J. Dairy Sci. 103:9730–9739
https://doi.org/10.3168/jds.2020-18302

MILK Symposium review: Microbiological quality and safety of milk from farm to milk collection centers in Rwanda*

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ABSTRACT

The aim of this study was to generate knowledge on the most important milk quality and safety attributes, including somatic cell count (SCC), total bacterial count (TBC), *Escherichia coli*, *Salmonella*, and *Brucella* spp. antibodies and antibiotic residues in milk in the chain from farm to milk collection center (MCC) in Rwanda. In addition, we investigated farm and management factors associated with high TBC, SCC, and *Salmonella* counts. Raw milk was sampled at the farm and MCC levels. Milk samples were taken from dairy farms linked to 2 selected MCC in each of the 4 provinces in Rwanda. In total, 406 bulk milk samples from 406 farms and 32 bulk milk samples from 8 MCC were collected and analyzed. Farm milk average SCC varied between 180 $\times$ 10^3 and 920 $\times$ 10^3 cells/mL, whereas average SCC in milk samples at MCC varied between 170 $\times$ 10^3 and 1,700 $\times$ 10^3 cells/mL. The mean milk TBC of different farms per MCC varied between 1.1 $\times$ 10^6 and 1.6 $\times$ 10^7 cfu/mL, whereas in milk samples from different MCC, the mean TBC ranged between 5.3 $\times$ 10^5 and 2.4 $\times$ 10^8 cfu/mL. The high TBC in milk from MCC suggests proliferation or recontamination of milk by bacteria during transportation. *Escherichia coli* was detected in 35 of 385 farm milk samples and ranged between 5 cfu/mL and 1.1 $\times$ 10^4 cfu/mL, whereas in milk samples from the MCC, it was detected in 20 out 32 samples varying between 5 cfu/mL and 2.9 $\times$ 10^3 cfu/mL. Overall farm prevalence of *Salmonella* in milk samples was 14%, but no milk samples from MCC were positive for *Salmonella*. Five out of 22 bulk milk samples from different MCC were positive for *Brucella* spp. antibodies, but no *Brucella* antibodies were detected in milk samples from farms. The prevalence of antibiotic residues as detected by the Delvotest SP NT (DSM, Delft, the Netherlands) was low: 1.3% in farm milk samples and undetected in MCC milk samples. Lack of a separate milking area was associated with high TBC, whereas offering of supplemental feeds, keeping data of past diseases, and an unhygienic milking area were associated with high SCC. Lack of teat washing before milking was the only factor associated with *Salmonella* contamination of milk at the farm level. This study indicated high TBC and SCC of milk samples at the farm and MCC levels, which indicates both microbial contamination of milk and poor udder health in dairy cows. Presence of *E. coli*, *Salmonella*, and *Brucella* antibodies in milk was common, but finding antibiotic residues in milk was uncommon.
Key words: raw milk, microbial contamination, dairy chain, public health, East Africa

INTRODUCTION

Raw milk from dairy cows may be contaminated by microorganisms originating from the udder (mastitis associated), by zoonotic pathogens shed from infected animals, or by other microorganisms from the environment. Environmental organisms could be transferred to the milk through poor hygiene of udder and teat surfaces and from uncleaned and unsanitized milking equipment (Elmoslemany et al., 2009), but also from milkers or other people handling the milk. Improper cooling of milk during transport can also influence bacterial count by increasing the rate of bacterial growth before the milk reaches milk collection centers (**MCC**) or processors. The total bacterial count (**TBC**) is used to evaluate the extent to which such processes have affected milk quality or safety. However, Murphy and

