



## Herd-level occurrence and risk factors associated with respiratory and enteric pathogens from dairy calves in Ontario: A cross-sectional study

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### ABSTRACT

This cross-sectional herd-level study aimed to determine the occurrence of and risk factors for pathogens associated with neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) on Ontario dairy farms. From April to August 2022, a convenience sample of 100 dairy farms was visited once. A questionnaire covering farm biosecurity, calving and colostrum management, preweaning nutrition, and housing was administered on-farm. At each farm visit, approximately 5 calves between 2 and 35 d old were randomly selected for fecal sampling. Furthermore, approximately 5 calves between 21 to 122 d old were randomly selected for nasopharyngeal sampling. In total, 363 fecal samples (from 83 dairy farms) and 390 nasopharyngeal swab samples (from 80 dairy farms) were collected. Fecal samples were analyzed individually using a multiplex PCR to identify bacterial and parasitic enteric pathogens. Nasopharyngeal swabs were analyzed as one pooled sample per farm using bacterial culture and real-time PCR. The most common enteric pathogens detected at herd-level were *Cryptosporidium parvum* (67.4%) and *Escherichia coli* K99+ (13.2%). The most common respiratory pathogens detected at herd-level were *Pasteurella multocida* (62.5%), bovine coronavirus (42.5%), and *Mycoplasma bovis* (21.2%). Multivariable logistic models were built to explore associations between the most common pathogens and herd-level predictors selected from the questionnaire. Herd positivity for *C. parvum* was positively associated with having more than 61 preweaning calves per year and feeding mainly whole milk to calves. The presence of *M. bovis* was positively associated with herds that

combined manual and automatic milk-feeding systems, and the presence of bovine coronavirus was positively associated with having more than 98 preweaning calves during the year. Univariable Poisson regression models were built to explore the association between the most common pathogens and preweaning calf mortality. Herds that were positive for *C. parvum*, *M. bovis*, or bovine coronavirus had a greater risk of preweaning calf mortality. These results provide insights for future research on pathogens associated with NCD and BRD and offer guidance for veterinarians and dairy farmers in implementing disease control measures in dairy calf herds.

**Key words:** diarrhea, pneumonia, surveillance, dairy youngstock

### INTRODUCTION

Neonatal calf diarrhea (NCD) and bovine respiratory disease (BRD) are the 2 most common diseases in dairy calves in North America (Windeyer et al., 2014; Urie et al., 2018b). In the United States and Canada, between 17% and 24% of calves are treated for NCD (Windeyer et al., 2014; Urie et al., 2018b) and between 10% and 21% are treated for BRD (Windeyer et al., 2014; Urie et al., 2018b). The percentage of preweaning deaths due to NCD is approximately 57%, whereas BRD accounts for almost 24% of preweaning deaths (NAHMS, 2021). Beyond the short-term consequences, such as reduced growth and elevated mortality, associated with these diseases, there are long-term effects, including reduced weight gain (Virtala et al., 1996; Aghakeshmiri et al., 2017), increased time to first calving (Stanton et al., 2012; Aghakeshmiri et al., 2017), and reduced first lactation milk production (Stanton et al., 2012; Aghakeshmiri et al., 2017) that result in significant economic losses to dairy producers.

Neonatal calf diarrhea is a complex syndrome commonly caused by an interaction between calf immunity

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The list of standard abbreviations for JDS is available at [adsa.org/jds-abbreviations-24](https://adsa.org/jds-abbreviations-24). Nonstandard abbreviations are available in the Notes.

and infection by individual or multiple pathogens, namely bovine rotavirus group A, bovine coronavirus (BCV), *Salmonella enterica* spp., *Escherichia coli* K99+, and *Cryptosporidium parvum* (Cho et al., 2013). Similarly, BRD in calves is a multifactorial condition caused by multiple agents, including *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, bovine viral diarrhoea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (BPI3V), bovine herpesvirus type 1 (BHV-1), and BCV (Pansri et al., 2020).

Published data are limited with respect to the herd-level prevalence of the pathogens associated with NCD and BRD in dairy calves. These studies often have small sample sizes, which can compromise the validity of the inferences reported in the source population (Barkley et al., 2021). Furthermore, the prevalence of pathogens is predominantly reported at the individual level, which does not consider the differences between herds (Guliksen et al., 2009b; Barkley et al., 2021; Garcia et al., 2022), or they are conducted on outbreaks, which can overestimate their occurrence (Pardon et al., 2020). Thus, the limited herd-level data and small sample sizes present significant challenges in drawing robust conclusions about the prevalence of pathogens associated with NCD and BRD. The focus on individual-level prevalence and outbreak data further complicates the understanding of pathogen distribution across herds, underscoring the need for more comprehensive research with larger, more representative sample sizes to accurately assess the epidemiology of these diseases at the herd level.

Animal health surveillance is the collection, analysis, interpretation, and dissemination of data on animal health to detect, monitor, and control diseases within animal populations (Stärk et al., 2018). Surveillance of pathogens associated with BRD and NCD in dairy calves allows for the measurement of the effects that current health and management interventions have against these diseases. Particularly, the detection of pathogens in asymptomatic individuals helps to describe the spread of infectious agents associated with BRD and NCD among dairy calf herds. Also, evaluating the presence of pathogens in asymptomatic and symptomatic individuals can provide insights into how diseases develop and identify factors that trigger the transition from a latent or carrier state to active disease. Information from pathogen surveillance can also contribute to describing the prevalence emergent pathogens and the trend of zoonotic agents (i.e., *Salmonella enterica* spp., *Cryptosporidium parvum*) in dairy herds. For the industry, this information is crucial for guiding resource allocation and effectively focusing on interventions to control emerging and endemic pathogens.

Differences in the management of calves and their environment can influence pathogen abundance within a

population. For example, dairy farms that did not have a designated calving pen were more likely to be positive for *M. bovis* compared with farms that did have a designated calving pen (Gille et al., 2018). Also, during winter months (December–March), BRSV was more frequently isolated from beef and dairy calves compared with other seasons of year (Pardon et al., 2020). Furthermore, management practices may not have the same effect on the presence of pathogens regionally. In dairy farms from southern Ontario, Canada, feeding milk replacer to dairy calves was associated with increased *C. parvum* shedding (Trotz-Williams et al., 2008), whereas in dairy farms from New York, it was associated with a decrease in the shedding of *C. parvum* (Mohammed et al., 1999). Thus, precise estimates of pathogens related to NCD and BRD and risk factors may be needed on a regional scale.

The objectives of this cross-sectional study were (1) to determine the occurrence of enteric and respiratory pathogens associated with NCD and BRD from a population of dairy calves within a convenience sample of dairy herds, (2) to describe herd-level risk factors associated with detection of enteric and respiratory pathogens associated with NCD and BRD in dairy calves, and (3) to describe the association between detection of pathogens and herd-level preweaning mortality.

## MATERIALS AND METHODS

This study was part of a larger project that also evaluated risk factors associated with preweaning calf mortality (Umaña Sedó et al., 2024) and *Salmonella Dublin* on Ontario dairy farms (Perry et al., 2023). A convenience sample of dairy farms in Ontario, Canada, were recruited based on their willingness to participate through contact with 4 veterinary clinics located in Southern and Eastern Ontario. Farms ( $n = 100$ ) were visited once between April 13, 2022, and August 26, 2022, to administer a questionnaire on management practices and collect fecal and nasopharyngeal samples. Human ethics (REB no. 22-02-029) and animal use (AUP no. 4770) approvals were received from the University of Guelph Research Ethics Board and Animal Care Committee, respectively, before beginning the study.

