The Microbe 9 (2025) 100605

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

Milk quality in a traditional buffalo milk production chain in Bangladesh

Salma Chowdhury [a,b,*,1], Shuvo Singha [b], Md. Mizanur Rahman [b,c], Sanjib Chandra Nath [b,d], Ovirup Bhushan Paul [b,e], Mohammad Abdul Mannan [b], Md. Ahasanul Hoque [b,c,h], Ylva Persson [b,f], Sofia Boqvist [b,g], Fabrizio Ceciliani [b,i]

[a] Department of Industrial Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh
[b] Participating member, Udder Health Bangladesh, Bangladesh
[c] Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh
[d] Global Health Development, Eastern Mediterranean Public Health Network, Amman, Jordan
[e] Buffalo Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka 1341, Bangladesh
[f] National Veterinary Agency, Uppsala 751 89, Sweden
[g] Department of Animal Biosciences, The Swedish University of Agricultural Sciences, Uppsala 750 07, Sweden
[h] One Health Institute, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh
[i] Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Via dell'Università 6, Lodi 26900, Italy

ARTICLE INFO

Keywords:
Bulk milk
Somatic cell
Bacterial counts
Correlation
Quality

ABSTRACT

The quality of milk is influenced by the microbial load related to the shelf life of milk and its spoilage. The water buffalo milk supply is a vital asset for the dairy chain in Bangladesh. However, no systematic study has been conducted to assess the quality of water buffalo milk. This cross-sectional study aims to cover this gap by measuring milk quality indicators such as the bulk milk somatic cell count (BMSCC), bacterial counts, and their correlations from farm to collection center along the milk supply chain. One hundred and thirty-two milk samples were collected from three different nodes of the milk supply chain: at the producer level from farms (n = 45), from middlemen (n = 42), and from collection centers (n = 45). Bulk milk somatic cell counts at the farm and different bacterial counts such as the total bacterial count (TBC), total *Staphylococcus aureus* count (TSA), total non-aureus *Staphylococcal* count (TNAS), total *Enterobacteriaceae* count (TEC) along the milk chain were determined. The geometric mean of BMSCC was 4.1×10^5 (95 % CI: 3.4×10^5 - 4.8×10^5) cells/ mL at the farm level. An increase in TBC, TSA, TNAS and TEC were observed in the milk collection center. TBC, TNAS and TEC were significantly increased ($P \leq 0.01$) along the milk supply chain, except TSA. Significant positive correlations for TNAS with TBC ($r = 0.55$, $P \leq 0.001$) and TNAS with BMSCC ($r = 0.35$, $P \leq 0.05$) at farm level; TEC with TBC ($r = 0.40$, $P \leq 0.05$) at middlemen; TNAS with TBC ($r = 0.31$, $P \leq 0.05$) and TEC with TBC ($r = 0.39$, $P \leq 0.05$) at the collection centers were observed. The study findings highlight the heavy contamination risk, emphasizing public health threat harboring pathogenic bacteria such as *Salmonella, E. coli, S. aureus* linked to foodborne illness. A continuous monitoring system is needed to ensure safe and quality milk.

1. Introduction

Water buffalo holds a strategic place in Asian countries as more than 98 % of the 205 million water buffaloes in the world reside in Asia (FAO, 2025). Water buffalo milk is well known for its high milk quality (Hao et al., 2021), and globally, it is second only to cows' milk in terms of popularity and consumption (Felice et al., 2021). Around 1.52 million buffalo heads reside in Bangladesh (Department of Livestock Services DLS, 2024), mostly are indigenous non-descriptive types where some cross breed with Murrah, Nili-Ravi, Surti and Jaffrabadi are distributed surrounding the Indian borders of Bangladesh (Hamid et al., 2016). Buffaloes are mainly reared by the traditional farming system. Being traditional, the hygienic quality of milk is often suboptimal due to inappropriate milking practices, handling, inadequate storage and

* Corresponding author at: Department of Industrial Biotechnology, Chattogram Veterinary and Animal Sciences University, Chattogram 4225, Bangladesh.
E-mail addresses: salma.chy@cvasu.ac.bd (S. Chowdhury), shuvosingha702@gmail.com (S. Singha), mizanuhcp@cvasu.ac.bd (Md.M. Rahman), sanjibcn@gmail.com (S.C. Nath), ovirup@blri.gov.bd (O.B. Paul), shmannan1997@gmail.com (M.A. Mannan), md.hoque@my.jcu.edu.au (Md.A. Hoque), ylva.persson@sva.se (Y. Persson), Sofia.Boqvist@slu.se (S. Boqvist), fabrizio.ceciliani@unimi.it (F. Ceciliani).
[1] https://orcid.org/0000-0001-9739-6942

https://doi.org/10.1016/j.microb.2025.100605
Received 26 April 2025; Received in revised form 4 October 2025; Accepted 31 October 2025
Available online 1 November 2025

cooling facilities, and transportation along the supply chain (Singha et al., 2023a, 2021a). Unlike milk, buffalo meat contributes about 0.94 % in Bangladesh. The popularity of meat is growing for its low cholesterol level and high quality. However, a large number of buffaloes are sold in the market in disguise of beef in a lower price in Bangladesh (Hamid et al., 2016).

Bulk milk somatic cell count (BMSCC) is a strong indicator and threshold for milk quality, and strong positive relationship was demonstrated between somatic cell count (SCC) and mastitis in different species like cattle, sheep, goat and buffalo (Moroni et al., 2006; Smistad et al., 2021; Bobbo et al., 2017; Özenc et al., 2011). The SCC increases mainly in response to intramammary infection (IMI) in the udder (Zecconi et al., 2019). Elevated SCC levels in milk can adversely affect its quality and safety, leading to reduced milk yield and decreased shelf-life of milk and its derived products (Troendle et al., 2017; Ma et al., 2000). Moreover, elevated bulk milk SCC may compromise the shelf life of pasteurized milk by increasing levels of heat-stable proteases and lipases originating from the animal with increasing milk SCC (Barbano et al., 2006). According to EU Regulation no. 853/2004 (European Union, 2004), for bulk milk, the SCC limit of 400,000 cells/mL has been determined solely for cow milk. In Italy, according to decree 9 may, 1991 n.185 regulation concerning hygiene requirements for raw milk intended for use in the production of high-quality pasteurized fresh milk, the raw milk must meet the following criteria such as bacterial count at + 30 °C (per mL): not more than 100,000 CFU/mL and SCC not more than 300,000 cells/mL, while different values are accepted in Canada 500,000 cells/mL (Barkema et al., 2013), and USA (750,000 cells/mL) (Norman et al., 2009). However, some states in the USA have recently lowered their state limits as significant variation in average BMSCC is observed across different states, and many dairy processors have already imposed stricter standards on their producers (Norman et al., 2011). Moreover, the average SCC for the herd participating in the dairy Herd Improvement Program in the USA declined to 200,000 cells/mL by 2012. Currently, there are no systematic assessments of BMSCC, penalties, or premiums depending on the level of SCC in bulk milk in the dairy industry of Bangladesh. But the level of informal awareness about milk quality, such as avoid milk from visible mastitis animal and reject abnormal milk, are often practiced by the buffalo farmers. However, it can become an indicator of quality milk in the future.

