

PRELIMINARY STUDY OF BOVINE COLOSTRUM QUALITY IN LATVIA

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Abstract

Bovine colostrum is a mammary gland secret which, due to its high immunoglobulin concentration, is necessary for the transfer of passive immunity to the calf, preventing diseases caused by microbial infections in the newborn ruminants. Colostrum, however, may contain pathogens and can be an infection transmitter, affecting morbidity and mortality rates of calves in the farms. Total plate count and immunoglobulin concentration are two main factors affecting colostrum quality, therefore the aim of the study was to analyse Latvian dairy herd colostrum quality.

Colostrum was collected from Holstein Black cows within the first six hours after calving, lactation period of animals ranged from 1st to 4th lactation. Colostrum samples (n=51, 50 mL) were collected from December 2018 to February 2019. Immunoglobulin concentration (n=51) was defined by colostrometer (COLOSTROMETER[™] Biogenics, USA), total solids content by optical refractometer (Model BX, UK). *Staphylococcus* spp. colony-forming unit (CFU) (LVS EN ISO 6888-1+A1:2007), the presence of *Listeria* spp. (LVS EN ISO 11290-1+A1:2007) and *Salmonella* spp. (LVS EN ISO 6579-1:2017) were examined in the colostrum samples (n=20). Despite the high immunoglobulin concentration in the analysed samples, our research findings demonstrate suboptimal colostrum quality received by calves. That indicates the necessity for regular colostrum quality control and better management practise providing on the farm.

Key words: bovine colostrum, immunoglobulins, *Staphylococcus* spp., *Listeria* spp.

Introduction

Bovine colostrum is a mammary gland secret which, due to its high immunoglobulin (Ig) content, is necessary for the transfer of passive immunity to the calf, preventing diseases caused by microbial infections in the newborn ruminants. It is crucial for newborns to ensure adequate immunoglobulin concentration in the blood during the first 12 to 36 hours (Hernández-Castellano *et al.*, 2015). The highest concentration of biologically active compounds in colostrum is collected at the first milking after calving (Hurley & Theil, 2011). According to Sacerdote *et al.* (2013), maximum Ig concentration in colostrum is in the first four hours after parturition, and six hours according to Borad & Singh (2018) data. Latvian researchers' results (Eihvalde, Kairisa, & Zagorska, 2012) confirmed that the lactation period influences immunoglobulin concentration, but the difference was not significant.

Contaminated colostrum, however, may contain pathogens and can be an infectious disease transmitter, affecting morbidity and mortality rates of animals in the farms (Stewart *et al.*, 2005; Morales-delaNuez *et al.*, 2011; Mohammed *et al.*, 2018). Bovine milk contains complex microbiota that affects quality and safety of the product. Total plate count in raw milk from healthy bovine can range from 10³ to 10⁵ CFU mL⁻¹ (Porcellato *et al.*, 2018).

Colostrum microbiological contamination can occur in different ways: milk can be contaminated with commensal bacteria from the teat skin, epithelial lining of teat canal, or via the lactiferous duct while it is being excreted; or due to the contamination of milk during production, collection, processing, handling, distribution and storage (Alegbeleye *et al.*, 2018).

Staphylococcus spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Corynebacterium* spp. and sometimes coliforms, are common bovine commensal bacteria (Alegbeleye *et al.*, 2018; Curone *et al.*, 2018). Some pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, pathogenic *Escherichia coli* (Stewart *et al.*, 2005; Elizondo-Salazar, Jayarao, & Heinrichs, 2010) may be excreted into milk if they are localized in the mammary gland or associated lymph nodes due to systemic disease (Alegbeleye *et al.*, 2018). Total plate count and Ig concentration are two main factors affecting colostrum quality, therefore the aim of the study was to analyse Latvian dairy herd colostrum quality.

Materials and Methods

Colostrum was collected from Holstein Black cows within the first six hours after calving. Analysed animals lactation ranged from 1st to 4th (see Table 1). Before calving (>49 days) 'Ceprevin dry cow' (LTD MSD, Netherlands) was used. Active component of 'Ceprevin dry cow' is cephalonium.

Colostrum samples (n=51, 50 mL) were collected at conventional farm 'X', located in Zemgale, from December 2018 to February 2019. Colostrum samples were collected according to LVS EN ISO 707:2011 Milk and milk products – Guidance on sampling. Samples were used at 20 °C for detection of Ig and total solid content immediately after collection (Baltrukova, Zagorska, & Eihvalde, 2019).