### Sample Size Calculations

The total number of dairy farms needed for estimating the proportion of farms with *C. parvum* was 62, for *M. bovis* was 62, for *P. multocida* was 35, and for BCV was 49 based on an expected herd-level prevalence of 80% (Trotz-Williams et al., 2008), 20% (Hurri et al., 2022), 90% (Francoz et al., 2015), and 23% (Studer et al., 2021) for each of the respective pathogens. All calculations were done in Excel (Version 2402, Microsoft, Redmond, WA)

and considered a confidence level of 95% with a precision estimate of 10% as proposed by Dooho et al. (2014).

### Questionnaire

During the farm visit, the person responsible for calf care was asked to answer questions posed by a research technician verbally, and their responses were entered into an online survey software (Qualtrics; <https://www.qualtrics.com/>). Technicians offered no advice or opinions while the questions were answered to avoid influencing the respondent. The questionnaire consisted of 114 multiple-choice and fill-in-the-blank questions focusing on herd demographics, within-herd and between-herd biosecurity, calving management, calf care, colostrum management, calf housing, and feeding, as well as dry and milking cow management. The questionnaire was designed in collaboration with another research project focused on *Salmonella* Dublin (Perry et al., 2023) and preweaning mortality (Umaña Sedó et al., 2024) on dairy farms in Ontario. The questionnaire was developed through a literature review of the main risk factors associated with *S. Dublin* infection and calf morbidity and mortality. Before beginning data collection, the questionnaire was pretested on 4 dairy farmers in the Guelph area. After pretesting, no changes were made to the questionnaire. A full version of the questionnaire is available at <https://borealisdata.ca/file.xhtml?fileId=661149&version=DRAFT>.

### Fecal and Respiratory Scoring and Sample Collection

At each dairy farm, a randomization software (Random.org, Randomness and Integrity Services Ltd., Dublin, Ireland) was used to select a maximum of 5 dairy calves for gastrointestinal evaluation and sample collection. Similarly, a maximum of 5 dairy calves were selected for respiratory health evaluation and sample collection. The authors agreed a priori to sample 5 animals per disease of interest (i.e., NCD and BRD) per farm to run pathogen identification analyses due to economical constraints. Upon arrival at the farm, calves that were contemporaneously treated with an antimicrobial or that were treated in the past 15 d with an antimicrobial were identified based on producer treatment records; these animals were not eligible for sampling. Thereafter, a list of the calves that were not treated contemporaneously with an antimicrobial and falling within the age range for sampling was obtained from the farm records or herd management software. Trained research assistants proceeded to randomly select calves from the list before physical inspection of the animals in the pens/hutches. If multiple pens were present, calves were randomly

selected again according to the number of pens; for example, if there were 2 pens available and 5 animals needed to be sampled, 2 calves were randomly chosen from each pen. Subsequently, to decide from which pen the last calf would be sampled, both pens were randomized using the same randomization software. Once the pen was assigned, a calf that was not previously sampled was then randomly selected from that pen.

**Fecal Scoring and Fecal Sampling.** Five dairy calves that were between 2 and 35 d old were randomly selected at each farm. The range for sampling ages was selected based on the premise that the occurrence of diarrhea in dairy calves can start as early as the first day of life, peaks between the second and third week of life, and decreases toward the end of the fifth week of life (Reiten et al., 2018; Urie et al., 2018b). If there were <5 calves within the targeted age range when visiting the farm, all calves within the age group were sampled. For each calf, fecal consistency was scored by a trained research assistant, and fecal samples were collected using a long cotton swab that was rotated inside the rectum. The samples were placed into individual 5-mL nonsterile tubes (SCT5ML, Axygen, Union City, CA), refrigerated, and transported to the University of Guelph on the same day and stored at  $-20\text{ }^{\circ}\text{C}$ . Fecal consistency was scored on a scale of 0 to 3, where 0 indicated normal consistency, 1 indicated semi-formed or pasty feces, 2 indicated feces that was runny, spreads easily, and 3 indicated feces that was liquid, devoid of solid material (Larson et al., 1977; Renaud et al., 2020). Those with a fecal score  $\geq 2$  were considered to have NCD (Renaud et al., 2020). If a farm had at least one sampled calf with diarrhea at the visit, it was classified as NCD-positive at the herd-level.

**Respiratory Scoring and Sampling.** Five dairy calves with a targeted age between 21 and 122 d of life were randomly selected at each farm. Briefly, a list of tag numbers of calves that were between 21 to 122 d was provided by the farmer and then each tag number was introduced to the randomization software. This range of age was selected based on the premise that the occurrence of pneumonia in dairy calves starts to increase at 3 wk of age, peaking between 5 to 6 wk of age and continuing after weaning (Reiten et al., 2018; Urie et al., 2018b); we chose 122 d as the upper limit due to safety concerns and limitations of animal restraint beyond these ages. Calves that were fecal-scored and sampled could have been also included in respiratory scoring and sampling. If, when visiting the farm, <5 calves were within the targeted age range, all calves within that age range were sampled.

The University of California, Davis, respiratory scoring system was used to evaluate each selected calf before nasopharyngeal sampling according to Love et al. (2014). Briefly, the scoring system was as follows: coughing (induced or spontaneous, 2 points), nasal discharge (any,

4 points), ocular discharge (any, 2 points), ear and head carriage (ear droop or head tilt, 5 points), fever ( $\geq 39.2^{\circ}\text{C}$ , 2 points), and respiratory quality (abnormal respiration, 2 points). Calves were categorized as “BRD-positive” if their total score was  $\geq 5$  (Love et al., 2014). If a farm had at least one sampled calf positive for BRD at the visit, it was classified as “BRD-positive” at the herd-level.

Nasopharyngeal sampling was conducted using two 68.5-cm cotton swabs guarded by a plastic guide with a guarded tip (Cotton swab KI-3000, Kalayjian Industries Inc., Signal Hill, CA). The animal’s head was restrained to collect the sample, and the nostrils were cleaned with a disposable cloth. The distance from the nostrils to the medial canthus of the eye was measured with the plastic guide to have the proper length to reach the nasopharynx; if needed, the length of the plastic guide was cut. The plastic guide was inserted into the ventral meatus of the nose and advanced according to the premeasured distance. Once secured, the long cotton swab was inserted into the plastic guide and advanced until contact with the guarded tip. The long cotton swab was pushed against the guarded tip, exposing it to the nasopharyngeal tissue. After that, the long cotton swab was vigorously rotated against the nasopharyngeal mucosa for 15 to 20 s and it was retracted out of the nasal cavity. Without removing the plastic guide, the second long cotton swab was inserted following the same method. With the use of cleaned scissors, the cotton tip of both swabs was cut. One swab was put into a red top tube with no anticoagulant (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) with 5 mL of sterile saline solution (Aspen Veterinary Resources Ltd., Loveland, CO) to submit for viral testing. The other swab was put into a collection and transport medium for bacterial testing (BBL Culture Swab Plus, Becton Dickinson Franklin Lakes, NJ). Samples were immediately refrigerated and transported to the Animal Health Laboratory at the University of Guelph (Guelph, ON, Canada), where they were submitted as a pooled sample for viral and bacterial bovine respiratory panels. The samples used for bacterial culture were not frozen before culture.

