only 25% of the calves were tested for these agents. Nonetheless, the regression coefficient for the overall *C. parvum* shedding status of calves (produced by the construction of a logistic model for diarrhea that included data on all of the enteropathogens obtained from the second-stage as fixed effects, as well as herd as a random effect) was adjusted using the method previously described. In comparison with the unadjusted coefficient and standard error for *C. parvum* (1.06 and 0.34, respectively), the adjusted values were farther from the null: 1.47 and 0.27, respectively. Corresponding unadjusted and adjusted odds ratios were 2.89 (95% CI 1.48, 5.70) and 4.35 (95% CI 2.22, 8.52), respectively.

4. Discussion

The prevalence of *C. parvum* shedding among the calves in this study was higher than figures published in other reports from areas within North America (Garber et al., 1994; Olson et al., 1997a,b; O'Handley et al., 1999; Sischo et al., 2000). Probable reasons for this apparent discrepancy include the fact that 4 weekly samples were taken from each animal instead of the single sample used in most other studies. Furthermore, farms were selected based on the criterion of a previous history of calf diarrhea. In addition, calves in our study were all under 1 month of age, and were therefore within the age group known to show the highest prevalence of *C. parvum* infection and diarrhea. Moreover, of the 1045 calves enrolled in this study, 584 were from a single large herd with a high prevalence of *C. parvum* shedding (91%) and diarrhea (79%); the overall prevalence of *C. parvum* shedding on the other 10 farms was 62%.

Multivariable modelling of *C. parvum* shedding (Table 3) showed that calves born in the summer months were more likely to shed *C. parvum*. The quantity of colostrum fed to calves in

Table 3
Multivariable generalized linear mixed model of *Cryptosporidium parvum* shedding in calves 0–30 days in south-western Ontario, regressed on management factors significant at the 25% level in univariable analysis

Variable	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Age of calf at sampling[a]	(0.91)	(0.81, 1.00)	<0.001
Age squared[a]	(−0.03)	(−0.04, −0.03)	<0.001
Season of calf's birth			
Winter	0		
Summer	1.58	1.17, 2.12	0.002
Quantity of colostrum fed to calf in first 24 h after birth[a]	(0.09)	(−0.01, 0.19)	0.067
Calf born in multi-cow calving pen			
No	0		
Yes	0.96	0.69, 1.33	0.800
Calf feed contained coccidiostat			
No	0		
Yes	0.67	0.49, 0.93	0.016
Dam of calf treated with ScourGuard or Ecostar[b]			
No	0		
Yes	0.30	0.20, 0.45	<0.001

[a] For continuous variables, regression coefficients are reported instead of odds ratios.
[b] Ecostar (Novartis, Mississauga, Ontario) or ScourGuard (Pfizer, Orangeville, Ontario).

the first 24 h after birth was also positively associated with the risk of *C. parvum* shedding. On the other hand, calves that received feed containing coccidiostats, and those born to dams that had been treated with calf-scour prophylactic medications (ScourGuard [Novartis, Ontario] or Ecostar [Pfizer, Ontario]) were significantly less likely to shed *C. parvum*. Those born in multi-cow calving pens also showed a slightly lower risk of shedding, though this variable appeared to be a confounder and was not significantly associated with shedding at the 5% level ($P = 0.800$). In a previous study, Frank and Kaneene (1993) found that the use of individual maternity stalls was significantly associated with an increased incidence of calf diarrhea in dairy herds with more than 200 cows in Michigan, USA. As is often the case with predictors found to be significantly associated with a response variable in a statistical model, the associations observed in our study may not reflect actual causal or protective relationships. Instead, the predictors included in the model may merely be indicators of other unknown management factors that are directly related to the risk of *C. parvum* infection and shedding in calves. For example, the negative association between use of calf-scour prophylactic medications in pregnant cows and the risk of shedding may reflect the impact of a generally higher standard of herd management in some herds on the risk of shedding, rather than a direct protective effect, as neither of the medications used is known to provide any protection against *C. parvum* in calves. Further, it is often difficult to distinguish between calf- and herd-level exposure factors. Indeed, the small number of herds used in this study increases the likelihood that the observed associations may be reflections of individual herd effects instead of true causal associations. Having said that, we believe that our findings will add to the current knowledge of potential risk factors for *C. parvum* shedding and diarrhea.

