# Effect of milking time and handling techniques on microbial quality and exposure assessment of cow's fresh milk consumption in Lilongwe, Malawi

**Albert Ngolombe[1*], Lizzie Saka[1], Elias Mwakilama[2]**

[1]Lilongwe University of Agriculture and Natural Resources,
Natural Resources Campus,
Lilongwe, Malawi

[2]Department of Mathematical Sciences,
University of Malawi, Malawi
[*]**Corresponding author Email:** *angolombe@gmail.com*

## ABSTRACT

Fresh cow milk is both a source of food nutrients and income to humans. However, if improperly handled at milking stage, it can be a source of bacterial pathogens which threaten health of human beings. This study investigated the effect of milking time and handling techniques on microbial quality and exposure assessment of consumption, using 52 fresh cow milk and corresponding water samples at the LUANAR-NRC dairy farm. Total bacterial count (TBC) was used as an indicator of the microbial quality of the milk, normalized by log-transformation and expressed in coliform plate counts (CFU/ml) as means ± standard deviations. One-way ANOVA was used to identify and assess the possible predictors of TBC. A cross-sectional survey of consumers around the farm was conducted to assess exposure effect at raw consumption. The major bacterial isolates from the milk samples were *Staphylococcus Spp*. (38%) and *E. coli* (34%). Consistent with records of below standard pH values (6.072 ± .0285), morning milk samples registered higher mean bacterial counts (6.0867±1.9334 log CFU/ml) than the afternoon (2.2001±2.8732 log CFU/ml) batch. Furthermore, milking time and handling techniques, combined, significantly ($p < 0.05$) contributed to the presence of bacteria. Unlike milk handling ($p > 0.05$), milking time alone significantly contributed ($p < 0.01$) to the high proportions of bacterial counts. The significant effect of both milking time and handling on raw cow's milk microbial quality calls for immediate actions along the dairy value chain to prevent the spread of foodborne diseases caused by bacterial hazards.

**Keywords:** *Fresh milk, milking time, handling technique, acidity level, microbial content.*

## 1. INTRODUCTION

Globally, milk plays an important role in diets because it is a complex mixture of macro and micro-nutrients, and a rich source of fats, proteins, carbohydrates, minerals and vitamins such as calcium, vitamin B-12, and riboflavin (Nyokabia et al., 2021). Fresh cow milk is particularly favourable because it is rich in proteins (3-4%), caseins (80%) and whey (20%) which are important for human body functions (Achchuthan & Kajananthan, 2012; Kon, 1972; Pandey & Voskuil, 2011). In addition, fresh cow milk contains fat (3-4%) and water (87-88%) and is also a good source of; Vitamin A, B-Riboflavin, vitamin D, Calcium, Phosphorus and Magnesium among other trace elements in the diet (Vaclavik & Christian, 2014; Walstra, 2006). Since proteins contain amino acid responsible for synthesizing lactose, they are regarded important for child's growth and development (Walstra, 2006). Proteins help to build and repair body tissues and to produce antibodies responsible for resisting infection. On the other hand, riboflavin, vitamin D and vitamin A help to promote healthy skin and eyes (Walstra, 2006). Because it is a low caloric food, milk is a relatively expensive source of energy (Kon, 1972). It also helps in body absorption of calcium and phosphorous, which are necessary for bone formation (Walstra, 2006). The aforementioned bioactive elements are attributed to the presence of enzymes which act at specific sites of targeted molecules under optimal conditions of pH and temperature (Vaclavik & Christian, 2014). Milk enzymes come from several sources including native milk, airborne bacterial contamination, fermented milk, or somatic cells present in raw milk (Nwankwo et al., 2015). When put into dairy farming, fresh cow milk becomes a source of poverty alleviation for most Africans (FAO, 1990; Uddin et al., 2012). Dairy farming is both a source of income (Nargunde, 2013) and employment (Kale et al., 2013; Uddin et al., 2012) in many rural parts of Africa. However, raw milk is also an ideal growth medium for several microorganisms (Ridwana et al., 2017).

Because of the aforementioned advantages, fresh cow milk is largely consumed in its fluid state or in form of other dairy products such as butter, cheese, ice cream, and confectionary (Brown, 2015; Kvoger et al., 1989). The dairy products are also a major source of milk fat. However, milk fat has suddenly become undesirable after scientific research which reported that saturated milk fat raises blood cholesterol levels that contribute to heart disease (McGee, 2004). Nonetheless, milk and milk products remain a good part of daily human diet such that research is now encouraged on paying much attention to ways of improving dairy production yield while optimally minimizing side effects (Harding, 1995). Improper way of handling fresh cow milk remains a leading source to pathogenic microorganisms such as *E.* coli, *Staphylococcus aureus*, *Campylobacter Spp*., *Listeria monocytogene* and *Salmonella Spp*. (Adugna et al., 2013). These food borne pathogens remain a serious threat to lives of people in low-and-middle income countries (LMICs) as they are responsible for approximately 33-90% cases of child deaths in Africa (Flint et al.,

2005). The conditions under which fresh cow milk is handled at farm level may affect both its quality and nutritive value (Nell et al., 2014; Shirima et al., 2003). Yet, such crucial information is less known despite the milk being of significance to public health.

