

Article

Colostrum Management and Its Effects on Bacterial Load, Passive Immunity Transfer, and Neonatal Diarrhea in an Italian Dairy Herd

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Abstract

Effective colostrum management is essential to ensure adequate transfer of passive immunity (TPI) and reduce neonatal morbidity in calves. This study evaluated the impact of pasteurization on colostrum microbiological and immunological quality and its association with TPI and calf health on a dairy farm in Italy. A total of 79 colostrum samples were included: 39 pasteurized (PC) and 40 non-pasteurized (NPC). Immunoglobulin concentration was estimated by Brix refractometry, while bacterial contamination was assessed by total bacterial count (TBC) and total coliform count (TCC). In a second phase of the study, 80 newborn calves received either pasteurized (C-PC, $n = 40$) or non-pasteurized colostrum (C-NPC, $n = 40$). TPI was evaluated at 48 h and calves were clinically monitored for 21 days. Overall, colostrum showed not only high Brix values but also elevated bacterial contamination. Pasteurization significantly reduced the microbial load in colostrum (TBC, 5.7 vs. 2.9 log CFU/mL; TCC 4.6 vs. 1.5 log CFU/mL) ($p < 0.0001$). Brix values differed significantly between PC and NPC colostrum ($p < 0.001$), although the magnitude of the difference was minimal (27.8% in NPC vs. 27.6% in PC), suggesting limited biological relevance. Calves receiving pasteurized colostrum showed significantly lower odds of developing neonatal diarrhea than calves receiving unpasteurized colostrum (C-PC: OR 0.1, 95% CI 0.0–0.3; C-NPC: OR 9.9, 95% CI 3.4–32.6). These findings indicate that colostrum pasteurization reduces contamination effectively even in colostrum with high bacterial loads without substantial impact on its Brix values and was associated with improved calf diarrhea outcomes in the investigated herd.

Keywords: colostrum; transfer of passive immunity; calf; heat treatment; bacteria; neonatal diarrhea



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1. Introduction

The transfer of passive immunity (TPI) is a crucial process in the early life of a calf, playing a vital role in preventing neonatal infections. Because the cow's synepitheliochorial placenta prevents in utero immunoglobulin (Ig) transfer, newborn calves are immunologically naive. Given that calves require 2 to 4 weeks to develop active immunity, their primary source of immune protection during this vulnerable period is the ingestion of colostrum [1]. A serum IgG concentration of less than 1 g/dL traditionally defines Failure

of Passive Transfer (FPT) in calves aged 1 to 7 days [2], although more recent studies suggest that higher cut-offs may be associated with better calf performance [3,4]. FPT, which is a major risk factor for the development of neonatal infections, is caused by the calf's inability to ingest or absorb an adequate amount of colostrum antibodies. Because colostrum IgG concentration is a reliable predictor of serum IgG levels in newborn calves, it is a key indicator for assessing the risk of FPT. A colostrum IgG concentration greater than 50 g/L is widely considered to indicate high-quality colostrum and is associated with a lower risk of FPT [5]. Radial immunodiffusion (RID) remains the gold standard for determining IgG concentrations in colostrum and serum; however, refractometers represent a practical, cost-effective, and reliable indirect method for rapid on-farm assessment. In this regard, several studies have investigated the optimal diagnostic cut-off values of refractometers across different breeds, including dairy cattle [6–8]. Many individual, environmental, and management variables can influence the IgG concentration in colostrum and consequently the transfer of IgG to the calf [9]. Among these, high bacterial contamination of colostrum has been shown to interfere with IgG absorption across the gut, potentially through direct binding [10]. Consequently, fresh colostrum is recommended to contain less than 100,000 CFU/mL of total bacteria count (TBC) and less than 10,000 CFU/mL of total coliform count (TCC) [5]. To achieve these standards, producers are advised to prevent contamination from the moment of harvest to feeding and to minimize bacterial growth during storage. Additionally, colostrum pasteurization is a suggested tool for reducing bacterial load [11]. Despite the knowledge of these optimal management strategies, the economic benefits of feeding pasteurized colostrum are often considered controversial by many farmers. Furthermore, predicting the effects of specific colostrum management choices under different field conditions remains challenging. Indeed, the risk factors for low colostrum quality and the development of FPT may vary considerably depending on the specific breeding and production system [9], making colostrum quality monitoring a critical step for preventing FPT. On this basis, the present study aimed to evaluate the effects of colostrum management and pasteurization on colostrum immunological and microbiological quality, as well as to examine their influence on TPI and on calf health and survival, on an Italian Parmigiano Reggiano production farm.

