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Original Article

Immunological and bacteriological quality of fresh cow colostrum and passive immunity transfer in selected dairy farms in Fars, Iran

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Abstract

Background: The quality of colostrum is affected by IgG level and microbial load. **Aims:** The quality of colostrum used in feeding dairy calves and passive immunity transfer in selected dairy farms in Fars province, Iran was investigated. **Methods:** A total of 75 colostrum and neonatal blood samples were collected from 11 herds. The immunological quality of colostrum was assessed using a Brix digital refractometer. The bacteriological quality was assessed by performing total plate count (TPC), total coliform count (TCC), spore-former count, fungi count, and species-specific PCR assay to detect some bacterial species. **Results:** The mean Brix of colostrum samples was 25.4% and 72% of the samples had a Brix score ≥22%. The mean serum Brix and the prevalence of failure of passive transfer (FPT) were 10% and 4%, respectively. The mean TPC, TCC, spore-former count, and fungi count were 3.6×10^5, 2.8×10^4, 3.2×10^4, and 1.1×10^4 CFU/ml, respectively. The results showed that 50, 5.9, and 4% of colostrum samples were positive for *Staphylococcus* spp., *Salmonella* spp. and *Maycobacterium paratuberculosis*, respectively. There was no evidence of contamination with *Brucella* spp., *Corynebacterium bovis* and *Mycoplasma bovis.* **Conclusion:** Considering all colostrum quality indicators comprehensively, only 37.3% of the studied samples met the industry standard. A large number of calves were at risk of receiving poor quality colostrum, especially in terms of microbial contamination. Further researches are needed to evaluate the colostrum management and the effect of bacterial contamination of colostrum on the health of neonate calves in this region.

Key words: Colostrum, FPT, IgG, MAP, TPC

Introduction

The immunoglobulin content of colostrum has been the subject of a huge body of research during the past decades. Dairy calves are born agammaglobulinemic and are relied on the passive transfer of immunoglobulins through colostrum for protection against infectious diseases early in life (Davis and Drackley, 1998; Weaver *et al*., 2000; Cuttance *et al*., 2018). Furthermore, while colostrum is more nutritious than milk, it also possesses ingredients such as cytokines and growth factors (Godden, 2008), important for growth of intestinal epithelial cells and development of intestinal functions (Buhler *et al*., 1998; Yang *et al*., 2015; McGrath *et al*., 2016). If immunoglobulin G (IgG) is absorbed in the intestine in sufficient amounts, its serum level exceeds the cut-off point of 10 g/L at 24 to 48 h of birth, and the passive transfer of immunity occurs successfully. Thereby, decreased risks of morbidity and mortality in the preweaning period and improved milk production later in life are anticipated (DeNise *et al*., 1989; Donovan *et al*., 1998). However, the bacterial contamination of colostrum may affect the transfer of passive immunity and probably the further performance of the animals. Although bacteria can cause diarrhea and septicemia, they may bind to immunoglobulins before absorption or to the nonspecific receptors of enterocytes, interfering with immunoglobulin uptake (Johnson *et al*., 2007). Colostrum may be a source of infectious agents that infect the animals early in life (Godden, 2008) and affect their performances later. Some of the bacteria that may be transmitted through colostrum are *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Godden *et al*., 2015), *Salmonella* spp. (Spier *et al*., 1991), *Escherichia coli* (Clark *et al*., 1989; Steele *et al*., 1997), *Staphylococcus aureus* and *Mycoplasma bovis* (Jayarao *et al*., 2004), *Listeria monocytogenes* (Doyle *et al*., 1987), and *Campylobacter* spp. (Lovett *et al*., 1983; Steele *et al*., 1997). Infectious agents may be shed from mammary glands or may contaminate the colostrum during milking, storage or feeding practices (McGuirk and Collins, 2004; Godden, 2008). Contamination of raw

milk with *Staphylococcus aureus*, *Corynebacterium bovis*, and *Mycoplasma bovis* has been reported from dairy farms in Fars province, Iran (Mohebbi-Fani *et al*., 2016).