Although milk production in LMICs such as Malawi is an important livelihood source for smallholder dairy farmers and a source of good health for the consumers, challenges of milk quality and safety occur due to unhygienic handling methods, time, and non-adherence to food safety standards (Garantjang et al., 2020; Mahari & Yemane, 2016; Nwankwo et al., 2015; Nyokabia et al., 2021; Swai & Schoolman, 2011; Tadesse et al., 2020). Moreover, unlike in the neighboring countries (Bacigale et al., 2023; Gwandu et al., 2018; Kivaria et al., 2006; Mosalagae et al., 2011; Nyokabia et al., 2021; Swai & Schoolman, 2011), reviewed dairy literature in Malawi shows that majority of studies focus on milk production (Baur et al., 2017). Very little is known about the microbial quality and profiles of fresh cow's milk at the milking sites. Therefore, this study aimed to investigate the effect of milking time and handling techniques on the microbial quality of fresh cow's milk at LUANAR-NRC dairy farm that serves part of the population of central Malawi. Specifically, the current study was set to (a) determine the bacterial composition of the fresh cow milk, (b) assess the pH of fresh cow milk, and (c) measure consumer's perception on fresh cow milk from LUANAR-NRC dairy farm.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

This study was conducted at LUANAR-NRC Dairy Farm, located in Likuni in Traditional Authority (TA) Malili around latitude 13° 55' 11" S and longitude 34° 4' 30" E in Lilongwe District in central Malawi (Figure 1). The LUANAR-NRC Dairy Farm serves an approximate population of at least 115,931 living in TA Malili (NSO, 2019) and its surrounding areas of Lilongwe rural. Moreover, TA Malili borders with Lilongwe City to the east (see Figure 1), meaning that the milk from the farm also reaches the population of Lilongwe city.

