Implications of differences in safety and hygiene control practices for microbial safety and aflatoxin M1 in an emerging dairy chain: The case of Tanzania

James Ledo [a], Kasper A. Hettinga [a], Jamal B. Kussaga [b], Pieternel A. Luning [a,*]

[a] Food Quality and Design Group, Wageningen University and Research, P. O. Box 17, 6700AA, Wageningen, the Netherlands
[b] Department of Food Technology, Nutrition and Consumer Sciences, Sokoine University of Agriculture, P. O. Box, 3006, Chuo Kikuu, Morogoro, Tanzania

A B S T R A C T

The varying performance of safety and hygiene control practices by chain actors can influence the consistent production of milk of good quality and safety in dairy chains. Therefore, the study aimed to investigate if differences in safety and hygiene control practices translate into distinctions in milk quality and safety at the farm, and to analyse the implications for actors further in the Tanzanian dairy chain. A previously developed diagnostic tool, customised for emerging dairy chains, was applied to assess and differentiate the performance of safety and hygiene control practices of actors from the farm to local retail shops. Based on interviews and on-site visits, each safety and hygiene control practice were differentiated into a poor, basic, intermediate or standard level. Milk samples were collected with a 7-day interval over three-time points to determine total bacterial counts (TBC), coliforms and *Staphylococcus aureus*. Besides, aflatoxin M1 (AFM1) occurrence was determined in farm milk as an indication of feed storage and monitoring practices. Data showed that none of the chain actors attained the standard level on any of the safety and hygiene control practices. Cluster analysis of on-farm safety and hygiene control practices generated two clusters, which differed mainly on the scores for udder and teat care, and disease detection practices. Differences in safety and hygiene control practices observed among farmers did not translate into differences in milk quality and safety. The analysis for AFM1 showed that 22% exceeded the maximum limit of the United States Food and Drug Authority Standard. Also, the microbial data showed that the farm milk already exceeded maximum limits of the East Africa Community (EAC) standard to the extent that no continued growth was observed further in the chain. The study demonstrates that improvements in milk quality and safety would require multiple practices to be upgraded to the standard level. Research is needed to advance the performance of control practices towards compliance with international standard requirements.

1. Introduction

Global milk production has increased by more than 50% over the last three decades (FAO, 2018). This growing trend would continue in emerging economies due to rapid population growth and improve income (Gerosa & Skoet, 2012; Kapaj & Deci, 2017). Simultaneously, reports of food safety issues associated with the consumption of fresh milk, and related products continue in developed and emerging dairy chains (Cheng, Mantovani, & Frazzoli, 2016; Johler et al., 2015; Van Asselt, van der Fels-Klerx, Marvin, Van Bokhorst-van de Veen, & Groot, 2017). Loopholes in the performance of safety and hygiene control practices, which create avenues for microbial and chemical contamination, have

been implicated in several of these food scares (Powell, Jacob, & Chapman, 2011; Todd et al., 2010; Van Asselt et al., 2017). In emerging dairy chains, the concern for food scares is magnified by the lack of uniformity in the implementation of food control systems (Kamana, Jacxsens, Kimonyo, & Uyttendaele, 2017; Ledo, Hettinga, Bijman, & Luning, 2019a). More so, when there is the direct sale of a large proportion of fresh milk to consumers (Grace, 2015) without any form of adequate milk cooling and pasteurisation.

At the same time, only a limited number of dairy processing industries in emerging dairy chains, have implemented the Hazard Analysis and Critical Control Point (HACCP) principles into their food safety management systems (FSMS) (Kussaga, Jacxsens, Tiisekwa, & Luning,

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* Corresponding author.
  E-mail address: pieternel.luning@wur.nl (P.A. Luning).

2014). Moreover, non-compliance with hygienic practices typifies the performance of these implemented systems (Kussaga, Luning, Tiisekwa, & Jacxsens, 2015; Opiyo, Wangoh, & Njage, 2013). On the farm, safety and hygiene control practices are still being performed at levels that demonstrate a lack of progress to standard requirements (Islam et al., 2018; Ledo et al., 2019a). Concerns about the adequate performance of safety and hygiene control practices by other actors such as milk traders, milk collection centres (MCCs) and local retail shops in the chain, continue to recur (Islam et al., 2018; Kamana, Ceuppens, Jacxsens, Kimonyo, & Uyttendaele, 2014). The underlying limitations in the performance of practices have implications for microbial and chemical milk safety risks. Bacteria and aflatoxin M1 (AFM1) contamination are common representatives of these type of risks (Vissers & Driehuis, 2009), as they are linked to multiple on-farm safety and hygiene control practices. Consequently, poor microbial quality and safety (Belli, Cantafora, Stella, Barbieri, & Crimella, 2013; Kunadu, Holmes, Miller, & Grant, 2018; Swai & Schoonman, 2011) and occurrence of AFM1 (Ahlberg, Grace, Kiarie, Kirino, & Lindahl, 2018; Iqbal, Jinap, Pirouz, & Faizal, 2015) above maximum limits in milk, continue to persist. The need to focus on AFM1 is necessary due to its common occurrence in emerging dairy chains in tropical countries, more so, when dairy farmers in these chains are often not aware of this specific risk. Hence, strategies to enhance the performance of safety and hygiene control practices and mitigate the recurring milk safety risks in emerging dairy chains are still necessary, as consumer demands for milk and milk products continue to increase.

Recently, we developed a customised assessment tool to support a systematic and differentiated analysis of safety and hygiene control practices and milk safety performance along the chain (Ledo, Hettinga, & Luning, 2019b). The tool describes crucial practices necessary to mitigate microbial and AFM1 contamination. It includes four different levels (i.e. poor, basic, intermediate and standard) to position the performance level of the practices accurately. A pilot study with the new tool in Tanzania demonstrated that many dairy farmers were performing the practices below the minimum standard level. Actual milk safety performance was, however, not assessed. Expanding the application of the tool to assess practices and milk safety performance during milk trading, collection/bulking, and retailing is essential, as this will give a better indication of the overall chain effectiveness to safeguard food quality and safety.

This study aimed to investigate if differences in safety and hygiene control practices translate into distinctions in milk quality and safety at the farm, and to analyse the implication for actors further in the chain, using the Tanzanian dairy chain as an example. The customised assessment tool for emerging dairy chains was applied to systematically distinguish practices of dairy chain actors from the farm to local retail shops. The practice assessment was followed by fresh milk sampling and laboratory analysis of microbial and aflatoxin M1 levels to investigate the possible relations between the level of practices along the chain with milk quality and safety.

2. Materials and methods

2.1. Study design

The study was designed based on the techno-managerial research approach previously outlined by Luning and Marcelis (2006, 2007) for food chains, to unravel the interrelatedness of technological and people-related conditions impacting milk safety in emerging dairy chains. The study covered two major parts. The first part comprised the application of a customised assessment tool previously developed by Ledo et al. (2019b) to evaluate the level of practice performance of farmers, milk traders, milk collection centres (MCCs), and local retail shops, using interviews and structured on-site observations. The second part involved the sampling of fresh milk from the actors along the chain to investigate the presence and levels of bacteria and aflatoxin M1, as an

Table 1
Characteristics of study respondents along the Tanzania dairy chain.