Recommendations for colostrum quality are IgG levels higher than 50 g/L, and total plate count (TPC) and total coliform count (TCC) less than 100,000 and 10,000 CFU/ml, respectively (McGuirk and Collins, 2004). However, many colostrums do not meet these quality standards (Fecteau *et al*., 2002; Morrill *et al*., 2012; Phipps *et al*., 2016). The prevalence of FPT in dairy calves has been estimated to be between 20 and 40% (Raboisson *et al*., 2016) in various countries: 19.2% in the US (Beam *et al*., 2009), 33% and 24.8% in New Zealand (Cuttance *et al*., 2017, Lawrence *et al*., 2017), 34.6% in the Czech Republic (Stanek *et al*., 2019), 31% in organic Norwegian and Swedish dairy herds (Johnsen *et al*., 2019), 26% in the United Kingdom (MacFarlane *et al*., 2015) and 38% in Australia (Vogels *et al*., 2013).

Little, if any information is available on the colostrum quality (IgG level; bacterial contamination) and passive transfer of immunity in dairy farms of Fars province. In the present study, we examined the quality of fresh colostrum just before being fed to the calves in terms of Brix value, which correlates with IgG concentration in both colostrum and serum, and bacterial contamination of colostrum in selected dairy farms with apparently acceptable production routines. We also determined the frequency of FPT in newborn calves.

Materials and Methods

Farms

Eleven commercial Holstein farms located in Fars province (south of Iran) were selected for this study that was performed from June to October, 2018. The farms were enrolled on the study based on 1) having constant, apparently acceptable, production routines and management protocols for newborn calves; 2) feeding unpasteurized fresh maternal colostrum to the newborn calves, 3) willing to participate in the study and to share farm data based on the designed protocol, and 4) being able to collect colostrum and blood samples by fully trained persons or to provide the accommodation facilities for such persons. The average number of milking cows in the farms was 121 (ranging from 40 to 340), and the average daily milk production was 33.81 kg (30 to 37 kg) (Table 1). This study was approved by Shiraz University and performed according to the guidelines of the Iranian Council on Animal Care (ICAC, 2005).

Sample collection

In each farm, 4 to 8 colostrum samples were taken from the first meal of colostrum. At the time of sampling, the cows and their calves were in good general health condition. No interventions were made in the routine farm practices such as the time of milking, the methods of collecting, transporting, storing, and feeding colostrum to calves. Just before feeding the first meal of colostrum to the calf, 50 ml of colostrum was taken directly from the nursing bottle using a disposable sterile syringe and transferred into a sterile falcon tube for evaluation of the Brix score and microbial analysis. A total number of 75 colostrum samples were collected. For each colostrum sample, a questionnaire including information on the dam, the newborn and the usual methods of colostrum management was answered by the persons directly responsible for caring of calves. The questionnaire was completed for 63 cows, and for the other cows (n=12), the parity of cows, the volume of the first milking colostrum, and the amounts fed to the calves were not clear. The samples were stored in the farm at -20°C until the sampling was completed. Then, samples were transferred cool for analysis. A single jugular blood sample (10 ml) was taken from each calf 24-48 h after birth. The samples were centrifuged and the separated sera were stored at -20°C until testing for Brix value.

Colostrum and serum refractometry test

The immunological quality of colostrum and passive immune transfer was assessed with a digital Brix refractometer (Misco, PA203X, USA; scale range: 0 to 85% Brix). The samples and the refractometer were placed in the laboratory for 30 min to reach room temperature (24 ± 2°C). The Brix refractometer was calibrated with distilled water. All samples were tested in triplicate.