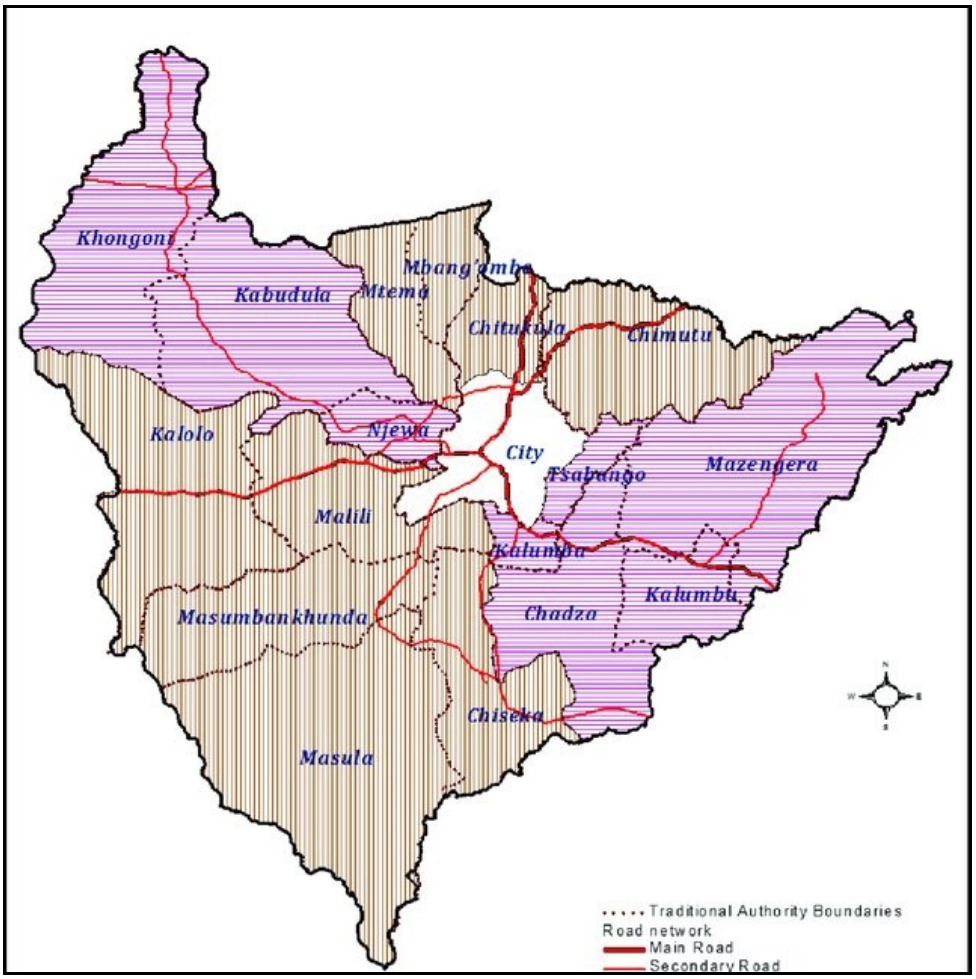


***Figure 7:*** *Map of Lilongwe in Malawi showing Tradtional Authorities (TAs) Source (Bell et al., 2017)*

## 2.2. Milking time, process, and handling techniques at LUANAR-NRC Dairy Farm

At the LUANAR-NRC Dairy Farm, the milking time occurs at two intervals: 3:00 am in the morning and 3:00 pm in the afternoon. The milking process from the dairy cows at the farm is done manually and the workers neither use the teat machines nor the robots. Procedurally, first, a dairy cow stands in the milking position with the help of the milking parlour attendant who then washes his/her hands with look warm water (38 ºC) and dries them with a sterile paper towel. Secondly, the cows' ulder is washed with warm water (38 ºC) and gets dried with a sterile paper towel as well. Afterwards, the parlour attendant sanitizes his/her hands with an 80% alcohol-based liquid in readiness for the milking process. The 2L jugs are often used as milking

collection tools from the ulder. From the jugs, the fresh milk is then filtered into the 10L chambers through a muslin Manton cloth for storage or transport by decanting method (Wang et al., 2009) to avoid contact with the milk. The fresh milk is then sold to individuals living within LUANAR-NRC dairy farm, including the NRC staff and students.

After the morning milking process, the chambers are washed at 9:30 am for an hour, leaving them open dry for a period of four and half hours before the afternoon milking time. However, cleaning of the milking jugs and chambers after the afternoon milking process occurs at 4:30 pm such that the period of open chamber drying lasts for about eleven hours. However, when such jars are not all ready for milk collection, other alternative plastic containers are used during the milking process.

### 2.3. Study design and Sampling

This was a mixed-methods research study (Walliman, 2021). Firstly, an experimental study was carried out to identify bacteria isolates from the milk samples. A total of 52 fresh cow milk samples were randomly collected from the LUANAR-NRC dairy farm for a period of six weeks at two different times: 4 am (morning) and 3 pm (afternoon) of each day from June to August 2019. The milk samples were obtained in two categories: firstly, the researcher collected milk samples directly from the cow teats; and secondly, samples were collected from the milk chambers. The former samples were labelled “un-sieved samples” whilst the latter were labelled “sieved samples”. These two sample collection times were chosen to match with the milking times at the farm. Immediately after acquisition, the samples were kept in a cooler box aided with ice blocks to keep temperatures below 10 $^{o}$C during 45 min drive from the dairy farm to Central Veterinary Laboratory (CVL) for analysis. This was done to minimize exposure to light and heat, thereby eliminating cases of fermentation and souring of the milk (Walstra, 2006).

### 2.4. pH and microbial analysis

Before taking the samples to CVL for bacterial analysis, all the collected milk samples were first tested for acidity through measures of pH on each harvesting day using a procedure described by Black and Barach (2015). This was done to ascertain any microbial activity which could reduce freshness of the milk through lactose fermentation. To ensure quality tests, the pH meter was first calibrated by dipping both the electrode and the temperature probe in buffer solutions of known pH (4, 7 and 10) and then rinsed with de-ionised water. However, to ensure that no milk sample was physically removed from the batch to eliminate possible chances of contamination, every sample in the cooler box was lifted a little and opened to allow the electrodes touch the milk and determine the acidic levels. After the taking the

reading on the meter screen, each sample bottle was instantly covered and left in the cooler box. In addition, the electrodes for the pH-meter were sterilized with an 80% alcohol liquid. All the milk pH tests were evaluated and interpreted using pH parameter ranges described in Table 1 (Walstra, 2006).

***Table 4:*** *Fresh milk pH specification, (Walstra, 2006)*

| Milk quality description | Standard pH |
|---|---|
| Poor quality milk | 5.4-5.8 |
| Mildly poor quality | 5.9-6.3 |
| Standard milk | 6.4-6.8 |
| Neutral milk | 6.9-7.3 |

At CVL, sample sub-culturing was done using MacConkey Agar HIMEDIA REF M081-500G and Blood Agar (Oxoid brand CM271) tests (Lagier et al., 2015). Plating was done in triplicate using sterilized streaked petri dishes, incubated in an inverted position at 37±1°C for 18-24 hours. Subsequently, samples were prepared using Peptone water (9 mls each) as diluent and each rubber cocked cuvette was sterilised at 121°C for 15minutes in an autoclave (Figure 2(a)). Then, an aliquot of 1ml was pipetted into 9ml sterile diluents to produce $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, to $10^{-10}$ dilutions. Upon running triplicates of $10^{-1}$, $10^{-3}$, $10^{-4}$, $10^{-7}$ dilutions, an optimal dilution factor of $10^{-3}$ was determined for colony counting throughout the study. UHT milk taken from the first-choice full cream milk long life brand (procured from nearby supermarket) was a source of study control samples. The UHT milk was chosen because lab tests proved that the milk was completely sterilized before the batches were released for sale.