Received February 4, 2020.
Accepted July 28, 2020.
*Presented at the MILK Symposium: Improving Milk Production, Quality, and Safety in Developing Countries at the ADSA Annual Meeting, Cincinnati, Ohio, June 2019.
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Boor (2000) indicated that TBC should be interpreted with caution because different types of bacteria can contaminate milk from various sources such as equipment, milk handlers, and different environmental niches. These microorganisms proliferate in milk because milk contains key nutrients and has high water activity and an ideal pH for their growth and development (Hassan and Frank, 2011). Numerous groups of bacteria can grow in milk, but *Escherichia coli* is particularly used as an indicator organism for fecal contamination of foodstuff (i.e., an indicator of hygiene) and it can be associated with foodborne outbreaks (Tryland and Fiksdal, 1998). The SCC in milk may be related to the immune reaction following an IMI. Subclinical mastitis is a situation in which leukocytes increase in milk without apparent visual changes in milk appearance, whereas in clinical mastitis, there are apparent changes in milk, sometimes in combination with local signs in the udder or systemic clinical signs that can be recognized by the farmer (Hillerton and Berry, 2005). A high leukocyte level, measured as SCC, and high TBC in milk may result in the production of enzymes that degrade milk components such as fats and proteins (Li et al., 2014; Baur et al., 2015), thus reducing the quality of milk and milk products. This will affect the shelf life and reduces consumer acceptance of these products (Elmoslemany et al., 2009). Moreover, mastitis bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae* can contaminate bulk milk and be a public health concern because they are zoonotic pathogens (Zadoks et al., 2011; Bi et al., 2016). Several other zoonotic pathogens, including *Brucella* spp. and *Salmonella* spp., may be found in infected animals and contaminate raw milk when milking techniques, hygiene, and handling during transportation are suboptimal at the farm or in the milk chain (Kamana et al., 2014; Habarugira et al., 2014; Rujeni and Mbanzamihigo, 2014).

Increasing milk quality and safety around the world is highly relevant because regulations that protect the health of consumers require adherence to key milk quality and safety guidelines such as low SCC. The maximum concentration of SCC allowed for commingled bulk milk destined for processing and for human consumption differs by region. For example, the European Union (2004) requires an SCC limit of bulk milk of 400×10^3 cells/mL, the United States has a limit of 750×10^3 cells/mL and Canada has a limit of 500×10^3 cells/mL (Schukken et al., 2003). The East African standard for SCC is 300×10^3 cells/mL (EAS 67:2006; East African Community, 2006) although this is not generally enforced. Although payment for milk volume is widely practiced in Rwanda, there are increasing calls for differentiated milk payment according to milk quality or safety because processors and consumers are paying more attention to quality and safety of milk and milk products. The use of antibiotics in food-producing animals has resulted in practical and cost-effective ways to control disease and improve animal welfare (Hillerton and Berry, 2005). On dairy farms, antibiotics are used for therapeutic purpose; for example, to treat mastitis, metritis, respiratory disease, and foot disease, and for prophylactic purposes; for example, for blanket dry-cow therapy and medicated milk replacer for calves (Redding et al., 2019). However, overuse or misuse of antibiotics can increase the risk of antibiotic residues in milk and contributes to the rise or selection of microorganisms that are resistant to antibiotics (Yan and Gilbert, 2004).

In Rwanda, milk is typically produced by smallholders and is generally transported, with unreliable refrigeration, using bicycles or motorcycles to MCC; individual large-scale farmers may also supply milk directly to the MCC. There are about 100 MCC functioning in Rwanda (IFAD, 2016). Hand milking is widely practiced, and smallholders are characterized by low productivity, insufficient use of modern farm technologies and practices, and challenges in accessing clean water and adequate training (Doyle et al., 2015; IFAD, 2016). Milk collection centers serve as centralized cooling and storage centers for milk from many producers before the milk is forwarded to kiosks selling fresh milk or to factories for processing (Miklyaev et al., 2017). Milk quality and safety testing is rare on farms in Rwanda; however, at the MCC, milk is typically tested for acidity and added water using an alcohol testing and a lactometer, respectively. Raw milk quality and safety in the chain from farm to MCC in Rwanda is important for both processors and consumers, and collecting basic data on key quality attributes is vital for problem-solving regarding farm hygiene and sanitization, mastitis control, and milk collecting hygiene. The aim of this study was to generate information on the most important milk quality attributes, including SCC, TBC, *E. coli*, *Salmonella*, and *Brucella* antibodies, as well as antibiotic residues in the farm-to-MCC milk chain. In addition, potential risk factors associated with TBC, SCC, and *Salmonella* were investigated. The knowledge generated in this project will be used to develop milk quality improvement programs for the dairy sector in Rwanda.