Characteristics of respondents	Farmers	Milk traders	MCC	Milk retail shops
	(n = 24)	(n = 3)	(n = 4)	(n = 4)
	n (%)	n (%)	n (%)	n (%)
Respondents from study sites				
Manyinga	6 (25)			1 (25)
Wamidakawa	6 (25)	2 (66.7)	2 (50)	1 (25)
Mwangoi	6 (25)		1 (25)	1 (25)
Ngulwi	6 (25)	1 (33.3)	1 (25)	1 (25)
Age				
<20 years				
21–30 years	2 (8)			
31–40 years	2 (8)	1 (33.3)	3 (75)	2 (50)
41–50 years	8 (34)	1 (33.3)	1 (25)	1 (25)
>50 years	12 (50)	1 (33.3)		1 (25)
Sex				
Male	18 (75)	3 (100)	3 (75)	1 (25)
Female	6 (25)		1 (25)	3 (75)
Education level				
Attended no school	3 (12)			
Primary school level	17 (71)	3 (100)	1 (25)	3 (75)
Secondary school level	4 (17)		3 (75)	1 (25)
Post-secondary certificate training				
Tertiary/higher education level				
Ability to read and write				
Yes	21 (88)	3 (100)	4 (100)	4(100)
No	3 (12)			
Water source used for hygiene activities				
Tap water	4 (17)	1 (33.3)	4 (100)	3 (75)
Borehole water	10 (42)	1 (33.3)		
Streams/rivers/dams	4 (17)	1 (33.3)		
Tap water & borehole water	1 (4)			1 (25)
Borehole & streams/rivers/dams	2 (8)			
Tap water & stored rainwater	3 (12)			
Who buys most of your milk?				
Milk traders	5 (21)			
MCC	7 (29)	3 (100)		
Neighbours/individuals	10 (42)			4(100)
Milk retail shops	1 (4)			
Neighbours and retail shops	1 (4%)			
Processing company			4 (100)	
Type of farming system				
Intensive (zero-grazing)	14 (58)			
Semi-intensive (Zero + free)	6 (25)			
Extensive (Free range)	4 (17)			
No. of milking cows				
1 - 3 cows	13 (54)			
4 - 6 cows	8 (34)			
>7 cows	3 (12)			

indication of milk quality and safety. The study was conducted in two selected milk-producing districts of Tanzania: Mvomero and Lushoto, located in the Morogoro and Tanga regions, respectively. The regions and districts were selected because they have been part of multiple dairy intervention programs with prominent dairy production and marketing activities, and representative of the Tanzanian dairy chain (Njehu & Omore, 2014). In each district, two study locations were selected, linked to our previous study (Ledo et al., 2019a).

2.1.1. Selection of study participants

The dairy farmers were contacted through livestock officers of the study locations from a register of farmers used in a previous study (Ledo et al. (2019a). Those willing to participate were followed up for
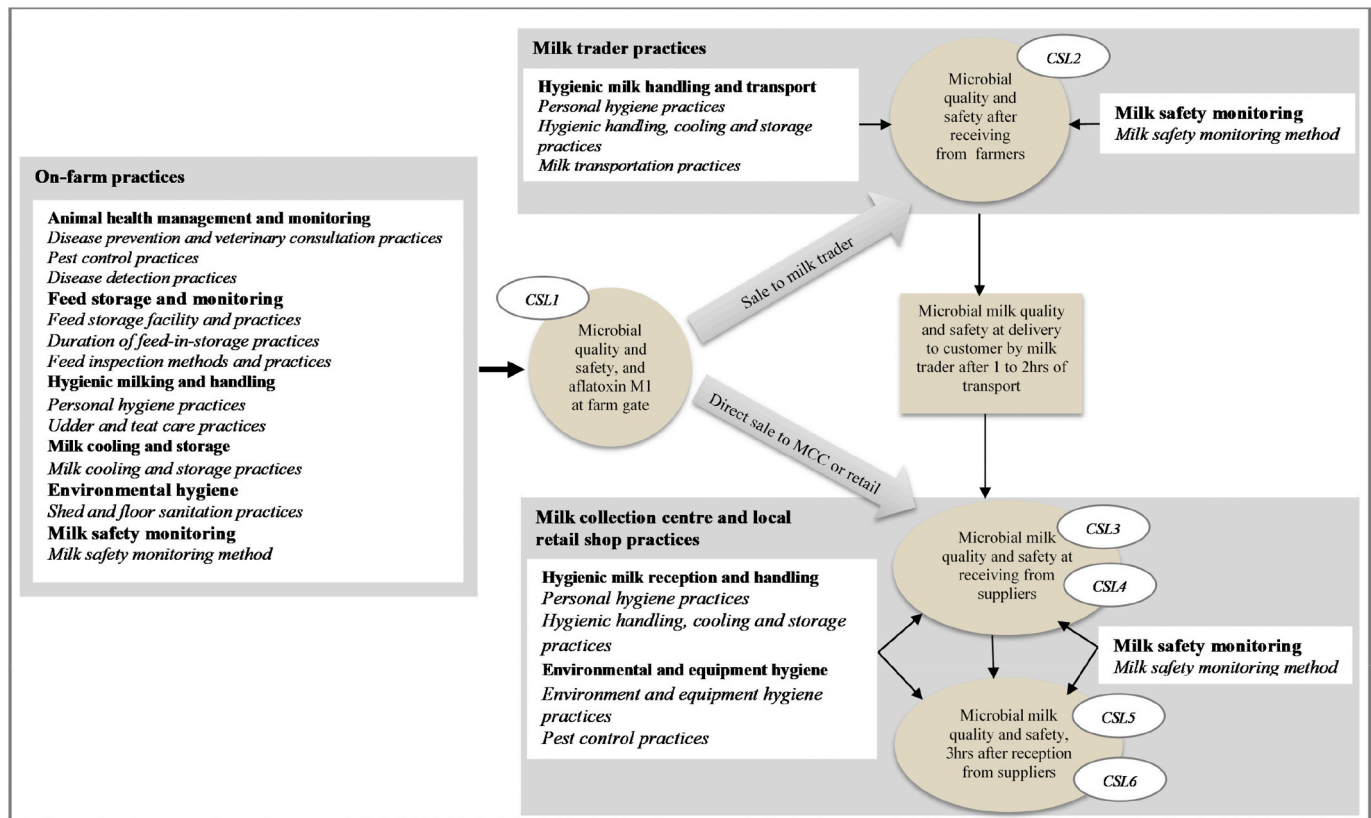
Fig. 1. Analytical framework showing the crucial safety and hygiene practice indicators, their relationship with milk safety (microbial and Aflatoxin M1) and the critical sampling locations (CSL) along the emerging dairy chain.

interviews, on-site observations and milk sampling for laboratory analysis. The milk traders, milk collection centres and milk retail points were identified through the snowball sampling technique (Biernacki & Waldorf, 1981), where the dairy farmers referred their customers of the fresh milk and were contacted to plan the interviews and on-site observations. All the dairy farmers were operating at a small-scale with at least one milking cow at the time of the study and over a year experience in dairy farming. All the milk traders and retailers were private local businesses. Two of the MCCs were individually owned while the other two were owned by farmer co-operative groups. Overall, 24 dairy farmers, three milk traders, four MCCs and four retail shops were included in the study (Table 1).