On the basis of the $10^{-3}$ optimal dilution factor, Nutrient Agar and the diluted milk samples were thoroughly mixed in independent plates and incubated in an inverted position at 37°C for 24 hours in readiness for colony counting. Microbial colony counting ($N$) per sample was guided by the categories [≤10 colonies-too few to count, 30-299 colonies-countable and > 300 colonies-innumerable], expressed in CFU/ml, using the formula (Markey et al., 2013)

$$\mathrm{N\ (CFU/ml)} = \frac{\mathrm{DF}}{V} \times T \quad (1)$$

where $DF$ is the dilution factor, $V$ is the volume plated and $T$ is the average of the triplicate. Then the specific bacteria identification was done through biochemical characterization and microscopic examination procedures (Ogodo et al., 2022) on selective media. Following the procedures described by (Markey et al., 2013),

biochemical tests were conducted to confirm bacteria species. These tests included Gram staining test (Figure 2(b)), Catalase test, Oxidase test, Motility test, Oxidative/Fermentative (O/F) test, Citrate utilization test and Coagulase test.

***Figure 8:*** *(a) Autoclaving process of diluent and media after Aga preparation and (b) Gram statining process*

During the aforementioned tests, the following standard reagents and chemicals were used; crystal violet stain, Lugol's iodine and acetone; Mannitol salt Agar (HG 000C26.500); hydrogen peroxide (H2O2); Tetra methyl-p-phenalainine Diamine dihydrochloride; Sodium Chloride, Di-potassium Phosphate, Peptone, Bromothynol blue, Pure Agar, Glucose; Simon Citrate Agar (Oxoid CM155); Olive oil and sheep plasma, all taken from the CVL.

Oxidase positivity outcome was determined by an instant purple colour change of a colony using a Tetra methyl-p-phenalainine Diamine dihydrochloride oxidase reagent. Bacterial motility was identified by the rolling and translational motions of bacteria. On other hand, catalase positivity was determined by the production of bulbs. Further, O/F results were achieved when the bacteria under test only bleached the O/F media in aerobic set up. Positive citrate utilisation was observed when colour changed from green to blue. The presence of coagulase enzymes was determined by a clumping or agglutination of the plasma. All the bacterial morphology observations (gram-positive/gram-negative) by Gram staining test were done under a light microscope (Olympus CH30, Germany) with 100X objective lens.

Limited by lack of laboratory equipment to carry out individual bacterial plating and isolation for specific bacterial specie counting in each sample, the prevalence of bacterial species was instead obtained through the formulae:

Proportion of bacterial specie *A* in all samples $(\%) = \frac{n_A}{N} \times 100$ (2)

where $n_A$ is the number of milk samples registered with bacteria of specie type $A$ and $N$ is the total number of milk samples under tests.

### 2.5. Cross-sectional survey

Secondly, a total of 63 purposively sampled individuals living within LUANAR-NRC dairy farm were recruited for a quality control satisfaction survey on the milk, farm surroundings, and milking utensils. To ensure representative results, the sample composition involved 20 agriculture study students, 20 animal health and reproduction study students, 20 households that consume NRC farm's fresh cow milk, and 3 NRC farm staff. The participants were subjected to a structured questionnaire which included general questions on dairy farm management practices, milk handling techniques, and consumers' perceptions towards the milk sourced from such farm.

### 2.6. Data analysis

Finally, to investigate the effect of milking handling time and techniques on microbial quality and pH levels, a generalized linear model (GLM) (McCullagh, 1989) was employed in accordance with the linear regression model (Kleinbaum et al., 2007)

$$Y = \mu + \beta_1 M_t + \beta_2 H_t + \beta_3 M_t H_t + \varepsilon \quad (3)$$

where $M_t$ is the milking time, $H_t$ is the milk handling technique, $\beta_j$'s are regression model coefficients, and $\mu$ and $\varepsilon$ are overall sample mean and model error, respectively. The regression model was run using SPSS software version 20 IBM for windows. Additionally, the survey data was descriptively analysed in Microsoft Excel 2013 to assess the perception of consumers on milk quality from the LUANAR-NRC dairy farm. All the analyses were considered at a 5% significance level. Results are presented using tables and graphs.