Colostrum microbial analysis

The colostrum samples were thawed, vortexed, serial decimal diluted and examined for TPC, TCC, spore-former count, and fungi count using corresponding culture media (Merck®, Darmstadt, Germany). To count spores, before serial dilution, the samples were heated at 62.8°C for 30 min to kill the vegetative bacteria. For TPC and spore count, plate count agar was used and the plates were incubated at 37°C for 24 h. For TCC, violet red bile dextrose agar was used and the plates were incubated at 37°C for 24 h. For fungi counts Sabouraud dextrose agar containing chloramphenicol (100 µg/L) was used and the plates were incubated at

Table 1: The characteristics of the studied herds (n=11)

Characteristics	Mean	SD	Min	Max
Herds size (milking cows)	121	101	40	340
Small ≤80 milking cows (n=6)	65.3	13.2	40	80
Large >80 milking cows (n=5)	188	110.1	100	340
Average daily milk production (kg)	33.8	2.1	30	37
Number of samples (colostrum/serum) in each herd	6.8	1.1	4.0	8.0
Lactation number of cows	2.2	1.4	1	6

Table 2: Details of PCR assays for identification of the bacteria

Bacterial spp.	Primer pairs (5´-3´)	Annealing temp. (°C)	Fragment size (bp)	Reference
Mycobacterium paratuberculosis	F: GAAGGGTGTTGGGGGCCGTCGCTTAGG R: GGCGTTGAGGTCGATCGCCCACGTGAC	59	413	Haghkhah *et al*. (2015)
Corynebacterium bovis	F: CGTTTAGTGTGTGCG R: GGCACGGAAATCGTGGAA	60	750	Mohebbi-Fani *et al*. (2016)
Brucella spp.	F: TCCGCAAGCTTCAAGCCTTCTATCC R: GCGTGTCTGCATTCAAGGTAACC	69	325	Abdali *et al*. (2020)
Mycoplasma bovis	F: AAGGTACACCAGCTAACCCAG R: AATGAAGCTACTGATCCAAG	52	319	Tenk *et al*. (2006)

Table 3: The weight (kg) of first milking colostrum produced by the cows and the amount of colostrum fed to the calves in the studied farms

Variables	No.	Mean	SD	Min	Max
Weight of the first milking colostrum (kg)	63	6.3	2.6	1.5	15.0
First parity	27	5.4[a]	2.3	1.5	11.5
Second parity	11	5.3[a]	1.5	3	8
Third parity or more	25	7.6[b]	2.9	2	15
Unknown parity	12	-	-	-	-
Low (<5 kg)	17	3.3	0.8	1.5	4.5
Medium (5-9.9 kg)	37	6.5	1.2	5	9
High (≥10 kg)	9	10.9	1.7	10	15
Unknown weight	12	-	-	-	-
Volume of colostrum fed to the calves (L)					
First meal	63	2.4	0.5	1.0	3.0
The first 6 h	63	3.5	1.0	1.0	5.0

[a, b] Significant difference in colostrum volume for parity (P<0.05)

25°C for 48 h. Following incubation, numbers of colonies that were developed on the plates were counted and reported as CFU/ml.

Isolation of *Staphylococci* and *Salmonella*

These bacteria were traced using corresponding culture media (Merck®, Darmstadt, Germany). In order to isolate *Staphylococci*, colostrum samples were processed by both enrichment and direct plating on Giolitti-Cantoni broth and Baird-Parker agar according to the method of Rahmdel *et al*. (2019). For isolation of *Salmonella*, the samples were cultured sequentially in lactose broth, selenium cysteine and Rappaport-Vassiliadis media, and XLD agar. Finally, biochemical tests were used to confirm *Salmonella*-suspected colonies (Broadway *et al*., 2021).

Detection of MAP, *Corynebacterium bovis*, *Brucella* spp., and *Mycoplasma bovis* using PCR assay

DNA extraction

A DNA extraction kit (Bioneer, South Korea) was used on a 100-µg colostrum sample, as was described. Briefly, the samples were initially transferred into 1.5 µL microfuge tubes, 200 µL lysis buffer and proteinase K were then added to the tube contents. Finally, 30 µL of DNAse free water was added to the DNA precipitate, which was kept at -20°C until further use.

PCR assays for amplification of 16 sRNA gene

Species-specific PCR assay was performed on MAP, *Corynebacterium bovis*, *Brucella* spp., and *Mycoplasma bovis*. Details are given in Table 2.