### Fecal Sample Analysis

Fecal samples were tested for the presence of *E. coli* K99+, *Salmonella enterica* spp., and *C. parvum*; viruses associated with NCD were not tested due to resource limitations. The multiple PCR Qiagen Multiplex DNA Kit (Qiagen, Venlo, the Netherlands) was used as per the manufacturer’s instructions (Qiagen, 2017). In brief, the initial sample DNA concentration was measured on a Nanodrop spectrophotometer. The DNA was then diluted using HyClone ultrapure water (Fisher Scientific, Waltham, MA) to a concentration of  $\leq 300$  ng/ $\mu\text{L}$ . The

PCR multiplex master mix consisted of 25  $\mu\text{L}$  of kit-provided 2X Qiagen Multiplex PCR Master Mix solution, 2  $\mu\text{L}$  of all forward and reverse primers (at a concentration of 10  $\mu\text{M}$  for a final concentration of 2  $\mu\text{M}$ ), 5  $\mu\text{L}$  of DNA template, and brought to a final volume of 50  $\mu\text{L}$  with RNase-free water (Qiagen, 2016). Thermocycler conditions were set as defaults recommended by the kit including: a 5-min activation step at  $95^{\circ}\text{C}$ , a 3-cycle sequence of 30 s at  $95^{\circ}\text{C}$ , 90 s at  $60^{\circ}\text{C}$ , and 30 s at  $72^{\circ}\text{C}$  repeated for 35 cycles, with a final 10-min extension step at  $68^{\circ}\text{C}$ .

Forward and reverse primer sequences for *E. coli* K99+, *Salmonella enterica* spp., and *C. parvum* were based on Cho et al. (2010). Lyophilized primer sequences were ordered from Integrated DNA Technologies and resuspended in ultrapure HyClone water (100  $\mu\text{M}$ ). For each batch of samples that underwent multiplex PCR, extracted pathogen control DNA was also run to ensure the PCR reaction conditions resulted in amplification. At the same time, a negative H<sub>2</sub>O control was run to confirm lack of contamination. For visual identification of DNA bands, samples and positive control reactions were run on 2.5% agarose gels. Multiplexed DNA from pathogens was run at both ends of the gel and visual confirmation of positive samples was performed via band alignment.

### Nasopharyngeal Sample Analysis

**Bacteriology Analysis.** Samples for bacterial culture were processed at the Animal Health Laboratory, University of Guelph (Guelph, ON, Canada), as per their standard operating procedure. Briefly, all samples were directly plated on blood (BA) and McConkey (MAC) agar plates. All plates were incubated at  $35^{\circ}\text{C}$ , with BA plates incubated in the presence of 5% CO<sub>2</sub> and MAC plates incubated aerobically. In addition, Rappaport-Vassiliadis (RV) broth (for *Salmonella* enrichment) was inoculated and incubated at  $42^{\circ}\text{C}$  aerobically. After overnight incubation, an aliquot from RV broth was plated on MAC and Hectoen agar plates, which were subsequently incubated at  $35^{\circ}\text{C}$  aerobically. All plates were read at 24 h and 48 h. Individual colonies were smeared onto stainless steel target plates and covered by  $\alpha$ -cyano-4-hydroxycinnamic acid. The bacterial identification was done using a MALDI Biotyper Sirius system (Bruker Daltonics Inc., Bremen, Germany).

**Virology Analysis.** Samples were processed at the University of Guelph Animal Health Laboratory using their standard operating procedures. Bovine viral diarrhea virus and adenovirus were run as a panel, as were BCV and rotavirus A and B. Briefly, nucleic acids were extracted using the MagMAX-96 Viral RNA Isolation Kit in a MagMAX Express-96 Magnetic Particle Processor (Thermo Fisher). Nucleic acids extracted from samples were reverse-transcribed and amplified using Ag Path-ID

One-Step RT-PCR Kit (Thermo Fisher). Reverse transcription, PCR amplification, and detection were carried out in 25- $\mu$ L reactions in a Light Cycler 480 (Roche).

Samples were tested for *M. bovis* using real-time PCR. Samples were also processed at the University of Guelph Animal Health Laboratory using their standard operating procedures and analyzed as suggested by Rossetti et al. (2010). For template DNA preparation, nasopharyngeal swabs were transferred into 500  $\mu$ L of lysis buffer (0.1 M Tris HCl, pH 8.5, 0.05% Tween 20, 0.24 mg/mL proteinase K) and incubated for 1 h at 60°C, followed by a 15-min denaturation step at 95°C. The sequence of the *uvrC* gene of *M. bovis* was retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>; accession number: AF003959.1). Primers and probes were designed with the Primer Express software 2.0 (Applied Biosystem, Foster City, CA). Real-time PCR were performed. Briefly, 25  $\mu$ L of PCR mixtures contained 10 mM Tris (pH 8.3), 50 mM KCl, 5 mM MgCl<sub>2</sub> (prepared from a 200 mM stock solution), 2.5 mM deoxynucleotide triphosphates, 400 nM of each primer, 80 nM fluorogenic TaqMan probe, 0.625 U AmpliTaq Gold, 0.25 U AmpErase UNG, and 2.5  $\mu$ L of lysate were used as template in the reaction. An exogenous internal positive control (Applied Biosystems) was added to each reaction, according to the manufacturer's protocol, to check for the presence of PCR inhibitors. The real-time PCR was performed on a 7500 real-time PCR system (Applied Biosystems). The PCR conditions were as follows: after an initial step at 50°C for 2 min and at 95°C for 10 min, PCR amplification was carried out for 40 cycles (denaturation at 95°C for 15 s and extension at 60°C for 1 min). The data were analyzed with the 7500 System Software version v1.2.3f2 with auto settings for baseline and cycle threshold values.

### Preweaning Mortality Data

The data regarding preweaning mortality were collected in parallel with Umaña Sedó et al. (2024) and Perry et al. (2023). These data were collected by a trained graduate student at every farm visit using the main herd management software used by the farm or their written records. For this project, we used individual female calf records for each dairy farm and records were evaluated for the period of a year before the date of the farm visit. We defined the preweaning period from  $\geq 48$  h to 60 d of life for all the farms analyzed. We chose this interval because, during the first 2 d of life, carry-over effects from birth and immediate perinatal care may cause mortality (Umaña Sedó et al., 2023). The upper limit of 60 d was considered because it has been reported to be the average age for weaning in Canada (Vasseur et al., 2010). After that, the individual records were evaluated to determine the number of female calves that started

and ended the preweaning period per farm, the number of female calves that left the herd during the preweaning period per farm, and the number of female calves that died during the preweaning period per farm. With this information, we calculated the preweaning mortality risk as follows:

Preweaning mortality risk

$$= \frac{\text{no. of female dairy calves dead during the preweaning period}_{ij}}{\text{initial NAR} - \frac{1}{2} \text{withdrawals during the preweaning period}_{ij}}$$

where NAR refers to the number of female dairy calves at risk at the beginning of the preweaning period; *i* represents each herd, and *j* represents the period of evaluation, which was 58 d (60 d – 2 d [perinatal period]).