2.1.2. Customised assessment tool

The tool consists of indicators that reflect the crucial practices, from farm to retail shops (Fig. 1), that may influence milk safety. For each indicator, four situational descriptions with a score were established (i.e. grids) describing a poor (score 1), basic (score 2), intermediate (score 3), and standard level (score 4) to differentiate the practice performance, as described in detail in (Ledo et al., 2019b).

2.2. Data collection approach

2.2.1. Questionnaire and observation checklist design

For each indicator, a set of open-ended questions were formulated to assess the performance level (and corresponding score) of the safety and hygiene control practice. The format of the open-ended question was chosen to allow respondents to detail how they perform their practices freely. Besides, an observation checklist was developed to verify the presence of cleaning and personal hygiene tools, milk handling and storage equipment, facility floor design, and extent of documentation unique to the performance of practices. The details of the specific question and the checklist for the on-site observations can be seen in

supplementary material III.

2.2.2. Face-to-face interviews and structured on-site observation

The face-to-face interviews and structured on-site observations were conducted for all identified respondents at the farm or the dairy business location. The scientific research approval was obtained from the Sokoine University of Agriculture, Tanzania, and written informed consent of each respondent was obtained. The questions for the face-to-face interviews were read out to respondents in their local language; their responses were written out and audio-taped at the same time. The structured on-site observation followed immediately after the interviews. On average, the visits took 1½ hours.

2.2.3. Milk sample collection

The fresh milk samples were collected under aseptic conditions into sterile falcon screw-capped vials of 50 mL. The samples were stored and transported to the laboratory in isolation boxes on blue ice packs at less than 4 °C, consistent with the sampling technique described by Chye, Abdullah, and Ayob (2004). The milk samples were transported immediately to the laboratory, stored at 0 °C and further analysed within 24 h. Overall, 72 milk samples were collected from the dairy farmers, nine samples from milk traders, 12 samples each from the MCCs and retail points. Altogether, the sampling and analysis were done over three months covering all the study locations. For sampling details and explanation, see section 2.2.4.

2.2.4. Microbial analysis of the fresh milk

The microbial analysis was performed based on a modification of the principles underpinning microbial assessment scheme (MAS) described by Jacxsens et al. (2009). Firstly, we identified critical sampling locations (CSL) along the chain (Fig. 1), which refers to points at each stage of the chain where microbial sampling provides an indication of practices performance (Jacxsens et al., 2009), as detailed in the customised

tool. CSLs were identified to assess on-farm microbial quality and safety (*CSL1*), during milk trading (*CSL2*), at the MCC (*CSL3 and CSL5*), and at the retail shops (*CSL4 and CSL6*). Secondly, we defined microbiological parameters, to enable judgement of the level of contamination in terms of the number of bacteria present (Jacxsens et al., 2009). We assumed that low bacterial counts with small variations are evidence of well-performed practices at that stage of the chain (Jacxsens et al., 2009; Ledo et al., 2019b). Total bacteria count (TBC) was selected as an indicator of the presence of aerobic mesophile bacteria (Robinson, 2005), which are the most abundant in raw milk, thus providing insights in overall contamination.

We also selected coliforms as an indicator of environmental and hygienic handling performance (Wanjala, Nduko, & Mwende, 2018), and *Staphylococcus aureus* as an indicator of udder health, hand hygiene and food safety performance (Jacxsens et al., 2009; Perin, Pereira, Bersot, & Nero, 2019). All microbial parameters were analysed at each CSL. Thirdly, a three-time sampling frequency was adopted at an interval of 7-days between samplings, to provide an insight into the microbial load profile overtime at each CSL. Finally, the sampling method and method of analysis were based on ISO standards and all the analyses were performed in the microbiology laboratory of Tanzania Official Seed Certification Institute (TOSCI). The details of the sampling method and method of analysis are described for each selected microbial parameter. Colony counts between 30 and 300 were used for calculating the number of colony-forming units (CFU) per mL of milk according to the formula, colony count = n * 1/V * 1/d (ISO, 1996). Where n is the number of colonies counted per plate, V is the volume of inoculum in each plate (mL), and d is the dilution factor used to determine the colony count. The average number of the countable colonies after the incubation time of the duplicate plates was used for the calculations.

2.3. Total bacterial count analysis

Total bacterial count (TBC) was enumerated, as stated by ISO 4833-1:2013 (ISO, 2013) using Plate Count Agar (PCA) prepared according to the manufacturer's direction (HIMEDIA M091, Mumbai, India). Serial dilutions of the fresh milk were made in peptone water (HIMEDIA MO28, Mumbai, India) prepared according to the manufacturer's specifications. Based on the suspected level of contamination, exactly 1 mL of $10^{-5}, 10^{-7}$ and 10^{-9} dilutions were pour plated with 15 mL of PCA in duplicate, allowed to set and incubated in inverted positions at 30 °C for 72 h.

2.4. Coliform analysis

The total coliform was enumerated based on the procedure described by (Wehr & Frank, 2004) using MacConkey agar consisting of 0.15% bile salts, crystal violet (CV) and sodium chloride (NaCl) (HIMEDIA M081, Mumbai, India). The MacConkey agar is a selective and differential medium to detect gram-negative bacteria. Serial dilutions of $10^{-2}, 10^{-4}$ and 10^{-6} were prepared based on the suspected level of contamination, 0.1 mL of each dilution was surface plated in duplicate and incubated in inverted positions at 37 °C for 48 h.

2.5. Staphylococcus aureus analysis

Staphylococcus aureus was identified and enumerated using Baird-Parker Agar (BPA) (HIMEDIA M043, Mumbai, India), as outlined by ISO 6888-1 and 2 (ISO, 1999a; 1999b). Serial dilutions of 10^{-3} and 10^{-5} of the fresh milk were spread plated and incubated at 37 °C for 48 h. Typical black colonies surrounded by clear zones and atypical colonies were picked, inoculated into 5 mL Brain Heart Infusion (BHI) (HIMEDIA M210, Mumbai, India) broth prepared according to the manufacturer's specification and incubated at 37 °C. After 24 h, 0.1 mL of the enriched broth was transferred into 0.3 mL of coagulase plasma (from Rabbit) (HIMEDIA FD 248, Mumbai, India) and incubated at 37 °C for another

Table 2
Microbiological criteria for classifying fresh milk quality and safety.