2. Materials and Methods

2.1. Animals and Management Practices

The study was conducted on a commercial dairy farm located in the Mantua province in northern Italy. The farm rears Holstein–Friesian cattle and produces milk for Parmigiano Reggiano PDO cheese production. At the time of the study, the farm housed 985 cattle, including 510 lactating animals; the number of calves born per year was 550. The feeding program adheres strictly to the Parmigiano Reggiano official specifications, focusing on a diet of natural, unfermented forage from the designated geographical area. A more comprehensive description of the diet can be found in a review article of Mordenti et al. [12]. The herd is subject to a robust vaccination schedule. Primiparous and multiparous cows are vaccinated annually against Bovine Herpesvirus 1 and Bovine Viral Diarrhea Virus. Additionally, 35–40 days before calving, cows are vaccinated against Bovine Parainfluenza-3 virus, Bovine Respiratory Syncytial Virus, *Mannheimia haemolytica*, Bovine Coronavirus, Bovine Rotavirus, and *Escherichia coli*. Calves receive an intranasal vaccine against Bovine Parainfluenza-3 virus and Bovine Respiratory Syncytial Virus within the first 8 days of life. At 3 weeks of age, a subcutaneous vaccine against Bovine Parainfluenza-3 virus, *Mannheimia haemolytica*, and Bovine Respiratory Syncytial Virus is administered, followed by a booster dose at 60 days of age. The farm implements a selective dry cow therapy strategy based on individual animal risk assessment. Cows are eligible for treatment if they exhibit

clinical signs of mastitis and/or have a somatic cell count exceeding 2×10^5 cells/mL (multiparous cows) or 1.5×10^5 cells/mL (primiparous cows) over the course of lactation. Animals meeting these criteria receive an intramammary antibiotic treatment at dry-off, while internal teat sealants and film-forming dip are applied to all cows at dry-off. Far-off and lactating cows are housed in straw-bedded cubicles, whereas pre-partum cows (approximately 20 days before calving) are housed on deep straw bedding. Calves are kept in individual hutches with straw bedding and have ad libitum access to fresh water and pre-weaning concentrate feed. To prevent colostrum suckling from the dam, newborn calves are removed from the calving pen immediately after birth. Cubicles are scraped three times a day for feces removal, while bedding replenishment and application of an alkaline disinfectant are performed twice weekly. In the calving area, the straw bedding is replenished every two days and treated with an alkaline disinfectant. Milking machines are cleaned twice daily with a detergent solution and disinfected twice weekly using nitric acid. The farm maintains a colostrum bank, where colostrum is collected into storage bags using a dedicated filling device and subsequently stored in a chest freezer at -20°C . The filling device, as well as the equipment used for colostrum administration (bottles and esophageal feeding tubes), undergo a routine two-step cleaning procedure consisting of an initial wash with a detergent followed by disinfection with a 30% (*w/w*) hydrogen peroxide solution containing an acetic acid adjuvant. The farm colostrum bank consists of colostrum collected from all cows after calving. Colostrum quality is evaluated on-farm using a Brix refractometer, and samples with Brix values below 20% are excluded from the colostrum bank. Colostrum bags typically contain 4 L of colostrum, a volume consistent with current recommendations for Holstein calves (10–12% of body weight). The bags are randomly allocated to calves receiving colostrum by bottle feeding, except in rare cases in which colostrum is administered via an esophageal tube at a volume equivalent to 10% of the calf's body weight. Colostrum bags were typically stored for a relatively short period (<1 month). Finally, the calf's umbilical cord is routinely disinfected with a 1% povidone-iodine solution immediately after birth. The farm has a history of neonatal diarrhea, with *Escherichia coli* being the most frequently identified agent.

2.2. Sample Size and Power Analysis

An a priori power analysis was conducted to determine the required sample size for evaluating the effect of colostrum pasteurization on the incidence of calf diarrhea. The calculation was based on a two-sided test comparing two independent proportions with a significance level (alpha) of 0.05 and a target statistical power of 80% ($1 - \beta = 0.80$). The baseline calf diarrhea occurrence in the control group (receiving unpasteurized colostrum) was assumed to be 60%. Colostrum pasteurization was hypothesized to achieve a 50% relative reduction in diarrhea occurrence, resulting in a projected incidence of 30% in the treatment group. To detect this difference, a minimum sample size of 79 is required.

2.3. Colostrum Protocols and Sample Collection

The study comprised two distinct experiments.