Samples for microbiological analysis immediately after collection were frozen (-19 ± 1 °C, 30 min) and delivered to the laboratory, stored for up to 30 days.

Table 1

The characteristics of analysed bovine colostrum samples

Lactation period	Number of samples	Period
1 st Lactation	21	18.12.2018 – 17.02.2019
2 nd Lactation	15	12.12.2018 – 12.02.2019
3 rd Lactation	10	16.12.2018 – 17.02.2019
4 th Lactation	5	23.12.2018 – 10.02.2019

The research was carried out at the Institute of Food Safety, Animal Health and Environment 'BIOR' at Food and Environmental Microbiology laboratory. Before microbiological tests, samples were removed from freezer, defrosted and homogenized in a water bath (45 ± 2 °C), after that samples preparation followed, according to: LVS EN ISO 6887-1:2017 'Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions' and LVS EN ISO 6887-5:2011 'Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products'.

Colostrometer (COLOSTROMETER™ Biogenics, USA) was used for Ig concentration determination, which is expressed as mg mL⁻¹. Percentage of total solids in colostrum (n=51) was measured using an optical refractometer (Model BX, UK) with a range of 0 to 34% Brix.

In colostrum samples (n=20) *Staphylococcus* spp. CFU mL⁻¹ was examined according to LVS EN ISO 6888-1+A1:2007 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium; medium used for microorganism isolation was B-P agar, MSA (Mannitol Salt Agar), TSA (Tryptic Soy Agar). Presence of *Listeria* spp. was examined according to 11290-1+A1:2007 Microbiology of the food chain – Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. – Part 1: Detection method; medium used for microorganism isolation was Half-Frazer broth, Frazer broth, ALOA (Agar *Listeria* acc. to Ottaviani & Agosti) agar, Blood agar. The presence of *Salmonella* spp. was detected according to LVS EN ISO 6579-1:2017 Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.; medium used for microorganism isolation was BPW (Buffered peptone water), RVS (Rappaport-Vassiliadis

Soy Peptone) broth, MKTTn (Muller-Kauffmann Tetrathionate-Novobiocin) broth, XLD (Xylose Lysine Deoxycholate) agar, Salmonella Chromogenic agar, Nutrient agar.

Isolated cultures (see Table 2) were identified using MALDI-TOF autoflex speed (Bruker, Germany) in Collection of Microorganism Culture of the Laboratory at the Institute of Food Safety, Animal Health and Environment 'BIOR'. For culture identifications Blood agar, Nutrient agar and TSA were used. All mediums in this study were purchased from 'Biolife' (Italy).

Descriptive statistics were used for data analysis, Duncan's test was calculated. The difference was considered statistically significant if $p < 0.05$.

Results and Discussion

Immunoglobulin concentration (mainly IgG and IgM) is considered as one of the most important immune variables (Hernández-Castellano *et al.*, 2015). Ig level in bovine colostrum varies considerably in different studies: 15 – 180 mg mL⁻¹ (Borad & Singh 2018), 1.4 – 204 mg mL⁻¹ (Dunn *et al.*, 2017), 1.8 – 200.2 mg mL⁻¹ (Morrill *et al.*, 2012), 60 – 100 mg mL⁻¹ (Sanchez *et al.*, 2004). According to the findings by Lago *et al.* (2018), high-quality colostrum contains more than 50 mg mL⁻¹ of Ig, providing calves with passive immunity.

In the analysed colostrum samples, Ig concentrations varied from 39 to 150 mg mL⁻¹ (Table 2). In the present study, mean Ig concentration (90.29 ± 3.33 mg mL⁻¹) was similar to the results obtained by Yaylak, Yavuz, & Özkaya (2017) – 91.00 ± 4.48 mg mL⁻¹ and it was higher than reported by Morrill *et al.* (2015) – 72.91 ± 33.53 mg mL⁻¹. Comparing immunoglobulin concentration among lactation period, a significant difference ($p < 0.05$) was established, the highest Ig concentration observed in colostrum obtained from cows after the 3rd calving, all samples contained ≥ 89 mg mL⁻¹ Ig and $\geq 25\%$ Brix. In the current study, the 1st lactation cows had higher Ig level (92.28 ± 4.44 mg mL⁻¹) than the 2nd lactation cows (77.20 ± 5.94 mg mL⁻¹), that corresponds to the results by Yaylak, Yavuz, & Özkaya (2017) – 94.1 ± 6.82 mg mL⁻¹ and 88.03 ± 5.66 mg mL⁻¹, respectively.