MATERIALS AND METHODS

Study Areas

The study was conducted in 8 selected MCC, 2 from each of the 4 provinces in Rwanda, and with the dairy farmers associated with these MCC. The MCC were

chosen to represent potential differences in agroecology conditions, milk handling practices, and cultures that are specific to each province. The MCC were located at the following sites: MCC1 and MCC2 were located in Rwamagana and Nyagatare in the eastern province, MCC3 and MCC4 in Nyankenke and Rubaya in the northern province, MCC5 and MCC6 in Mudende and Rubengera in the western province, and MCC7 and MCC8 in Rugobagoba and Muyira in the southern province. Inclusion of each MCC was based on a mean receiving capacity of at least 4,000 L of milk per day. Because we could not obtain an official list of all dairy farmers associated with each MCC, the linear snowball sampling method, as described by Balinas (2014) and Etikan et al. (2016), was used. The MCC technicians and milk transporters guided the research team to enlist farmers located in all provinces (east, south, west, and north) relative to the MCC. Dairy farmers included in the study per MCC corresponded to farmers whose lactating cows were previously screened for subclinical mastitis and described in a study by Ndahetuye et al. (2020). Based on these estimations, the number of dairy farmers included in the study were 50 in MCC1, 14 in MCC2, 64 in MCC3, 55 in MCC4, 58 in MCC5, 56 in MCC6, 45 in MCC7, and 64 in MCC8.

Milk Sample Collection

The first sampling was done at the farm level, and each farm was sampled once from May to September 2017. The second sampling was done at the level of the MCC where farmers delivered their milk. Each MCC was sampled on 4 occasions, approximately every 4 mo in total, spanning 16 mo during 2017 and 2018. Aseptic collection of milk samples at the farm and MCC levels was done according to National Mastitis Council (NMC, 2017) guidelines. Before milk collection, milk in the bulk tank at the MCC or in bulking containers on farms was agitated for 10 min and samples were collected from the top of the bulk tank using a clean, sanitized dipper, transferred to sterile test tubes, and then placed in an ice-cooled box for immediate transport to the microbiology laboratory at the University of Rwanda, College of Agriculture, Animal Sciences and Veterinary Medicine, Busogo Campus, Rwanda. Somatic cell count was analyzed in fresh milk within 24 h after collection. The remaining milk was stored at −20°C until further analyzed.

Somatic Cell Count

The SCC was determined in milk samples from farms (n = 393) and from MCC (n = 32) using an electronic portable somatic cell counter (DeLaval Cell Counter, DCC, DeLaval, Sweden). A cut-off level of 300×10^3 cells/mL was used to compare levels of SCC, representing the standard used in East African region (East African Community, 2006; EAS 67:2006).

Total Bacterial Count

To determine TBC in farm milk samples (n = 386) and MCC milk samples (n = 32), 1 mL of the milk sample was mixed with 9 mL of diluent (sterilized peptone physiological saline solution) and the mixture vortexed thoroughly. Then, serial dilutions (10^{-1} to 10^{-9}) were prepared. From each dilution starting from the highest, 0.1 mL of test sample was inoculated onto plate count agar (Titan Biotech Ltd., Rajasthan, India) plates in duplicate. The sample was spread evenly on the surface of the plate using a sterile spreading glass rod. Samples were incubated at 37°C for 24 h. At the end of the incubation period, plates with between 30 and 300 colonies were counted. The number of colony-forming units was then converted, considering the dilution factor and the plated sample volume, into colony-forming units per milliliter of raw milk.

Escherichia coli

Enumeration of β-glucuronidase-positive *E. coli* in bulk milk samples from farm (n = 385) and samples from MCC (n = 32) was performed according to ISO (2001; 16649-1:2001). The milk sample (100 μL) was inoculated directly onto tryptone bile x-glucuronide (TBX) medium (BioMérieux, Marcy l'Etoile, France) plates in duplicate and spread evenly. Plates were incubated at 44°C for 24 h. At the end of incubation period, plates with between 30 and 300 colonies were counted.

Salmonella

The ISO 6579:2002-A1 2007 method (ISO, 2007) was followed to detect *Salmonella* in milk samples from the farm (n = 313) and MCC samples (n = 22). For each sample, 4.1 mL of each milk sample was added to 9 mL of peptone water (BiolaZrt, Budapest, Hungary) and the mixture was incubated at 37°C for 24 h for pre-enrichment. Then, 0.1 mL of suspension was added to 10 mL of modified semisolid Rappaport-Vassiliadis agar (Oxoid, Basingstoke, UK) and the mixture was incubated at 41.5°C for 48 h. Suspected *Salmonella* colonies were subcultured on xylose lysine deoxycholate (BiolaZrt). Final verification of *Salmonella* was done using the Oxoid Salmonella Latex Test (Oxoid) following the manufacturer's instructions.