In experiment 1, a total of 79 mechanically harvested colostrum samples, based on the sample size calculation, were included. Of the 79 samples, 39 were assigned to the pasteurized colostrum group (PC), whereas the remaining 40 were assigned to the non-pasteurized control group (NPC). Allocation was performed by simple randomization using cow identification numbers. An aliquot of each sample was collected and stored at -20°C . Pasteurization was performed at 60°C for 60 min using a ColostroMAT pasteurizer (Förster-Technik GmbH, Engen, Germany). Following pasteurization, an additional aliquot of each treated sample was collected and frozen. All colostrum samples were submitted to

the Laboratory of the Department of Veterinary Medicine, University of Perugia for indirect determination of immunoglobulin concentration by digital (Brix) and optical refractometers and bacterial count.

In experiment 2, the same colostrum samples included in experiment 1 were thawed in a water bath set to 50 °C and randomly administered to 80 newborn calves born between August 2023 and January 2024. Accordingly, calves were assigned to two groups based on the type of colostrum received: the C-NPC group (n = 40), receiving non-pasteurized colostrum, and the C-PC group (n = 40), receiving pasteurized colostrum. At 48 h after feeding, calves were weighed and a 10 mL blood sample was collected via jugular venipuncture into a sterile vacutainer tube without anticoagulant as part of routine farm procedures for the assessment of passive transfer by refractometry. For this purpose, serum was obtained by centrifugation of whole blood at 4000× g for 5 min. All calves underwent a clinical examination every 24 h during the first 21 days of life to monitor the occurrence of diarrhea and mortality.

Data recorded included cow-related variables: parity, dry cow therapy, colostrum yield, and interval between calving and colostrum collection; and calf-related variables: sex, birth weight, breed, volume of colostrum ingested, method of colostrum administration (bottle vs. esophageal tube), interval between birth and first colostrum feeding, and occurrence of diarrhea and mortality in the first 21 days of life.

2.4. Colostrum and Serum Assessment by Refractometers

Colostrum and serum samples were analyzed at room temperature using both the digital refractometer (DR) Palm Abbe n. PA201 (MISCO®, Solon, OH, USA) and an optical refractometer (OR) (Sigma-Aldrich, St. Louis, MO, USA). Prior to each evaluation, the refractometers were calibrated with water and wiped clean. Each sample was first analyzed with the OR to prevent investigator bias. For the OR, one to two drops of the sample were placed on the prism, covered with the glass lid, and the value was read from the line separating the light and dark areas through the viewfinder. For the DR, 300 µL of the sample was placed on the prism. The refractometer then determined the score by transmitting visible light through the sample and recording the index of refraction as a refractometry percentage.

2.5. Bacterial Count

Bacteriological analysis was performed on PC and NPC colostrum samples. For the PC group, aliquots were collected from each sample both before and after pasteurization to assess the change in bacterial burden. Serial dilutions of each colostrum sample were prepared in sterile phosphate-buffered saline (PBS) and cultured using the pour plate method. TBC was determined using Plate Count Agar (Liofilchem, Roseto degli Abruzzi, Italy), and TCC was determined using Violet Red Bile Agar (Liofilchem, Roseto degli Abruzzi, Italy). One mL of each dilution was added to a sterile Petri dish, followed by the addition of molten agar (40–45 °C). The mixture was gently swirled to ensure even distribution and allowed to solidify at room temperature. TBC plates were incubated at 37 °C for 48 h, while TCC plates were incubated at 37 °C for 24 h. Results are expressed as colony forming units per milliliter (CFU/mL).

2.6. Data Analysis

Statistical analysis was performed using JMP 17 software (SAS Institute Inc, Cary, NC, USA). Spearman correlation coefficients (r_s) were calculated to evaluate correlations between the values read by optical and digital refractometers. The correlation was interpreted as previously reported [13,14]: r_s = 0.00–0.30, negligible; 0.30–0.50, low; 0.50–0.70, moderate; 0.70–0.90, high; and 0.90–1.00, very high. The study's primary goal was to evaluate the