Controlling for other variables included in the model shown in Table 4, the odds of diarrhea in calves born in the summer months were 69% higher than in those born during the winter, and this

Table 4
Multivariable generalized linear mixed model of diarrhea in calves 0–30 days in south-western Ontario, regressed on management factors significant at the 25% level in univariable analysis

Variable	Odds ratio (or regression coefficient)[a]	95% Confidence interval	*P*
Age of calf at sampling[a]	(0.148)	(0.101, 0.195)	<0.001
Age squared[a]	(−0.004)	(−0.006, −0.003)	<0.001
Season of calf's birth			
Winter	0		
Summer	1.69	1.37, 2.08	<0.001
Calf shedding *Cryptosporidium parvum* at the time of sampling			
No	0		
Yes	5.30	4.36, 6.45	<0.001
Calf allowed to remain with dam for more than 1 h following birth			
Yes	0		
No	1.39	1.15, 1.69	<0.001

[a] For continuous variables, regression coefficients are reported instead of odds ratios.

association was highly significant. This finding is in contrast to results published by Waltner-Toews et al. (1986), Curtis et al. (1988) and Frank and Kaneene (1993), who found higher incidences of calf diarrhea in the winter in New York, Ontario and Michigan dairies, respectively. However, none of these studies investigated the association between *C. parvum* shedding and calf diarrhea. Our study demonstrated a strong association between *C. parvum* shedding and diarrhea, with shedding calves having more than five times the odds of diarrhea than non-shedding calves. No such association was found between the risk of diarrhea and any of the other enteropathogens investigated. Indeed, the adjustment of the coefficient for overall *C. parvum* status of calves using the method described by Cain and Breslow (1988) and the data collected on other enteropathogens for the second stage sample, resulted in an increased value (farther from the null) for the coefficient. It appears, therefore, that *C. parvum* was the enteropathogen most strongly associated with diarrhea, as well as, perhaps, the most common cause of calf diarrhea, within our study population. The low predicted probabilities of diarrhea in *C. parvum* negative calves (Fig. 2), computed from coefficients of the model in Table 4, highlight the importance of the parasite as a cause of scouring, since the overall prevalence of diarrhea in the calves was much higher than the predicted probabilities of scouring in non-shedding calves shown in the graph. The association between *C. parvum* and diarrhea in calves in Ontario has previously been reported (Trotz-Williams et al., 2005a), and the results of the present study confirm the importance of the parasite as a cause of calf scours in this region. As the risk of *C. parvum* shedding was significantly higher in the summer months, it follows that the risk of *C. parvum*-associated diarrhea would also be higher in these calves at that time of the year. It is possible that the cases of diarrhea observed in the calves used in previous studies (Waltner-Toews et al., 1986; Curtis et al., 1988; Frank and Kaneene, 1993) were predominantly caused by enteropathogens other than *C. parvum*. Furthermore, the fact that the farms used in our study were selected based on a history of calf diarrhea or cryptosporidiosis may have contributed to some of the differences between the findings reported here and those of other researchers.

The length of time for which calves remained with their dams after birth was significantly associated with diarrhea; the odds of diarrhea in calves remaining with their mothers for more than 1 h were 39% higher than the odds of diarrhea in those separated from the dam within an

hour of birth (Table 4). Using an experimental approach, Quigley et al. (1994) found that, in a study population of 96 Jersey calves, the prevalence of *Cryptosporidium* was significantly higher in calves 1 week of age that had been allowed to nurse their dams, than in calves of the same age that had been separated from their dams before they could nurse, and fed colostrum by bottle. However, in our study, no statistically significant association was observed between the risk of shedding and length of time with the dam, either in univariable or in multivariable analysis. The reason for the apparent association between length of stay with dam and the risk of diarrhea in our study may therefore be related to factors other than infection with *C. parvum*.

Controlling for other variables included in the model summarized in Table 5, the odds of diarrhea in calves shedding *C. parvum* that were raised in the summer months were 64% higher than the odds of diarrhea in the shedding calves that were raised in the winter. This finding may have been due to better survival of oocysts in the environmental conditions that existed in the summer months, resulting in greater levels of challenge for the animals. Alternatively, other unknown and unmeasured factors that predisposed *C. parvum*-infected animals to the development of diarrhea may have been more prevalent in the warmer months of the year, either directly or indirectly because of seasonal conditions. Not surprisingly, among the *C. parvum*-positive calves, the odds of diarrhea in animals shedding more than 2.2×10^5 oocysts per gram of feces were more than six times the odds in calves shedding fewer than 2.2×10^5 oocysts per gram of feces. This finding implies that the number of oocysts shed in the feces of infected calves may be a reliable indicator of the intensity of infection.

