



## A calf-level study on colostrum management practices associated with adequate transfer of passive immunity in Québec dairy herds

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### ABSTRACT

The objective of this study was to identify the calf-level colostrum management practices associated with an adequate transfer of passive immunity (TPI; defined as serum Brix refractance  $\geq 8.4\%$  in the first week of life) in small-sized herds. A total of 818 calves from 61 commercial Holstein dairy farms were included in this observational cross-sectional study. For each calf, sex, colostrum delivery method, colostrum volume fed at first meal, and time to first feeding (delay between birth and first colostrum meal) were noted. Blood and colostrum samples were collected to estimate the serum and colostrum quality using Brix refractometry. To quantify the level of bacterial contamination in colostrum samples, total bacteria count and total coliform count (TCC) were measured using the Petrifilm (3M, St. Paul, MN) culture system. In this study, 68% of calves had an adequate TPI ( $\geq 8.4\%$ ). For data distribution, the 25th, 50th, and 75th percentiles were 1.3, 2.8, and 3.3 L for the colostrum volume fed at the first meal; 20.9, 23.5, and 26.5% Brix; and 1.1, 3.1, and 6.5 h for the time to first feeding of colostrum, respectively. The odds of adequate TPI were 2.6 times higher in calves receiving  $\geq 2.5$  L colostrum at their first meal, 2.9 times higher in calves receiving colostrum with  $\geq 24.5\%$  Brix, and 1.6 times higher in calves receiving colostrum within 3 h after birth, than in calves not meeting these criteria. In the present study, median bacterial contamination distribution (interquartile range) in the first colostrum meal was 14,000 cfu/mL (3,000–83,000 cfu/mL) for total bacteria count, and 0 cfu/mL (0–1,000 cfu/mL) for TCC. Total bacteria count and TCC were not associated with the odds of adequate TPI in the final model. Overall, these results suggest that specific calf-level colostrum management practices are associated with adequate TPI in small- to medium-sized dairy herds.

**Key words:** Brix refractometer, colostrum, contamination, transfer of passive immunity

### INTRODUCTION

Newborn calves are almost agammaglobulinemic at birth. They need to ingest immunoglobulins from colostrum, which can facilitate a transfer of passive immunity (TPI). This provides temporary protection to reduce the risk of mortality and morbidity at a young age (Donovan et al., 1998; Tyler et al., 1998; Raboisson et al., 2016). Adequate TPI is generally declared when serum IgG concentration is  $\geq 10$  g/L, a recognized threshold within the dairy industry (Besser et al., 1991; Furman-Fratczak et al., 2011; Buczinski et al., 2018), even if recent studies have suggested that this number may not be the optimal threshold for preventing neonatal diseases (Windeyer et al., 2014; Urie et al., 2018; Lombard et al., 2020). The gold standard test for the quantification of IgG in bovine colostrum and serum samples is radial immunodiffusion (Godden et al., 2019). This technique is sensitive and accurate, but it is expensive (Lee et al., 2008), provides delayed results (72 h; Dawes et al., 2002), and is complex because it requires laboratory expertise that renders it unusable directly on the farm. An alternative to this technique is the use of indirect IgG measurement tools such as the Brix refractometer, which is less complex and more suitable for on-farm use. Brix scores are highly correlated ( $r = 0.87$  to  $0.93$ ) with serum IgG concentrations (Morrill et al., 2013; Deelen et al., 2014). Also, a threshold of  $\geq 8.4\%$  Brix to maximize the sensitivity (Se) and the specificity (Sp) of the test to discriminate adequate TPI ( $\geq 10$  g/L IgG) from inadequate TPI ( $< 10$  g/L) have been suggested by Deelen et al. (2014; Se = 89%, Sp = 89%,  $n = 400$ , prevalence of inadequate TPI = 4.75%) and Mugnier et al. (2020; Se = 87%, Sp = 84%,  $n = 245$ , prevalence of inadequate TPI = 31%). An advantage of the Brix refractometer is that it is also correlated with colostrum IgG concentration ( $r = 0.64$  to  $0.94$ ; Quigley et al., 2013), and a good level of accuracy is obtained (combined Se and Sp) when it

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is used to detect colostrum samples with  $\geq 50$  g/L IgG concentration (Buczinski and Vandeweerd, 2016).