effect of colostrum pasteurization on key outcome variables, including colostral TBC, TCC, and calf health (specifically, the incidence of calf diarrhea and mortality). TBC and TCC values were analyzed as continuous data, transforming them on a base-10 logarithmic scale. In addition, the microbial load values obtained were divided into dichotomous categories using a cut-off of 100,000 CFU/mL for TBC (normal TBC vs. high TBC) and 10,000 CFU/mL for TCC (normal TCC vs. high TCC) according to Šlosárková et al. [15]. For TBC, TCC, and Brix, an initial value and a final value were available: the initial value was the one before pasteurization, while the final value corresponded to that after pasteurization for pasteurized colostrum and was equal to the initial value for unpasteurized colostrum. Differences in initial Brix, TBC, and TCC values between the PC and NPC groups were assessed using ANOVA, while differences in the proportions of high TBCs and high TCCs between the PC and NPC groups were assessed using the chi-square test. For the final values, aiming to establish the association between pasteurization and Brix, TBC, and TCC, three mixed linear models were created. Brix, TBC and TCC were used as dependent variables in the respective models, while pasteurization (PC/NPC), month/year of calving, and parity (1, 2, 3+) were used as fixed-effect independent variables; in each model, the initial TBC, TCC, and Brix values were used as covariates and the cow ID as a random effect. Least Squares Means (LSMeans) were calculated for PC and NPC. Pairwise comparisons of the LSMs were performed using Student's t-tests to identify significant differences. For experiment 2, serum Brix values were used as a continuous variable and were classified into categories (Poor, Fair, Good, Excellent) according to Godden et al. [16]. Differences in baseline characteristics between pasteurization groups were assessed using ANOVA for continuous variables (birth weight, volume of colostrum ingested, and time between birth and first feeding) and the chi-square test for categorical variables (sex and colostrum administration method). Least-squares means for calf serum Brix were estimated for calves receiving pasteurized colostrum (PC) and non-pasteurized colostrum (NPC). To this end, a linear regression model was built using colostrum type (PC vs. NPC), time from calving to colostrum administration in min, method of colostrum administration (bottle feeding vs. esophageal tube feeding), birth weight, and volume of colostrum administered as fixed-effect independent variables. To assess the association between colostrum pasteurization and the odds of developing diarrhea or death, two logistic regression models were built using Diarrhea (Yes/No) and Death (Yes/No) as binomial dependent variables and the same independent variables used for the serum Brix model. Results from the linear models were reported as least-squares means with corresponding 95% confidence intervals, whereas results from the logistic regression models were reported as odds ratios with corresponding 95% confidence intervals. Statistical significance was set at $p < 0.05$ and trends toward significance at $p < 0.10$.

3. Results

3.1. Effects of Colostrum Pasteurization on Colostrum Brix and Bacterial Count

Descriptive statistics are reported for both colostrum donor cows and recipient calves (Supplementary material, Tables S1 and S2). Since the correlation between the optical and digital (Brix) refractometers was found to be very high ($r_s = 0.990$ for colostrum; $r_s = 0.973$ for serum), only Brix values were used for statistical analyses.

The colostrum dataset included 79 donor cows, 17 primiparous cows, 20 parity cows, and 42 cows in their third or greater lactation, with calving dates ranging from August 2023 to January 2024. Thirty-four cows (43.0%) received intramammary antibiotic treatment at dry-off, whereas forty-five cows (57.0%) did not. Colostrum yield at first milking ranged from 3.0 to 12.0 L, with an average value of 5.4 ± 2 L. Primiparous cows produced 4.8 ± 1.5 L of colostrum, with 29.4% producing less than 4 L (typical volume of colostrum

recommended for a newborn calf). Cows in their second lactation produced 5.6 ± 1.9 L, of which 5.0% produced less than 4 L. Cows in their third or greater lactation produced 5.5 ± 2.2 L of colostrum, with 23.8% yielding less than 4 L. Colostrum was collected at a mean of 202 ± 148 min after calving (median = 180 min). The interval between calving and colostrum collection varied widely, ranging from 30 min to 20.0 h, with most samples collected within the first 6 h postpartum. Early collection (<6 h) was observed in a large proportion of cows, consistent with recommended colostrum management practices, although delayed collection occurred in several cases. No significant differences were observed between the experimental groups PC and NPC with respect to parity, calving month, percentage of cows treated with antibiotics at dry-off, and the time interval between calving and milking.

To evaluate the effect of pasteurization on colostrum Brix, TBC, and TCC, samples from the PC and NPC groups were compared. Supplementary Tables S3 and S4 report the results for Brix and TBC/TCC, respectively. Table 1 reports the initial values for Brix, TBC, and TCC, as well as the proportion of colostrum samples with high TBC (>100,000 CFU/mL) and high TCC (>10,000 CFU/mL) in both groups. Apart from the proportion of samples with initial high TCC, which was higher in PC (79.5%) than in NPC (62.5%), the two groups were otherwise comparable at baseline. To estimate the effect of heat treatment on these parameters, linear mixed-effects models were fitted using normalized values based on the initial measurements. Figure 1a–c presents the least-squares means, 95% confidence intervals, and statistical significance for PC and NPC derived from linear mixed-effects regression models including pasteurization, month/year of calving, and parity. Pasteurization significantly reduced the microbial load in colostrum ($p < 0.0001$). TBC was $5.7 \log \text{CFU/mL}$ (95% CI: 5.0–6.3) in NPC and $2.9 \log \text{CFU/mL}$ (95% CI: 2.2–3.5) in PC, whereas TCC was $4.6 \log \text{CFU/mL}$ (95% CI: 3.8–5.3) in NPC and $1.5 \log \text{CFU/mL}$ (95% CI: 0.8–2.3) in PC. Following heat treatment, all samples showed microbial contamination below the established cut-off values. Brix least-squares-means differed significantly between NPC and PC colostrum (27.8% vs. 27.6%, respectively; $p = 0.001$); however, the magnitude of the difference was very small, suggesting limited biological relevance.