Brucella spp. Antibodies

Antibodies to *Brucella abortus* and *Brucella melitensis* were analyzed by ELISA (Svanovir Brucella-Ab, Boehringer Ingelheim, Uppsala, Sweden) in milk samples from farms (n = 313) and samples from MCC (n = 22). Test kit specificity for milk samples was reported by the manufacturer to be 99 to 100%. Relative test kit sensitivity for the Rose Bengal test is 89.6% and that for the complement fixation test is 100% (Svanova, 2009). Milk samples were thawed at room temperature, and ELISA was performed according to the manufacturer's protocol for milk samples. On each ELISA plate, positive and negative control sera were included to ensure accuracy of the test, and all samples and controls were run in duplicate. Skanlit Software for Thermo Scientific Multiskan FC (Thermo Scientific, Ratastie, Finland) was used to read the ELISA plates and to calculate sample optical density (**OD**) values. Percent positivity (PP) was calculated as (OD of sample or negative control/OD of positive control) × 100. A milk sample with PP ≥10% was considered positive according to the manufacturer's instructions.

Antibiotic Residues

The prevalence of antibiotics, as detected by Delvotest SP NT kit (DSM, Heerlen, the Netherlands), was evaluated in milk by incubating 100 μL of homogenized milk sample for 2 to 3 h at 64°C and observing a color change of the lower two-thirds of the test panel to yellow (negative test) or completely purple for positive. According to the manufacturer, this test can detect more than 40 antibiotics. The kit has been previously validated and its sensitivity were found to be 1.5 ng/g for penicillin G, 2.5 ng/g for amoxicillin, 3.0 ng/g for ampicillin, and 5.8 ng/g for cephapirin (Hennart and Faragher, 2012). In total, 372 and 32 milk samples from farms and MCC, respectively, were tested for antibiotic residues.

Questionnaire

Data collection and observations on dairy husbandry practices at the farms were done by the research team using a semi-structured questionnaire. Milking practices, housing, and hygiene routines were recorded. Variables included in the questionnaire are presented in Table 1. The hygiene concepts referred to in Table 1 (e.g., good/poor, slightly dirty/very dirty) were taken from mastitis studies such as Schreiner and Ruegg (2003) or Abrahmsén et al. (2014) and modified for our study. To classify farm environment as having good or poor hygiene or to describe milking as slightly or very dirty was based on whether these environment were visually completely free of dirt (i.e., good hygiene or clean), partially loaded with dirt (i.e., slightly dirty), or full of dirt (i.e., poor hygiene or very dirty). Data collectors were trained to ensure consistent scoring of hygiene. The interviews were conducted after milking.

Data Analysis

Prevalence of *E. coli*, *Salmonella*, or *Brucella* spp. antibodies was calculated as the number of positive samples against the total number of samples analyzed at the farm and MCC levels, respectively. Mean and median of TBC and SCC of data from farms within MCC and in different MCC were calculated and tabulated accordingly. The TBC and SCC were transformed on a $\log_{10}$ basis to achieve a normal distribution before analysis. Thereafter, associations between TBC or SCC and potential risk factors were analyzed by linear regression analysis as follows. To evaluate on-farm risk factors associated with TBC or SCC, unconditional associations between each independent variable and the dependent variable, first with TBC, and subsequently in a separate analysis with SCC, were investigated using univariable linear or univariable mixed-effect linear regression analysis, including MCC as random factor. Statistical significance in this step was assessed at $P < 0.20$. Factors that were significant in the univariable analyses were then investigated using Spearman rank correlation to assess collinearity; if 2 variables showed high collinearity ($r \geq 0.70$), the one with the lowest P-value was then offered to the multivariable regression models. If MCC as a random factor was not significant ($P \geq 0.05$), an ordinary linear regression model was used. The multivariable models were reduced using a manual, stepwise backward variable selection procedure where the initial model included all independent variables (with P-value <0.20 in the univariable analysis) as main effects. Variables with a significant association ($P \leq 0.05$) with the dependent variable were kept in their respective final models. In each model, all variables with $P \leq 0.20$ for TBC and SCC in the univariable analyses were then retested one at a time in their respective final model and kept in the model if they were significantly associated with the dependent variable. In parallel, confounding was checked if removal of a variable in final multivariable models changed the regression coefficients of the remaining variables (>25%). All plausible 2-way interactions between the significant main effects were tested in all final models. Model fit was assessed by determination of multiple correlation coefficient (R) and coefficient of determination (R^2). Risk factors associated with *Salmonella* in bulk milk were analyzed in similar manner but using