Together with the SCC, other bacterial indicators including Total Bacterial Count (TBC), total *Staphylococcus aureus* count (TSA), total non-aureus *Staphylococcal* count (TNAS), total *Enterobacteriaceae* count (TEC), are important when ranking the hygienic quality of milk (Anderson et al., 2011; Berhe et al., 2020). TBC is an internationally accepted microbial indicator for milk quality and is widely used for grading milk to ensure safe consumption and processing of dairy products (Wang et al., 2023). An increase in TBC is related to environmental contamination, such as unclean milking equipment, unclean udder and teats, and IMI-causing organisms (Bava et al., 2011; Pantoja et al., 2009). It is often associated with increased enzyme activity that can result in textural and flavor defects in milk and processed dairy products (Murphy et al., 2016). The acceptable limit for TBC in Bangladesh is $< 2 \times 10^4$ per mL of milk (Bangladesh Standards and Testing Institute (BSTI), 1009:1982). But its enforcement in local markets, particularly for raw buffalo milk or informal milk chain is limited. Similarly, *Staphylococcal* contamination can occur from raw milk produced by milked cows suffering from mastitis, food handlers, or poor hygiene practices. In contrast, *Enterobacteriaceae* indicates direct or indirect fecal contamination of food (O'Brien et al., 2009). Moreover, buffalo milk naturally carries different organisms. In a recent study in India on microbial diversity of milk revealed the abundance of *Staphylococcus, Enterococcus, Escherichia, Streptococcus*, *Lactococcus* spp. in clinical, subclinical and healthy milk samples of Jafarabadi buffalo (Patel et al., 2019). In Pakistan, study on Nili Ravi buffalo reported the high prevalence of *Staphylococcus* in both healthy and mastitis buffalo milk along with other dominant bacterial genera such as *Streptococcus, Coryenebacterium, Bacillus, Herbaspirillum* (Salman et al., 2023).

Staphylococcus and *Enterococcus* spp. were also found to be significantly higher in mastitis buffalo milk samples (Li et al., 2024). These reports indicated different organisms that causes foodborne illness may enter the milk chain directly from buffalo. In staphylococcal foodborne illness, enterotoxin produced by *S. aureus* is a major cause of food poisoning which is mainly associated with a form of gastroenteritis, vomiting and diarrhea (Argudín et al., 2010). In case of *E. coli.,* pathogenic *E. coli.* reported to cause illness like diarrhea and abdominal pain in humans (Kashima et al., 2021; Lim et al., 2020).

The above mentioned quality indicators are found correlated in some study findings. Correlation among the BMSCC and other bacterial counts have been reported in the milk of the dairy cows (Pantoja et al., 2009), goats (de Geus et al., 2024; Koop et al., 2010) and sheep (Gonzalo et al., 2006) but not in water buffaloes yet. Moreover, the unique buffalo production system, traditional milking technique with minimum hygiene intervention, and warm climate may influence the somatic cell and bacterial counts in the milk chain. Therefore, investigating the correlation between BMSCC and bacterial counts is critical to understand the udder health and related risk in buffalo milk chain.

A better understanding of the milk BMSCC, bacterial counts, and their relationships would help troubleshoot the milk quality problem. The water buffalo population in Bangladesh is growing. Yet, the value chain remains with limited regulations and checkpoints. The milk safety is critical as there is limited surveillance of microbial contamination in informal milk supply chain. Creating knowledge on quality milk is crucial in assisting farmers to decrease BMSCC and other counts to meet the standards. Assessing their correlation is required to address underlying risk factors. This study aims to estimate the BMSCC, bacterial counts, and their correlations at the buffalo milk supply chain from the farm to collection center/shop level from where the milk reaches directly to the consumer.

2. Materials and methods

2.1. Study area and study design

This cross-sectional study was conducted at Subarnachar upazila in the Noakhali district (22.8246°N, 91.1017°E) of Bangladesh in April 2021. Noakhali district is one of the concentrated buffalo farming areas in the Southeastern part of Bangladesh, where buffalo are mainly reared in bathan (free range) and semi-free range farming system and some scattered households (Habib et al., 2017; Singha et al., 2021b).

In this study, three nodes of the milk supply chain (farms, middlemen, and milk collection centers) were selected. The farm selection mechanism has been detailed in two previous studies in this project (Singha et al., 2023a, 2021b). During sample collection, one hundred and thirty-two milk samples were collected from three different points: farm (n = 45), middlemen (n = 42), and collection center (n = 45).

2.2. Sample collection, preservation, and transportation

Farms were selected by making a comprehensive list of farmers who had at least 3 lactating buffalo with the help of regional livestock officers, local farmers, and owners of the different milk collection centers. After that, a convenient selection procedure was performed by using the list of farmers and considering the buffalo-concentrated areas. Farmers were contacted to identify the farm location and number of lactating buffaloes. The detailed selection procedure was explained in our previous study (Singha et al., 2023b). Middlemen samples were collected within 1 h after collection from the household/ semi-free-range farm, and final mixed bulk milk samples were collected from each collection center/shop approximately 1 h after delivery by farmers or middlemen. No missing data was obtained during sampling.

Bulk milk was thoroughly mixed, and from each farm, middlemen,

and collection center, 10 mL of milk was collected aseptically in a sterile screw-capped falcon tube for bacterial counts. BMSCC was performed immediately after collection. Due to the remote buffalo concentrated study area of the bacteriology lab, the milk samples were stored immediately in the icebox (4°C) and transferred to the freezer (-10 to −15 °C) available in the nearby UVH after each day collection. After that samples were transported to the Udder Health Bangladesh laboratory at Chattogram Veterinary and Animal Sciences University and stored at −20 °C until for further bacterial analysis. Quantification of bacteria was performed 24 hr after the samples were stored. However, previous studies suggest that the temperature might cause a little variation on bacterial counts during long storage condition (Alrabadi, 2015).

2.2.1. Bulk milk somatic cell count

BMSCC was performed at the farm level (n = 45) by using the DeLaval cell counter (DCC) (DeLaval Group, Stockholm, Sweden; Sensitivity 88 % and Specificity 80 %) immediately after collecting the milk samples. The DCC displays the BMSCC results as cells/ mL of milk. Analyses followed manufacturer instructions immediately after bulk milk collection at the farm level. Briefly, a small amount of milk (around 60 μL) was loaded into the cassette and inserted into the DCC. The DCC determines SCC optically on a cassette by staining with a DNA-specific fluorescent reagent. With the help of a digital camera, pictures of the cell nuclei were taken individually, and the SCC results were displayed immediately on the screen within 45 s. The DCC displayed the BMSCC results as cells/ mL milk minutes after inserting the cassette.

2.3. Quantification of bacteria

2.3.1. Total bacteria count

The pour plate technique was carried out following ISO 4833–1 standard (International Organization for Standardization ISO, 2013) described by Singha et al (Singha et al., 2023a). to estimate the TBC. Briefly, milk samples were serially diluted up to 10^{-7} using 0.9 % sterile normal saline. Then, from each dilution, 1 mL aliquots of each of the ten-fold dilutions were mixed with 15–20 mL of molten plate count agar (PCA) (Oxoid Ltd, Basingstoke, Hampshire, UK), and the petri dish was rotated for 5–10 s to mix thoroughly with the media and left to solidify. After that, the plates were incubated aerobically at 30 °C for 72 h. Bacterial counts were made on up to five inoculated dilutions on the plates containing between 30 and 300 colonies, and the last countable dilution was considered as a result and expressed as CFU/ mL by using the following formula: Colony counts in the final countable dilution x dilution factor/ mL of milk × 1.1 = CFU/ mL of the original culture.