Statistical analysis

In this study, a total number of 75 individual colostrum samples of Holstein cows and 75 serum samples of calves from 11 dairy farms were analyzed. The studied farms were divided into small (≤80 milking cows) and large (>80 milking cows) categories. Cows were divided into three groups based on the parity number: first (n=27), second (n=11), and third or greater parity (n=25). Twelve cows had unknown parity numbers. The cows were also divided into three groups based on the volume of the first milking colostrum: low (<5 kg), medium (5-9.9 kg), and high (≥10 kg) producers. The data (mean±SD) were analyzed using the SPSS statistical software (Illinois, USA, version 16). The volume and the Brix score of the colostrum were compared in the described parity and volume groups using one-way ANOVA. The Brix scores of the serum of calves were grouped into four categories: poor (<8.5%), fair (8.5-8.8%), good (8.9-9.3%), and excellent (≥9.4%) according to Godden *et al*. (2019). The averages of these categories were compared with the cut-off level of 8.5% using the one-sample t-test. The correlations between different studied indicators were assessed by Pearson's correlation test. In all cases, the P-value of ≤0.05 was set as the level of significance.

Results

The average weight of the first milking colostrum produced by cows was 6.3 kg (1.5 to 15 kg; Table 3).

The cows with 3 or more parities produced significantly (P<0.05) more colostrum than the cows in the first and the second parities (Table 3). Most of the cows produced medium volumes of colostrum. The average amount of colostrum fed to the calves at the first meal and during the first 6 hours after birth were 2.4 and 3.5 kg, respectively.

Colostrum Brix reading

The Brix value of colostrum samples ranged from 13.8% to 37.3% with an overall mean of 25.4%. According to the cut-off point of 22% (Godden *et al*., 2019), corresponding to IgG concentration of 50 g/L, 54 out of 75 colostrum samples (72%) had IgG levels above 50 g/L (Table 4). The low (<5 kg) and medium (5-9.9 kg) levels of first milking colostrum did not differ in the Brix score but both of them had higher scores (P<0.05) compared to the high volumes (≥10 kg). The cows in the 3rd parity or more had significantly higher colostrum Brix scores (P<0.05) compared to the cows in the first and second parities. The lowest colostrum Brix score was detected in the second parity (P<0.05), but there was no difference between the first and the second parities.

Serum Brix reading

The mean serum Brix scour of the newborn calves was 10% with a range of 7.6% to 12.8% (Table 4). According to the cut-off level of 8.5% (Godden *et al*., 2019), corresponding to IgG level of 10 g/L, the prevalence of FPT was 4% (3 calves out of 75) (Table 5). These 3 calves had a mean Brix level of 7.9% (poor serum Brix level). The reminder of calves had excellent

Table 4: Mean (±SD) of Brix score and TPC, TCC, spore-former, and fungi count of colostrum samples and serum Brix scores of newborn calves

Variables	No.	Mean	SD	Min	Max	Max limit (CFU/ml)	Met the standard * No. (%)
Colostrum Brix (%)	75	25.4	4.9	13.8	37.3	-	54 (72)
Low volume (<5 kg)	17	26.7[a]	5.3	17.8	37.3	-	14 (82.4)
Medium volume (5-9.9 kg)	37	24.4[a]	4.1	13.8	31.9	-	27 (73.0)
High volume (≥10 kg)	9	21.2[b]	3.1	14.6	25.1	-	3 (33.3)
First parity	27	24.1[A]	3.3	17.0	31.5	-	18 (66.7)
Second parity	11	22.5[A]	5.4	13.3	31.0	-	5 (45.5)
Third parity or more	25	25.9[B]	5.2	14.0	37.3	-	18 (72)
Unknown volume/parity	12	29.7[cC]	4.0	23.2	37.3	-	12 (100)
Calves' serum Brix (%)	75	10.0	1.1	7.6	12.8	-	72 (96)
Low volume (<5 kg)	17	10.0	1.0	8.0	12.3	-	16 (94.1)
Medium volume (5-9.9 kg)	37	9.9	1.1	7.6	12.8	-	35 (94.6)
High volume (≥10 kg)	9	9.8	0.9	8.6	11.3	-	9 (100)
First parity	27	9.8	1.1	7.6	12.8	-	25 (92.6)
Second parity	11	10.0	0.8	8.6	11.8	-	11 (100)
Third parity or more	25	10.0	1.0	8.0	12.4	-	24 (96)
Unknown volume/parity	12	10.2	1.2	8.6	12.4	-	12 (100)
Microbial analysis							
TPC[1] (CFU/ml)	75	3.6×10^5	6.5×10^5	200	3.3×10^6	10^5	43 (57.3)
TCC[2] (CFU/ml)	75	2.8×10^4	6.2×10^4	0	2.9×10^5	10^4	50 (66.7)
Spore-former (CFU/ml)	75	3.2×10^4	8.5×10^4	0	6.5×10^4	-	-
Fungi (CFU/ml)	75	1.1×10^4	2.1×10^4	0	1.1×10^5	-	-