## 3. RESULTS AND DISCUSSIONS

### 3.1. Microbial profile analysis of fresh cow milk

Results of the total bacterial counts from both the morning (un-sieved vs. sieved) and afternoon (un-sieved vs. sieved) milk samples, as obtained by using equation (1), are shown in Figure 3 and Figure 4, respectively.

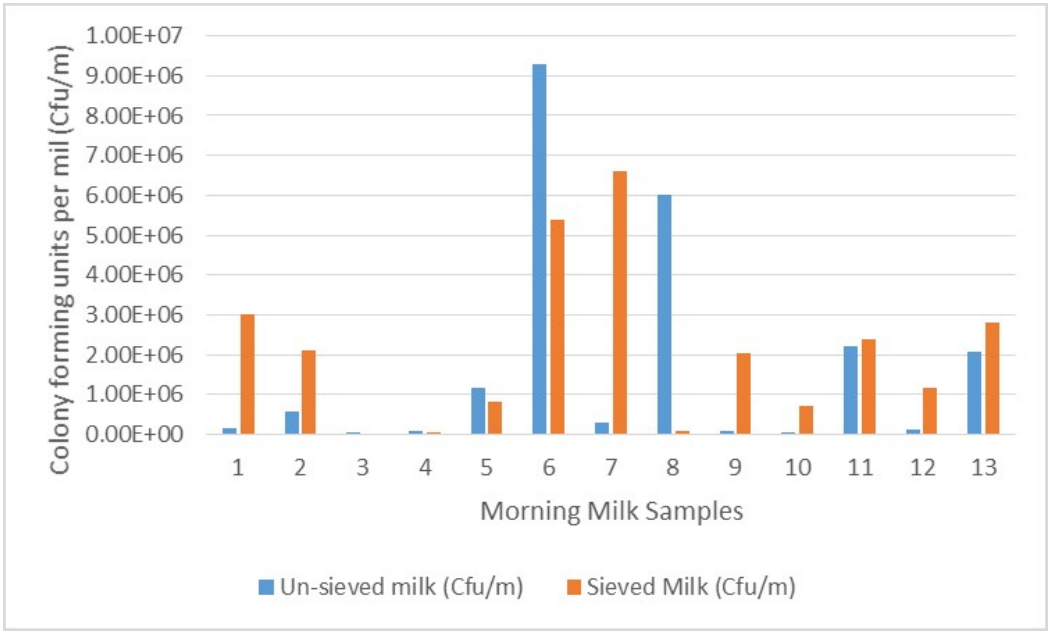


***Figure 9:*** *Total bacteria counts from the morning milk samples*

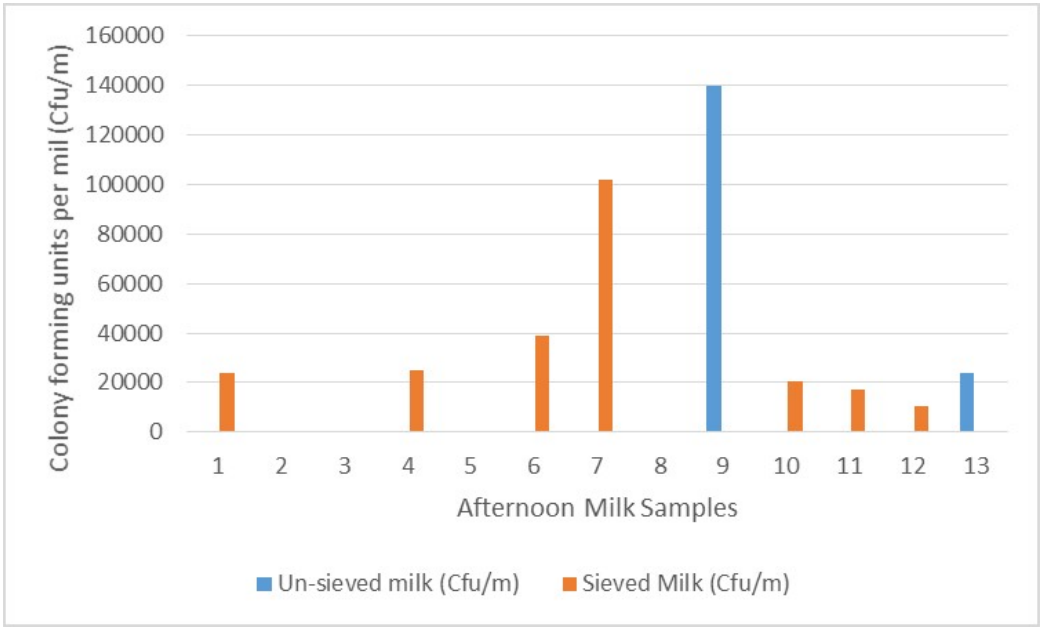


***Figure 10:*** *Total bacteria counts from the afternoon milk samples*

Compared with the afternoon samples (see Figure 4), overall, the graphs show that the more bacteria colonies were found in the morning milk samples (see Figure 3) and the mean difference was very significant ($t\alpha_{/2} = 2.06, p < 0.01$). Moreover, despite that the outlier bacteria colony counts were found in the un-sieved milk from both sample collection times (sample 6 in the morning and sample 9 in the afternoon), overall, the colony forming counts are higher in the sieved than in the un-sieved milk samples. Thus, both results suggest that the improper way of handling milking jags and chambers is the possible source of contamination.