2.3.2. Total staphylococcal count

The *Staphylococci* count was determined using the surface plate technique following Viçosa et al (Viçosa et al., 2010). Briefly, samples were serially diluted up to 10^{-5}. For each sample, 0.1 mL was vortexed, and diluted milk/curd samples from each 10-fold dilution of the sample were spread evenly over the solidified Baird-parker agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37 °C for 48 h. Counts were made for *S. aureus* (TSA) and Non-aureus Staphylococcus (TNAS) on the plates with < 300 colonies to enable counting. The counts were converted into CFU/mL by following the formula. Colony count in the final countable dilution x dilution factor/ mL of milk sample= CFU/ mL of the original culture

2.3.3. Total enterobacteriaceae count

Total *Enterobacteriaceae* count was performed using the pour plate technique following 5th ed. NMKL-144 (Nordic Committee on Food Analysis) standards. Briefly, samples were serially diluted up to 10^{-5} dilution. A 1.0 mL vortexed diluted milk/curd sample from each 10-fold dilution was mixed with 10 – 15 mL VRBG agar in a petri dish and rotated for 5–10 s for proper mixing. The agar plate was left to solidify, and an overlay of 5–10 mL VRBG was added. The agar plates were incubated at 37°C for 48 h. Counts were made on up to four inoculated dilutions (10^{-2} - 10^{-5}) on the plates with counts < 300. The last countable dilution was considered and converted into CFU/mL using the formula: Colony counts in the final countable dilution x dilution factor/ mL of milk sample= CFU/ mL of the original culture.

An oxidase test was performed to differentiate the non-Enterobacteriaceae from the Enterobacteriaceae family. Five prominent colonies were randomly selected for an oxidase test while counting bacteria from each agar plate.

2.4. Statistical evaluation

Data collected from different farms and milk nodes and results from bacteriological analyses were entered into the Microsoft Excel spreadsheet for 2013 (MicrosoftCorp., Redmond, WA, USA). Data cleaning, coding, and integrity were thoroughly checked in MS Excel before exporting to STATA-IC-13 (StataCorp, Texas, USA) for statistical analysis. Summary statistics were performed for different bacterial counts (TBC, TSA, TNAS, TEC) for every node of the milk value chain (farm level, middlemen, collection center), and results were expressed as a mean median. Correlation between bacterial counts was performed using the Pearson correlation test to investigate potential correlations between BMSCC and bacterial counts. The P value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Bulk milk somatic cell count

The distribution of BMSCC in studied buffalo farms (n = 45) is presented in Fig. 1. The geometric mean of BMSCC was 4.1×10^5 (95 % CI: 3.5–4.9) cells /mL of milk whereas the arithmetic mean of BMSCC was 4.8×10^5 (95 % CI: 4.0–5.5) cells /mL of milk at the farm level. The median BMSCC and its quartiles are presented in Table 1.

3.2. Bacterial counts along the milk chain

The distribution of bacterial counts along the milk chain is presented in Fig. 2. The tested sample was 100 % positive for TBC (n = 132), 99 % positive for TNAS (n = 91), 54–55 % (n = 72) positive for TEC, and 29–30 % (n = 39) positive for TSA.

The mean TBC was 5.5 $\log_{10}$ CFU per mL (95 % CI: 5.3–5.7) at the farm level which increased to 6.8 $\log_{10}$ CFU/mL (95 % CI: 6.5–7.0) at the collection center. The mean TNAS observed at the farm level was 2.6 $\log_{10}$ CFU/mL (95 % CI: 2.4–2.9) increased to 3.9 $\log_{10}$ CFU/mL (95 % CI: 3.5–4.1) at the collection center. The increasing TEC also observed such as 2.9 $\log_{10}$ CFU/mL (95 % CI: 2.4–3.4) at the farm level increased to 3.9 $\log_{10}$ CFU/mL (95 % CI: 3.5–4.3) at the collection center. Overall bacterial counts showed a significant and progressive increase (P ≤ 0.01) along the milk supply chain with the exception of TSA. Although TSA count also increased from 1.7 $\log_{10}$ CFU/mL (95 % CI: 1.2–2.1) at the farm to 1.8 $\log_{10}$ CFU/mL (95 % CI: 1.4–2.1) at the middlemen and 2.1 $\log_{10}$ CFU/mL (95 % CI: 1.6–2.6) at the collection center, the difference was not statistically significant.

3.3. Pair-wise correlation (r) among the BMSCC and bacterial counts

A higher correlation was observed between TBC and TNAS (r = 0.55; p ≤ 0.001) while BMSCC showed a significant correlation with TNAS (r = 0.35; p ≤ 0.05), Other pairwise combinations of bacterial counts showed weaker correlations. Pair-wise correlation (r) of different bacterial counts at other nodes (farm, middlemen, and collection center) is presented in Table 2.

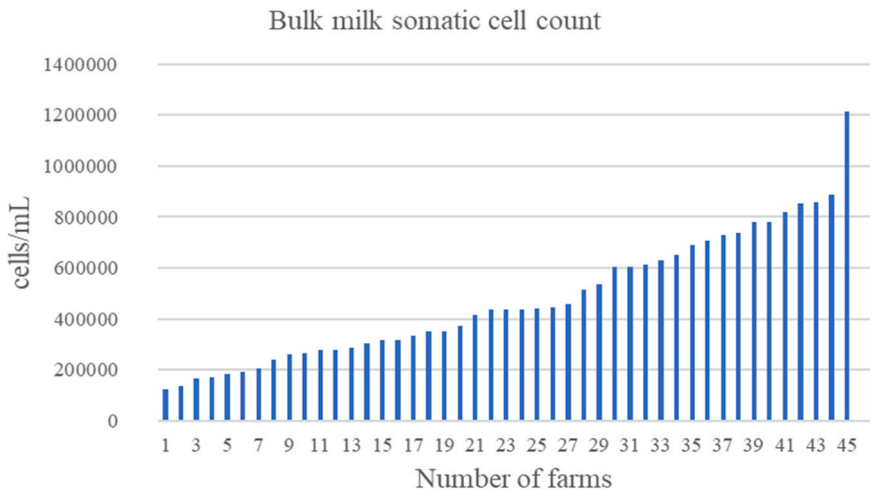

Fig. 1. Distribution of Bulk milk somatic cell count at 45 studied buffalo farms in Noakhali district, Bangladesh.

Table 1
Mean bulk milk somatic cell count obtained from buffalo farms (n = 45) in Noakhali district, Bangladesh.