Achieving adequate TPI in calves depends on the amount of IgG ingested and its ability to be absorbed into the bloodstream (Morin et al., 1997; Godden et al., 2019). The main factors affecting the mass of Ig consumed by the calf are the IgG concentration of the colostrum (colostrum quality) and the colostrum volume fed at first meal (Godden et al., 2019). Another important factor affecting the absorption of Ig molecules into the bloodstream is the delay between birth and the first colostrum meal, as the permeability of the small intestine epithelium decreases rapidly after birth (Baumrucker and Bruckmaier, 2014). Colostrum cleanliness (bacterial contamination) is a further factor affecting IgG absorption to consider (Johnson et al., 2007).

The standard test for measuring colostrum bacterial contamination is bacteriological count performed in the laboratory. This technique is relatively expensive (CAD \$20; = US\$15) and requires samples to be tested in a laboratory. An alternative to traditional bacterial culture counts is the Petrifilm system, which can be carried out on the farm or at a veterinary clinic (Morin et al., 2021a).

In general, studies recommend that colostrum containing  $\geq 50$  g/L IgG, a total bacteria count (**TBC**)  $\leq 100,000$  cfu/mL, and a total coliform count (**TCC**)  $\leq 10,000$  cfu/mL should be given within the first few hours after birth (Morin et al., 1997; McGuirk and Collins, 2004). Although these recommendations are commonly used, they have not been determined based on associations between these criteria and the subsequent TPI status of the calf (i.e., the dependent variable we ultimately want to predict in calves). There is therefore a need and interest to challenge these criteria because they have not been determined with the objective of maximizing the chances of a calf having a serum IgG concentration  $\geq 10$  g/L (i.e., adequate TPI). Furthermore, it can be assumed that the recommended thresholds may be affected by the farming context or regional area where they are intended to be used (such as general practices and prevalence of adequate TPI). Thus, these general recommendations may not be appropriate or realistic for the small dairy farm context found in Eastern Canada. It has been previously shown that the calf and heifer management of these herds can differ from large American dairy farms, from which most of these recommendations and thresholds come (Vasseur et al., 2010; Winder et al., 2018). In this regard, the objective of this study was to identify the colostrum management practices associated with adequate TPI ( $\geq 8.4\%$ ) in calves from small- to medium-sized farms in Québec, Canada.

## MATERIALS AND METHODS

A cross-sectional observational study was conducted on commercial Holstein dairy herds located near the bovine ambulatory clinic of the Faculté de médecine vétérinaire of the Université de Montréal (St-Hyacinthe, QC, Canada). The herds were selected by convenience from the ambulatory bovine clinic clients list ( $n = 135$ ) enrolled in a preventive medicine program (visited at least once a month). The Animal Care Committee of the Université de Montréal approved this study (protocol number 16-Rech-1854).

In participating herds, calves were enrolled in the study beginning as soon as the producer agreed to participate. Between November 2016 and January 2018, a total of 1,524 Holstein calves were recruited in this study from 68 herds (1 to 22 calves per herd). Calves that received frozen colostrum or colostrum replacer were not allowed to be enrolled in the study. On farms where producers managed colostrum feeding between male calves and females differently, only females were enrolled.

### Assessment of TPI

During each farm visit, a team of researchers and an animal health technician collected blood samples from 1- to 7-d-old calves (Wilm et al., 2018) by jugular venipuncture using a plain blood collection tube (Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ) followed by serum harvesting by centrifugation ( $1,750 \times g$  for 10 min at  $20^\circ\text{C}$ ) within 24 h of collection. The TPI was estimated by refractance (Brix score) using an automatic temperature compensation digital refractometer previously validated in calves (PA203, MISCO, Cleveland, OH; Deelen et al., 2014). At the beginning of each series of analyses, the refractometer was calibrated with distilled water. Calves were classified as having an adequate TPI when the Brix score was  $\geq 8.4\%$  (Deelen et al., 2014).