Microorganisms	East Africa Community (EAC) standards (EAS 67:2006)	European Union (EU) standards (Regulation (EC) No 853/2004)
	Log $_{10}$ CFU/mL	Log $_{10}$ CFU/mL
Total bacteria count (TBC)		
Grade 1	<5.3	5.0
Grade 2	5.3–6.0	5.6
Grade 3	6.0–6.3	
Beyond grade 3	>6.3	
Coliforms		
Very good	<3.0	
Good	3.0–4.7	
Below good	>4.7	
Staphylococcus aureus[a]		
Within range		4–5
Outside range		> 5

[a] We used the EU criteria for raw milk intended for cheese making (Regulation (EC) No 2073/2005).

24 h. Clotted tubes were identified, and the presence of coagulase-positive *Staphylococcus aureus* was confirmed. Confirmed typical and atypical colonies were used to determine the count of *Staphylococcus aureus*.

2.5.1. Aflatoxin M1 analysis of fresh milk

The level of contamination of fresh milk samples with aflatoxin (AFM1) was determined using Aflasensor Quanti 0.5ppb-KIT078 (Unisensor, 2016). This is a rapid assay in dipstick format to visualise and quantify AFM1 which does not require any sample processing, cleaning or extraction. The kit consists of 96 dipsticks and microwells, a heat sensor DUO-APP032 for incubation and a read sensor-APP038 (Unisensor, 2016). Exactly 200 μL of fresh milk was added to one reagent microwell using a specific micropipette of 200 μL, mixed thoroughly to homogenise, placed in the heating block in the heat sensor and incubated at 25 °C for 10 min. The dipstick drops down automatically into the microwell after the first incubation time and incubates for another 10 min at 25 °C. The dipstick was observed to detect the presence or absence of AFM1 after the incubation. Positive and negative controls were used to confirm the colour changes of the dipsticks to verify presence or absence of AFM1 in the milk samples. The quantitative value was then determined by inserting the dipstick into the dipstick reader which was programmed for AFM1. The dipsticks were tailored to read actual values from the lower limit of 0.2 μg/L up to the maximum limit of 0.75 μg/L regarding the United States Food and Drug Administration (USFDA) standard. The USFDA standard of 0.5 μg/L (Iqbal et al., 2015) was preferred over the European Union (E.U.) standard to provide a wider spectrum for quantification of AFM1. Analyses were done in duplicate for each fresh milk sample, and the average AFM1 value was calculated.

2.3. Data processing and interpretation

Interview responses depicting poor, basic, intermediate and standard practice performance were assigned scores 1, 2, 3 and 4 as described in detail in Ledo et al. (2019b). The assigned scores were entered in Microsoft Excel and imported into IBM SPSS statistics version 25 for windows for descriptive statistical analysis. To determine the frequency of occurrence of AFM1 in the farm milk, the number of milk samples tested (144) with their corresponding AFM1 values were compared with the detection range of the dipstick method (0.2 μg/L to 0.75 μg/L) and with the USFDA maximum limit of 0.5 μg/L. Also, to gain insight into the overall microbial quality and safety regarding TBC, coliforms and *Staphylococcus aureus* for farmers (72 samples), milk traders (9 samples), MCCs (12 samples) and local retail shops (12 samples), the log CFU/mL were calculated. These were compared with the microbiological criteria
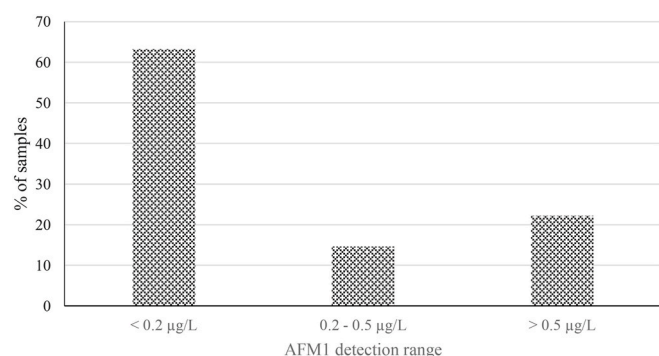
Fig. 2. Occurrence of aflatoxin M1 in all tested farm milk samples of dairy farmers. Milk samples (n = 72) were analysed in duplicate; Dipstick lower detection limit = 0.2 µg/L, USFDA maximum limit = 0.5 µg/L.

(Table 2) of the East Africa Community (EAC, 2006) and the European Union (E.C., 2004, 2005), and the corresponding frequencies for each parameter were determined. Hierarchical and K-means cluster analyses were performed with R version 3.5.0 using Ward's method and Euclidean distance (Kassambara, 2017) to determine the cluster number and pattern that best fitted the dairy farmers data set using the assigned scores of the safety and hygiene control practices. The mode scores for each practice was determined for both clusters and these were used to construct the spiderwebs. For each cluster, the average $\log_{10}$ CFU/mL were determined for each farmer regarding TBC, coliforms and *Staphylococcus aureus* of the three-time points. For the AFM1, the average of the three-time points was computed for each farmer of the two clusters for interpretation.

3. Results and discussion

3.1. Occurrence of Aflatoxin M1 in farm milk

Fig. 2 shows the occurrence of AFM1 in farm milk sampled from dairy farmers as an indication of the level of performance of feed storage and monitoring practices, and the extent of risk for the chain. Overall, the majority (63%) of the farm milk samples (91/144) were below the lower detection limit (0.2 µg/L) of the dipstick method, while 14% were between the lower detection limit and the maximum limit of the USFDA legal standard (0.5 µg/L). About 22% of the farm milk samples (32/144) exceeded the USFDA maximum limit. A previous study by Mohammed, Munissi, and Nyandoro (2016) in Tanzania, and Gizachew, Szonyi, Tegegne, Hanson, and Grace (2016) in Ethiopia, also found that 16% (6/37) and 26% (29/110) respectively, of all milk samples, exceeded the USFDA maximum limit for AFM1. The majority of the milk samples were below the USFDA maximum limit of AFM1. Still, even at lower levels, AFM1 poses a risk to consumers given that processing does not remove or reduce AFM1 in milk (Roze, Hong, & Linz, 2013). More so, long-term exposure to aflatoxins can lead to chronic health effects such as liver damage for both cows and humans (Liu, Chang, Marsh, & Wu, 2012; Wu, Groopman, & Pestka, 2014). In the Tanzanian context, this is crucial as, Magoha et al. (2014) found that infant growth could be impaired through the exposure of AFM1 in the breast milk of their mothers. Appropriate steps are needed to mitigate the risk AFM1 to safeguard public health.