Unit of measurement	Geometric Mean BMSCC ($\times 10^5$ cells/mL) (95 %CI)	BMSCC ($\times 10^5$ cells/mL)
	4.1 × 10^5 (95 % CI: 3.48–4.88)	
Lowest 25 % farms		2.8
Lowest 50 % farms		4.37
Lowest 75 % farms		6.53

BMSCC = Bulk milk somatic cell count/mL of milk, CI= Confidence Interval

4. Discussions

BMSCC is considered a key indicator of milk quality and udder health. Monitoring the production level is needed to ensure milk quality throughout the chain. The geometric mean of farm BMSCC was 4.1 × 10^5 cells/mL of milk, which was higher than previously reported buffalo studies in Bangladesh (Singha et al., 2023a, 2021b) and other buffalo studies in different countries (Moroni et al., 2006; Dhakal, 2006). High BMSCC in the farm has been reported to be associated with the presence of an IMI (Moroni et al., 2006), and several other factors such as practicing foremilk, milking mastitic cow first, using water to wash teat have also been reported (Tadich et al., 2003). BMSCC is also found associated with milk yield and quality traits (Costa et al., 2020). Moreover, BMSCC than the threshold level may reflect the udder health infection, thermal stress due to hot humid temperature in this region, seasonal based rearing system and poor management practices which may lead to economic loss and reduce milk quality (Singha et al., 2023a). Moreover, IMI is considered to be the most critical factor for increasing BMSCC, suggesting that improving buffalo udder health might reduce BMSCC (Singha et al., 2023a). IMI in buffaloes are mainly caused by *Staphylococcus* spp. such as *S chromogenes, S. hyicus, and S. epidermidis, S. aureus* etc. which may possess antimicrobial resistance. Other risk factors include the cleanliness of the hind quarter, body condition score, udder symmetry, rearing system, milking hygiene, frequency of milking per day, number of milkers during milking are also found associated with the buffalo IMI (Singha et al., 2023a, 2021b).

Buffalo is reared in semi-bathan conditions, primarily on grazing fields with few wallowing facilities. Due to the tropical environment, this stressful condition could be attributable to increasing BMSCC. Farmers in the study area neglected hand and udder washing pre and post-dipping of teat before milking; likely contributing to IMI and thus increasing BMSCC. However, using teat dipping and practicing pre and post-dipping significantly decreased BMSCC (Jayarao et al., 2004).

This study demonstrated that bacterial count increased along the milk chain, posing a potential threat to milk quality for consumption. The mean TBC observed at different nodes of the milk chain (farm, middleman and collection center) was higher than the acceptable threshold of 4.6 $\log_{10}$ CFU/mL set by the Bangladesh Food Safety Authority. The findings of high TBC were similar to some previously documented studies in Bangladesh (Singha et al., 2023a; Islam et al., 2018). These results indicate inadequate milking, milk handling hygiene, improper storage and transportation of raw milk along the supply chain cause high loads of bacteria into the milk chain (Kalmus et al., 2015; Ndahetuye et al., 2020). At farm level, raw milk TBC is primarily driven by the herd factors such as herd size, milking and udder hygiene, milking container, water source, storage temperature etc. which may influence the increase TBC at farm level. At middlemen, improper storage and transportation and collection centers hygienic status of the milk can, storage may affect the increase bacterial counts in milk (Singha et al., 2023a; Piepers et al., 2014). Elevated TBC directly associated with the presence of pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella*, *Listeria*, and *Campylobacter* causing gastrointestinal illness, systemic infections, and severe hemolytic-uremic syndrome and meningitis (Berhe et al., 2020). The findings of TNAS might be due to the higher BMSCC recorded in the study, as NAS was the predominant IMI-associated bacterial species found in previous buffalo studies (Kashima et al., 2021; Singha et al., 2024). NAS can be also found on bovine skin, teat canals, as well as animal farm's environment and may lead to contamination of milk (Hamel et al., 2020). While NAS is considered less virulent but it acts as a reservoir for antibiotic resistance genes in milk and dairy equipment (Pizauro et al., 2019). *S. aureus* can produce heat-stable enterotoxins causing food borne illness (Kadariya et al., 2014). Several staphylococcal foodborne outbreaks had been reported in several countries like Vietnam in 2018 (Le et al., 2021), Italy in 2015 (Vitale et al., 2015), Zimbabwe in 2014 (Gumbo et al., 2015) and Australia in 2012 (Pillsbury et al., 2013).

The increasing TEC from farm to collection center reported in this study is similar to previous studies (Islam et al., 2018; Knight-Jones et al., 2016). This finding also indicates that contamination may increase along the supply chain and rise above the threshold level involving some factors. The presence of *Enterobacteriaceae* in milk, for example, is related to fecal contamination and unhygienic milk handling practices along the milk chain. However, others have reported that some herd management practices were associated with *E. coli.* contamination, such as milking hygiene, milk handlers hygiene (Piepers et al., 2014; Mhone et al., 2011) at farm and other nodes of the milk chain. The