### Assessment of Colostrum

A 10-mL sample of the first colostrum meal of each calf was collected by the producer or farm employee directly from the feeding bottle, bucket or esophageal feeding tube immediately before feeding. This sample of colostrum was considered representative of the quality of the first colostrum meal received by the calf. The samples were frozen ( $-18^\circ\text{C}$ ) and kept frozen until the bacteriological analysis and colostrum quality estimation by Brix refractometer had been completed at the in-house laboratory of the bovine ambulatory clinic by and the animal health technician (Université de

Montréal, St-Hyacinthe, QC). For bacteriological testing, the samples were thawed, thoroughly mixed, and transferred to sterile plastic tubes for dilution (1:1,000) with sterile water. One milliliter of the diluted solution was placed on an aerobic count plate (3M Petrifilm Aerobic Count Plate, 3M, St. Paul, MN) and a coliform count plate (3M Petrifilm Coliform Count Plate, 3M; McCarron et al., 2009). The plates were incubated at 38°C for 48 h in the case of the aerobic count plate, and 24 h in the case of the coliform count plate. The colony-forming units were then quantified (TBC, TCC) according to the manufacturer's recommendations (3M Food Safety, 2017a,b).

### **Data Recorded on the Farm About First Colostrum Meal and Farm Characteristics**

The producer or the person in charge of calf management was asked to record on a specific form the following information for each newborn calf enrolled in the study: sex, dam and calf identification, colostrum volume fed at first meal, feeding method, and time to first feeding (delay between birth and first colostrum meal). Furthermore, data on the main farm characteristics were noted, such as herd size and dry period length, to describe the study population.

### **Sample Size Estimation**

Considering that we potentially had 7 exploratory variables, we estimated the need for a minimum of 70 calves with TPI failure using the 10 event per variable rule (Moons et al., 2014). Thus, with a prevalence of adequate TPI from 70 to 90%, we would require between 233 and 700 calves to recruit. We recruited a higher number of calves to account for potential incomplete data entry due to farmers' participation and to account for calves clustered within farms when using mixed models.

### **Statistical Analysis**

Statistical analysis was conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC) and R (R Core Team, 2020, Vienna, Austria). Descriptive statistics were performed to describe colostrum management practices at the calf level. Descriptive statistics (minimum, median, 25th and 75th percentiles, and maximum) were obtained for each continuous independent variable (colostrum volume fed at first meal, time to first feeding, colostrum quality, TBC, and TCC) and the dependent variable (adequate TPI) using Proc MEANS in SAS. The categorical variables (sex and

feeding method) were described using frequency tables (PROC FREQ in SAS).

### **Determination Colostrum Management Practices Associated with Adequate TPI**

Continuous variables were dichotomized using multiple threshold values; colostrum volume fed at first meal (2, 2.5, and 3.0 L), time to first feeding (1 to 6 h by 0.5 h increment), colostrum quality (21% to 26.5% by 0.5% increment), TBC (3,000 to 5,000 cfu/mL by 1,000 cfu/mL increment, 10,000 to 80,000 cfu/mL by 10,000 cfu/mL increment, and 83,000 cfu/mL), and TCC (1,000 cfu/mL). These potential threshold values were defined within the interquartile range of the data set. Information about these thresholds are presented in Supplemental Tables S1 to S4 (<https://doi.org/10.3168/jds.2020-19475>).

Subsequently,  $2 \times 2$  frequency tables were obtained based on the outcomes of the calves (adequate TPI if  $\geq 8.4\%$  Brix; inadequate TPI if  $< 8.4\%$  Brix). Sensitivity, Sp, positive predictive value (PPV), and negative predictive values (NPV) were computed. For each specific predictor, the threshold value that provided the highest sum of Se + Sp to predict an adequate TPI was retained as the optimal threshold value to include in the multivariable analysis.