Table 2

Ig and total solids concentration in bovine colostrum

Parameter	Quantity of samples, %	mean	SD	minimum	maximum
Mean analysed bovine colostrum sample					
Ig (mg mL ⁻¹)	100.0	90.29 ^b	3.33	39	150
Brix (%)		25.04 ^B	0.48	19	32
1 st Lactation					
Ig (mg mL ⁻¹)	41.2	92.28 ^b	4.44	53	121
Brix (%)		25.29 ^B	0.66	19	32
2 nd Lactation					
Ig (mg mL ⁻¹)	29.4	77.20 ^d	5.94	40	118
Brix (%)		22.80 ^C	0.73	20	30
3 rd Lactation					
Ig (mg mL ⁻¹)	19.6	109.40 ^a	5.82	89	150
Brix (%)		28.60 ^A	0.76	25	32
4 th Lactation					
Ig (mg mL ⁻¹)	9.8	83.00 ^{ac}	12.26	39	112
Brix (%)		23.6 ^C	0.93	21	26

Duncan's test: different letters (a–d, A–C) in the column indicate significant difference among samples ($p < 0.05$).

Total solids content in colostrum varied from 19 to 32%. Mean concentration was $25.04 \pm 0.48\%$, which is higher than reported by Lago *et al.* (2018) – $20.3 \pm 2.9\%$, by Morrill *et al.* (2015) – $21.24\% \pm 4.43\%$, by Quigley *et al.* (2013) – $23.8\% \pm 3.5\%$ and lower than the value reported by Yaylak, Yavuz, & Özkaya (2017) – $26.61 \pm 0.84\%$.

Quigley *et al.* (2013) recommended 21% Brix solids content to be considered as the breakpoint for high-quality bovine colostrum, which corresponds to Ig concentration of ≥ 50 mg mL⁻¹ in colostrum. Morrill *et al.* (2015) specified Ig concentration of ≥ 50 mg mL⁻¹ in colostrum exactly for Holstein Black breed,

analysed in the current research. Based on these recommendations, only in 3.9% colostrum samples Ig concentrations were lower than 50 mg mL⁻¹, but other 96.1% samples contained more than 53 mg mL⁻¹ Ig. In comparison, Lago *et al.* (2018) showed 80% ($n=53$) sample quality conformity, Morrill *et al.* (2012) reported 70.6% ($n=827$) and Dunn *et al.* (2017) – 56% ($n=1239$).

Ig concentration was moderately positively correlated ($r=0.76$) with solids content % Brix (Figure 1). Morrill *et al.* (2015) reported similar results, e.g. $r=0.79$, results by Yaylak, Yavuz, & Özkaya (2017) reported $r=0.70$.

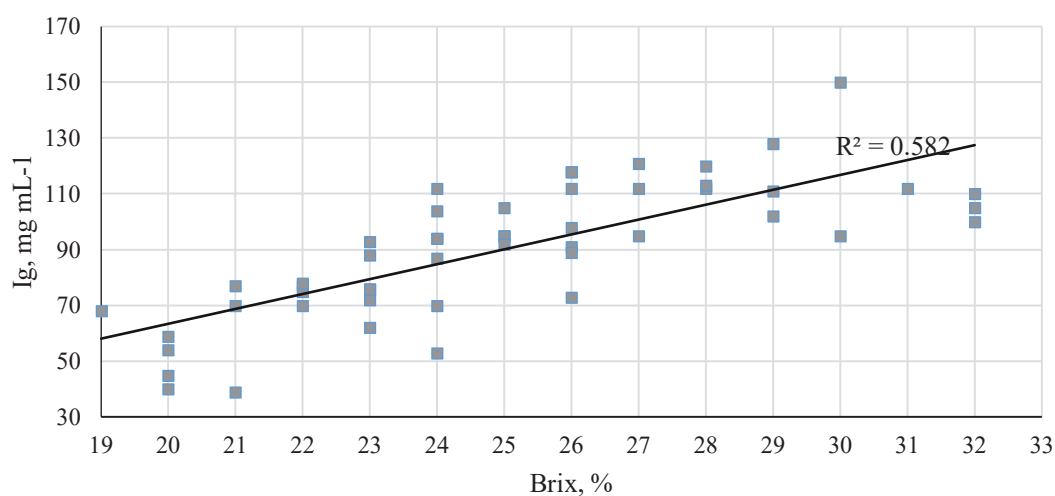


Figure 1. Correlation among solids content % Brix and Ig concentration in colostrum samples ($n=51$).