Table 1. Factors analyzed at the farm level (n = 406) in 4 regions in Rwanda

Variable	Category
Type of cattle kraal	Individual, grouped, or no kraal
Type of floor of cow housing	Concrete, earthen, or raised wood
Type of bedding materials	Sawdust, grass, or none
Wet bedding	Yes or no
Frequency of bedding material replacement	Once a week or twice a week
Grazing type	Zero grazing, semi-grazing, or free grazing
Separate calving area; separate milking area	Yes or no
Farm hygiene	Good or poor
Milking area hygiene	Clean, slightly dirty, or very dirty
Frequency of cleaning milking area	Before every milking; once per day; once, twice, or thrice per week; other
Technique of milking	Stripping or full hand
Milking frequency	Once or twice daily
Who milks the cow	Owner, worker, or child
Hand washing before milking	With water only, with water and soap, or no hand washing
Teat and udder washing before milking; teat and udder drying; use of clean towel for drying	Yes or no
Premilking teat dipping; postmilking teat dipping	Yes or no
Foremilk stripping; performing California Mastitis Test regularly; milking mastitic cows last; culling chronically infected cows	Yes or no
Feed cows after milking	Yes or no
Feeds sometimes concentrates	Yes or no
Knowledge of clinical/subclinical mastitis	Yes or no
Dry-cow therapy	Yes or no
Availability of veterinary service; fly control; data record of past diseases	Yes or no

logistic regression models. The statistical analyses were performed using Stata 15 (Stata Corp LLC, College Station, TX).

RESULTS

Somatic Cell Counts

The average milk SCC of farms varied between 180 $\times$ 10^3 and 920 $\times$ 10^3 cells/mL, whereas average milk SCC in all MCC varied between 170 $\times$ 10^3 and 1,700 $\times$ 10^3 cells/mL. The median SCC of milk at the farm level varied between 85 $\times$ 10^3 and 760 $\times$ 10^3 cells/mL, whereas that at the MCC level varied between 105 $\times$ 10^3 and 1,091 $\times$ 10^3 cells/mL (Table 2). The results of the final multivariable mixed-effect linear regression analysis showed that feeding concentrates, keeping records of past diseases, and unhygienic milking area were associated with a high SCC in milk at the farm level (Table 3).

Total Bacterial Count

Results of the TBC analyses are found in Table 4. Average TBC in farm milk varied between 1.1 $\times$ 10^6 and 1.6 $\times$ 10^7 cfu/mL, whereas average TBC of milk at the MCC varied between 5.3 $\times$ 10^5 and 2.4 $\times$ 10^8 cfu/mL. The farm milk median TBC varied between 7 $\times$

Table 2. Somatic cell counts ($\times 10^3$ cells/mL) of bulk milk from farms (n = 406) and milk collection centers (MCC; n = 8) in 4 provinces in Rwanda

	SCC at farm level					SCC at MCC level				
MCC	Mean	SD	Median	Q1[1]	Q3[1]	Mean	SD	Median	Q1	Q3
1	340	470	170	62.5	503	480	190	485	305	652
2	440	350	270	213	604	450	210	406	285	670
3	430	530	190	107	547	1,700	1,800	1,091	540	3,692
4	920	1,100	760	220	1,274	450	360	437	124	802
5	360	410	190	68.5	529	680	360	618	372	1,057
6	180	270	85	31	182	170	190	105	36.5	373
7	350	380	170	52.5	619	450	140	433	326	604
8	360	500	150	47	327	350	140	304	260	497

[1]Q1, Q3 = first and third quartiles, respectively.

Table 3. On-farm factors associated with SCC in bulk milk from farms (n = 406) in 4 regions in Rwanda

Factor	Regression coefficient	SE	*P*-value	95% CI
Sometimes feeds concentrates				
No	Referent			
Yes	0.22	0.08	0.007	0.38–0.59
Data record of past diseases				
No	Referent			
Yes	0.32	0.14	0.02	0.58–0.05
Milking area hygiene			0.001	
Clean	Referent			
Slightly clean	0.26	0.08	0.001	0.11–0.42
Very dirty	0.30	0.09	0.001	0.11–0.48
Intercept	5.54	0.15	<0.001	5.24–5.83

10^3 and 1.1×10^6 cfu/mL, whereas that of milk at MCC varied between 2.5×10^5 and 1.4×10^8 cfu/mL (Table 4). The variable "lack of separate milking area" was significantly ($P < 0.05$) associated with higher TBC levels at farm level. The TBC was 0.49 cfu/mL higher (95% CI = 0.15–0.88, $P = 0.005$) in milk samples from farms without a separate milking area than in those from farms with a separate milking area.