Receiver operating characteristic curves were built. All variables with  $P \leq 0.20$  in the univariable analysis were selected for the multivariable model building. A multivariable mixed logistic regression model (Proc GLIMMIX in SAS) accounting for the herd as a random effect was then built. Multivariable modeling was performed using a backward elimination strategy. The modeling was stopped once all the remaining variables had a value of  $P < 0.05$ . The least squares means were used to compare the probability of having a successful TPI among calves with and without the identified factors while controlling for other covariates. Variance inflation factor and tolerance (Proc REG in SAS) were calculated between all predictors that were considered for inclusion in the final model to avoid issues associated with collinearity. Consequently, if the variance inflation factor or tolerance coefficient between 2 variables had a value  $> 10$  or  $< 0.1$ , respectively, only the variable with the most biological relevance was included in the multivariable model-building process. Possible confounding factors and biologically possible 2-way interactions were evaluated. Interaction terms that were found to be statistically significant ( $P \leq 0.05$ ) were added to the model, and confounding factors were maintained as fixed effects if their effect on the regression coefficients of the other predictors was greater than 10%

(Maldonado and Greenland, 1993). The goodness of fit was evaluated with Pearson chi-squared statistic (Proc GLIMMIX in SAS). Intraclass correlation coefficients (ICC) were calculated for the final model to determine the amount of total variance of the outcome that could be attributed to variation between herds and between calves. The ICC was calculated by the simulation method described by Goldstein et al. (2002).

## RESULTS

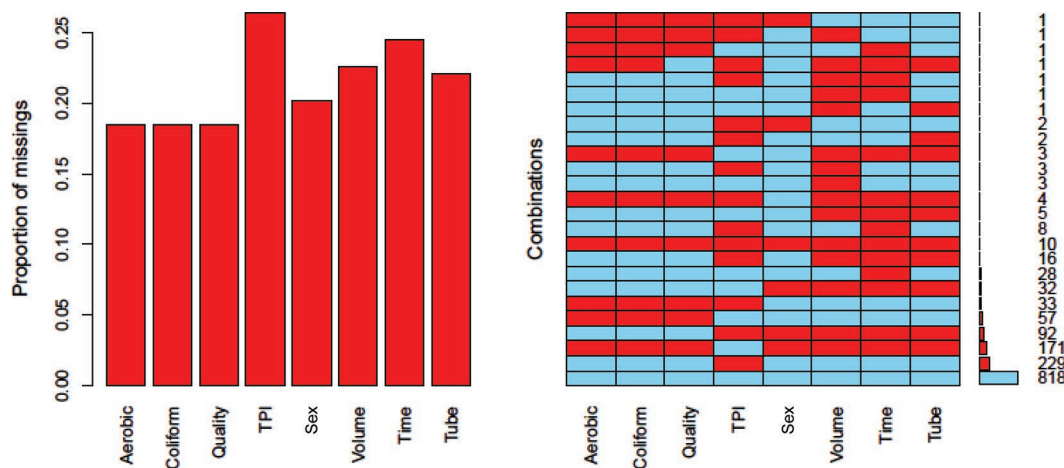
Between November 2016 and January 2018, 1,524 Holstein calves from 68 commercial herds were recruited. Due to missing information for one or more of the colostrum management practices to be investigated, 706 calves were excluded from the study (Figure 1). A total of 818 calves from 61 farms (1 to 22 calves/farm) were used for analysis. The calves enrolled in the study came from herds ranging in size from 27 to 300 lactating cows (median = 60 lactating cows) and having a dry period length between 45 and 73 d (median = 60 d). Most female calves were sampled (66%). Most calves received their first colostrum meal through a bottle (51%), whereas smaller proportions received their colostrum via a nipple bucket (8%), a bucket (15%), or an esophageal tube (26%).

Descriptive statistics for continuous variables are listed in Table 1. In this study, only 68% of the calves ( $n = 818$ ) had an adequate TPI ( $\geq 8.4\%$ ). The threshold values of independent variables that provided the highest sum of Se + Sp to predict an adequate TPI were  $\geq 2.5$  L for the colostrum volume fed at first meal,  $\leq 3$  h for the time to first feeding,  $\geq 24.5\%$  Brix for colostrum