Table 2

Bacteria species isolated from analysed bovine colostrum samples (n=20)

Microorganisms	Species	Number of samples
<i>Listeria</i> spp.	<i>L. innocua</i>	5
<i>Staphylococcus</i> spp.	<i>S. aureus</i>	1
	<i>S. epidermidis</i>	5
	<i>S. haemolyticus</i>	4
	<i>S. capitis</i>	3
	<i>S. chromogenes</i>	4
	<i>S. cohnii</i>	1
	<i>S. xylosus</i>	1
<i>Enterococcus</i> spp.	<i>E. faecium</i>	4
	<i>E. faecalis</i>	4
<i>Bacillus</i> spp.	<i>B. clausii</i>	1
<i>Macrococcus</i> spp.	<i>M. caseolyticus</i>	1

Microbiological quality of colostrum is a very important factor, which can have a significant effect on calf health. Our previous study showed poor microbiological quality of obtained colostrum due to high total plate count $5.65 \log_{10}$ CFU mL⁻¹ (Baltrukova, Zagorska, & Eihvalde, 2019). Previous studies (Dunn *et al.*, 2017) detected a wide range of pathogens in colostrum, therefore in the current study samples were examined for *Listeria* spp. (*L. monocytogenes*), *Salmonella* spp. presence and *Staphylococcus* spp. count (Table 2). They are considered the most common pathogens in bovine milk, that increase newborn calves mortality rate, as well as, it could be dangerous for humans if raw colostrum has been consumed without thermal treatment (Stewart *et al.*, 2005; Alegbeleye *et al.*, 2018; Mohammed *et al.*, 2018).

In this study, *Salmonella* spp. and *Listeria monocytogenes* presence was not detected in any of the analysed colostrum samples. *Listeria* spp. was isolated from 25% of colostrum samples and identified as *L. innocua*; it is one of the non-pathogenic *Listeria* species, which is common in habitat and may contaminate farm environment, animal feed and water (Osman *et al.*, 2014).

Two *Enterococcus* species were found on Blood agar and TSA: four colostrum samples contained *E. faecium* and the same number of samples contained *E. faecalis*; on Blood Agar *Bacillus clausii* and in TSA *Macrococcus caseolyticus* were detected.

Despite the 'Cepvavin dry cow' use before calving, in the current study coagulase-negative *Staphylococcus* was the most common microorganism in bovine colostrum (Figure 2), which represents 100% of isolated microorganisms. Other studies showed similar results – *Staphylococcus* spp. were among the most frequent of the isolated bacteria in 57.7% (Fecteau *et al.*, 2002) and 47.9%

(Garedew *et al.*, 2015) of cases. Coagulase-negative *Staphylococcus* species such as *S. epidermidis*, *S. haemolyticus*, *S. capitis*, *S. chromogenes*, *S. cohnii*, *S. xylosus* were identified in all analysed colostrum samples. The number of microorganisms varied from 10² to 10⁴ CFU mL⁻¹, and all samples contained more than one *Staphylococcus* specie. The number of microorganisms of the most frequently identified *S. epidermidis* and *S. haemolyticus* species varied from 10² to 10³ CFU mL⁻¹, but the least common *S. cohnii* varied from 10¹ to 10² CFU mL⁻¹, *S. xylosus* below 10¹ CFU mL⁻¹. *S. epidermidis* and *S. haemolyticus* are normal inhabitants of bovine skin and mucous membranes, but their strains show resistance to various antibiotics that may decrease curing effect of cow mastitis (Susan, Obansa, & Anthony, 2014). *S. epidermidis* and *S. aureus*, isolated from milk samples in cows (Da Silva Chagas *et al.*, 2017) and sheep (Vasil *et al.*, 2017) with mastitis, have a role in biofilm production at different surfaces (milking and other equipment). Biofilms are a major form of microbial growth, they are considered to be responsible for the high resistance of microorganisms to sanitizers, allowing pathogenic and spoilage bacteria to survive the sanitization process (Flach *et al.*, 2014).