### Statistical Analyses

**Data Cleaning.** Explanatory variables selected from the questionnaire plausibly linked to a pathogen of interest, individual calf records and health scores, and bacteriology and virology results per farm were exported into Microsoft Excel (Version 2402, Microsoft, Redmond, WA) for cleaning. The categories of the explanatory variables that contained <5% of total responses were collapsed into broader categories. Cleaned data were exported into Stata/SE 17.0 (StataCorp, College Station, TX) for further analyses.

For fecal sampling, we did not sample calves on 17 farms; due to the farm not having calves within the age criteria to be tested (*n* = 6), having one calf available for sampling, or having calves with the required age undergoing antimicrobial treatment (*n* = 10), or not knowing the age of the animals (*n* = 1). In total, fecal sampling and fecal consistency scoring were performed on calves from 83 farms. If a farm had at least one calf positive for an enteric pathogen of interest, the farm was considered positive for that enteric pathogen. In total, 80 herds had a pooled sample from calf nasopharyngeal swabs collected during the visit. The remaining 20 farms were omitted from sampling due to economic constraints. In addition, the same 80 farms had respiratory scoring completed on all the calves that had a nasopharyngeal swab collected. If a farm had at least one calf positive for a respiratory pathogen of interest, the farm was considered positive for that pathogen. Regarding the individual female records per farm for preweaning mortality, we did not include individual female calf data from 19 dairy farms due to incomplete female records (*n* = 8), no records were available on the farm on the day of the visit (*n* = 9), or animals were not identified individually before weaning (*n* = 2). This left a total of 81 farms included in the univariable Poisson model analyses.

**Table 1.** Results of final multivariable logistic regression models evaluating risk factors associated with the presence in the herd of *C. parvum*, *P. multocida*, *M. bovis*, and bovine coronavirus from a single interview and sampling, and 1-yr count data of 100 dairy farms in Ontario

Pathogen	Variable	Description	n (farms)	OR <sup>1</sup>	95% CI	P-value
<i>Cryptosporidium parvum</i>	Number of preweaning calves during the year	≤39	12	Referent		
		40–60	17	2.9	0.4–18.0	0.20
		61–97	19	22.3	2.9–195.9	<0.01
		≥98	20	7.5	1.1–1.4	0.03
	Most used source of milk	Milk replacer	43	Referent		
<i>Pasteurella multocida</i>	BRD herd status (at least 1 calf positive for BRD)	Whole milk	48	5.5	1.3–22.6	0.01
		Negative	29	Referent		
		Positive	51	4.2	1.1–16.0	0.03
<i>Mycoplasma bovis</i>	Milk-feeding system	Manual	60	Referent		
		Automatic	8	0.7	0.07–6.4	0.70
		Both	10	7.5	1.7–31.5	<0.01
Bovine coronavirus	Number of preweaning calves per year	≤39	13	Referent		
		40–60	17	1.3	0.2–7.2	0.6
		61–97	16	2.5	0.5–13.1	0.2
		≥98	19	5.7	1.2–28.1	0.03

<sup>1</sup>Results are reported as odds ratio.

**Model Building.** Descriptive statistics were generated for all explanatory variables in the dataset. Only pathogens (*C. parvum*, *M. bovis*, *P. multocida*, and BCV) present in ≥20% of the farms and previously associated in the literature with NCD and BRD in dairy calves were considered in the statistical analyses. We chose 20% to ensure a sufficient number of observations in each category of the predictors for the statistical analyses to improve the detection of meaningful associations. A causal diagram was created to illustrate the hypothesized relationship between the dependent and the explanatory variables (Supplemental Figures S1 and S2; see Notes).

A multivariable logistic model was used to explore the association of explanatory variables and each pathogen selected for analysis; the information about the type of explanatory variables and their number of observations can be found in Supplemental Tables S1 and S2 (see Notes). Collinearity was tested using Spearman rank coefficients for the explanatory variables selected. If the correlation coefficient between 2 variables was ≥0.6, only the variable with more biological plausibility was retained. Explanatory variables were tested for univariable associations with the dependent variable, and variables with a  $P < 0.2$  were offered to a multivariable model. Continuous variables were assessed for the assumption of linearity with each outcome variable by introducing their respective quadratic term and using Q-Q plots. In the case of a significant ( $P \leq 0.05$ ) quadratic term and visual quadratic relationship as assessed graphically, the variable with its quadratic term was included in the model. A preliminary multivariable logistic regression model was built using stepwise backward elimination, with variables retained if (1) they were significant at  $P \leq 0.05$ , (2) were deemed a confounder (based on a change of >25% in the coefficient of a significant variable in the model when the variable was removed), or (3) had a

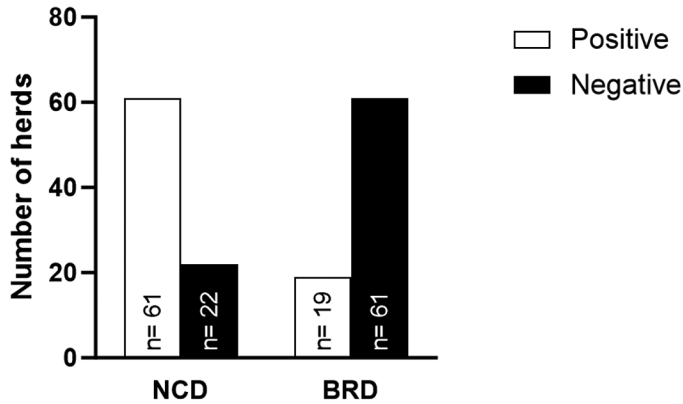
significant ( $P \leq 0.05$ ) interaction term. Multiple comparisons between categories of the predictor variables in the final model were made by changing the referent category of the predictor variable of interest while keeping the other predictor variables constant. Categorical variables were considered significant if at least one comparison of their levels was significant. The final model for each pathogen is presented in Table 1. Model fit was evaluated using Pearson  $\chi^2$  and deviance goodness-of-fit tests and examined graphically using deviance and Pearson residuals for potential outliers. If outliers were found, they were explored to determine the characteristics of the observations that made them outliers and ensure data were not erroneous. The logistic regression models report the results as odds ratios (OR).

A univariable Poisson regression model was created to explore the association between herd-level positivity for each pathogen and herd-level preweaning mortality. All univariable models included the total number of female calves during the preweaning period as the exposure factor and the total number of dead females during the preweaning period as the dependent variable. The Poisson models report results as incidence rate ratios (IRR; Table 2).

## RESULTS

### Farm Characteristics

From April 13, 2022, to August 26, 2022, 100 Ontario dairy farms were visited. Overall, 81 dairy farms were located in southern Ontario, and 19 in eastern Ontario. The 100 included dairy farms, which, on average, milked 126 dairy cows (median = 96, range = 37–770) and had an average of 281 total animals (median = 214, range = 67–1,842), including cows, heifers, steers, and bulls, housed on the premises at the time of visit. The average number



**Figure 1.** Number of herds positive and negative for NCD and BRD from a single interview and single sample collection from a convenience sample of 100 Ontario dairy farms. A farm was categorized as positive for NCD when a calf had a diarrhea score  $\geq 2$ . A farm was categorized as positive for BRD when a calf had a California BRD score  $\geq 5$ .

of dairy calves (i.e.,  $<60$  d) in our sample of dairy farms was 81.2 (median = 60, range = 13–530). Individual calf housing was used in 46 dairy farms, whereas 54 used group housing. Manual milk feeding was used in 80 of the farms, whereas 9 used automatic milk feeding alone and 11 used a combination of manual and automatic milk feeding. Forty-three farms used milk replacer as their most common source of milk, whereas 48 farms mainly used whole milk. Equal use of milk replacer and whole milk was practiced in 2 farms, and 4 and 2 of the farms mainly used waste milk and acidified milk, respectively.