quality,  $\leq 20,000$  cfu/mL for TBC, and  $\leq 1,000$  cfu/mL for TCC (Table 2). Specific threshold values tested for each variable are presented in Supplemental Tables S1 to S4 (<https://doi.org/10.3168/jds.2020-19475>). There was very little variation between quartiles in the colostrum coliform counts. Thus, only one threshold could be tested in  $2 \times 2$  frequency table, which is reported in Table 2. The univariable analyses are described in Table 3. As such, the variables retained for the multivariable analysis were  $\geq 2.5$  L for the colostrum volume fed at first meal,  $\leq 3$  h for the time to first feeding,  $\geq 24.5\%$  Brix for colostrum quality,  $\leq 20,000$  cfu/mL for TBC,  $\leq 1,000$  cfu/mL for TCC, and sex. The results from the multivariable mixed model are presented in Table 4 and Figure 2. The Pearson chi-squared statistics (goodness of fit) was 0.93, indicating that there was no overdispersion problem in the final model. Only 3 variables were associated with successful TPI in the final model; no confounding variable and no biological 2-way interactions were retained. Based on this final model, the odds of adequate TPI were 2.6 times greater in calves receiving  $\geq 2.5$  L colostrum at the first meal, 2.9 times higher in calves receiving colostrum with  $\geq 24.5\%$  Brix, and 1.6 times higher in calves receiving colostrum within 3 h after birth, than in calves not meeting these criteria. The amounts of total variance of the outcome that could be attributed to variation between herds and between calves were 0.89 and 0.11, respectively.

## DISCUSSION

This study was conducted on a large number of calves ( $n = 818$ ) and herds ( $n = 61$ ), to include dif-



**Figure 1.** Distribution of missing data and missing data patterns on this study. The proportion of missing data for each specific study variable is indicated in the first panel. The specific patterns of missing data are represented in the second panel as well as the number of cases. Missing data are presented in red boxes. Aerobic: aerobic count; Coliform: coliform count; IgG: information on colostrum quality (Brix); TPI: transfer of passive immunity; Sex: calf sex; Volume: information on volume at first feeding; Time: information on delay between birth and first colostrum meal; Tube: tube feeding used at first meal.



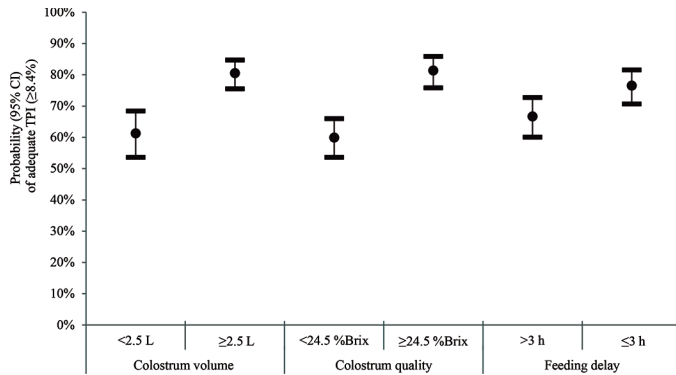
**Table 1.** Descriptive statistics of individual data from Holstein dairy calves (n = 818 from 61 commercial herds) enrolled in an observational study on colostrum management practices for the first colostrum meal

Variable	Minimum	25th percentile	50th percentile	75th percentile	Maximum	Mean
Transfer of passive immunity (% Brix)	5.9	8.2	8.7	9.4	12.4	8.8
Colostrum volume fed at first meal (L)	0	1.3	2.8	3.3	6.0	2.7
Colostrum quality (% Brix)	8.4	20.9	23.5	26.5	38.9	23.6
Time to first colostrum feeding (h)	0	1.1	3.1	6.5	21.3	4.3
Colostrum aerobic bacterial contamination (cfu/mL)	0	3,000	14,000	83,000	1,000 × 10 <sup>6</sup>	723 × 10 <sup>6</sup>
Colostrum coliform bacterial contamination (cfu/mL)	0	0	0	1,000	1,000,000	12,094

**Table 2.** Optimal thresholds for volume of colostrum fed at first meal, colostrum quality, time to first feeding (delay between birth and first colostrum feeding), and colostrum bacterial contamination (aerobic and coliform counts), based on the maximum sum of sensitivity (Se) and specificity (Sp) to predict an adequate transfer of passive immunity (≥8.4% Brix in serum) in 818 calves from 1 to 7 d of age in 61 commercial dairy herds