Escherichia coli and Salmonella

Escherichia coli was detected in 8.5% of farm milk samples (range: 5.0 cfu/mL to 1.2×10^4 cfu/mL) and in 63% (20/32 samples) from MCC milk samples (range: 5.0 cfu/mL to 2.9×10^3 cfu/mL). Overall, *Salmonella* prevalence in farm milk samples was 14.0%. No *Salmonella* were detected in milk samples from MCC. The only on-farm factor remaining after the multivariable mixed-effect linear regression analysis was "lack of teat washing before milking." Farms that did not wash cows' teats before milking had a significantly higher odds of also having a higher level of *Salmonella* in milk samples (odds ratio = 2.22, 95% CI = 1.13–4.36, $P = 0.02$).

Brucella Antibodies in Milk

No *Brucella* antibodies were detected in farm bulk milk samples. Five of 22 bulk milk samples from different MCC were positive for *Brucella* spp. antibodies. The positive samples came from the 2 MCC in the eastern province: MCC2 was positive on 2 occasions and MCC3 was positive on 3 occasions.

Antibiotic Residues in Milk

Antibiotic residues were found in 5 of 372 screened farm bulk milk samples as detected by Delvotest SP NT, yielding a prevalence of 1.3%. No antibiotic residues were detected in MCC milk samples.

DISCUSSION

The milk chain from farm to MCC is the cornerstone of the formal dairy market in Rwanda. This study showed milk to have high SCC and TBC and to be contaminated with *E. coli*.

Table 4. Total bacterial count (TBC; $\times 10^4$ cfu/mL) of milk from farms (n = 406) and milk collection centers (MCC; n = 8) in 4 provinces in Rwanda

	TBC at farm level					TBC at MCC level				
MCC	Mean	SD	Median	Q1[1]	Q3[1]	Mean	SD	Median	Q1	Q3
1	320	650	110	26	443	250	300	174	17	579
2	140	550	0.7	0.09	4.3	53	20.3	25	10	136
3	1,600	4,900	86	7.5	888	7,200	13,000	416	34	21,190
4	160	460	35	2	143	2,000	3,800	157	49	5,879
5	110	260	10	1.8	42	5,800	6,100	6,078	265	11,183
6	150	460	7.8	2.3	47	2,800	5,200	341	48	8191
7	200	840	9.4	1.8	31	24,000	3,500	14,230	234	62,921
8	450	180	10	2.7	88.4	6,100	11,000	534	53	17,863

[1]Q1, Q3 = first and third quartiles, respectively.