[1] TPC: Total plate count, [2] TCC: Total coliform count, * Met the standard: Cut-off points of Brix score 22% for colostrum and 8.5% for serum (Godden *et al*., 2019), and cut-off points for microbial analysis: 10^5 CFU/ml for TPC and 10^3 CFU/ml for TCC. [a, b, c] Significant difference for volume, and [A, B, C] Significant difference for parity, (P<0.05)

Table 5: Levels of passive transfer of immunity estimated 24 to 48 h after birth by serum Brix score in the studied herds (categories according to Godden *et al*., 2019)

Herd	No. of samples	Calves' serum Brix category			
		Excellent (≥9.4%) No. (%)	Good (8.9-9.3%) No. (%)	Fair (8.5-8.8%) No. (%)	Poor (<8.5%) No. (%)
1	7	5 (71.4)	1 (14.3)	1 (14.3)	0
2	8	7 (87.5)	0	1 (12.5)	0
3	7	7 (100)	0	0	0
4	7	2 (28.6)	3 (42.9)	2 (28.6)	0
5	7	4 (57.1)	1 (14.3)	0	2 (28.6)
6	8	5 (62.5)	1 (12.5)	2 (25)	0
7	7	7 (100)	0	0	0
8	7	5 (71.4)	0	2 (28.6)	0
9	4	3 (75 %)	1 (25.0)	0	0
10	7	5 (71.4)	1 (14.3)	0	1 (14.3)
11	6	5 (83.3)	0	1 (16.7)	0
Total	75	55 (73.3)	8 (10.7)	9 (12.0)	3 (4.0)

(10.4 ± 0.9%), good (9.1 ± 0.2%), and fair (8.7 ± 0.1%) serum Brix values with an overall value of 10 ± 1%. In all cases, the differences with the cut-off level of 8.5% were highly significant (P<0.001; P<0.002 for the fair category). Serum Brix categories of excellent, good and fair (Godden *et al*., 2019) were found respectively in 73.3, 10.7, and 12% of the calves (Table 5).

Bacteriological quality of the colostrum samples

The mean TPC of colostrum for all farms was 3.6 × 10^5 CFU/ml, ranging from 200 to 3.3 × 10^6 (Table 4). Four farms out of 11 (36.4%) and 43 colostrum samples out of 75 (57.3%) met the industry recommendation for TPC of below 10^5 CFU/ml. The mean TCC for all farms was 2.8 × 10^4 CFU/ml ranged from 0 to 2.9 × 10^5 (Table 4). Considering the industry recommendation, 6 farms out of 11 (54.6%) and 50 colostrum samples out of 75 (66.7%) met the standard of 10^4 CFU/ml. The mean Spore-former and Fungi counts were 3.2 × 10^4 and 1.1 × 10^4 CFU/ml, respectively (Table 4).

The results showed that 8/10 of farms (80%) and 30/68 of colostrum samples (44.1%) were positive for *Staphylococcus* spp., and 2/10 of farms (20%) and 4/68 of samples (5.9%) were positive for *Salmonella* spp. The presented data of isolation of *Staphylococci* and *Salmonella* belongs to 10 herds and the data of one herd were missed. There was no evidence of contamination with *Brucella* spp., *Corynebacterium bovis*, and *Mycoplasma bovis*. However, 3/75 of colostrum samples (4%) were positive for MAP (Fig. 1).