3.2. Feed storage and monitoring related practices about Aflatoxin M1 in farm milk

The performance of dairy farmers on feed storage and monitoring practices was analysed to relate it to the occurrence of AFM1 in the farm milk samples. Cluster analysis using the scores for the feed storage
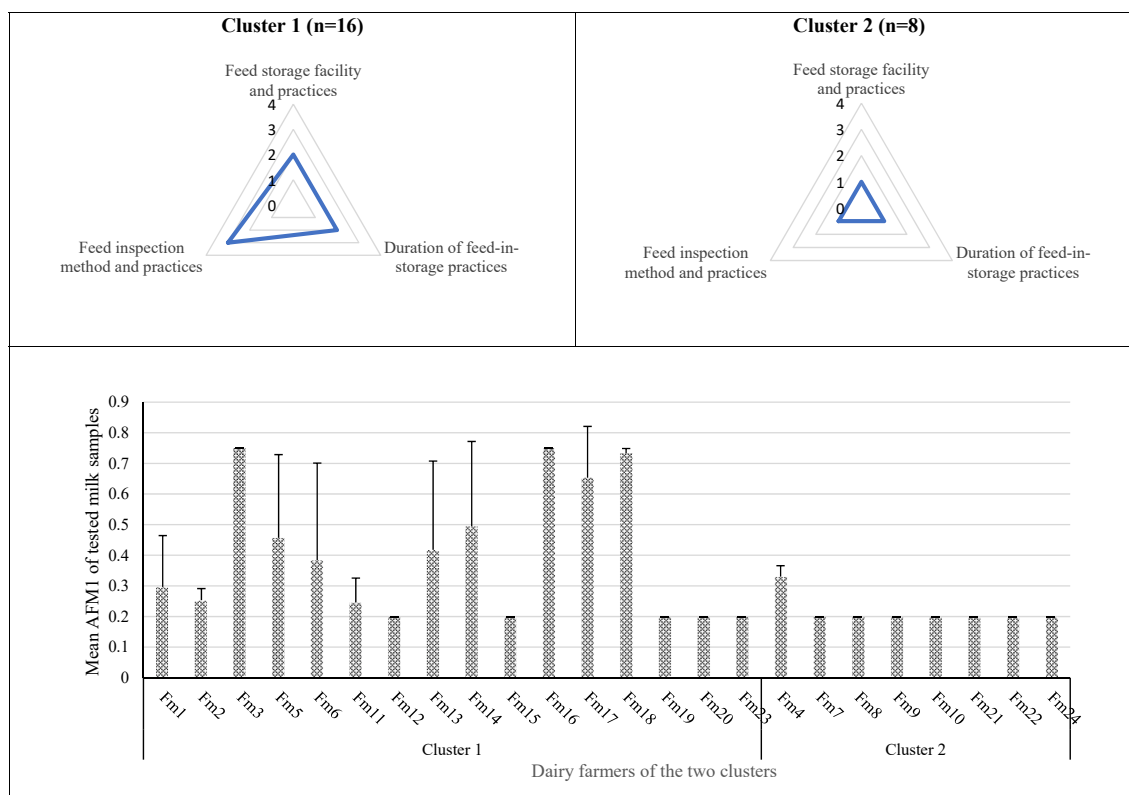
Fig. 3. Spiderwebs depicting mode scores of clusters of feed storage and monitoring related practices of dairy farmers and corresponding AFM1 concentrations detected.
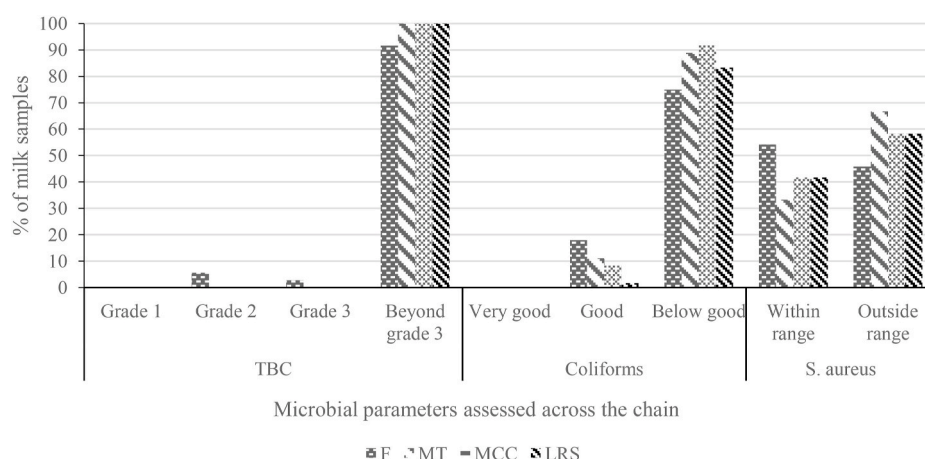
Fig. 4. Classification of microbial contamination of milk samples taken over three-time points along the dairy chain. **TBC (EAC):** Grade 1: <5.3 $\log_{10}$ CFU/mL, Grade 2: 5.3–6.0 $\log_{10}$ CFU/mL, Grade 3: 6.0–6.3 $\log_{10}$ CFU/mL, Beyond grade 3:>6.3 $\log_{10}$ CFU/mL; **Coliforms (EAC):** Very good: 0–3.0 $\log_{10}$ CFU/mL, Good: >3.0–4.7 $\log_{10}$ CFU/mL, Below good:>4.7 $\log_{10}$ CFU/mL; *S. aureus* **(EU):**Within range: 4–5 $\log_{10}$ CFU/mL, Outside range:>5 $\log_{10}$ CFU/mL; **F:**farmers (n = 72 samples), **MT:** milk traders (n = 9samples), **MCC:** milk collection centres (n = 12samples), **LRS:** local retail shops (n = 12samples).

facilities, feed inspection method and duration of feed-in-storage practices, yielded two clusters of dairy farmers. Fig. 3 shows the spiderwebs of mode scores for these feed storage and monitoring practices and the occurrence of AFM1 of farmers in these clusters. Overall, none of the farmers performed at the standard level (score 4) for any of the practices. Nevertheless, farmers in cluster 1 performed better on feed storage and monitoring practices than farmers in cluster 2. Most of the farmers in cluster 1 performed at the intermediate level (score 3) for feed inspection method and practices, whereas for feed storage facility and duration of feed-in-storage practices, they performed at the basic level (score 2). A basic level corresponds overall with the use of basic facilities, irregular practices with oral instructions, no documentation and ad hoc data collection. In contrast, most of the farmers in cluster 2 performed at the poor level (score 1) on all feed storage and monitoring practices. A poor level overall indicates that farmers exposed feed to all weather conditions, did not separate new feed from the old feed, did not monitor storage time, did not inspect for or remove mould, and did not keep records. Underlying this low score was that most of these farmers did not have a dedicated feed storage facility, which limits temperature and moisture control when feed would be stored. The resulting exposure of feed to the temperature range of 10–40 °C and the relative humidity of about 70% (Lanyasunya, Wamae, Musa, Olowofeso, & Lokwaleput, 2005), typical of tropical countries like Tanzania, would easily favour mould growth. Interestingly, Fig. 3 reveals that for most of the milk samples from farmers in cluster 2, the AFM1 levels were below the USFDA maximum limit (0.5 μg/L). The poor level of practices was expected to reflect in a higher occurrence of AFM1 in the farm milk. The absence of this relationship is likely because the on-site observations showed that most of these farmers took their cows to graze on the open fields and rarely used concentrates as feed. Flores-Flores, Lizarraga, de Cerain, and González-Peñas (2015) showed in their review that cows fed by grazing had lower AFM1 levels than those fed on concentrates. Thus, our data indicate that the mode of feeding may be more important in explaining AFM1 in milk than the performance level of feed storage and monitoring practices.