SCC at the Farm and MCC Levels

The MCC included in the study did not regularly screen milk for SCC and were therefore unable to enforce the SCC standard for threshold limits, whether it concerned requirements for acceptance or rejection or payment incentives (e.g., premium payment for a high-quality product or penalty for low-quality product). Milk samples from farms and MCC (7/8 MCC) had average SCC >300 $\times$ 10^3 cells/mL, which is the limit for raw milk set by the East African Community (2006; EAS 67:2006). This standard is stricter than those in the European Union and the United States, likely because it was adopted directly from ISO 13366 (ISO, 2006) without consideration of local conditions. The high SCC levels in milk indicate udder health problems in the cows. We observed considerable variation between the lowest and the highest recorded SCC in milk from farms: the lowest recorded SCC was 2 $\times$ 10^3 cells/mL and the highest was 7,900 $\times$ 10^3 cells/mL. This considerable variation demonstrates the difficulty in setting and complying with a relevant threshold for milk acceptance or rejection or for quality compensation. Our results showed that 36% of the farms had a bulk milk SCC >300 $\times$ 10^3 cells/mL, which is lower than that found in a study of smallholder farms in Lusaka, Zambia, where 61.4% of the milk samples had SCC above the recommended limit of 300 $\times$ 10^3 cells/mL (Kunda et al., 2016). To give good advice on how to lower the bulk milk SCC at the farm level, an understanding of the factors that affect bulk milk SCC is needed. Our results showed that the variables "sometimes feeding concentrates," "keeping records of diseases," and "unhygienic milking area" were associated with high bulk milk SCC. Improvement of these factors could result in lowering bulk milk SCC. It is not clear why feeding concentrate was associated with high bulk milk SCC. It could be that it is more common to feed concentrates to high-yielding cows, and these cows are more commonly Holsteins, a breed shown to have a higher risk of mastitis in Rwanda (Ndahetuye et al., 2019). It is not known whether feed manufacturers in Rwanda add selenium and vitamin A and E to feeds during feed formulation; these supplements are known to minimize mastitis incidence in dairy cows (Sandholm et al., 1995). Similarly, it is not clear why keeping records was associated with higher SCC. It is possible that farmers who keep records are those who have recently experienced mastitis in their farms and therefore want to keep records on the cases. An explanation to why farms with cleaner milking areas in this study had lower bulk milk SCC is that good hygienic conditions prevent and reduce transmission of mastitis bacteria from one cow to another (Philpot, 1979). The latter author stated that if transmission of mastitis pathogens is prevented by good hygiene, a parallel decrease in incidence of IMI will occur. By applying best practices, several of these issues can be mitigated or overcome. Bearing in mind that cattle owners, compared with farmers rearing other animal species, are more likely to adopt innovations, management technologies, and practices and that cattle are prioritized before other species in preventive health care and veterinary treatments (Amadou et al., 2012). Thus, the potential exists to increase and improve milk production and quality in Rwanda by inexpensive and simple means, such as application of the 10-point mastitis control plan (Middleton et al., 2014) and other best practices.

TBC at the Farm and MCC Levels

Except for 2 MCC, a higher TBC was detected in MCC milk samples than in farm milk samples. This suggests proliferation of bacteria in milk during transportation in unrefrigerated equipment. This agrees with Doyle et al. (2015), who detected an increase in total microbial load in the chain from farm through milk transporters to MCC and finally consumers in Rwanda. The same trend was reported in Uganda, where a 150-fold proliferation of bacteria occurred in milk from the farm level through transportation to consumers (Grimaud et al., 2007). In our study, TBC recorded in milk samples from farms were very high, suggesting that mixing such milk with milk of better quality at the MCC would increase the overall TBC of the milk at the MCC. Therefore, there is a need for infrastructure and equipment to separate out low-quality milk as early as possible in the milk chain, or to introduce economic incentives for farmers to produce and deliver milk with very low TBC. We speculate that the reason why 2 MCC did not experience an increase in TBC from farm to MCC was because the farmers were located close to the MCC and milk delivery took less time, allowing less opportunity for proliferation of microorganisms in the milk. The highest recorded TBC at the farm level (1.6 $\times$ 10^7 cfu/mL) was comparable to that reported in Zimbabwe (6.7 $\pm$ 5.8 $\log_{10}$ cfu/mL) in raw milk samples (Mhone et al.,2011), and comparable to the 7.08 $\log_{10}$ cfu/mL reported in milk samples from chilling centers in Sri Lanka (De Silva et al., 2016). The lowest median TBC (7 $\times$ 10^3 cfu/mL) was recorded in milk from MCC2, in Nyagatare, where farmers are known to have received more training on dairy husbandry and milk handling (TechnoServe Rwanda, 2008). In this study, we found that the lack of a separate milking area (such that farmers milk in the same place where the cow is housed) was significantly associated with increased risk of contamination of milk with environmental mi-

croorganisms, reflected by high TBC. Hence, a recommendation that farmers do not milk cows in the same place where cows are housed would likely improve the hygienic quality of milk.

Escherichia coli at the Farm and MCC Levels

Detection of *E. coli* was less frequent at the farm level than at the MCC level, suggesting contamination during handling at MCC or proliferation of bacteria during transport to MCC. Potential routes of contamination at the MCC level include personnel, equipment, and tools, whereas contamination at the farm level may be due to animal feces or poor hygienic level of animal husbandry practices (Kateřina et al., 2016). Our results agree with those of Grimaud et al. (2007), who reported high *E. coli* counts (2×10^6 cfu/mL) in raw milk samples at the farm level in Uganda.