Variable	Threshold	Proportion of calves (%)	Se, % (95% CI)	Sp, % (95% CI)	PPV, <sup>1</sup> % (95% CI)	NPV, <sup>2</sup> % (95% CI)	P-value
Colostrum volume fed at first meal	≥2.5 L	61	68 (64–72)	55 (49–61)	76 (72–80)	45 (39–50)	<0.01
Colostrum quality	>24.5% Brix	42	50 (45–54)	74 (69–79)	80 (76–84)	41 (36–45)	<0.01
Time to first colostrum feeding	≤3 h	50	55 (51–59)	63 (57–69)	76 (72–80)	40 (35–45)	<0.01
Aerobic bacterial contamination of colostrum	≤20,000 cfu/mL	56	59 (55–63)	52 (46–58)	73 (68–77)	37 (32–43)	<0.01
Coliform contamination of colostrum	≤1,000 cfu/mL	77	79 (76–82)	29 (23–35)	70 (67–74)	39 (32–46)	0.01

<sup>1</sup>PPV = positive predictive value.<sup>2</sup>NPV = negative predictive value.



**Figure 2.** Least squares means of the probability (95% CI) of adequate transfer of passive immunity (TPI;  $\geq 8.4\%$  Brix;  $n = 818$ ) stratified by colostrum volume fed at first meal, colostrum quality, and feeding delay between birth and first colostrum meal.

ferent colostrum management practices from Québec dairy herds. In this study, we also tried to determine the optimal threshold values that were adapted to our specific study population (i.e., herds with a relatively low number of milking cows) to compare them with traditional thresholds in the North American dairy industry.

### TPI

The prevalence of calves with adequate TPI (68%) in this study was lower than that reported in a previous study conducted in herds from Eastern Canada. As a comparison, Renaud et al. (2018) reported that 79%

of the 149 Holstein male calves sampled on arrival at an Ontario milk-fed veal farm had an adequate TPI. Another study conducted in herds regularly involved in research projects in Ontario reported a prevalence of adequate TPI of 95% (Deelen et al., 2014). Our prevalence of adequate TPI was also lower than the percentages reported in American studies, specifically 81% in 2007 (Beam et al., 2009) and 88% in 2014 (Shivley et al., 2018). This can be explained by the fact that colostrum management differs regionally due to specific dairy calf management and farm size.

It is important to consider that the prevalence of adequate TPI may be influenced by the fact that females were more frequently sampled (66%) than males in our study. It is likely that the true prevalence of adequate TPI in dairy farms was overestimated in our study compared with the overall general calf population (males and females together), because male calves that were treated differently from females were excluded from the present study to ensure a more representative portrait of colostrum management for animals to be kept as replacement. A previous Canadian study has shown that male management may differ from female management, with 9% of farms not always feeding colostrum to male calves (Renaud et al., 2017). Another study carried out in Québec on 80 lots of veal calves reported a prevalence of adequate TPI of only 38% (A. Abdallah, D. Francoz, S. Dufour, J. Berman, and S. Buczinski, University of Montreal, Montreal, QC, Canada, unpublished data). For this reason, we did not want to include males from farms that managed

**Table 3.** Univariable associations (logistic regression models) between various colostrum management practices and adequate transfer of passive immunity ( $\geq 8.4\%$  Brix in serum) in 818 calves from 1 to 7 d of age in 61 commercial dairy herds

Factor	$\beta$ -estimate (95% CI)	P-value
Colostrum volume fed at first meal		
<2.5 L	Referent	
$\geq 2.5$ L	1.0 (0.5 to 1.3)	<0.01
Time to first colostrum feeding		
>3 h	Referent	
$\leq 3$ h	0.6 (0.3 to 1.0)	<0.01
Colostrum quality		
<24.5% Brix	Referent	
$\geq 24.5\%$ Brix	1.1 (0.7 to 1.4)	<0.01
Aerobic bacterial contamination of colostrum (cfu/mL)		
>20,000 cfu/mL	Referent	
$\leq 20,000$ cfu/mL	0.4 (0.01 to 0.7)	0.04
Coliform contamination of colostrum		
>1,000 cfu/mL	Referent	
$\leq 1,000$ cfu/mL	0.3 (−0.2 to 0.7)	0.31
Sex		
Male	Referent	
Female	−0.2 (−0.6 to 0.2)	0.31
Use of esophageal tube		
No	Referent	
Yes	0.1 (−0.4 to 0.6)	0.56