Table 1. Initial Brix values, total bacterial count (TBC), total coliform count (TCC) (mean $\pm$ standard deviation [SD]) and percentages of samples exceeding 100,000 CFU/mL for TBC and 10,000 CFU/mL for TCC in colostrum designated for pasteurization (PC) (pre-pasteurization values) and non-pasteurized colostrum (NPC). p values refer to comparisons between the two groups using ANOVA, while differences in the proportions of samples with high TBC and high TCC between PC and NPC were determined using the chi-square test.

	NPC	PC	p
Colostrum Brix (% $\pm$ SD)	28.3 ± 3.2	27.5 ± 2.9	0.23
TBC (log CFU/mL $\pm$ SD)	5.8 ± 0.9	6.0 ± 0.9	0.37
High TBC (%)	85.0% (n = 34)	84.6% (n = 33)	0.25
TCC (log CFU/mL $\pm$ SD)	4.4 ± 1.0	4.7 ± 1.2	0.96
High TCC (%)	62.5% (n = 25)	79.5% (n = 31)	0.09

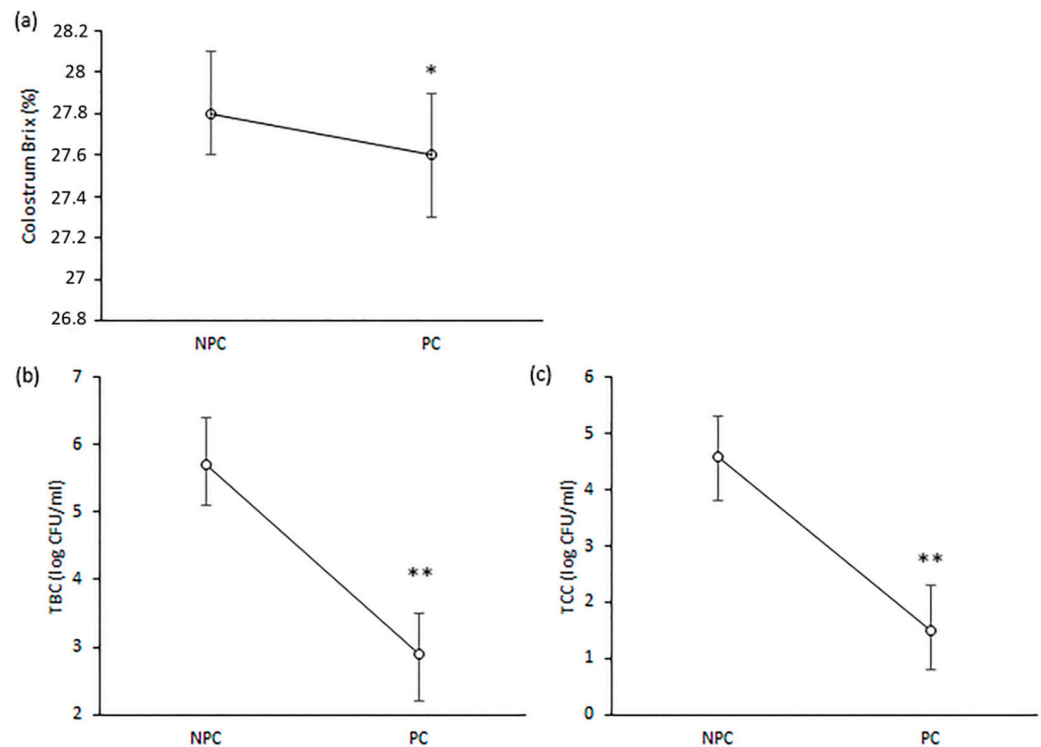


Figure 1. Least-squares means for colostrum Brix (a), total bacterial count (TBC) (b), and total coliform count (TCC) (c), with 95% confidence intervals, for pasteurized (PC) and non-pasteurized (NPC) colostrum, estimated from three linear mixed-effects models. Symbols: * $p < 0.01$; ** $p < 0.001$.