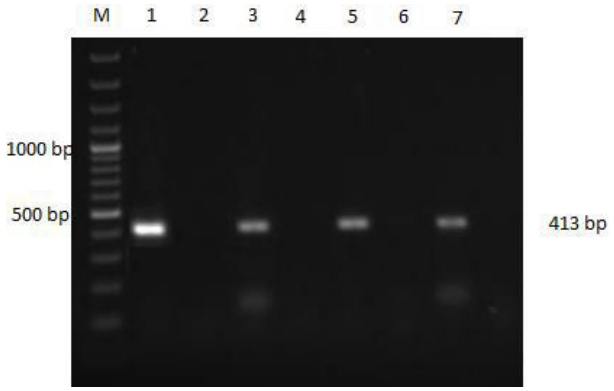

Fig. 1: Gel electrophoresis of IS900 PCR product from three MAP positive colostrum samples. Lane M: 100 bp DNA ladder. Lanes 1, 3, and 5: Positive samples, Lane 2: Negative control (no template), Lane 7: Positive control, and Lanes 4 and 6: Negative samples

Correlations

There was a negative correlation between colostrum volume and its Brix score (r=0.353; P=0.005). A positive correlation (r=0.323; P=0.01) was detected between colostrum volume and parity. There was a significant positive correlation (r=0.388; P=0.002) between the volume of colostrum consumed in the first 6 h after birth and the serum Brix score of calves. Such a correlation was not detected for the first meal colostrum (r=0.218; P=0.087).

Positive correlations were detected between TPC and TCC (r=0.356; P=0.002), spore-forming bacteria (r=0.385; P=0.001), fungi (r=0.280; P=0.0.015), and the volume of colostrum (r=0.255; P=0.043). The fungal count was correlated with spore forming bacteria (r=0.681; P=0.001), and TCC (r=0.481; P=0.001). TCC and spore forming bacteria were also related (r=0.496; P=0.001). None of the indices of microbial quality were correlated with colostrum or serum Brix values.

Discussion

To our knowledge, this is the first comprehensive study on the quality of fresh colostrum in terms of IgG concentration, microbial contamination, and transfer of passive immunity in Fars province dairies. As the most prominent quality index, the IgG level of colostrum should be at least 50 g/L, (Godden, 2008), equal to a Brix score of ≥22% (Godden *et al*., 2019). Various studies have validated the use of Brix refractometers to estimate IgG levels of colostrum (Bielmann *et al*., 2010; Quigly *et al*., 2013; Bartier *et al*., 2015). In our study, the average Brix reading of colostrum was 25.4%, consistent with Baltrukova *et al*. (2019; 25.04%), Bielmann *et al*. (2010; 26.3%), and Bartier *et al*. (2015; 24.3%), accounting for 70-80 g/L IgG (Quigley *et al*., 2013). However, some fewer values have been reported by Quigley *et al*. (2013; 23.8%), Morrill *et al*. (2015; 21.24%), and Phipps *et al*. (2016; 20.78%). In our study, the highest colostrum IgG level was observed in cows of third parity or more. The lowest IgG level was detected in the second parity, consistent with Gulliksen *et al*. (2008), MacFarlane *et al*. (2015), Reschke *et al*. (2017), and Baltrukova *et al*. (2019). The higher production level in the second parity (versus first parity), may have diluted the increased IgG concentrations that occur with increased age. The increased age-related IgG concentrations may be due to more exposure to antigens during lifetime (Morrill *et al*., 2012). Therefore, the current paradigm that the colostrum of primiparous cows cannot transfer sufficient immunity to the calf may not be valid in all circumstances. In the present study, the Brix score decreased with increased volume of the first milking colostrum, in accordance with Pritchett *et al*. (1991). Baumrucker *et al*. (2010), however, reported no relationship between colostrum IgG_1 concentration and colostrum volume.