S. chromogenes was detected in three samples and the numbers ranged from 10¹ to 10⁵ CFU mL⁻¹. One colostrum sample contained *S. aureus* 4.0·10² CFU mL⁻¹ that did not exceed incremental norms (<5.0·10² CFU mL⁻¹). *S. aureus*, *S. chromogenes*, *E. faecalis*, *E. faecium* are drug resistant (Susan, Obansa & Anthonomy, 2014; Garedew *et al.*, 2015) mastitis causative agents, which can be found in colostrum from diseased bovines (Alegbeleye *et al.*, 2018).

Summarizing study results can be concluded that some measures should be applied for improving colostrum quality, as a significant part of identified

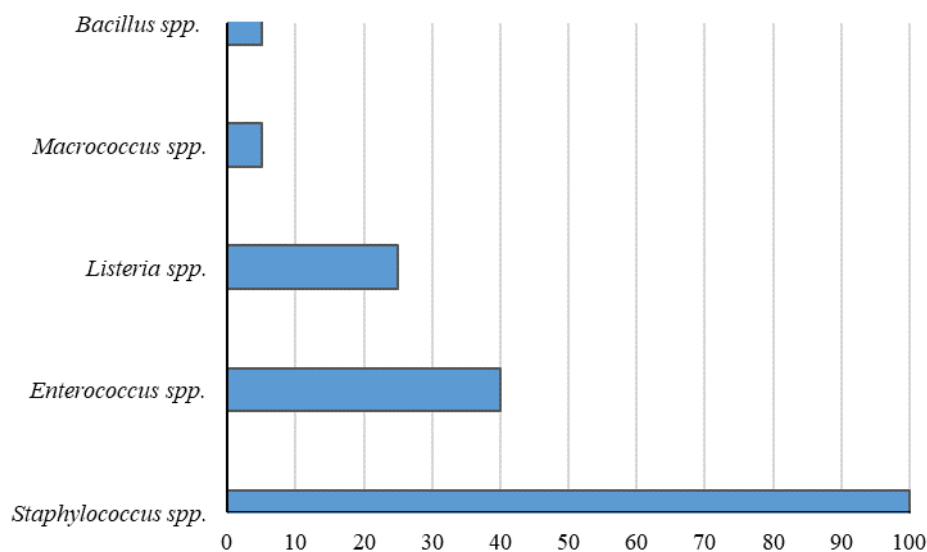


Figure 2. Distribution of isolated microorganism genus in bovine colostrum samples (%).

microorganisms are pathogenic, antibiotic resistant, and biofilm producing species (Susan, Obansa, & Anthonomy, 2014; Da Silva Chagas *et al.*, 2017; Vasil *et al.*, 2017). Enhanced animal health and hygienic conditions on dairy farms can minimize colostrum bacterial contamination (Alegbeleye *et al.*, 2018). One more possible solution for minimizing bacterial contamination of colostrum and decreasing calf mortality rate is heat treatment. The results of a study by Elizondo-Salazar, Jayarao, & Heinrichs (2010) indicate that heat treatment of bovine colostrum at 60 °C for 30 to 60 min may be used as an optimal regime to observe a less denaturation in IgG concentration and a significant decrease in plate count in samples (Elizondo-Salazar, Jayarao, & Heinrichs, 2010). That confirms the study by Malmuthuge *et al.* (2015) which showed that heat treatment of colostrum could serve as an effective method for reducing pathogen exposure to newborn calves. Further researches are needed to obtain high quality colostrum and explaining interaction among immunoglobulins (class, concentration) and specific pathogens. Thermal treatment importance for colostrum quality should be evaluated as well.

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Conclusions

According to Ig concentration and total solids content, analysed colostrum samples belong to high-quality colostrum group.

In the current study improper microbiological quality of analysed colostrum was highlighted, still *Staphylococcus* genus was the most frequently identified in the analysed samples. Pathogen *Staphylococcus* specie – *S. aureus* was determined in one colostrum sample. *L. innocua* was identified in 25% of analysed colostrum samples. *Salmonella* spp. and *L. monocytogenes* were not detected.

Despite high immunoglobulin concentration in the analysed samples, our research findings demonstrate suboptimal colostrum quality received by calves. That indicates the necessity for regular colostrum quality control and better management practise providing on the farm.

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