Interestingly, failure of passive transfer (FPT) was not significantly associated with the risk of diarrhea in the population of calves used in this study. As passive immunity has not been proven important in imparting resistance to the parasite in calves, the lack of an association with *C. parvum* shedding in multivariable analysis was not surprising. However, it is generally accepted that FPT has an impact on calf morbidity and mortality. It should be noted that the lack of association in our study may not be reflective of the relationship between FPT and diarrhea in the wider population of dairy calves in the region.

Table 5
Multivariable generalized linear mixed model of diarrhea in calves 0–30 days in south-western Ontario shedding *Cryptosporidium parvum*, regressed on management factors significant at the 25% level in univariable analysis

Variable	Odds ratio (or regression coefficient)[a]	95% Confidence interval[a]	*P*
Age of calf at sampling[a]			
Age squared[a]	(0.196)	(0.141, 0.250)	<0.001
	(−0.006)	(−0.008, −0.005)	<0.001
Season of calf's birth			
Winter	0		
Summer	1.64	1.27, 2.11	<0.001
Number of oocysts per gram of feces			
Low	0		
High[b]	6.11	4.77, 7.84	<0.001
Calf allowed to remain with dam for more than 1 h following birth			
Yes	0		
No	1.42	1.12, 1.80	0.003

[a] For continuous variables, regression coefficients are reported instead of odds ratios.
[b] High, more than 2.2×10^5 oocysts per gram of feces. Categorization based on grouping of number of oocysts using the 'lintrend' option in Stata 9.0, with number of oocysts grouped into six groups of similar sample size.

Generally, associations reported here between predictor variables and *C. parvum* oocyst shedding or diarrhea, though in many instances biologically plausible as discussed above, should be interpreted with caution. This would be prudent especially because this is the first published study of its kind conducted in Ontario, and because a large number of potential risk factors were investigated, increasing the likelihood of identifying spurious relationships between variables. Predictors that were significant only at the 25% level but not at the 5% level are particularly likely to be variables that have no true causal association with the outcomes. In addition, many of the variables investigated in this study showed little variation among calves within herds, that is, they were essentially herd-level variables. In view of the fact that only 11 purposively selected farms were used in the study, these variables were therefore less suitable for the identification of calf-level risk factors than the 'true' calf-level variables. As mentioned previously, most of the variation in the risk of infection was between herds, as opposed to within herds. With this in mind, and since many management factors were common to most calves within farms, a separate study specifically designed for the investigation of herd-level factors, and including herds with and without histories of calf diarrhea and cryptosporidiosis, would more effectively disclose any associations that may exist between such factors and the outcomes.

5. Conclusion

The results of this study show that *C. parvum* is common among calves under 1 month of age in south-western Ontario dairy herds experiencing problems with calf diarrhea. In addition, the parasite appears to be a primary cause of diarrhea among calves under 1 month of age group in the herds used in this study. The work described here has identified some calf-level, as well as a few herd-level, management practices that are potential predictors of the risk of *C. parvum* shedding and diarrhea among dairy calves in herds experiencing calf diarrhea problems. Further investigation of management practices identified here as predictors of *C. parvum* shedding and diarrhea in Ontario is warranted, and may be of benefit to producers and practitioners involved in the control of this parasite.

Acknowledgements

The authors thank Grazyna Adamska-Jarecka for diagnostic support. Data collection and processing of samples were carried out by Lotje Kouwenberg, Greg Young, Jen Wilstra, Erin Vernooy and Nicole Perkins. The assistance of the dairy practitioners and producers in recruitment of herds and data collection is much appreciated. This work was funded by Dairy Farmers of Canada, Ontario Ministry of Agricuture and Food, National Sciences and Engineering Research Council of Canada and Dairy Farmers of Ontario.

Funding agencies: Ontario Ministry of Agriculture and Food (OMAF), Dairy Farmers of Canada, Dairy Farmers of Ontario and National Sciences and Engineering Research Council of Canada (NSERC).

References

Cain, K.C., Breslow, N.E., 1988. Logistic regression analysis and efficient design for two-stage studies. Am. J. Epidemiol. 128, 1198–1206.

Calloway, C.D., Tyler, J.W., Tessman, R.K., Hostetler, D., Holle, J., 2002. Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves. J. Am. Vet. Med. Assoc. 221, 1605–1608.