For farmers in cluster 1, intensive and semi-intensive dairy farming was the dominant systems and storing feed for the dry seasons was common, which explains their basic level performance (score 2). A basic level means that feeds are kept on raised platforms covered with plastic bags, temperature and moisture fluctuates, feeds are stored for more than six months, and without a structured system to separate new from an old feed. An intermediate level (score 3) on feed inspection method and practices may compensate for this shortfall. The farmers in cluster 1, perform weekly visual observations of the stored feed and physically remove mouldy feed based on their experience. Although manually inspecting and discarding contaminated feed is a useful measure to control mould growth (Golob, 2007; Kabak, Dobson, & Var, 2006), it can be time-consuming and not always thorough. The latter is substantiated

by our finding that the AFM1 levels in several milk samples from farmers in cluster 1 exceeded the maximum USFDA limit of 0.5 μg/L with some samples being higher than 0.7 μg/L. Moreover, the variation between the three-time points was relatively large (Fig. 3), indicating that the inconsistent performance of the practices leads to variable AFM1 levels in milk. Nevertheless, the AFM1 concentrations in milk samples of some farmers in this cluster were at the lower detection limit (0.2 μg/L), suggesting that additional factors such as the amount of AFB1 ingested from the contaminated feed and the carryover of AFM1 into the milk could have contributed to the pattern seen. Several studies (Battacone, Nudda, Palomba, Mazzette, & Pulina, 2009; Gonçalves et al., 2017; Xiong, Wang, Nennich, Li, & Liu, 2015) have demonstrated the direct relationship between the amount of AFB1 intake in naturally contaminated concentrate feed and the occurrence of AFM1 in dairy farm milk. While our study could not be conclusive on the actual intake of contaminated feed concerning the occurrence of AFM1 in milk, the variability in AFM1 levels does demonstrate the complexity of AFM1 contamination in farm milk. Thus, awareness of these underlying factors and progression on all feed storage and monitoring practices to the 'standard level' is necessary for farmers that store feed.

3.3. Microbial load of fresh milk samples along the chain

Fig. 4 shows the classification of microbial load of the milk samples over the three-time points for TBC, coliforms and *Staphylococcus aureus* as an indication of milk quality and safety along the chain. Overall, most of the milk samples exceeded the maximum microbial limit (grade 3) for TBC and over 70% were over the maximum limit for coliforms according to the East Africa Community (EAC) standard for all the chain actors (Fig. 4). This is consistent with the study of Ngasala, Nonga, Madundo, and Mtambo (2015) in Tanzania, who reported that 91% of fresh milk samples analysed across the milk chain, exceeded the maximum limit for TBC. Likewise, other studies reported that up to 60% of fresh milk samples exceeded the maximum limit for TBC at the farm (Gwandu et al., 2018; Nonga et al., 2015). Also, a study by Swai and Schoonman (2011) reported that 83% of fresh milk samples were over the maximum limit for coliforms along the milk chain in Tanzania, which is comparable with the findings of our study. The high microbial load of milk samples over the maximum limit at all stages of the chain compares closely with other emerging dairy chains, like in Bangladesh (Islam et al., 2018) and Rwanda (Kamana et al., 2014). Collectively, the high TBC and coliforms load indicates poor production, handling and environmental hygiene practices (Perin et al., 2019). For *Staphylococcus aureus*, more than half of the tested samples from milk traders, MCCs and local retail shops were above the limit of the E.U. standard, whereas this was slightly below half for the farmers (Fig. 4). This corresponds to 33% of milk samples that were found to be contaminated with *Staphylococcus aureus* over the maximum E.U. limit in a study by Ngasala et al. (2015) in
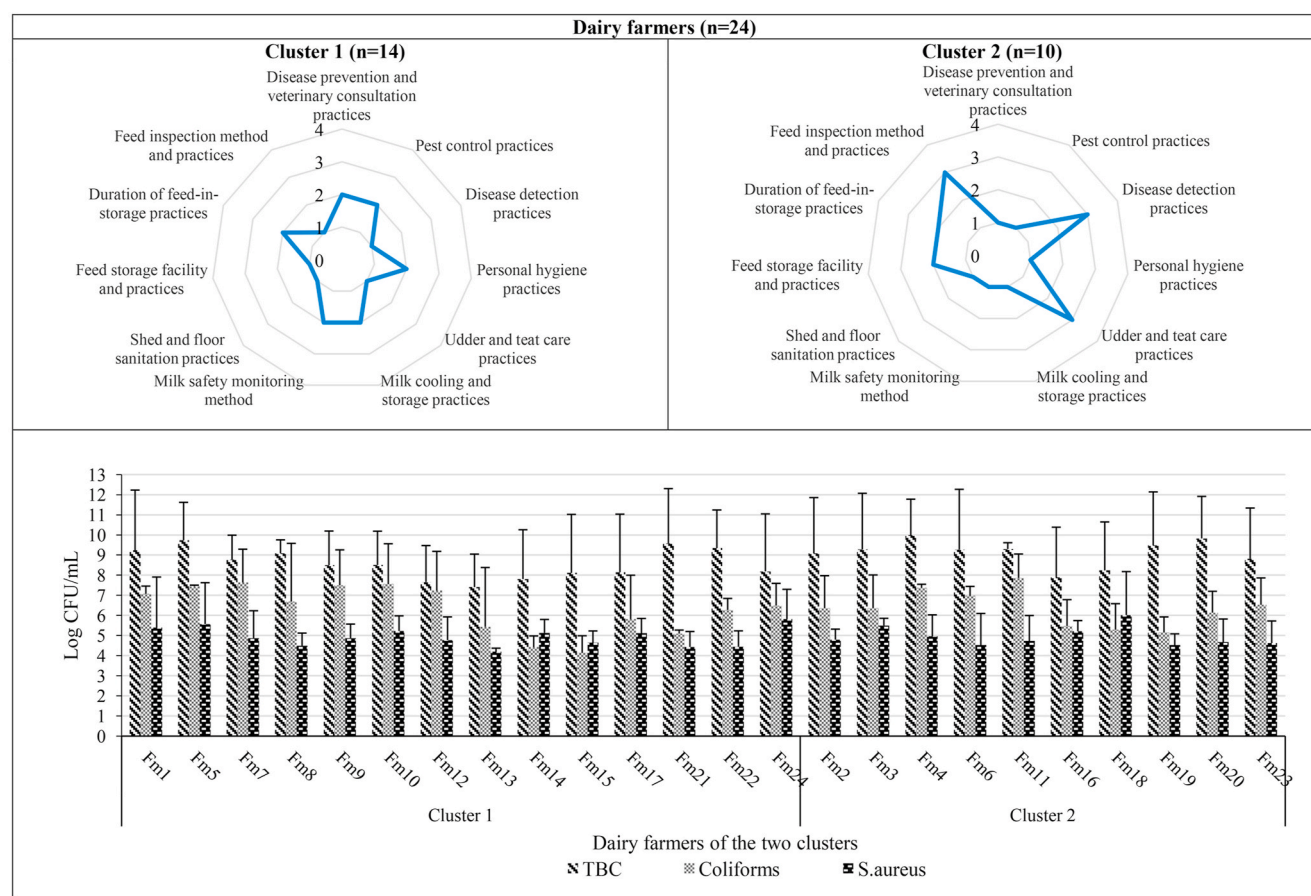
Fig. 5. Spiderweb depicting mode scores of safety and hygiene control practices of dairy farmer clusters and the corresponding microbial quality and safety.