#### Fecal Samples, Fecal Consistency, and Prevalence of NCD

A total of 363 samples were collected on 83 farms: 5 samples were collected on 56 farms, 4 samples on 12 farms, 3 samples on 11 farms, and 2 samples on 4 farms. Sampled calves were, on average, 19 d old (median = 18, range = 2–35), 322 were female and 41 were male, and all had a fecal consistency score measured. At the individual level, 32.7% (119/363) of calves were positive for NCD (i.e., a calf with a fecal score  $\geq 2$ ), and 61 of 83 herds had at least one calf positive for NCD (73.4%; Figure 1).

Out of the 363 fecal samples, 355 fecal samples (female = 324, male = 31) were analyzed because 8 samples lacked a sufficient amount of nucleic acid for testing. For *E. coli* K99+, 4.7% of calves were positive (15/363), resulting in a herd-level positivity of 13.2% (11/83). For *Salmonella enterica* spp., 0.5% (2/363) of calves were positive, resulting in a herd-level positivity of 2.4% (2/83). Finally, for *C. parvum*, 32.2% (117/363) of calves were positive, resulting in a herd-level positivity of 67.4% (56/83).

**Table 2.** Results of univariable Poisson regression models evaluating the association of *C. parvum*, *P. multocida*, *M. bovis*, and bovine coronavirus with preweaning mortality based on a single interview and sampling, and 1-yr count data of 100 dairy farms in Ontario

Pathogen	IRR <sup>1</sup>	95% CI	P-value
<i>Cryptosporidium parvum</i>	1.7	1.1–2.7	0.01
<i>Pasteurella multocida</i>	1.2	0.8–1.5	0.30
<i>Mycoplasma bovis</i>	1.4	1.1–1.9	0.03
Bovine coronavirus	1.7	1.2–2.4	$<0.01$

<sup>1</sup>IRR = incidence rate ratios.

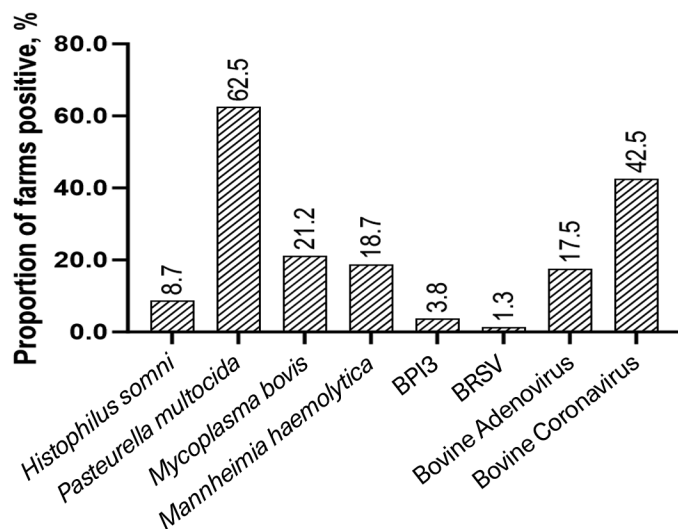
#### Respiratory Samples and Scoring and Prevalence of BRD

A total of 390 calves from 80 farms were sampled: 5 samples were collected from 72 farms, 4 samples from 6 farms, and 2 samples from 2 farms. Calves were, on average, 70 d old (median = 65, range = 21–122); 367 were female and 23 were male. The calf-level positivity for BRD (clinical score  $\geq 5$ ) was 6.9% (27/390), with 19 farms (23.7%, 19/80) having at least one BRD-positive calf (i.e., respiratory health score  $\geq 5$ ; Figure 1).

The results of the respiratory pathogens are reported at the herd-level because the nasopharyngeal swabs were processed as pooled samples. Regarding respiratory bacterial pathogens, 62.5% (51/80) of the farms were positive for *P. multocida*, 21.2% (17/80) for *M. bovis*, 18.7% (15/80) for *M. haemolytica*, and 8.7% (7/80) for *H. somni* (Figure 2). None of the nasopharyngeal swabs tested positive for *Salmonella enterica* spp. As for viral pathogens, none of the herds were positive for BVDV or BHV-1, whereas 42.5% of farms (34/80) were positive for BCV, 17.5% (14/80) for bovine adenovirus, 1.2% for BRSV (1/80), and 3.8% (3/80) for BPI3V (Figure 2).

#### Herd-Level Risk Factors Associated with *Cryptosporidium parvum*

Two factors were associated with herd positivity for *C. parvum*: the number of preweaning calves on the farm per year and the source of milk fed. Herds that had 61 to 97 (OR = 22.3, 95% CI = 2.5–195.9,  $P = 0.005$ ) and  $\geq 98$  (OR = 7.5, 95% CI = 1.1–49.9,  $P = 0.03$ ) preweaning calves in the year before the farm visit had higher odds of being positive for *C. parvum* compared with herds with  $\leq 39$  preweaning calves per year (Table 1; Figure 3). No difference was found among herds having  $\leq 39$  preweaning calves and 40 to 60 preweaning calves (OR = 2.9, 95% CI = 0.48–18.1,  $P = 0.20$ ; Figure 3). Compared with herds with 40 to 60 preweaning calves, herds with 61 to 97 calves had higher odds of being positive for *C. parvum* (OR = 7.5, 95% CI = 1.2–48.6,  $P = 0.03$ ), whereas no differences were found compared with herds with  $\geq 98$  preweaning calves over a year (OR = 2.5, 95%



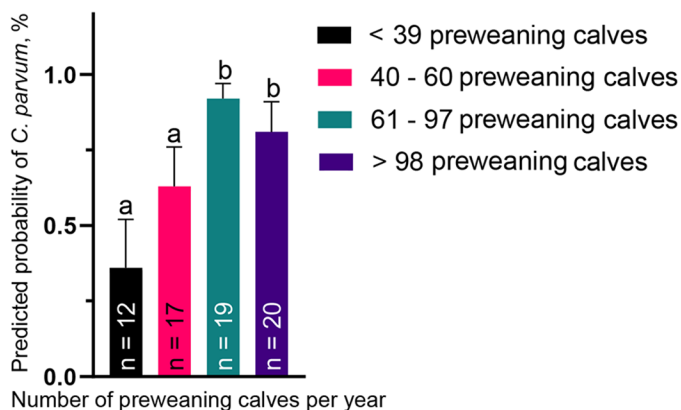
**Figure 2.** Proportion of farms positive for selected bacteria and viruses associated with BRD based on testing of pooled nasopharyngeal swab samples from a convenience sample of 80 Ontario dairy farms. *Salmonella enterica* spp. and bovine herpesvirus 1 and BVDV were not detected.