3.2. Influence of Colostrum Pasteurization on Passive Transfer of Immunity

Experiment 2 included 80 recipient calves, consisting of 37 males (46.3%) and 43 females (53.8%). Calves that were fed pasteurized colostrum included 22 females and 18 males, whereas those that received non-pasteurized colostrum included 21 females and 19 males. Birth weight ranged from 28 to 56 kg, with most calves weighing between 35 and 45 kg, indicating a relatively homogeneous neonatal population. Average birth weight was 40.4 ± 4.6 kg for females and 41.8 ± 5.4 kg for males. There were no significant differences in calf birth weight according to colostrum type. The volume of colostrum fed ranged from 3.0 to 4.1 L, with the same mean value (3.8 ± 0.3 L) in both the pasteurized and non-pasteurized colostrum groups. Colostrum was administered via an esophageal tube in 15% ($n = 6$) of cases for pasteurized colostrum and in 7.5% ($n = 3$) of cases for non-pasteurized colostrum. Colostrum was administered 226 ± 86 min after birth, with 94% of calves receiving colostrum within the first 6 h of life and no significant differences according to colostrum type. No significant differences were observed between the experimental groups C-PC and C-NPC with respect to sex, birth weight, breed, volume of colostrum ingested, method of administration (bottle vs. esophageal tube), and interval from birth to colostrum intake. Table 2 summarizes calf data by colostrum type.

The influence of colostrum pasteurization on TPI and calf health was evaluated. Based on serum Brix levels, calves were categorized as follows: in the C-PC group, 0.0% (0/40) poor, 15.0% (6/40) fair, 2.5% (1/40) good, and 82.5% (33/40) excellent; in the C-NPC group, 0.0% (0/40) poor, 10.0% (4/40) fair, 2.5% (1/40) good, and 87.5% (35/40) excellent, suggesting a minimal impact of the thermal process on serum indicators of passive transfer. This result was further supported by the multivariable linear model, which showed no statistically significant associations with any of the independent variables, including pasteurization (Table 3).

Table 2. Characteristics of calves and colostrum feeding according to the type of colostrum.

	C-PC		C-NPC		<i>p</i>
	F	M	F	M	
Sex (n.)	22	18	21	19	0.82
Birth weight (mean $\pm$ SD)	41.3 $\pm$ 5.5		40.8 $\pm$ 4.6		0.64
Volume of colostrum ingested (mean $\pm$ SD)	3.8 $\pm$ 0.3		3.8 $\pm$ 0.3		0.80
Minutes between birth and first feeding (mean $\pm$ SD)	232 $\pm$ 85		220 $\pm$ 88		0.55
Administration with esophageal tube (% , n.)	15% (6/40)		7.5% (3/40)		0.48

Table 3. Least-squares mean serum Brix values and 95% confidence intervals (CIs) for calves receiving non-pasteurized (C-NPC) or pasteurized colostrum (C-PC), along with serum Brix category, risk of diarrhea and mortality during the first 21 days of life according to the type of colostrum administered, and corresponding odds ratios (ORs) with 95% confidence intervals and significance levels.

	C-NPC	C-PC	<i>p</i>
Serum Brix (least squares mean, 95% CI)	10.7 (10.3–11.2)	10.6 (10.2–11.0)	0.66
Serum Brix categories (Poor, Fair, Good, Excellent) (%)	0.0; 10.0; 2.5; 87.5	0.0; 15.0; 2.5; 82.5	
Risk of diarrhea (OR, 95% CI)	9.9 (3.4–32.6)	Referent	0.001
Risk of death (OR, 95% CI)	2.14 (0.18–51.5)	Referent	0.55

Nonetheless, a significant difference was observed regarding calf health. In particular, calves receiving pasteurized colostrum showed a lower incidence of neonatal diarrhea (6/40, 15.0%) compared with calves receiving unpasteurized colostrum (25/40, 62.5%; $p = 0.001$). Logistic regression analysis confirmed a significant association between colostrum treatment and the occurrence of neonatal diarrhea. Calves receiving pasteurized colostrum had substantially lower odds of developing neonatal diarrhea than calves receiving unpasteurized colostrum (Table 3). Mortality did not differ significantly between the two groups; however, it was numerically lower in the C-PC group, with 1/40 calves (2.5%) in the pasteurized-colostrum group and 2/40 calves (5.0%) in the unpasteurized-colostrum group. These findings are summarized in Table 3.