Tanzania. These results imply that the risk for consumers is persistently high, as direct sale of raw milk is prevalent in the Tanzanian dairy chain. Moreover, poor microbial quality can significantly alter the composition, quality and yield of processed dairy products, such as cheese (Murphy, Martin, Barbano, & Wiedmann, 2016; Velázquez-Ordoñez et al., 2019), thus leading to losses if milk would be further processed in a formal production chain.

3.4. On-farm safety and hygiene control practices and microbial load of farm milk

A cluster analysis was performed with the scores for on-farm safety and hygiene control practices and the microbial data of farm milk to gain insight into possible relations between the level of practices and microbial load. The hierarchical cluster analysis yielded two distinct clusters of farmers. Fig. 5 shows the spiderweb profiles made of the mode scores of farmers' control practices for the two clusters. Overall, the dominant low levels (scores 1 and 2) of dairy farmers' safety and hygiene practices in both clusters reflect that rudimentary practices commonly reported in previous studies related to developing countries (Islam et al., 2018; Kamana et al., 2017; Ledo et al., 2019a), still persevere.

However, obvious differences can be seen for the udder and teat care, and disease detection practices of the two clusters where farmers in cluster 1 performed mainly at the poor level (score 1) compared to the intermediate level (score 3) for farmers in cluster 2. Poor performance on the udder and teat care implied no adherence to pre-/post-milking routines where the calves suckle on the teats without cleaning before milking. Also, no fore-stripping, no California Mastitis Test (CMT), and no records for diseases identified or treated depicts disease detection practices of similarly poor performance (Ledo et al., 2019b). Good dairy

production measures are lacking, which magnifies cow health and microbial risks. For farmers in cluster 2, the intermediate performance on the same practices indicates that the type of equipment used, the actual practices, documentation of protocols and data are much better but still not compliant with the 'standard level'. For instance, they apply fore-stripping and teat cleaning with a dedicated towel to clean the udder and teats; however, they do not apply post-dipping. Incomplete records on disease detection and treatment are kept, while the California Mastitis Test is sometimes, but not always, performed to identify subclinical signs of mastitis. Yet, the absence of post-dipping, particularly when shed and floor sanitation, and personal hygiene practices are performed at a poor level (score 1), can expose the cows to mastitis and microbial risks (Baumberger, Guarín, & Ruegg, 2016; Klostermann et al., 2010).

The microbial data of both clusters indicate that the average counts were over the maximum limits for TBC (>6.3 log CFU/mL) and coliforms (>4.7 log CFU/mL) according to the EAC criteria (Fig. 5). Furthermore, the average counts of *Staphylococcus aureus* were close to the maximum limit (5 log CFU/mL) according to the E.U. criteria, and these levels were equally high for both clusters. The high TBC (Cluster 1: 8.5 log CFU/mL; Cluster 2: 9.1 log CFU/mL), and high level of coliforms (Cluster 1 and 2: 6.3 log CFU/mL) and high counts for *Staphylococcus aureus* demonstrated that the poor dominating safety and hygiene practices created avenues for microbial contamination as demonstrated in several previous studies (Elmoslemany, Keefe, Dohoo, & Jayarao, 2009; Mhone, Matope, & Saidi, 2011; Tolosa et al., 2016). For instance, coliforms in farm milk have been associated with poor shed and floor sanitation practices that can lead to faecal contamination (Belbachir, Khamri, & Saalaoui, 2015; Wanjala et al., 2018). Also, the prevalence of *Staphylococcus aureus* in the farm milk is indicative of its possible presence in the udders of the dairy cow (Abebe, Hatiya, Abera, Megersa, &

Table 3

Mode scores of safety and hygiene control practices along the dairy chain.

Practice indicators	Farmers (n = 24)[a]										Milk traders (n = 3)[a]					MCC (n = 4)[a]					Local retail shops (n = 4)[a]				
	Cluster 1 (n = 14)					Cluster 2 (n = 10)																			
	1	2	3	4	Mode	1	2	3	4	Mode	1	2	3	4	Mode	1	2	3	4	Mode	1	2	3	4	Mode
Practices exclusive to the farm																									
Disease prevention and veterinary consultation practices	2	12	0	0	**2**	6	**4**	0	0	**1**															
Disease detection practices	**8**	3	3	0	**1**	1	3	**6**	0	**3**															
Feed storage facility and practices	**8**	6	0	0	**1**	1	**5**	4	0	**2**															
Duration of feed-in-storage practices	6	**8**	0	0	**2**	1	**7**	2	0	**2**															
Feed inspection method and practices	7	3	**4**	0	**1**	2	2	**6**	0	**3**															
Udder and teat care practices	10	4	0	0	**1**	4	0	**6**	0	**3**															
Practices performed by all actors in the chain																									
Personal hygiene practices	0	**13**	1	0	**2**	**5**	0	**5**	0	**1**[b]	1	**2**	0	0	**2**	0	1	**3**	0	**3**		0	**2**	**2**	0	**2**[b]
Milk safety monitoring method	1	**13**	0	0	**2**	**9**	0	1	0	**1**	1	1	1	0	**1**[b]	0	2	2	0	**2**	**4**	0	0	0	**1**
Hygienic milk handling, cooling and storage practices	1	**11**	1	0	**2**	**6**	0	4	0	**1**	**2**	1	0	0	**1**	1	**2**	1	0	**2**	**2**	**2**	0	0	**1**[b]
Practices exclusive to some actors in the chain																									
Pest control practices	6	**8**	0	0	**2**	**9**	1	0	0	**1**						**4**	0	0	0	**1**	**2**	**2**	0	0	**1**[b]
Shed and floor sanitation/Environmental and equipment hygiene practices	**8**	3	3	0	**1**	**6**	0	4	0	**1**						0	**3**	1	0	**2**	0	**2**	**2**	0	**2**[b]
Milk transportation practices											**2**	1	0	0	**1**										

[a] MCC = milk collection centre; 1 = Poor level, 2 = Basic level, 3 = Intermediate level 4 = Standard level.