Further analytical results, using equation (2), showed that fresh cow milk collected at both times was contaminated with different bacterial species (Figure 5). Out of the six identified bacterial isolates from the milk samples, *Staphylococcus Spp*. (38%), *E. coli* (34%), and *Streptococcus Spp*. (20%) were major, consistent with (Ansary, 2014) and others (Brown, 2015; Hussain, 2010; Kivaria et al., 2006; Nwankwo et al., 2015; Nyokabia et al., 2021; Shirima et al., 2003; Singh & Prakash, 2008; Tamine & Robinson, 2007). The microscopic picture of gram-positive bunches of cocci (*Staphylococcus. Spp.*) inside the milk sample is shown in Figure 4. *Staphylococcus Spp*. are common in dust, respiratory system, water, human skin and even in clothing, leading cause of neonatal deaths and mastitis (Flint et al., 2005). Hussain (2010) indicated that the prevalence of *Corynebacterium* in milk is low as compared to its isolation in other foods such as beef (Hussein, 2007) and goat (Tanganyika et al., 2017) carcasses.

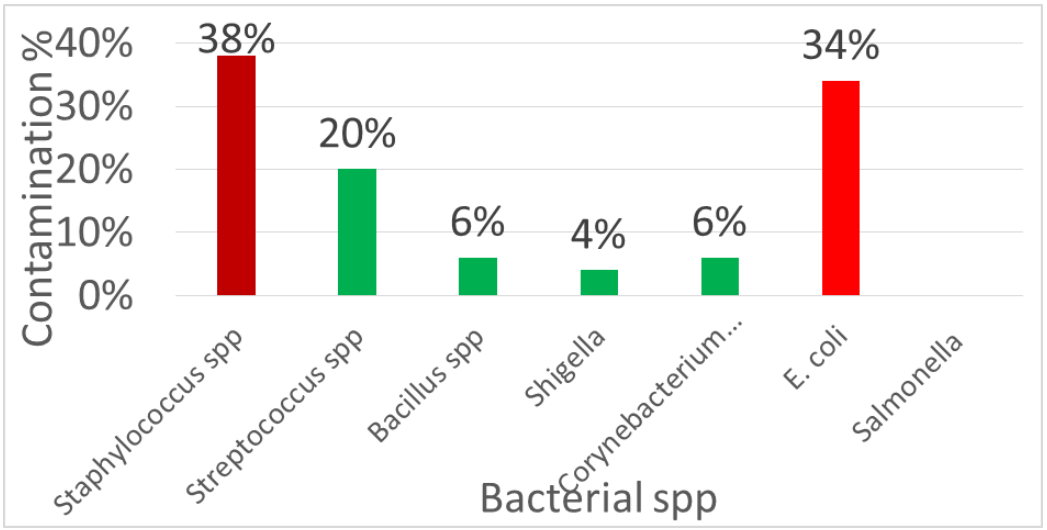


***Figure 11:*** *Proportion of the bacterial profiles detected in the samples*

Similarly, only six percent of *Corynebacterium Spp* were identified from the current study. This is an indication, that *Corynebacterium Spp* is more prevalent in the milk after several multiple contaminations. On the other hand, the highest proportion (38 %) of *Staphylococcus Spp.* amongst the 52 milk samples is an indication that the milk is externally contaminated. The result is consistent with other studies that also showed that the use of unclean attire or protective equipment by milking men contributes to high incidences of *Staphylococcus* strains in foods (Munthali, 2000; Nyokabia et al., 2021; Tanganyika et al., 2017). Furthermore, the gram negative small rod shaped enteric bacteria *E.coli*, which is most prevalent in the fecal matter, cause both human and animal diarrhoea because they produce exotoxins which increases secretion of water and electrolyte within the gut. For example, more than 62 cases of food borne outbreaks believed to have been associated with consumption of *E.coli* contaminated raw milk, were reported in East Africa between 2003 and 2007 (Yilmaz et al., 2009). In the present study, Figure 3 also shows that the proportion of *E.coli* bacteria among all the 52 milk samples was also high (34 %). The presence of *E.coli* bacteria in the milk is attributed to the unhygienic condition of the cows' resting area where the physical contact between cows ulder and cow dung facilitates the easy transfer of *E.coli* from the fecal matter to the teats.