The cut-points of ≥22% and ≤18% for Brix reading identify good- and poor-quality colostrum, respectively (Buczinski and Vandeweerd, 2016). The intermediate values are usually not suitable for the first meal unless fortified with colostrum supplements. In this study, the proportions of good-, intermediate-, and low-quality samples were 70.67, 24, and 5.33%, respectively. Therefore, 29.33% of colostrum samples had a Brix score <22%. The good quality colostrum (IgG >50 g/L) has been reported to range from 10 to 90% (Gulliksen *et al*., 2008; Bielmann *et al*., 2010; Morrill *et al*., 2012; Quigley *et al*., 2013; Phipps *et al*., 2016; Denholm *et al*., 2017; Reschke *et al*., 2017; Shivley *et al*., 2018). Such a wide range, resulted from numerous factors (some un-

controllable) necessities the estimation of the colostrum IgG level in the farm.

Radial immunodiffusion assay, the standard procedure for assessing calves' serum IgG levels, is costly and requires equipped laboratories (Weaver *et al*., 2000). Brix refractometers are alternative tools for indirect assessing serum IgG levels and diagnosing FPT (Zakian *et al*., 2018; Godden *et al*., 2019). In this study, the serum Brix score of 8.5% was used as the cut-off point to differentiate IgG levels above or below 10 g/L. Although FPT prevalence of 19 to 43.5% has been reported (Filteau, 2003; Beam, 2009; Vogels, 2013; McFarlane, 2015; Lawrence, 2017; Reschle, 2017; Johnsen, 2019; Stanek, 2019), we found a low prevalence of 4%, consistent with Deelen *et al*. (2014; 4.75% in 5 Canadian dairy farms). Methods of FPT assay and management, source, first feeding time, volume, and IgG level of colostrum and calves' age at sampling (Shivle *et al*., 2018) may cause such variations. The extremely low frequency of FPT in our study might be due to the appropriate management of preparturient cows and quality and volume of colostrum. Although evaluation of colostrum quality was not a management routine in the farms, the mean Brix score of first milking colostrum was 25.4%, approximating 70-80 g/L IgG (Quigley *et al*., 2013). Averagely, the calves received 2.4 L of such colostrum soon after birth and 3.5 L within the first 6 h. Serum Brix categories of poor (<8.5%), fair (8.5-8.8%), good (8.9-9.3%), and excellent (≥9.4%) (Godden *et al*., 2019) were found in 4, 12, 10.67, and 73.33% of calves, respectively. Similarly, Shivley *et al*. (2018) showed that 73.3% of the US calves had excellent passive transfer of immunity (serum IgG >15 g/L with an average of 21.6 g/L).

In this study, the volume of colostrum consumed within the first 6 h of birth was correlated to serum Brix score, in accordance with Reschke *et al*. (2017). Therefore, the total IgG intake and consequently, the serum IgG concentration can be increased by increasing the volume of colostrum fed and/or using colostrum with high IgG concentration. In our study, 2 out of 3 calves with FPT received good quality colostrum (Brix score ≥22%), but at a low volume (1.25 L). On the other hand, 27.8% of calves with good passive transfer of immunity (Serum Brix ≥8.5%) had received poor quality colostrum (Brix score <22%), but at a higher volume (3.89 L). Thus, the volume of colostrum fed in the first 6 h of birth could have a key role in passive transfer of immunity to calves.

Despite the desirable results for colostrum and serum Brix readings, the microbial examination of colostrum samples revealed potential dangers to the calves' health. Qualified colostrum should have TPC $<10^5$ and TCC $<10^4$ CFU/ml at the time of feeding. In our study, 57.3% and 66.7% of colostrum samples met these recommendations, respectively. Similar or better results have been reported for TPC by Fecteau *et al*. (2002; 64.1%), Houser *et al*. (2008; 62%), Morrill *et al*. (2012; 54.8%), and Phipps *et al*. (2016; 58%). Increased milk TPC correlates with poor milking hygiene (Chambers, 2002; Mohebbi-Fani *et al*., 2016). Phipps *et al*. (2016) showed that 94% of colostrum samples had TCC $<10^4$ CFU/ml. The presence of coliforms indicates fecal contamination and/or washing milking equipment with contaminated water (Jayarao *et al*., 2004). In our study, only 37.3% of samples achieved desirable conditions for all TPC, TCC, and Brix score indices. This finding was close to that of Morrill *et al*. (2012; 39.4%) but higher than what was reported by Phipps *et al*. (2016; 23%). This suggests that high proportions of calves may receive low-quality colostrum, which can expose them to infectious agents or potentially impair the transfer of passive immunity.