Curtis, C.R., Erb, H.N., White, M.E., 1988. Descriptive epidemiology of calfhood morbidity and mortality in New York Holstein herds. Prev. Vet. Med. 5, 293–307.

de Graaf, D.C., Vanopdenbosch, E., Ortega-Mora, L.M., Abassi, H., Peeters, J.E., 1999. A review of the importance of cryptosporidiosis in farm animals. Int. J. Parasitol. 29, 1269–1287.

Deregt, D., Carman, P.S., Clark, R.M., Burton, K.M., Olson, W.O., Gilbert, S.A., 2002. A comparison of polymerase chain reaction with and without RNA extraction and virus isolation for detection of bovine viral diarrhea virus in young calves. J. Vet. Diag. Invest. 14, 433–437.

Dohoo, I.R., Martin, S.W., Stryhn, H., 2003. Model-building strategies. In: Veterinary Epidemiologic Research, AVC Inc., Charlottetown, Prince Edward Island, Canada, pp. 317–334.

Frank, N.A., Kaneene, J.B., 1993. Management risk factors associated with calf diarrhea in Michigan dairy herds. J. Dairy Sci. 76, 1313–1323.

Garber, L.P., Salman, M.D., Hurd, H.S., Keefe, T., Schlater, J.L., 1994. Potential risk factors for *Cryptosporidium* infection in dairy calves. J. Am. Vet. Med. Assoc. 205, 86–91.

Larson, L.L., Owen, F.G., Albright, J.L., Appleman, R.D., Lamb, R.C., Muller, L.D., 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60, 989–992.

Maldonado-Camargo, S., Atwill, E.R., Saltijeral-Oaxaca, J.A., Herrera-Alonso, L.C., 1998. Prevalence and risk factors for shedding of *Cryptosporidium parvum* in Holstein Freisan dairy calves in central México. Prev. Vet. Med. 36, 95–107.

Mohammed, H.O., Wade, S.E., Schaaf, S., 1999. Risk factors associated with *Cryptosporidium parvum* infection in southeastern New York State. Vet. Parasitol. 83, 1–13.

Nydam, D.V., Wade, S.E., Schaaf, S.L., Mohammed, H.O., 2001. Number of *Cryptosporidium parvum* oocysts or *Giardia* spp cysts shed by dairy calves after natural infection. Am. J. Vet. Res. 62, 1612–1615.

O'Handley, R.M., Cockwill, C., McAllister, T.A., Jelinski, M., Morck, D.W., Olson, M.E., 1999. Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. J. Am. Vet. Med. Assoc. 214, 391–396.

Olson, M.E., Thorlakson, C.L., Deselliers, L., Morck, D.W., McAllister, T.A., 1997a. *Giardia* and *Cryptosporidium* in Canadian farm animals. Vet. Parasitol. 68, 375–381.

Olson, M.E., Guselle, N.J., O'Handley, R.M., Swift, M.L., McAllister, T.A., Jelinski, M.D., Morck, D.W., 1997b. *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. Can. Vet. J. 38, 703–706.

Quigley Jr., J.D., Martin, K.R., Bemis, D.A., Potgieter, L.N.D., Reinemeyer, C.R., Rohrbach, B.W., Dowlen, H.H., Lamar, K.C., 1994. Effects of housing and colostrum feeding on the prevalence of selected infectious organisms in feces of Jersey calves. J. Dairy Sci. 77, 3124–3131.

Schaubel, D., Hanley, J., Collet, J., Boivin, J., Sharpe, C., Morrison, H.I., Mao, Y., 1997. Two-stage sampling for etiologic studies: sample size and power. Am. J. Epidemiol. 146, 450–458.

Sischo, W.M., Atwill, E.R., Lanyon, L.E., George, J., 2000. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Prev. Vet. Med. 43, 253–267.

Trotz-Williams, L.A., Jarvie, B.D., Martin, S.W., Leslie, K.E., Peregrine, A.S., 2005a. Prevalence of *Cryptosporidium parvum* infection in south-western Ontario and its association with diarrhea in neonatal dairy calves. Can. Vet. J. 46, 349–351.

Trotz-Williams, L.A., Martin, S.W., Martin, D., Duffield, T., Leslie, K.E., Nydam, D.V., Jamieson, F., Peregrine, A.S., 2005b. Multiattribute evaluation of two simple tests for the detection of *Cryptosporidium parvum* in calf faeces. Vet. Parasitol. 134, 15–23.

Waltner-Toews, D., Martin, S.W., Meek, A.H., 1986. Dairy calf management, morbidity and mortality in Ontario Holstein herds. II. Age and seasonal patterns. Prev. Vet. Med. 4, 125–135.