4. Discussion

This study investigated the effects of colostrum handling and pasteurization on its immunological properties and microbiological profile, and evaluated their impact on TPI, as well as on calf health and survival, in an Italian dairy farm producing milk for Parmigiano Reggiano cheese. To our knowledge, this is the first study conducted in Italy addressing this topic. The study describes a farm scenario characterized not only by adequate colostrum Brix values, but concurrently high levels of bacterial contamination as well. Regarding the immunological characteristics of the analyzed colostrum samples, overall colostrum quality on the investigated farm was high, with a mean refractometry value of $27.9 \pm 3.1\%$ Brix. This value was even higher than the mean colostrum quality reported for samples collected from 25 dairy farms in Northern Italy ($24.3 \pm 4.41\%$ Brix), highlighting that, even within the same production area, colostrum quality may vary depending on farm-specific management practices [17]. As a general guideline, a Brix value above 22% is commonly

considered indicative of high-quality colostrum [18]. This result was not unexpected, as the evaluation of the herd management practices indicated that most colostrum donor cows were managed according to recommended standards in the study farm.

Despite the implementation of appropriate management practices and generally satisfactory hygienic conditions, elevated levels of bacterial contamination were observed in raw colostrum. This finding suggests the presence of critical deficiencies in colostrum handling protocols, which might be attributable to a lack of compliance with the milking machine cleaning procedures. Nevertheless, heat treatment at 60 °C for 60 min proved to be an effective intervention, yielding a statistically significant reduction in both TBC and TCC. This thermal process consistently reduced microbial loads to well below recommended thresholds, even in samples characterized by high initial contamination levels. At the same time, pasteurization was associated with a slight decrease in colostrum Brix values, used here as an indirect estimate of IgG concentration. These findings are consistent with previous studies reporting minimal loss of IgG following similar pasteurization protocols [11]. In contrast, exposure to higher temperatures may negatively affect IgG concentration [19]. These findings are in strong agreement with previous work demonstrating that on-farm pasteurization can reduce bacterial contamination without compromising colostrum quality [20,21]. Bacterial contamination of colostrum has been shown to impair IgG absorption through direct binding or competition for intestinal receptors [11], thereby potentially influencing calf health [22]. Additionally, unpasteurized colostrum may serve as a source of fecal enteropathogens and may also harbor antibiotic resistance determinants, as demonstrated for fecal extended-spectrum β -lactamase (ESBL)- and AmpC-producing *Escherichia coli* [23]. A previous field study reported that calves fed colostrum treated at 60 °C for 60 min, as in the present study, exhibited significantly higher serum IgG concentrations at 24 h after birth than calves fed raw colostrum, presumably as a result of a reduced bacterial load, thereby minimizing interference with the intestinal absorption of antibodies [24]. Conversely, in the present study, serum Brix values did not differ between calves fed pasteurized versus unpasteurized colostrum. Nevertheless, the incidence of neonatal diarrhea was markedly lower in calves receiving pasteurized colostrum, suggesting that the health benefits of colostrum pasteurization may extend beyond TPI alone.

Previous studies have consistently reported comparable reductions in morbidity among calves fed pasteurized colostrum, even in the absence of significant differences in serum IgG concentrations [20,21]. Collectively, these findings support the hypothesis that colostrum pasteurization lowers pathogen load to a degree sufficient to reduce disease risk, thereby enhancing calf health independently of the efficiency of IgG absorption. However, because no fecal pathogen identification was performed, a direct causal relationship between colostrum pasteurization and reduced disease occurrence cannot be established. According to the farm anamnesis, neonatal diarrhea had historically been reported as a recurrent issue, with *Escherichia coli* reported as the most common etiological agent. In this context, the marked reduction in coliform counts achieved through pasteurization may represent a biologically plausible factor contributing to the lower incidence of diarrhea.

The lack of association between bacterial contamination and serum Brix may be explained by the high colostrum Brix values observed in this farm, which could have mitigated the effects of contamination through antigen binding. This hypothesis is supported by previous studies [25]. It is important to emphasize that the C-PC and C-NPC groups were comparable with respect to variables known to influence serum Brix values, including colostrum volume administered, method of administration, and timing of colostrum feeding. However, a limitation of the present study is that passive transfer status was assessed exclusively by refractometry, an indirect method that, although widely used and validated under field conditions including in Italian Holstein dairy herds [6,26,27], does not

provide the analytical accuracy of RID, the reference method for serum IgG quantification. Furthermore, the high proportion of calves achieving excellent passive transfer may have reduced the ability to detect subtle differences between treatment groups. Therefore, the lack of differences observed between calves fed pasteurized and unpasteurized colostrum should be interpreted with caution.