CI = 0.5–14.1,  $P = 0.20$ ; Figure 3). No difference in the odds of being positive for *C. parvum* was found between herds with 61 to 97 preweaning calves and herds with  $\geq 98$  preweaning calves over a year (OR 0.3, 95% CI = 0.0–2.6,  $P = 0.30$ ; Figure 3). Regarding the type of milk fed, farms where mainly whole milk was fed to calves had higher odds of being positive for *C. parvum* (OR = 5.6, 95% CI = 1.3–22.6,  $P = 0.04$ ) compared with farms where mostly milk replacer was fed (Table 1).

### Herd-Level Risk Factors Associated with Respiratory Pathogens

The herd status for BRD was associated with the detection of *P. multocida*. Herds positive for BRD (respiratory score  $\geq 5$  in one or more of the sampled calves) had higher odds (OR = 4.2, 95% CI = 1.1–16.1,  $P = 0.03$ ) of being positive for *P. multocida* than those not positive for BRD based on the respiratory score. No other variables were associated with herd positivity for *P. multocida*.

Herds that used a combination of manual and automatic milk feeding had higher odds of being positive for *M. bovis* compared with herds using only a manual milk-feeding system (OR = 7.5, 95% CI = 1.7–31.5,  $P = 0.006$ ); however, no statistical differences were found in the odds of being positive to *M. bovis* compared with herds using only an automatic milk-feeding system (OR = 10.5, 95% CI = 0.9–121.3,  $P = 0.06$ ). No differences in the odds of being positive for *M. bovis* were detected among herds using only manual milk-feeding systems



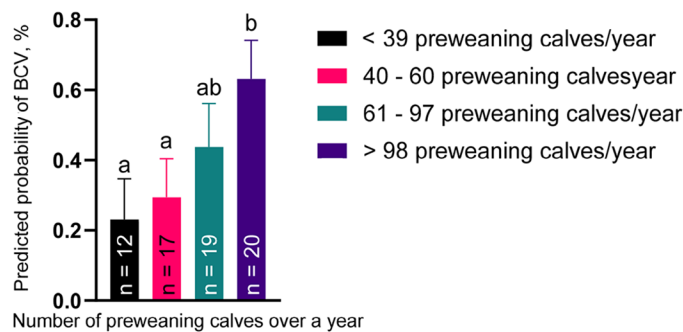
**Figure 3.** Predicted probability of being a positive herd to *Cryptosporidium parvum* related to the number of preweaning calves per year from a single interview and single sample collection from a convenience sample of 100 dairy farms. Fewer than 39 preweaning calves per year ( $n = 12$ ), 40 to 60 preweaning calves per year ( $n = 17$ ), 61 to 97 preweaning calves per year ( $n = 19$ ), and more than 98 preweaning calves per year ( $n = 20$ ). For all variables with the same lowercase letter (a,b), the difference between the means is not statistically significant ( $P > 0.05$ ). The error bars represent SEM.

and those using only automatic milk-feeding systems (OR = 0.7, 95% CI = 0.7–6.4,  $P = 0.70$ ).

The detection of BCV in the herd was associated with a higher number of preweaning calves per year (Table 1; Figure 4). Herds with  $\geq 98$  preweaning calves had higher odds of being positive for BCV compared with herds with  $\leq 39$  preweaning calves (OR = 5.7, 95% CI = 1.2–28.1,  $P = 0.03$ ), and 40 to 60 preweaning calves (OR = 4.1, 95% CI = 1.1–16.6,  $P = 0.04$ ); whereas no differences were found compared with herds with 61 to 97 preweaning calves (OR = 2.2, 95% CI = 0.5–8.5,  $P = 0.20$ ; Figure 4). No differences were detected among herds with  $\leq 39$  and 40 to 60 preweaning calves (OR = 1.3, 95% CI = 0.4–7.8,  $P = 0.69$ ) and 61 to 97 preweaning calves (OR = 2.5, 95% CI = 0.5–13.1,  $P = 0.25$ ). Furthermore, no difference was found among herds with 40 to 60 and 61 to 97 preweaning calves (OR = 1.8, 95% CI = 0.44–7.8,  $P = 0.39$ ; Figure 4).

### Preweaning Calf Mortality

As reported in a parallel study (Umaña Sedó et al., 2024), for the 81 dairy farms with complete individual records, the herd-level preweaning mortality risk ranged from 0% to 15.9%, with an average of 2.8% (SD = 3.8%) and a median of 1.5% (interquartile range [IQR] = 4.1). The total number of female calves that started the preweaning period was 6,584, with the number of female calves ranging from 13 to 530 (mean = 81.2 [SD = 74.0]) per farm. The total number of female calves that started the preweaning period and left the herd before 60 d of



**Figure 4.** Predicted probability of being a positive herd to BCV related to the number of preweaning calves per year from a single interview and single sample collection from a convenience sample of 100 dairy farms. Fewer than 39 preweaning calves per year ( $n = 13$ ), 40 to 60 preweaning calves per year ( $n = 17$ ), 61 to 97 preweaning calves per year ( $n = 19$ ), and more than 98 preweaning calves per year ( $n = 20$ ). For all variables with the same lowercase letter (a,b), the difference between the means is not statistically significant ( $P > 0.05$ ). The error bars represent SEM.

age was 311 (4.7%). Furthermore, the total number of female calves that died during the preweaning period was 200 (3.0%).

There was a higher risk of preweaning mortality in herds with detection (vs. no detection) of *C. parvum* (IRR = 1.7, 95% CI = 1.1–2.7,  $P = 0.01$ ). Similarly, a greater risk of preweaning mortality was associated with detection (vs. no detection) of *M. bovis* (IRR = 1.4, 95% CI = 1.1–1.9,  $P = 0.03$ ), or BCV (IRR = 1.7, 95% CI = 1.2–2.4,  $P = 0.001$ ). No association between preweaning mortality and herds positive to *P. multocida* was observed (IRR = 1.2, 95% CI = 0.–1.5,  $P = 0.3$ ).

## DISCUSSION

This study describes the current herd-level positivity burden of pathogens associated with NCD and BRD in dairy calves on a convenience sample of Ontario dairy farms. Additionally, this study describes herd-level factors associated with pathogens of interest for NCD and BRD in Ontario dairy farms

### Neonatal Calf Diarrhea and Enteric Pathogen Herd Prevalences

The calf-level prevalence of NCD in our sample was 14.4%. In Austria, a study that sampled 100 dairy farms reported a calf-level prevalence of NCD of 39.0% (79/205; Klein-Jöbstl et al., 2014), whereas in Chile, the calf-level prevalence of NCD from 29 farms was 13.7% (94/686). Furthermore, on 120 Irish dairy farms, the calf-level prevalence of NCD was 4.7%, and, on 139 Norwegian dairy farms, the calf-level prevalence of NCD was reported to be 4.7% (191/4,041; Gulliksen et al., 2009a; Calderón-amor and Gallo, 2020; McCarthy et al., 2021).

These variations could be attributed to different dairy systems (pasture vs. confined); however, within the studies, many different case definitions were used for NCD. At the herd level, we found that approximately 73% of the herds had at least one case of NCD in sampled calves. This information is unsurprising, because NCD is the disease with the highest morbidity in preweaning dairy calves (Urie et al., 2018b).