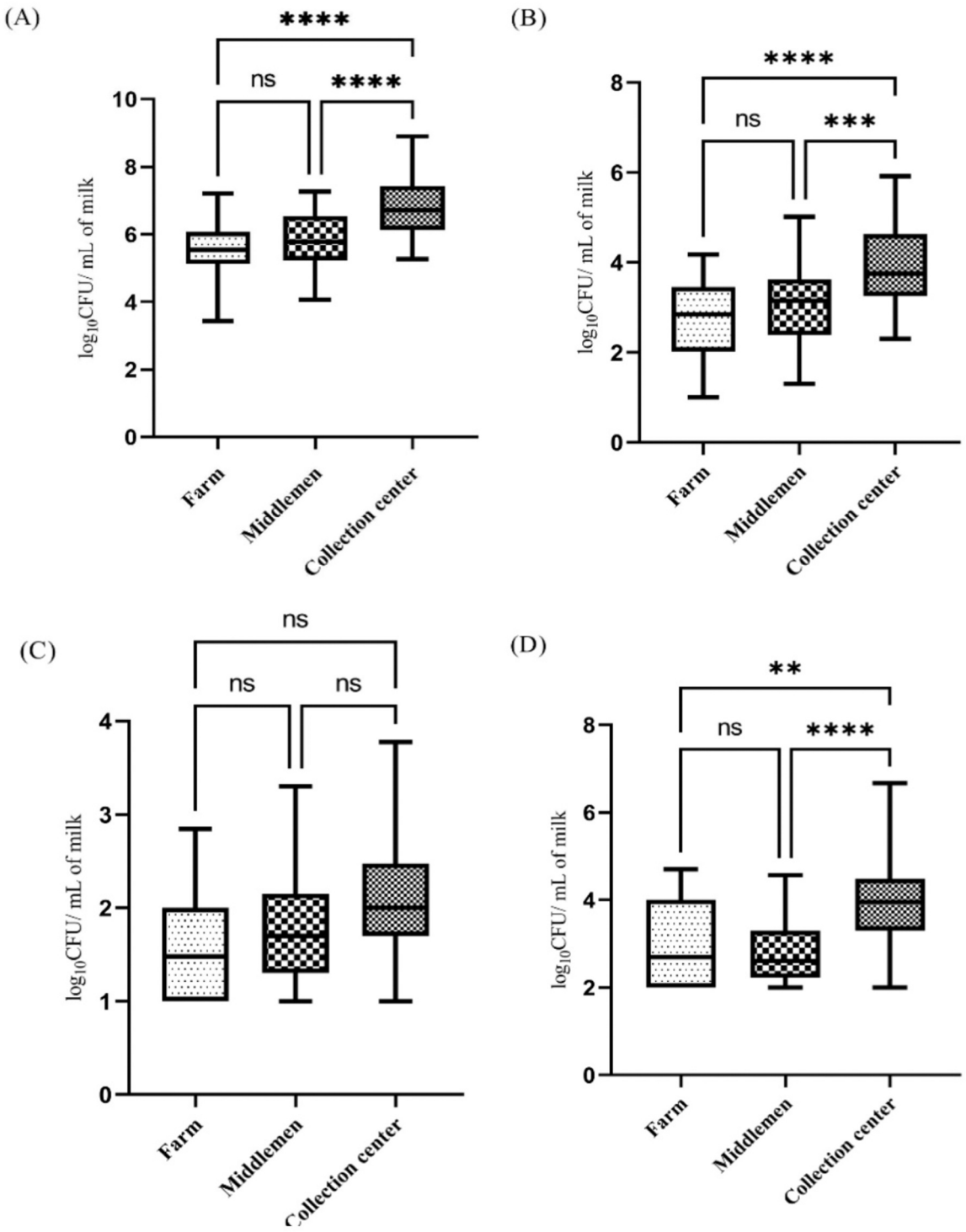

Fig. 2. Bacterial counts at three different nodes of the buffalo milk supply chain at Noakhali district Bangladesh, represented in the box plot where (A) represents the total bacterial count; (B) the total non-aureus staphylococci count; (C) the total *Staphylococcus aureus* count, and (D) represents the total Enterobacteriaceae count with the level of significance: ** =P ≤ 0.01; *** =P ≤ 0.001; **** =P ≤ 0.0001, ns=not significant.

enteropathogenic *E. coli.* are associated with enterohemorrhagic diseases in human. Enteropathogenic toxin-producing *E. coli.* (STEC) occur in children, likely through household livestock fecal contamination through foodborne routes (Lambrecht et al., 2022). Enterobacteriaceae often harbor resistance genes, posing antibiotic treatment challenges (Nahar et al., 2023). Moreover, several diarrheal disease outbreaks had been reported in different countries like Japan in 2020 (Kashima et al., 2021), South Korea in 2018 (Lim et al., 2020), United Kingdom in 2014 (Newitt et al., 2016) and Germany in 2011 (Buchholz et al., 2011).

The current study observed a significant correlation of BMSCC with TNAS (r = 0.35), which was in line with the findings of Koop et al (Koop et al., 2010). This indicates that the number of *Staphylococci* reflects, to some extent, the udder health status of a herd (Koop et al., 2010). Jayarao et al (Jayarao et al., 2004). also reported that an increase in the frequency of isolation of *Staphylococci* was significantly associated with an increased BMSCC. A previous study also reported that, in specific

Table 2
Pair-wise correlation of BMSCC and bacterial counts at different nodes (farm, middlemen, and collection center) of buffalo milk chain in Bangladesh with significance level: * = P ≤ 0.05; *** =P ≤ 0.001.

	Item	[1]*TBC*	*TNAS*
Farm	BMSCC		0.35*
	[2]*TNAS*	0.55 ***	
Middlemen	[3]*TEC*	0.40 *	
Collection center	*TNAS*	0.31*	
	TEC	0.39*	

BMSCC: Bulk milk somatic cell count, [1]TBC: Total bacterial count, [2]TNAS: Total non-aureus Staphylococci count, [3]TEC: Total *Enterobacteriaceae* count.

NAS associated IMI, BMSCC may remain low. Such as IMI with *S. xylosus, S. cohnii*, and *S. equorum* were associated with low SCC, while a high SCC IMI associated with *S. capitis, S. gallinarum, S. hyicus, S. agnetis,* or *S. simulans* (Condas et al., 2017). A positive significant correlation was also observed between TBC and TEC(r = 0.40) and also for TBC and TNAS (r = 0.55) at different nodes, which was also following some other reported findings (Pantoja et al., 2009; Pyz-Łukasik et al., 2015; Marshall et al., 2016). A significant correlation between TBC and prevalence of *S. aureus* (P < 0.01) and *E. coli*. (P < 0.01) were reported by Lan et al (Lan et al., 2017). This suggests that more attention should be paid to *S. aureus* and *E. coli* when TBC counts of milk are unusually high. This association between TBC and TEC might be explained by considering potential sources of *Enterobacteriaceae*, such as coliforms commonly found in fecal matter and dairy cows' environments. *Enterobacteriaceae*, such as coliform, are widely found in fecal matter. In the environment of farms, identifying some risk factors for increased TEC in bulk milk might also identify sources of increased TBC (Pantoja et al., 2009).

In Bangladesh, the buffalo milk chain is not regulated and poorly controlled and milk reaches consumers without checking quality standards. The bacterial counts found in this study highlight the risk to food safety in terms of milk quality and shelf life along the buffalo milk supply chain. BMSCC and bacterial count-related parameters help troubleshoot farm-based milk quality problems, such as investigating sources of IMI, bacterial contamination in bulk milk, milking hygiene, and milking machines or milkers' sanitation. Therefore, a milk traceability system should be introduced in the buffalo milk supply chain. Regular BMSCC should be incorporated into farm routine management and bacterial counts should be routinely monitored at the consumers level to ensure safe and quality milk. Bangladesh food safety authority should propose threshold for BMSCC and bacterial counts to enforce regular BMSCC and TBC, TNAS, TSA and TEC quality check for buffalo milk at market place based on regional study findings to ensure consumers' safety. Buffalo milk chain contamination point and associated risk should be identified properly to minimize the level of contamination.

5. Conclusions

This study evaluated the milk quality indicators such as the bulk milk somatic cell count, bacterial counts and their correlations from farm to collection center along the buffalo milk supply chain in Bangladesh. Monitoring the production level is needed to ensure milk quality throughout the chain. The buffalo milk value chain with limited regulations and checkpoints in these regions contributed to high bacterial counts. Moreover, high BMSCC in the farm associated with the intramammary infection and its correlation with the bacterial counts deteriorates the milk quality along the supply chain. The findings highlighted the increased bacterial contamination risk along the milk supply chain, emphasizing the potential threat to public health. Continuous monitoring of food borne bacteria and associated risk should be measured. This study calls for the implementation of strict monitoring of BMSCC and other quality indicators and introducing a milk traceability system in the buffalo milk chain in Bangladesh. Low cost farmers training on hygienic milk production such as cleanliness of milkers' hands and udder before milking, using sanitary containers and lid, proper storage and cooling are recommended to ensure safe milk. Farmers might also be encouraged to adopt better practices by offering premium price or support from the government and cooperatives for ensuring continuous supply of good quality milk. A specific policy should be introduced by Bangladesh food safety authority to enforce acceptable limit for BMSCC and TBC, TNAS, TSA and TEC for buffalo milk at market place based on regional study findings as well as aligning with international standards to ensure consumers safety. Moreover, the cross- sectional study has inability to determine causality which may slightly influence the interpretation of the study.

Limitations of the study

Due to the remote buffalo-concentrated study area of the bacteriology lab, it was impossible to culture the milk samples immediately after collection. This could be attributed to the mild alteration of the bacterial results in the collected samples. A convenient selection process was performed due to some highly concentrated buffalo areas, which might have affected the outcome.

CRediT authorship contribution statement

Sofia Boqvist: Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis. **Fabrizio Ceciliani:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Salma Chowdhury:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shuvo Singha:** Methodology, Investigation, Formal analysis, Data curation. **Md. Mizanur Rahman:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Sanjib Chandra Nath:** Investigation, Data curation. **Ovirup Bhushan Paul:** Investigation, Data curation. **Mohammad Abdul Mannan:** Investigation, Data curation. **Md. Ahasanul Hoque:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Ylva Persson:** Supervision, Project administration, Funding acquisition.