Prevalence of *Salmonella* at the Farm and MCC Levels

The prevalence of *Salmonella* in milk from farms in this study was 14% but no MCC samples tested positive. It is possible that due to the dilution effect, *Salmonella* concentrations in MCC milk samples were below the detection limit of the method used. This prevalence at the farm level is higher than results from Rwanda reported by Kamana et al. (2014), who found a prevalence of *Salmonella* of 5.2% in raw milk samples from dairy farms, MCC, and milk shops. Our results are in the range of those reported in a study in Tanzania, where a prevalence of 10.1% was found in raw milk (Schoder et al., 2013). Farm environment is likely where reservoirs and vehicles for the *Salmonella* can be found (Quintana et al., 2020). It is possible that the *Salmonella* found in milk originated from milkers' hands, which may have touched reservoirs of *Salmonella* such as infected calves, shedding cows, or contaminated water (Marth, 1969). Our study revealed that a lack of teat washing before milking was associated with *Salmonella* contamination of bulk milk. Because shedding of *Salmonella* is common in cattle (Wells et al., 2001), poor hygiene through lack of teat washing will facilitate the transmission of the pathogen from the cow to the milk.

Prevalence of *Brucella* Antibodies at the Farm and MCC Levels

No sample tested positive for *Brucella* antibodies among farm bulk milk samples, but antibodies were detected in 3 samples in MCC bulk milk. These antibodies may have come from farm milk that was not sampled because we did not visit all farmers associated with the MCC. It may also imply that some cows could be harboring the *Brucella* pathogen and zoonotically infecting themselves and humans in the region around the MCC. This type of transmission of brucellosis between animals and humans has been suggested in Uganda, where the presence of *Brucella* antibodies in humans was associated with *Brucella* antibodies in milk samples from cattle (Miller et al., 2016). The level of detection of *Brucella* antibodies in milk at the MCC level in this study (22.72%) was markedly higher than the level (11%) reported in Gulu in Uganda (Rock et al., 2016).

Antibiotic Residues at the Farm and MCC Levels

Detecting antibiotic residues in bulk milk was not common in the present study. Antibiotic residues were detected only in milk samples from farm delivering milk that had high SCC levels, suggesting that treating mastitis with antibiotics without observing the withholding period could explain the presence of antibiotic residues in the milk samples. The consequences of antibiotic residues in milk are severe; for example, antibiotic residues can prevent optimum growth of starter cultures during processing of dairy products, and β-lactam antibiotics, if present, can cause allergic reactions in some individuals (Dewdney et al., 1991; Griffiths, 2019). Our results showed a markedly lower prevalence of antibiotic residues in farm bulk milk than has been reported by others: 44.5% reported in Kenya (Teresiah et al., 2016) in a study that used a kit similar to the one used here; 30% in Zambia (Kunda et al., 2016), using the Copan milk test (Copan Italia spa, Brescia, Italy); and 36% reported in Tanzania (Kurwijila et al., 2006), using the Charm AIM-96 (Charm Sciences Inc., Lawrence, MA) antimicrobial inhibition assay.

CONCLUSIONS

Milk delivered to MCC in Rwanda by farmers or intermediaries had high microbial contamination and SCC, which contributes to high TBC and SCC of milk at the MCC. Improved testing and separating low- from high-quality milk, followed by rejection of milk with high TBC and SCC upon receipt at the MCC is recommended. Overall, the increase in TBC from farm to MCC suggests bacterial proliferation during transport, emphasizing the need for refrigeration and proper handling during transport. Contamination of milk with *E. coli* seemed to be more frequent at the MCC level, suggesting that conditions were less hygienic at milk bulk collection sites. The 14% prevalence of *Salmonella* on dairy farms suggests that it is a key pathogen, and prevention and control measures are required to

safeguard public health from the risk associated with consumption of *Salmonella*-contaminated milk. Antibiotic residues were rarely detected but *Brucella* spp. antibodies were common in milk samples from MCC.

ACKNOWLEDGMENTS

The authors acknowledge funding from the Swedish International Development Agency (SIDA; Stockholm, Sweden), within the University of Rwanda-Sweden program for research, higher education and institutional advancement, subprogram agricultural sciences, project no. 20290000. This work was also funded in whole or part by the United States Agency for International Development (USAID; Washington, DC) Bureau for Food Security under Agreement #AID-OAA-L-15-00003 as part of Feed the Future Innovation Lab for Livestock Systems (University of Florida, Gainesville). Travel to the meeting was funded by the American Dairy Science Association. Any opinions, findings, conclusions, or recommendations expressed here are those of the authors alone. The authors have stated that they have no conflicts of interest.

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