**Table 4.** Final multivariable mixed logistic regression model describing the relationship between various colostrum management practices and adequate transfer of passive immunity ( $\geq 8.4\%$  Brix in serum) in 818 calves from 1 to 7 d of age in 61 commercial dairy herds

Colostrum management practice	$\beta$ -estimate (95% CI)	Odds ratio (95% CI)	P-value
Intercept	-0.3 (-0.7 to 0.05)		<0.01
Colostrum volume fed at first meal			
<2.5 L	Referent		
$\geq 2.5$ L	1.0 (0.6 to 1.3)	2.6 (1.8 to 3.8)	<0.01
Time to first colostrum feeding			
>3 h	Referent		
$\leq 3$ h	0.5 (0.1 to 0.8)	1.6 (1.2 to 2.3)	<0.01
Colostrum quality			
<24.5% Brix	Referent		
$\geq 24.5\%$ Brix	1.1 (0.7 to 1.4)	2.9 (2.1 to 4.2)	<0.01

colostrum differently depending on the calf's sex. The main focus of our study was to quantify what is done on Québec commercial dairy farms with females, or at least males that are managed like females. This should be taken into account when comparing prevalence data from various studies.

#### Colostrum Volume Fed at First Meal

In this study, the first colostrum volume fed at first meal threshold that was associated with adequate TPI was  $\geq 2.5$  L. The odds of adequate TPI were 2.6 times higher in calves receiving  $\geq 2.5$  L of colostrum at their first meal than in calves receiving a lower volume. This volume threshold was lower than the current recommendation, which is to feed 10 to 12% of BW at first feeding (4 L for a 43-kg calf; Godden et al., 2019). In the present study, only 19% of calves received  $\geq 4$  L at first meal. This observation is similar to the results reported by Vasseur et al. (2010), these authors finding that only 25% of calves received 4 L in the first 12 h in a study conducted on 111 Québec dairy herds. Although our data suggest that feeding  $\geq 2.5$  L is associated with greater odds of adequate TPI, it is important to understand that this is after accounting for the other variables presented in the final model (Table 4). Therefore, any general recommendation of this specific threshold should be made carefully.

#### Time to First Colostrum Feeding

According to our data, the odds of adequate TPI were 1.6 times higher in calves receiving their colostrum within 3 h of birth than in calves fed later. The first few hours after birth are a critical period for calves, for the reason that IgG absorption in the intestine rapidly decreases over time (Godden et al., 2019). However, we observed that only 50% of the calves received their first colostrum meal in the first 3 h after birth, whereas 75%

of the calves received their colostrum within the first 6 h. It is important to consider that this variable (time to first feeding) likely reflected the period from which the calf was discovered by the farmer after birth, rather than the time of birth itself. The inaccuracy surrounding the actual time of calving is part of the reality of Québec dairy farms, where calving supervision is not continuous during a day because farm sizes are small (Vasseur et al., 2010). Interestingly, one study reported that calves fed more than 4 h after birth had 2.7 times greater odds of inadequate TPI than calves fed earlier (Beam et al., 2009).

#### Colostrum Quality

In the current study, colostrum quality using  $\geq 24.5\%$  Brix was the optimal threshold associated with adequate TPI in calves. Specifically, we found that the odds of adequate TPI were 2.9 times higher in calves receiving colostrum  $\geq 24.5\%$  than in calves receiving colostrum <24.5%. This threshold is higher than that reported ( $\geq 22\%$  Brix) in a systematic review and meta-analysis study (Buczinski and Vandeweerde, 2016). However, it is important to keep in mind that these 2 thresholds were not determined according to the same objective. The threshold  $\geq 22\%$  Brix demonstrated the greatest accuracy to detect colostrum samples with radial immunodiffusion values  $\geq 50$  g/L of IgG (Buczinski and Vandeweerde, 2016), whereas our threshold was based on the relationship between colostrum quality and adequate TPI in calves. Thus, it is not surprising that these thresholds are not exactly the same.