*Streptococcus Spp*. are gram positive chains of cocci of varied lengths in morphology that are able to ferment glucose and cause beta hemolysis in blood, thereby leading causes of urinary infections, sore throat, fever as well as Chronic and Bovine Mastitis (Markey et al., 2013). Hence an increase in the carry over effect of *Staphylococcus Spp.* present in the milk (Figure 5) suggests that there was little sanitisation process of the utensils during the early morning milking process at the farm. Although they were reported in low proportion (6 %), *Bacillus Spp.* are gram positive short rods, widely common in the soil, water and air but resistant to heat change. Because of the latter scenario, *Bacillus Spp.* mostly infect the respiratory system causing nasal lesions and inflammations which are then characterised by shock, low blood pressure and sudden deaths (Clarence et al., 2009). Moreover, other studies (Parekh & Subhash, 2008) have indicated that most of the pathogens that we identified in the milk samples (Figure 3) contribute to almost 90 percent of all dairy related diseases (Flint et al., 2005; Kivaria et al., 2006; Schmidt et al., 2003; Sivapalasingams et al., 2004; Yilmaz et al., 2009). The high proportions of *Staphylococcus Spp*. and *E. coli* (Figure 5) in the raw milk samples, consistent with Gume et al. (2023), are indicators of poor hygiene and sanitation, thereby attesting to the increased CFU counts in the sieved milk.

### 3.2. Effect of milking time and handling techniques on microbial levels in fresh milk

According to Adugna et al. (2013), milk becomes contaminated with bacteria during or after milking process. In other instances, milk gets contaminated through adulteration or udders infected with mastitis (Kivaria et al., 2006; Sindani, 2012; Walstra, 2006). In a few instances, exposure to drug or chemical residuals coming from the treatment of animals may also put milk at risk of contamination (Swai & Schoolman, 2011). Figure 3 results suggest that fresh cow milk of LUANAR-NRC dairy farm gets contaminated both during and after the milking process, as more evidenced by the results (Table 2) of the regression model. Milking time $M_t$, significantly ($p < 0.05$) contributes to the higher proportions of bacterial counts.

***Table 5:*** *Results of ANOVA and Multivariate tests on Mean Log CFU/ml of milking time and milk handling technique*

| Variable(s) | Treatments | Mean±SD LogCFU/ml | P-Value |
|---|---|---|---|
| $M_t$ | Morning milk yield | 6.0867±1.9334 | |
| | Afternoon milk yield | 2.2001±2.8732 | 0.0001 |
| $H_t$ | Non-sieved milk | 3.6769±3.2735 | |
| | Sieved milk | 4.6100±2.9463 | 0.285 |
| $M_t * H_t$ | | 6.027±1.8330 * 2.011±2.8431 | 0.044 |

Table 2 also shows that on average, the morning milk samples had a higher log count (6.0867±1.9334 log CFU/ml) than afternoon milk samples (2.2001±2.8732 log CFU/ml), higher than the acceptable ranges of unsatisfactory level among consumable samples at retailers (Gume et al., 2023b; MBS, 2022). This could be attributed to the carry over effect from the previous batch in the milking containers (Adugna et al., 2013; Nyokabia et al., 2021; Sindani, 2012), since a short period of time of cleaning that is done between the end of morning batch and beginning of the afternoon yield signifies less carry over effect as compared to the long open dry exposure time between the afternoon cleaning of jugs time and the morning milking time. Thus, milking utensils interact less with the environment between the end of morning batch and when the afternoon milking is done. The long open drying time of milking jugs exposes the jugs to any possible contamination.

Conversely, non-sieved (3.6769±3.2735 log CFU/ml) and sieved milk (4.6100±2.9463 log CFU/ml) yields both showed mean log CFU counts that are within standards of specification. Although the milking handling process $H_t$ did not significantly ($p > 0.05$) contribute to the presence of bacteria in the milk, the sieved milk had a higher CFU count than non-sieved milk. This result suggests that there was some possible cross contamination at the sieving unit operation which could be linked to the less frequent dry washing of the Manton cloth. On the other hand, use of alternative utensils to collect milk after sieving might have contributed to the possible contamination process (Igumbor et al., 2000). Besides milking time $M_t$, the results also show that the interaction effect $M_t * H_t$ significantly ($p < 0.05$) contributes to an increase in proportion of microbes inside the milk samples (Table 2). The findings suggest that a combination of the two effects significantly affects the quality of the milk at the farm, consistent with Swai and Schoolman (2011).