Ethical approval statement

Ethical approval was obtained from the SAU research system (SAU/Ethical Committee/AUP/21/06) of Sylhet Agricultural University, Bangladesh. Consent to participate: Written and informed consent was given by the buffalo farmers, middlemen, and milk collection center before participation in this study.

Funding

This work was supported by the Swedish Research Council (Grant numbers 2018–03583).

Declaration of Competing Interest

We declare no conflicts of interest.

Acknowledgements

We thank the Swedish Research Council for funding this project, "Climate change mitigation by a sustainable water buffalo dairy chain in Bangladesh." We also thank the UHB bacteriology laboratory of Chattogram Veterinary and Animal Sciences University and the University of Milan, Italy, for their technical support. We also thank the Department of Livestock Services, Bangladesh, and the buffalo farmers of Noakhali district for cooperating during data collection and providing accommodation during the field visit. We also thank all members of the UHB

for their immense support during research.

Data availability

Data will be made available on request.

References

FAO, 2025. Dairy Production and Products: Buffaloes. ⟨https://www.fao.org/dairy-production-products/dairy/buffaloes/en⟩.

Hao, M., Jiang, J., Zhang, Y., Wang, S., Fu, G., Zou, F., Xie, Y., Zhao, S., Li, W., 2021. Transcriptional profiling of buffalo mammary gland with different milk fat contents. Gene 802, 145864. https://doi.org/10.1016/j.gene.2021.145864.

Felice, V.D., Owens, R.A., Kennedy, D., Hogan, S.A., Lane, J.A., 2021. Comparative structural and compositional analyses of cow, buffalo, goat and sheep cream. Foods 10, 2643. https://doi.org/10.3390/foods10112643.

Department of Livestock Services (DLS), Livestock economy at a glance, 2024. ⟨https://dls.portal.gov.bd/sites/default/files/files/dls.portal.gov.bd/page/ee5f4621_fa3a_40ac_8bd9_898fb8ee4700/2024-08-13-10-26-93cb11d540e3f853de9848587fa3c81e.pdf⟩.

Hamid, M.A., Siddiky, M.N.A., Rahman, M.A., Hossain, K.M., 2016. Scopes and opportunities of buffalo farming in Bangladesh: a review. SAARC J. Agric. 14 (2), 63–77. https://doi.org/10.3329/sja.v14i2.31246.

Singha, S., Ceciliani, F., Rahman, M.M., Mannan, M.A., Chowdhury, S., Nath, S.C., Paul, O.B., Persson, Y., Boqvist, S., 2023a. Factors influencing somatic cell counts and bacterial contamination in unpasteurized milk obtained from water buffalo in Bangladesh. Trop. Anim. Health Prod. 55, 242. https://doi.org/10.1007/s11250-023-03644-x.

Singha, S., Persson, Y., Dutta, P.K., Ceciliani, F., Rahman, M.M., 2021a. Traditional buffalo milk chain in Bangladesh. IDF Anim. Health Rep. 15, 24–26. https://doi.org/10.13140/RG.2.2.15576.98564.

Moroni, P., Sgoifo Rossi, C., Pisoni, G., Bronzo, V., Castiglioni, B., Boettcher, P.J., 2006. Relationships between somatic cell count and intramammary infection in buffaloes. J. Dairy Sci. 89, 998–1003. https://doi.org/10.3168/jds.S0022-0302(06)72165-8.

Smistad, M., Sølverød, L., Inglingstad, R.A., Østerås, O., 2021. Distribution of somatic cell count and udder pathogens in Norwegian dairy goats. J. Dairy Sci. 104 (11), 11878–11888. https://doi.org/10.3168/jds.2021-20549.

Bobbo, T., Ruegg, P.L., Stocco, G., Fiore, E., Gianesella, M., Morgante, M., Pasotto, D., Bittante, G., Cecchinato, A., 2017. Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows. J. Dairy Sci. 100 (6), 4868–4883. https://doi.org/10.3168/jds.2016-12353.

Özenc, E., Şeker, E., Acar, D.B., Birdane, M.K., Darbaz, İ., Doğan, N., 2011. The importance of staphylococci and threshold value of somatic cell count for diagnosis of subclinical mastitis in Pirlak sheep at mid-lactation. Reprod. Domest. Anim. 46 (6), 970–974. https://doi.org/10.1111/j.1439-0531.2011.01768.x.

Zecconi, A., Vairani, D., Cipolla, M., Rizzi, N., Zanini, L., 2019. Assessment of subclinical mastitis diagnostic accuracy by differential cell count in individual cow milk. Ital. J. Anim. Sci. 18, 460–465. https://doi.org/10.1080/1828051X.2018.1533391.

Troendle, J.A., Tauer, L.W., Gröhn, Y.T., 2017. Optimally achieving milk bulk tank somatic cell count thresholds. J. Dairy Sci. 100, 731–738. https://doi.org/10.3168/jds.2016-11578.

Ma, Y., Ryan, C., Barbano, D., Galton, D., Rudan, M., Boor, K., 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83, 264–274. https://doi.org/10.3168/jds.S0022-0302(00)74873-9.

Barbano, D.M., Ma, Y., Santos, M.V., 2006. Influence of raw milk quality on fluid milk shelf life. J. Dairy Sci. 89 (1), E15–E19. https://doi.org/10.3168/jds.S0022-0302(06)72360-8.

European Union (EU), Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004; specific hygiene rules for food of animal origin, 2004. ⟨https://eur-lex.europa.eu/eli/reg/2004/853/oj⟩ (accessed 9 November 2024).

Barkema, H.W., De Vliegher, S., Piepers, S., Zadoks, R.N., 2013. Herd level approach to high bulk milk somatic cell count problems in dairy cattle. Vet. Q. 33, 82–93. https://doi.org/10.1080/01652176.2013.799791.

Norman, H.D., Miller, R.H., Ross Jr, F.A., 2009. Somatic cell counts of milk from Dairy Herd Improvement herds during 2008. AIPL Res. Rep. SCC 10. ⟨https://www.ars.usda.gov/research/publications/publication/?seqNo115=236874⟩.

Norman, H.D., Lombard, J.E., Wright, J.R., Kopral, C.A., Rodriguez, J.M., Miller, R.H., 2011. Consequence of alternative standards for bulk tank somatic cell count of dairy herds in the United States. J. Dairy Sci. 94, 6243–6256. https://doi.org/10.3168/jds.2011-4645.

Anderson, M., Hinds, P., Hurditt, S., Miller, P., McGrowder, D., Alexander-Lindo, R., 2011. The microbial content of unexpired pasteurized milk from selected supermarkets in a developing country. Asian Pac. J. Trop. Biomed. 1, 205–211. https://doi.org/10.1016/S2221-1691(11)60028-2.

Berhe, G., Wasihun, A.G., Kassaye, E., Gebreselasie, K., 2020. Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. BMC Public Health 20, 1–8. https://doi.org/10.1186/s12889-020-09016-6.

Wang, M., Bai, Z., Liu, S., Liu, Y., Wang, Z., Zhou, G., Gong, X., Jiang, Y., Sui, Z., 2023. Accurate quantification of total bacteria in raw milk by flow cytometry using membrane potential as a key viability parameter. LWT 173, 114315. https://doi.org/10.1016/j.lwt.2022.114315.