#### Bacterial Contamination of Colostrum

Bacteria found in colostrum may interfere with IgG intake (James et al., 1981) by binding to nonspecific receptors of neonatal enterocytes, thereby decreasing the number of receptors available for IgG intake

(Staley and Bush, 1985). To provide some guidelines, McGuirk and Collins (2004) have recommended that TBC should be  $\leq 100,000$  cfu/mL and TCC  $\leq 10,000$  cfu/mL when using standard bacteriological laboratory tests. Interestingly, the use of the Petrifilm bacteriological culture system has been shown to be an accurate alternative to standard bacteriological tests (Morin et al., 2021a). However, it is important to keep in mind that different threshold values were recommended to yield the same test performance (Morin et al., 2021a). As such,  $\leq 24,000$  cfu/mL for TBC and  $\leq 4,000$  cfu/mL for TCC were found to demonstrate the greatest accuracy (TBC: Se = 69%, Sp = 86%; TCC: Se = 90%, Sp = 93%) for identifying heavily contaminated colostrum samples (based on the usual reference test thresholds of  $\leq 100,000$  and  $\leq 10,000$  cfu/mL, respectively, when performed in a laboratory). Surprisingly, TBC and TCC were not associated with adequate TPI when accounting for other important covariates in our study. It is important to keep in mind that in our study, the proportions of heavily contaminated colostrum (greater than the aforementioned thresholds) were only 44 and 24% for TBC and TCC, respectively. In our mind, this finding does not mean that bacterial contamination has no effect on the probability of adequate TPI; perhaps the situation would have been different if our data had a greater prevalence and magnitude of bacterial contamination. This finding should be explored in the future.

### Calf vs. Herd Variance

The ICC from our final model showed that the variation of calves within a herd was proportionally smaller than the variation between herds itself. This result implies that colostrum management programs should be based on a herd approach instead of on an individual calf approach. This supports the need for more population-based studies to make appropriate herd-level management decisions on farms. Therefore, it is important to consider exploring herd-level risk colostrum management practices concerning colostrum management (Morin et al., 2021b).

### Study Limitations

Herds were selected based on convenience. This approach was chosen to complete this project within a reasonable time frame. It was also an appropriate method considering that this project required the physical involvement of dairy farmers in some part of the data collection process; hence, we needed a sufficient degree of willingness and involvement from them. However, this type of sampling carries a risk of selection

bias. For example, participating herds were all clients of the bovine ambulatory clinic (Université de Montréal, St-Hyacinthe, QC, Canada). These herds are regularly enrolled in research projects and were all involved in a preventive herd health medicine program (visited at least once a month). It is therefore possible that these herds were above-average herds in our area regarding general farm and possibly calf management. One may also speculate that willingness to participate might have been greater for farmers having recurrent health problems with their calves or the most progressive ones. In both cases, convenience sampling may have resulted in farmers being recruited from both extremes, potentially biasing our estimations. It remains unclear whether this choice affected our results, so these should be interpreted with this fact kept in mind.

Because estimation of IgG in colostrum and serum samples was done using an indirect method that is known not to be as accurate as the gold standard test (radial immunodiffusion), potential measurement bias or misclassification should be considered when interpreting the current study results. It has also been reported that in dehydrated calves the measurement of TPI with a Brix refractometer can be biased, since the refractive index of the serum is affected by dehydration, which concentrates blood components, resulting from an abnormally high total protein concentration (Tyler et al., 1999). Unfortunately, the state of health and hydration of the enrolled calves was not systematically evaluated. Based on this limitation, it is possible that the prevalence of adequate TPI may slightly be overestimated in our data, even if a recent study did not find an association with failure of TPI and overall health score (Renaud et al., 2020).

Finally, another limitation when interpreting the results of this study is that the information regarding the colostrum volume fed at first meal and the time to first feeding was collected by the producers or farm employees, which could have affected the accuracy of the reports. It is also possible that participants' behavior and farm management practices changed positively during the study period (in comparison with before the start of the study) as a result of agreeing to participate in the study and knowing that their own practices were evaluated (Hawthorne effect; McCambridge et al., 2014).

## CONCLUSIONS

The results of this study suggest that various calf-level colostrum management practices were associated with adequate TPI, with relevant colostrum management practices including the colostrum volume fed at first meal, colostrum quality, and time to first feeding



of colostrum. Interestingly, colostrum bacterial contamination was not associated with adequate TPI, a finding that requires further investigation. Future studies should consider exploring herd-level risk colostrum management practices concerning colostrum management to provide novel and practical information for herd managers and calf health consultants.

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