The majority of calves in the present study received approximately 4 L of colostrum, which is consistent with the recommended volume for Holstein calves (10–12% of body weight) [16]. In contrast, Pavesi et al. reported a mean colostrum intake of 2.74 L in Holstein calves in Northern Italy [17], further highlighting variability in colostrum management practices among geographically associated farms with the same production orientation. The standardized feeding protocol observed in this study likely minimized potential differences attributable to the method of administration, which has previously been reported to affect serum IgG concentrations. In particular, some studies have suggested that esophageal tube feeding may reduce IgG absorption efficiency compared with bottle feeding [28]. However, this effect has primarily been observed when relatively small volumes of colostrum (e.g., 1.5 L) were administered. Conversely, when adequate volumes of high-quality colostrum (≥ 3 L) are provided, the method of administration appears to be of secondary importance [28].

The mean timing of colostrum ingestion did not differ substantially between the two groups. It is well established that intestinal permeability to immunoglobulins declines rapidly after birth due to the physiological process of gut closure, which typically begins approximately 4 h postpartum [29]. Therefore, administration of colostrum within the first 2–3 h of life is recommended to maximize IgG absorption, although national guidelines establish 6 h after birth as the maximum recommended time frame for colostrum intake in calves (Italian legislative decree No. 126 of 7 July 2011). Although a subset of calves was fed colostrum more than 4 h after birth, both groups exhibited a high proportion of calves classified as having excellent passive transfer based on serum Brix values (82.5% in the C-PC group and 87.5% in the C-NPC group) (Table 3).

The statistical analysis did not account for the potential effects of freezing on the immunological and microbiological characteristics of the colostrum. Previous studies have reported that freezing may reduce colostrum Brix values by 4.1–6.2% compared with fresh colostrum. IgG concentrations appear to be affected only after prolonged storage (32 weeks at -20 °C), whereas IgM and IgA concentrations remain largely unchanged. From a microbiological perspective, frozen storage has been shown to decrease colostrum TCC after 4 to 52 weeks of storage, while TBC appears unaffected for up to one year [30]. Because colostrum storage on the study farm was limited to a short period (<1 month), it is unlikely that freezing had a substantial impact on Brix values, TBC, or TCC. Furthermore, all colostrum samples were handled and stored under the same conditions in both the NPC and PC groups, thereby minimizing potential bias and reducing the likelihood that freezing-related effects influenced the observed between-group differences.

Although the findings of the present study may support the use of heat-treated colostrum, no cost–benefit analysis was performed, and the available literature on the economic implications of this practice remains limited. This may represent a barrier to its widespread adoption on commercial dairy farms. Among the few studies addressing this aspect, Jamaluddin et al. reported an economic benefit associated with feeding pasteurized colostrum, which was attributed to reduced calf mortality and a lower incidence of health disorders [31]. These findings appear to support the implementation of colostrum pasteurization under conditions similar to those of the present study. Nevertheless, additional research is warranted to evaluate its economic sustainability across different herd sizes and management systems before broad recommendations can be made.

5. Conclusions

On-farm colostrum pasteurization may represent an effective management strategy to improve microbiological safety and reduce early-life morbidity, especially in herds such as the one included in the present study where colostrum has adequate immunoglobulin concentrations but exhibited high bacterial contamination. The observed association between colostrum pasteurization and reduced diarrhea incidence may suggest a possible beneficial effect on calf welfare, with potential implications for reducing the need for treatments. However, the results of the present study should be interpreted in the context of its design, as data were derived from a single commercial dairy farm. Given that management practices, hygienic conditions, colostrum handling procedures, and feeding strategies may differ among farms, the observed effects of colostrum pasteurization may not be fully generalizable across different production settings. Further multicenter studies are needed to validate these findings and to more comprehensively assess the cost–benefit profile and long-term impacts of colostrum pasteurization on calf health.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/dairy7040055/s1>. Table S1: Characteristics of colostrum donor cows; Table S2: Characteristics of receiving calves; Table S3: Evaluation of colostrum quality and calf serum by optical and digital refractometers; Table S4: Total Bacterial Count (TBC) and Total Coliform Count (TCC) in untreated and heat-treated colostrum samples.

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Informed Consent Statement: Informed consent was obtained from the owner for the use of the data collected on the farm.

Data Availability Statement: Data is contained within the article or Supplementary Material.

Conflicts of Interest: Author Carra S. declares a personal relationship with the owner of the farm where the study was conducted (family relationship). This relationship did not influence the study design, data collection, analysis, or interpretation of the results. Author Guadagnini M. is an employee of Axiota Animal Health and declares no conflicts of interest.

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