[b] Bimodal situation; we used the lower scores for the discussion.

Asmare, 2016; Viguier, Arora, Gilmartin, Welbeck, & O'Kennedy, 2009) and an indication of inadequate hygiene practices during milking (Perin et al., 2019). However, the observed differences in the performance of practices between farmers in cluster 1 and 2, were not reflected in clear differences in the microbial load. Small transitions from the low to basic to intermediate level are not sufficient to substantially improve the microbial safety of milk. Progress to the standard level should thus be the minimum level aimed for. Nevertheless, the farmers in cluster 2 that perform the udder and teat care, and disease detection practices at the intermediate level are at a better position to advance towards the standard level.

3.5. Safety and hygiene control practices and microbial load of fresh milk along the chain

Fig. 6 shows how the microbial load in the milk, directly from the farm, evolved along the dairy chain and Table 3 shows the mode scores of the safety and hygiene control practices of the actors. Overall, the microbial load of all fresh milk samples exceeded the maximum limit for TBC and coliforms and *Staphylococcus aureus* levels were equally high. For all actors, the scores for safety and hygiene control practices were below 4, so none of them performed according to international requirements (i.e. the standard level). Most practices were dominated by a poor (score 1) to a basic level (score 2), except for milk safety monitoring method and personal hygiene practices at the milk collection centres (Table 3). The intermediate level (score 3) for milk safety monitoring method, implies that standardised tests are performed whereas the Resazurin test for bacteria presence is limited. For personal hygiene, it indicates that a dedicated facility for hand hygiene exists, handwashing occurs before and after milk handling, but work protocols are not described completely. Because the MCCs are involved in bulking milk for onward transfer to dairy processors, these practices are performed at a higher level to meet their quality demands. However, these measures are not sufficiently comprehensive. For instance, we observed that some cooling tanks missed an available or calibrated thermometer at the MCCs. While containers used by traders and retail shops for storage of fresh milk lacked hygienic design with wide necks and stainless steel for proper cleaning. Also, a poor level (score 1) for milk transportation practices indicates that the transport vehicle used by milk traders is not clean and cannot maintain a specific low temperature during transportation. A rapid increase in microbial load is inevitable as there is no control of temperature. This limited proper transportation is characteristic of milk traders in Tanzania (Gwandu et al., 2018; Schoder, Maichin, Lema, & Laffa, 2013) and in other emerging dairy chains, such as Gambia (Washabaugh, Olaniyan, Secka, Jeng, & Bernstein, 2019) and Ethiopia (Tolosa et al., 2016).

Fig. 6 shows that even though the microbial load of the farm milk was already high, it remained stable in the milk samples taken across the other actors in the chain. The observation in this study differs from other studies in Tanzania (Nonga et al., 2015; Schoder et al., 2013; Swai & Schoonman, 2011) and other emerging dairy chains (Islam et al., 2018; Kamana et al., 2014; Millogo, Svennersten-Sjaunja, Ouédraogo, & Agenäs, 2010), which showed amplification of microbial load beyond the farm. The high contamination level at the farm may have created a limiting effect for further rapid bacterial proliferation (Li et al., 2018; Quigley et al., 2013), which may explain why there is no further increase even though the safety and hygiene practices were performed below the 'standard level'.

4. Conclusions

In this study, we demonstrated that differences in low and basic levels of safety and hygiene control practices performed did not translate into clear distinctions in milk quality and safety along the chain. The microbial load in milk samples at the farm, as well as along the chain, remained stable even though their safety and hygiene practices were also below the standard level. The transition in multiple practices towards the standard level should be aimed for to achieve a significant reduction in the occurrence of AFM1 and microbial contamination in milk as improvement in isolated practices do not seem to translate into significant outcomes in milk quality and safety. Nevertheless, practices performed at the intermediate level are at a better position to advance towards the standard level. Further research into appropriate interventions to help farmers and chain actor's progress toward the standard level is necessary.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

James Ledo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Kasper A. Hettinga:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Jamal B. Kussaga:** Supervision, Writing - review & editing. **Pieternel A. Luning:** Conceptualization, Methodology, Supervision, Writing - review & editing, Funding acquisition.
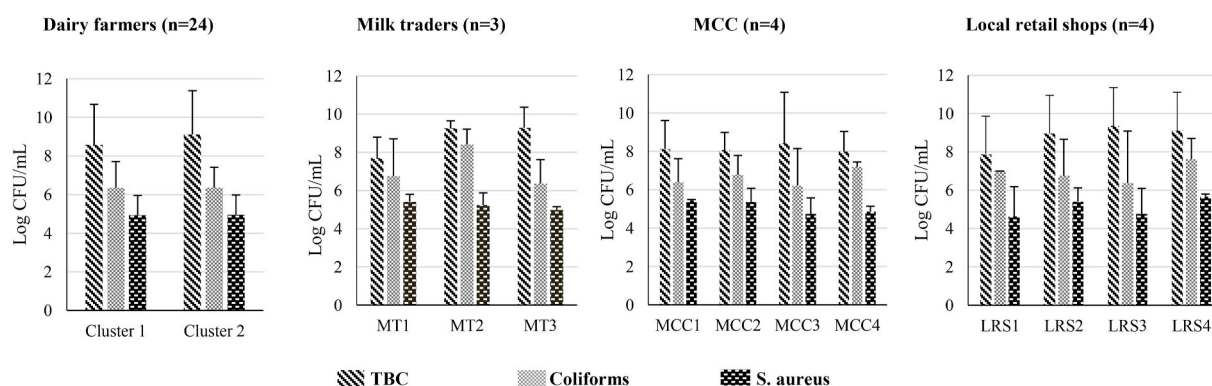
Fig. 6. Microbial milk quality and safety along the dairy chain. MT = Milk traders, MCC = Milk collection centres, LRS = Local retail shops; TBC (EAC): Grade 1: <5.3$\log_{10}$ CFU/mL, Grade 2: 5.3–6.0 $\log_{10}$ CFU/mL, Grade 3: 6.0–6.3 $\log_{10}$ CFU/mL, Beyond grade 3:>6.3 $\log_{10}$ CFU/mL; Coliforms (EAC): Very good: 0–3.0 $\log_{10}$ CFU/mL, Good: >3.0–4.7 $\log_{10}$ CFU/mL, Below good:>4.7 $\log_{10}$ CFU/mL; *Staphylococcus aureus* (EU):Within range: 4–5 $\log_{10}$ CFU/mL, Outside range:>5 $\log_{10}$ CFU/mL.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2020.107453.

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