In general, the number of herds with at least one sampled calf positive for *C. parvum* (i.e., 67.4%) was slightly lower than reported in other countries and previously in Canada. In France, the herd prevalence of *C. parvum* was reported to be 93.8% (91/97) in a study that collected fecal samples of 10 calves from 7 to 21 d old per farm (Delafosse et al., 2015), whereas in Argentina it was reported to be 89% (48/54) in a study that sampled all calves below 60 d old (Lombardelli et al., 2019). In Canada, 2 prior studies have been conducted that reported that the herd-level prevalence was 77% (92/117) in Ontario (Trotz-Williams et al., 2008) and 88.7% (448/505) in Québec (Ruest et al., 1998). Because our study found fewer herds with at least one calf positive for *C. parvum* than previously reported, it suggests that control of *C. parvum* in dairy farms may have improved. However, there are differences in the methodology from the studies of Trotz-Williams et al. (2008) and Ruest et al. (1998) compared with our study. Dairy calves between 7 and 28 d old were sampled in the study of Trotz-Williams et al. (2008). Furthermore, Ruest et al. (1998) sampled 50 farms in each of the 12 agricultural regions of the province of Québec, resulting in a more representative sample size. Also, both studies used a sucrose wet mount test to diagnose animals positive for *C. parvum*, a more practical but less accurate test than the one used in our study (Ruest et al., 1998; Trotz-Williams et al., 2008). Hence, the observed differences between our current study and those from 1998 and 2008 may potentially be underestimated due to the higher test sensitivity of our current method and our nonrepresentative sample.

The number of herds positive for *E. coli* K99+ was similar to that reported in other countries. For example, in New Zealand, the herd prevalence of *E. coli* K99+ was 11.8% (11/93; Al Mawly et al., 2015), whereas, in the Netherlands, 9% (10/108) of dairy herds were positive for *E. coli* K99+ (Bartels et al., 2010). Our result is considerably lower than the 41% (33/78) herd prevalence of *E. coli* K99+ reported previously in Ontario (Waltner-Toews et al., 1986). However, Waltner-Toews et al. (1986) included 110 dairy farms that were randomly selected, only sampled 2 calves that were <15 d old per farm, and *E. coli* K99+ was diagnosed using bacterial cultures. By contrast, in our study, only 36.9% of the calves that had a fecal sample collected were <15 d old; thus, our result likely underestimates the occurrence of *E. coli* K99+

because this pathogen is reported to affect most calves during the first week of life (Younis et al., 2009).

Very few calves were identified with *Salmonella enterica* spp. leading to a herd positivity of 2.4%. This is close to what has been reported in countries with eradication programs for *Salmonella enterica* spp., such as Norway (Gulliksen et al., 2009a). Our result is considerably lower than the 21.8% herd prevalence reported by Waltner-Toews et al. (1986) in Ontario. However, as outlined previously, our results might not be comparable to Waltner-Toews et al. (1986) due to the methodological differences. Due to the considerable mortality (4.8%–100%; Casaux et al., 2023) that results with some strains of *Salmonella enterica* spp. and the importance that this pathogen has in human health (Nichols et al., 2022), constant surveillance and future research should focus on providing an accurate update of the true prevalence of *Salmonella enterica* spp. in preweaning calves of Ontario dairy farms.

### **Bovine Respiratory Disease and Respiratory Pathogen Herd Prevalences**

The calf-level prevalence of BRD in this study was 6.9%. Dubrovsky et al. (2019) reported a higher calf-level prevalence of BRD of 22.8% (2,620/11,470) from 5 dairy farms located in different regions of California. However, Dubrovsky et al. (2019) only included dairy calves from 0 d old to weaning, which differs from the age criteria of our study. Comparing BRD data between studies is challenging due to the use of different scoring systems, including the Wisconsin Calf Respiratory Scoring system, the California BRD scoring system, or thoracic ultrasonography. Regarding herd-level prevalence, 23.7% of the herds in our study were classified as BRD-positive, which differs from reports in other regions. Karle et al. (2019) used the California BRD scoring to determine 3 different areas of California reported a herd-level prevalence of BRD of 4.5% (95% CI = 3.3–6.1%; northern San Joaquin Valley), 7.3% (95% CI = 5.7–9.3%; greater Southern California), and 9.3% (95% CI = 7.7–11.2%; Northern California; Karle et al., 2019). In Québec, Canada, the herd-level prevalence of BRD was reported to be 8.0% (IQR = 0%–22%) in summer and 15.0% (IQR = 0–35%) in winter (Buczinski et al., 2018). Also, an Irish study reported a median herd prevalence of BRD of 10% (95% Bayesian credible interval; 1%, 23%; Donlon et al., 2023). These variations can be attributable to each study's distinct methodologies and diagnostic criteria. Although Karle et al. (2019) defined a case of BRD based on the California BRD scoring system, their study averaged BRD prevalence across dairy farms. Buczinski et al. (2018) also averaged BRD prevalence

across dairy farms and defined a case of BRD based on thoracic ultrasound. Donlon et al. (2023) used information from both clinical scoring and thoracic ultrasound in a hierarchical Bayesian model to make a true prevalence estimate. These differences highlight the importance of standardizing methods and definitions in future research to facilitate more meaningful comparisons across different studies and regions.

Our study found that *P. multocida*, *M. bovis*, and BCV were the most commonly identified BRD pathogens. It is our understanding that herd-level occurrence of BRD pathogens has not been published previously. However, studies recording data at the individual-level have reported *P. multocida*, *M. bovis*, and BCV as common pathogens causing BRD (Francoz et al., 2015; Studer et al., 2021), which aligns with our findings.

### **Herd-Level Risk Factors Associated with Enteric Pathogens and Respiratory Pathogens**

We found that a larger number of preweaning calves per year was associated with increased odds of a herd being positive for *C. parvum*, which is similar to previous reports in the United States (Mohammed et al., 1999; Urie et al., 2018a,b). Conversely, in Ontario, Trotz-Williams et al. (2008) found no association between herd size and within-herd prevalence of *C. parvum*. However, herd size was defined as the number of milking cows, whereas in our case, we considered the total number of preweaning calves per year as herd size, which is more biologically related to the animal population of interest (Umaña Sedó et al., 2023). Having a greater number of preweaning calves over a year likely increases the number of calves shedding *C. parvum*, while also having more calves susceptible to *C. parvum* infection, as speculated previously (Urie et al., 2018a). We also found that *C. parvum* was positively associated with herds feeding whole milk instead of milk replacer. If not managed hygienically, whole milk can be a reservoir of pathogens, including *C. parvum* (Harp and Goff, 1998), whereas milk replacer is less likely to be contaminated. However, the effect of feeding milk replacer on *C. parvum* is inconclusive within the literature (Brainard et al., 2020). For example, Trotz-Williams et al. (2008) found that feeding milk replacer was associated with an increased prevalence of *C. parvum*; whereas Mohammed et al. (1999) found that feeding calves with milk replacer decreased the prevalence of *C. parvum*. Furthermore, Urie et al. (2018a,b) did not find differences between using milk replacer and whole milk on the odds of being positive for *C. parvum*. Thus, the effect seen in our study may be due to other management factors that we did not capture, for example, cleaning methods for feeding equipment or whole milk pasteurization.