Bava, L., Zucali, M., Sandrucci, A., Brasca, M., Vanoni, L., Zanini, L., Tamburini, A., 2011. Effect of cleaning procedure and hygienic condition of milking equipment on bacterial count of bulk tank milk. J. Dairy Res. 78, 211–219. https://doi.org/10.1017/s002202991100001x.

Pantoja, J.C., Reinemann, D.J., Ruegg, P.L., 2009. Associations among milk quality indicators in raw bulk milk. J. Dairy Sci. 92, 4978–4987. https://doi.org/10.3168/jds.2009-2329.

Murphy, S.C., Martin, N.H., Barbano, D.M., Wiedmann, M., 2016. Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield. J. Dairy Sci. 99, 10128–10149. https://doi.org/10.3168/jds.2016-11172.

O'Brien, M., Hunt, K., McSweeney, S., Jordan, K., 2009. Occurrence of foodborne pathogens in Irish farmhouse cheese. Food Microbiol. 26, 910–914. https://doi.org/10.1016/j.fm.2009.06.009.

Patel, R., Kunjadia, P., Koringa, P., Joshi, C., Kunjadiya, A., 2019. Microbiological profiles in clinical and subclinical cases of mastitis in milking Jafarabadi buffalo. Res. Vet. Sci. 125, 94–99. https://doi.org/10.1016/j.rvsc.2019.05.012.

Salman, M.M., Nawaz, M., Yaqub, T., Mushtaq, M.H., 2023. Exploring the milk microbiota of healthy and mastitic Nili Ravi buffalo using 16S rRNA gene-based metagenomic analysis. Animals 13, 2298. https://doi.org/10.3390/ani13142298.

Li, L., Miao, W., Li, Z., Huang, L., Hau, E., Khan, M.F., Liu, Q., Zeng, Q., Cui, K., 2024. Meta-genomic analysis of different bacteria and their genomes found in raw buffalo milk obtained in various farms using different milking methods. Genes 15, 1081. https://doi.org/10.3390/genes15081081.

Argudín, M.Á., Mendoza, M.C., Rodicio, M.R., 2010. Food poisoning and Staphylococcus aureus enterotoxins. Toxins 2, 1751–1773. https://doi.org/10.3390/toxins2071751.

Kashima, K., Sato, M., Osaka, Y., Sakakida, N., Kando, S., Ohtsuka, K., Honda, A., 2021. An outbreak of food poisoning due to Escherichia coli serotype O7:H4 carrying astA for enteroaggregative E. coli heat-stable enterotoxin 1 (EAST1). Epidemiol. Infect. 149, e244. https://doi.org/10.1017/S0950268821002338.

Lim, M.A., Kim, J.Y., Acharya, D., Bajgain, B.B., Park, J.H., Yoo, S.J., Lee, K., 2020. A diarrhoeagenic enteropathogenic Escherichia coli (EPEC) infection outbreak among elementary school children in Yeongsangbuk-Do province of South Korea associated with consumption of water-contaminated food items. Int. J. Environ. Res. Public Health 17, 3149. https://doi.org/10.3390/ijerph17093149.

de Geus, Y., Scherpenisse, P., Smit, L.A.M., Bossers, A., Stegeman, J.A., Benedictus, L., Spieß, L., Koop, G., 2024. Total bacterial count and somatic cell count in bulk and individual goat milk around kidding: Two longitudinal observational studies. J. Dairy Sci. 107, 5427–5437. https://doi.org/10.3168/jds.2023-24574.

Koop, G., Dik, N., Nielen, M., Lipman, L.J., 2010. Short communication: repeatability of differential goat bulk milk culture and associations with somatic cell count, total bacterial count, and standard plate count. J. Dairy Sci. 93, 2569–2573. https://doi.org/10.3168/jds.2009-2705.

Gonzalo, C., Carriedo, J.A., Beneitez, E., Juárez, M.T., De La Fuente, L.F., San Primitivo, F., 2006. Short communication: bulk tank total bacterial count in dairy sheep: factors of variation and relationship with somatic cell count. J. Dairy Sci. 89, 549–552. https://doi.org/10.3168/jds.S0022-0302(06)72117-8.

Habib, M.R., Haque, M.N., Rahman, A., Aftabuzzaman, M., Ali, M.M., Shahjahan, M.J.A. Jo.M., Research, B., 2017. Dairy buffalo production scenario in Bangladesh: a review. Asian J. Med. Sci. 3, 305–316. https://doi.org/10.3329/ajmbr.v3i3.34518.

Singha, S., Ericsson, C.D., Chowdhury, S., Nath, S.C., Paul, O.B., Hoque, M.A., Boqvist, S., Persson, Y., Rahman, M.M., 2021b. Occurrence and aetiology of subclinical mastitis in water buffalo in Bangladesh. J. Dairy Res. 88, 314–320. https://doi.org/10.1017/s0022029921000698.

Singha, S., Koop, G., Ceciliani, F., Derks, M., Hoque, M.A., Hossain, M.K., Howlader, M. M.R., Rahman, M.M., Khatun, M., Boqvist, S., Persson, Y., 2023b. The prevalence and risk factors of subclinical mastitis in water buffalo (Bubalis bubalis) in Bangladesh. Res. Vet. Sci. 158, 17–25. https://doi.org/10.1016/j.rvsc.2023.03.004.

Alrabadi, N.I., 2015. The effect of freezing on different bacterial counts in raw milk. Int. J. Biol. 7 (4), 9–12. https://doi.org/10.5539/ijb.v7n4p9.

International Organization for Standardization (ISO), Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony counts at 30 °C by the pour plate technique, ISO 4833-1 (2013). ⟨https://www.iso.org/standard/53728.html⟩.

Viçosa, G.N., Moraes, P.M., Yamazi, A.K., Nero, L.A., 2010. Enumeration of coagulase and thermonuclease-positive Staphylococcus spp. in raw milk and fresh soft cheese: an evaluation of Baird-Parker agar, Rabbit Plasma Fibrinogen agar and the Petrifilm Staph Express count system. Food Microbiol. 27, 447–452. https://doi.org/10.1016/j.fm.2009.12.007.

Dhakal, I.P., 2006. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. J. Vet. Med. B. Infect. Dis. Vet. Public Health 53, 81–86. https://doi.org/10.1111/j.1439-0450.2006.00918.x.

Tadich, N., Kruze, J., Locher, G., Green, L., 2003. Risk factors associated with BMSCC greater than 200 000 cells/mL in dairy herds in southern Chile. Prev. Vet. Med. 58, 15–24. https://doi.org/10.1016/S0167-5877(03)00002-3.

Costa, A., Neglia, G., Campanile, G., De Marchi, M., 2020. Milk somatic cell count and its relationship with milk yield and quality traits in Italian water buffaloes. J. Dairy Sci. 103 (6), 5485–5494. https://doi.org/10.3168/jds.2019-18009.

Jayarao, B.M., Pillai, S.R., Sawant, A.A., Wolfgang, D.R., Hegde, N.V., 2004. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J. Dairy Sci. 87, 3561–3573. https://doi.org/10.3168/jds.S0022-0302(04)73493-1.

Islam, M.A., Roy, S., Nabi, A., Solaiman, S., Rahman, M., Huq, M., Siddiquee, N.A., Ahmed, N., 2018. Microbiological quality assessment of milk at different stages of the dairy value chain in a developing country setting. Int. J. Food Microbiol. 278, 11–19. https://doi.org/10.1016/j.ijfoodmicro.2018.04.028.