Staphylococcus spp., the most common bacteria isolated from dairy cows' milk (Wald *et al*., 2019) and the probable cause of delayed mastitis in milking cows (Jayarao *et al*., 2004) was detected in 44.1% of samples. Fecteau *et al*. (2002) showed that 57.7% of colostrum samples were positive for *Staphylococcus* spp. *Salmonella* spp was detected in 5.9% of samples in our study, consistent with Jayarao *et al*. (2004) for bulk milk samples (6.1%). Houser *et al*. (2008) detected *Salmonella* in 15% of colostrum samples. Baltrukova *et al*. (2019) did not detect *Salmonella* spp. in colostrum samples. MAP, the cause of Johne's disease, was detected in 4% of colostrum samples. Streeter *et al*. (1995) reported that 6.3% and 2.4% of cows shed MAP in colostrum and milk, respectively. Sweeney *et al*. (1992) showed that 12% of subclinically infected cows had positive milk cultures.

Spore forming bacteria, resistant to pasteurization and causes of spoilage in raw and pasteurized dairy products (Griffiths *et al*., 1992; Magnusson *et al*., 2007; Gopal *et al*., 2015) were detected to be 3.2×10^4 CFU/ml in colostrum. Contamination of raw milk with *B. cereus* spores is prominent in grazing cows due to the higher risk of teat contamination with soil (Christiansson *et al*., 1999). Contaminated feed may be a source of raw milk contamination with *B. cereus* spores during housing period (Magnusson *et al*., 2007).

In our study, 97.4% of colostrum samples were contaminated with fungi (mean: 1.1×10^4 CFU/ml). Santos *et al*. (2017) from Brazil reported a mean of 39.8 CFU/ml for yeasts in colostrum. While very low yeast contaminations of 0.8% of samples have been reported from Canadian dairy herds (Fecteau *et al*., 2002) and, high fungal contaminations of 46-100% have been reported from Serbia (Pesic-Mikulec *et al*., 2005). Gulbe and Valdovska (2014) showed that 63.1 and 44.2% of milk samples were contaminated with yeasts and molds, respectively. The fungal contamination of raw milk has been reported to be affected by many factors, including the health of dairy cows and the hygienic methods of the milking process (Gulbe and Valdovska, 2014).

We found positive correlations between various microbiological indicators, indicating common source(s) of contamination. Contaminations usually occur during milking, storage and feeding of colostrum and can be influenced by the health of dairy cows and the environment hygienic level (Godden *et al*., 2019).

Microbial transmission occurs routinely from the cow to the milking parlor and then to the milk (Gulbe and Valdovska, 2014). Manure and soil are known sources of bacterial and fungal contamination (Christiansson *et al*., 1999; Jayarao *et al*., 2004). Contaminated feed may also be a source of fungal contamination (Torkar and Vengust, 2008).

In our study, there was no significant difference between the quality of colostrum in small and large farms, consistent with Houser *et al*. (2008). Phipps *et al*. (2016), however, reported less microbial contamination of colostrum samples in large herds compared with small ones.

The frequency of FPT in studied farms was very low, which could be due to the high IgG level in colostrum and proper volume of colostrum fed during the first few hours after birth. However, only 37.3% of fresh colostrum achieved all of the quality indicators including IgG concentration, TPC and TCC. Contaminations with *Staphylococci* were prominent and contaminations with *Salmonella* and MAP also existed. Therefore, a large number of calves may be at risk of receiving poor quality colostrum, which may expose them to a variety of infectious agents early in life with probable early and delayed consequences. However, more studies are needed in herds with different management routines. The effect of bacterial contamination of colostrum on the further performance of the animal needs to be studied. Evaluation of the routines of colostrum collection and management regards to bacterial contamination should also be investigated.

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Conflict of interest

The authors declare that they have no conflict of interest.

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