In farms that use a combination of manual and automatic milk-feeding systems, the odds of being positive for *M. bovis* were greater than for those using only manual feeding systems. Using automatic milk-feeding systems has been found to increase the risk of infection for *M. bovis* (Arcangioli et al., 2021). This is likely due to using one nipple for multiple animals when using automatic milk feeders, which increases the risk of oral infection for *M. bovis* (Arcangioli et al., 2021). The fact that herds using automated milk-feeding systems were not associated with greater odds of being positive compared with manual systems in the present study was unexpected. This could be due to other management factors; for example, isolation of sick calves has been recommended previously as a measure of control of *M. bovis* (Haapala et al., 2021). Isolation of sick calves, in our study, was practiced in approximately 71.2% (57/80) of the farms using manual milk-feeding systems, 55.5% (5/9) of farms using only automatic feeding systems, and 27.2% (3/11) of farms using both feeding systems. Also, we considered that farms that were combining both systems were moving calves from manual milk-feeding systems to auto feeders and maybe the grouping of calves at an older age could be a risk factor for *M. bovis*. However, in our questionnaire, we did not request information about the age of transition from manual to automatic feeding systems.

For the detection of *P. multocida*, the only associated risk factor was herd positivity for BRD. *Pasteurella multocida* is an opportunistic pathogen in the respiratory tract of calves (Centeno-Martinez et al., 2022); its abundance in the nasal microbiome between BRD-positive and healthy calves was reported not to be different (Centeno-Martinez et al., 2022). In addition, we did not target our sampling to animals with BRD, thus, this could suggest that other herd-level factors not included in our study, rather than individual factors, potentially have a more significant effect on its prevalence.

Similar to *C. parvum*, BCV was associated with a higher number of preweaning calves per year. Bovine coronavirus is a highly transmissible pathogen via the fecal-oral and respiratory (aerosol) routes of infection (Saif, 2010). In agreement with our result, Pardon et al. (2020) found that BCV was associated with a higher total number of animals (herd size) in dairy farms, probably due to infection by an increased number of direct and indirect contacts (Oma et al., 2016; Pardon et al., 2020).

### **Preweaning Mortality and Specific Pathogen Associations**

Herds that were positive for *C. parvum*, *M. bovis*, and BCV were associated with higher herd-level preweaning mortality. *Cryptosporidium parvum* damages the

enteric epithelium resulting in malabsorption of nutrients, increasing the chances of severe diarrhea (i.e., diarrhea score 3; Renaud et al., 2021) and subsequent death due to dehydration. *Mycoplasma bovis* is a bacterial pathogen known for its ability to potentially initiate persistent infections in organs such as the lungs (Maunsell et al., 2011). Also, *M. bovis* has been previously associated with antimicrobial resistance (Heuvelink et al., 2016; Nobrega et al., 2021), which can lead to treatment failure. Observational studies have identified BCV as a common infection in calves with BRD (Pardon et al., 2020). Furthermore, BCV has been identified in lung and trachea lesions of calves with BRD (Rahe et al., 2022), suggesting that BCV plays a role in the development of BRD. The identification of these pathogens and their association with preweaning mortality in dairy farms presents the possibility to further study their epidemiology. This exploration could aim to formulate strategies for preventing their infection and spreading among dairy calves.

### **Limitations**

The participant farms in our study corresponded to 3.1% of the total number of dairy farms in Ontario (n = 3,233; Agriculture and Agri-Food Canada, 2024), and our convenience sample may have characteristics or management factors that differ from the rest of Ontario dairy farms. Thus, the external validity of our results may be influenced by the recruitment strategy used in this study. As a questionnaire-based study, it is possible that responses to the questionnaire may not necessarily reflect actual practices on the farm. This may in part have been due to the timing of the questionnaire relative to the timing of calvings and different management factors, which may introduce recall bias. Also, survey responders could have provided answers according to industry or society expectations instead of their own beliefs or experience, which could have introduced social desirability bias. Based on a small number of calves on each farm ( $\leq 5$  calves), it is possible that we did not get an accurate representation of the NCD or BRD status, especially when a farm was deemed positive for these diseases if one calf exhibited symptoms of the disease. It is also noteworthy that the number of calves available for sampling per dairy farm could have varied in our study due to differences in herd size; thus, in some farms a maximum sample of 5 calves sampled and health scored per disease of interest was not possible to obtain. Nevertheless, we considered that it is still possible to make inferences about a dairy farm with fewer than 5 animals sampled because this is related to the herd size. Also, variation on herd size through time in Canadian dairy farms is limited because they are under a quota system

that does not allow them to produce more milk than is needed for Canada (Agriculture and Agri-Food Canada, 2024). Thus, it is likely that the farms in our study would only have a fixed number of calves to be sampled even if we had visited them at different points of the year. Regarding our statistical analyses, testing a large number of variables may have introduced a risk of having a type I error (or false-positive results). Furthermore, several risk factors were not evaluated as part of the survey, such as methods used to treat BRD or NCD, pasteurization of whole milk, preweaning pen density, post-weaning feeding and housing, and the use of precalving vaccination or medications, which could have influenced the results of this study. A dilution effect, which could have resulted in greater false negative results, may have also been present in this study because nasopharyngeal swabs were pooled by farm for analysis. We also failed to capture any seasonality effect that could have modified the herd-level estimates of NCD, BRD, and associated pathogens.

## CONCLUSIONS

This study found much higher herd- and animal-level prevalences of NCD than BRD in young calves across a convenience sample of Ontario dairy farms. Approximately three-quarters of herds and one-third of calves were affected by NCD, suggesting room for improvement in control methods. Because *C. parvum* was found on two-thirds of farms and linked to higher prewarning mortality, control programs should enhance management of this parasite. High herd prevalence of BCV and *M. bovis* was also concerning due to their association with preweaning mortality. *Pasteurella multocida* was commonly found with BRD, though not tied to mortality. Larger farms were at higher risk for certain pathogens raising concerns for larger operations. Conflicting findings on milk feeding and pathogen detection suggest a need for controlled studies. Veterinarians and dairy advisers could use these insights for farms with high NCD or BRD rates, and further research is needed on pathogen links to NCD and BRD.

## NOTES

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able at <https://borealisdata.ca/file.xhtml?fileId=661151&version=DRAFT> (Figure S1); <https://borealisdata.ca/file.xhtml?fileId=661153&version=DRAFT> (Figure S2); <https://borealisdata.ca/file.xhtml?fileId=661152&version=DRAFT> (Table S1); and <https://borealisdata.ca/file.xhtml?fileId=661154&version=DRAFT> (Table S2). Human ethics (REB no. 22-02-029) and animal use (AUP no. 4770) approvals were received from the University of Guelph Research Ethics Board and Animal Care Committee, respectively, before beginning the study. The authors have not stated any conflicts of interest.

**Nonstandard abbreviations used:** BA = blood agar; BCV = bovine coronavirus; BHV-1 = bovine herpesvirus type 1; BPI3V = bovine parainfluenza virus type 3; BRD = bovine respiratory disease; BRSV = bovine respiratory syncytial virus; BVDV = bovine viral diarrhoea virus; IQR = interquartile range; IRR = incidence rate ratios; MAC = McConkey agar; NCD = neonatal calf diarrhoea; OR = odds ratio; RV = Rappaport-Vassiliadis.

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