Kalmus, P., Kramarenko, T., Roasto, M., Meremäe, K., Viltrop, A., 2015. Quality of raw milk intended for direct consumption in Estonia. Food Control 51, 135–139. https://doi.org/10.1016/j.foodcont.2014.11.018.

Ndahetuye, J.B., Artursson, K., Båge, R., Ingabire, A., Karege, C., Djangwani, J., Nyman, A.K., Ongol, M.P., Tukei, M., Persson, Y., 2020. Milk symposium review: microbiological quality and safety of milk from farm to milk collection centers in Rwanda. J. Dairy Sci. 103, 9730–9739. https://doi.org/10.3168/jds.2020-18302.

Piepers, S., Zrimšek, P., Passchyn, P., De Vliegher, S., 2014. Manageable risk factors associated with bacterial and coliform counts in unpasteurized bulk milk in Flemish dairy herds. J. Dairy Sci. 97 (6), 3409–3419. https://doi.org/10.3168/jds.2013-7203.

Singha, S., Koop, G., Rahman, M.M., Ceciliani, F., Howlader, M.M.R., Boqvist, S., Cremonesi, P., Hoque, M.N., Persson, Y., Lecchi, C., 2024. Foodborne bacteria in milk and milk products along the water buffalo milk chain in Bangladesh. Sci. Rep. 14, 16708. https://doi.org/10.1038/s41598-024-67705-3.

Hamel, J., Zhang, Y., Wente, N., Krömker, V., 2020. Non-S. aureus staphylococci (NAS) in milk samples: Infection or contamination? Vet. Microbiol 242, 108594. https://doi.org/10.1016/j.vetmic.2020.108594.

Pizauro, L.J.L., de Almeida, C.C., Soltes, G.A., Slavic, D., de Ávila, F.A., Zafalon, L.F., MacInnes, J.I., 2019. Short communication: Detection of antibiotic resistance, mecA, and virulence genes in coagulase-negative Staphylococcus spp. from buffalo milk and the milking environment. J. Dairy Sci. 102 (12), 11459–11464. https://doi.org/10.3168/jds.2018-15920.

Kadariya, J., Smith, T.C., Thapaliya, D., 2014. Staphylococcus aureus and staphylococcal food-borne disease: An ongoing challenge in public health. Biomed. Res. Int. 2014 (1), 827965. https://doi.org/10.1155/2014/827965.

Le, H.H.T., Dalsgaard, A., Andersen, P.S., Nguyen, H.M., Ta, Y.T., Nguyen, T.T., 2021. Large-scale Staphylococcus aureus foodborne disease outbreak among primary school children. Microbiol. Res. 12, 43–52. https://doi.org/10.3390/microbiolres12010005.

Vitale, M., Scatassa, M.L., Cardamone, C., Oliveri, G., Piraino, C., Alduina, R., Napoli, C., 2015. Staphylococcal food poisoning case and molecular analysis of toxin genes in Staphylococcus aureus strains isolated from food in Sicily, Italy. Foodborne Pathog. Dis. 12, 21–23. https://doi.org/10.1089/fpd.2014.1760.

Gumbo, A., Bangure, D., Gombe, N.T., Mungati, M., Tshimanga, M., Hwalima, Z., Dube, I., 2015. Staphylococcus aureus food poisoning among Bulawayo City Council employees, Zimbabwe, 2014. BMC Res. Notes 8, 485. https://doi.org/10.1186/s13104-015-1490-4.

Pillsbury, A., Chiew, M., Bates, J., Sheppeard, V., 2013. An outbreak of staphylococcal food poisoning in a commercially catered buffet. PMID: 24890941 Commun. Dis. Intell. Q. Rep. 37, 144–148. https://doi.org/10.33321/cdi.2013.37.20.

Knight-Jones, T.J., Hang'ombe, M.B., Songe, M.M., Sinkala, Y., Grace, D., 2016. Microbial contamination and hygiene of fresh cow's milk produced by smallholders in Western Zambia. Int. J. Environ. Res. Public Health 13. https://doi.org/10.3390/ijerph13070737.

Mhone, T.A., Matope, G., Saidi, P.T., 2011. Aerobic bacterial, coliform, Escherichia coli and Staphylococcus aureus counts of raw and processed milk from selected smallholder dairy farms of Zimbabwe. Int. J. Food Microbiol. 151 (2), 223–228. https://doi.org/10.1016/j.ijfoodmicro.2011.08.028.

Lambrecht, N.J., Wilson, M.L., Bridges, D., Eisenberg, J.N., Adu, B., Baylin, A., Folson, G., Jones, A.D., 2022. Ruminant-related risk factors are associated with Shiga toxin–producing Escherichia coli infection in children in southern Ghana. Am. J. Trop. Med. Hyg. 106 (2), 513–520. https://doi.org/10.4269/ajtmh.21-0550.

Nahar, A., Islam, A.A., Islam, M.N., Khan, M.K., Khan, M.S., Rahman, A.A., Alam, M.M., 2023. Molecular characterization and antibiotic resistance profile of ESBL-producing Escherichia coli isolated from healthy cow raw milk in smallholder dairy farms in Bangladesh. Vet. World 16 (6), 1333. https://doi.org/10.14202/vetworld.2023.1333-1339.

Newitt, S., MacGregor, V., Robbins, V., Bayliss, L., Chattaway, M.A., Dallman, T., Hawker, J., 2016. Two linked enteroinvasive Escherichia coli outbreaks, Nottingham, UK, June 2014. Emerg. Infect. Dis. 22, 1178–1184. https://doi.org/10.3201/eid2207.152080.

Buchholz, U., Bernard, H., Werber, D., Böhmer, M.M., Remschmidt, C., Wilking, H., Kühne, M., 2011. German outbreak of Escherichia coli O104:H4 associated with sprouts. N. Engl. J. Med. 365, 1763–1770. https://doi.org/10.1056/nejmoa1106482.

Condas, L.A., De Buck, J., Nobrega, D.B., Carson, D.A., Roy, J.P., Keefe, G.P., DeVries, T. J., Middleton, J.R., Dufour, S., Barkema, H.W., 2017. Distribution of non-aureus staphylococci species in udder quarters with low and high somatic cell count, and clinical mastitis. J. Dairy Sci. 100 (7), 5613–5627. https://doi.org/10.3168/jds.2016-12479.

Pyz-Łukasik, R., Paszkiewicz, W., Tatara, M.R., Brodzki, P., Bełkot, Z., 2015. Microbiological quality of milk sold directly from producers to consumers. J. Dairy Sci. 98, 4294–4301. https://doi.org/10.3168/jds.2014-9187.

Marshall, J.C., Soboleva, T.K., Jamieson, P., French, N.P., 2016. Estimating Bacterial Pathogen Levels in New Zealand Bulk Tank Milk. J. Food Prot. 79, 771–780. https://doi.org/10.4315/0362-028x.Jfp-15-230.

Lan, X.Y., Zhao, S.G., Zheng, N., Li, S.L., Zhang, Y.D., Liu, H.M., McKillip, J., Wang, J.Q., 2017. Microbiological quality of raw cow milk and its association with herd management practices in Northern China. J. Dairy Sci. 100 (6), 4294–4299. https://doi.org/10.3168/jds.2016-11631113.