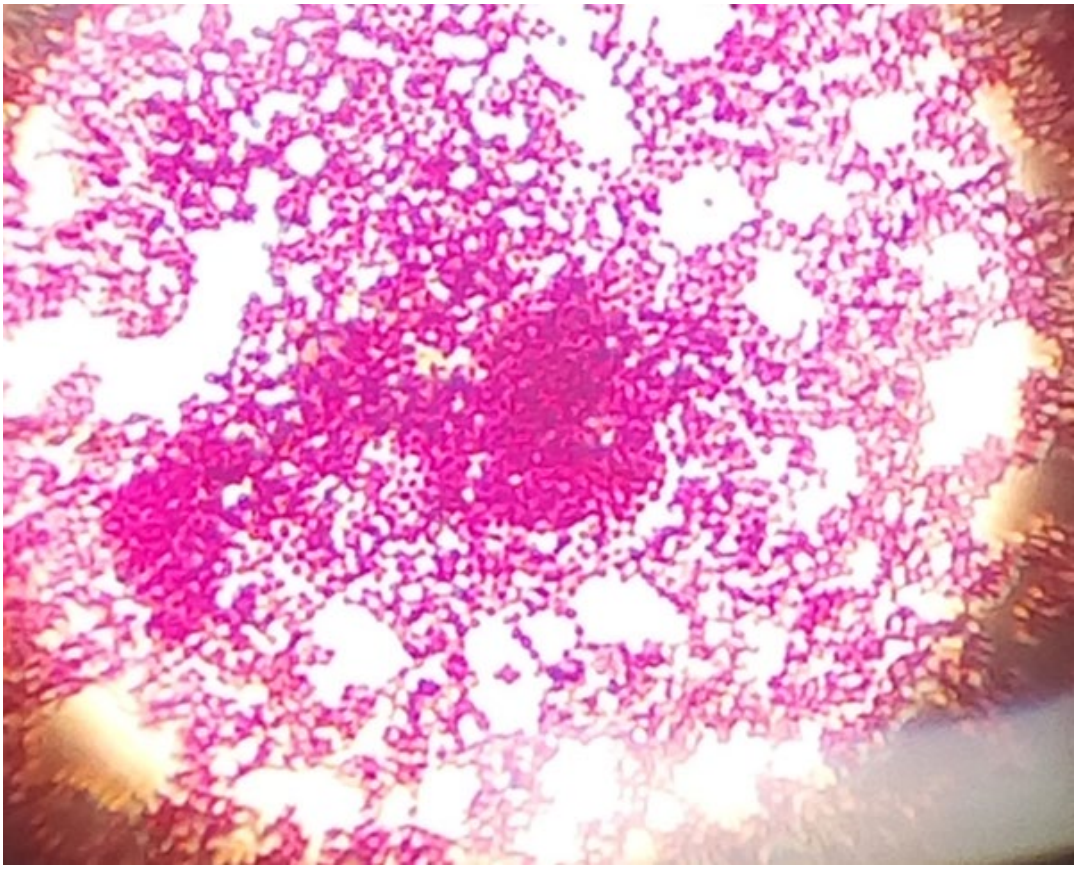

***Figure 12:*** *A microscopic picture of bunches of gram positive bacterial (Staphylococcus. Spp.)*

### 3.3. Physiochemical analysis of fresh cow's acidity level

Walstra (2006) reported that good fresh milk pH should be between 6.4 and 6.8 (Table 1). On the contrary, this study found that (Table 3) mean pH for the morning samples (6.072 ± .0285) were outside the recommended range, suggesting that morning milk samples had fallen in the rage of mildly poor quality (see Table 1). The results suggest a reduced freshness of the milk and an indication of the microbial activity inside the milk. Moreover, milk coming from morning batch also had a high proportion of bacterial counts than its counterpart, evidenced by the below standard pH values.

***Table 6:*** *Mean pH values of milk samples in relation to harvesting time*

| Milk harvesting time | N | pH mean ±SD |
|---|---|---|
| Morning | 26 | 6.072±.0285 |
| Afternoon | 26 | 6.436±.358 |

On the other hand, a mean pH value of 6.436±.040 for the afternoon milk was within the acceptable limits of good quality fresh milk (see Table 1). The observed decrease of pH for the morning sampled milk is in good agreement with the significant presence of bacteria as reported (Table 2) earlier on. In addition, the presence of *E. coli* in the samples (Figure 5) could be the likely cause of the decrease in pH (Chekabab et al., 2013), attributed to natural fermentation process due to the presence of lactose fermenters (Vaclavik & Christian, 2014; Walstra, 2006).

### 3.4. Raw milk consumption and exposure assessment

A socio-demographic analysis of 63 raw milk consumers and handlers revealed that 36 were females (56.7 %) and 45 (71.6 %) were people of age category 21-25 years (Figure 7). The results of the survey are summarised in Figure 8. On raw milk consumption preference, a majority of the respondents (38.3 %) preferred taking hot boiled milk directly. Generally, such approach reduces probability of infection at consumption because, except for the *Bacillus Spp*., most of the identified pathogens do not survive in high temperatures. However, for those (36.7 %) who preferred boiling and waiting for the milk to cool down before consuming had probably created some room for the infestation of the milk with *Staphylococcus* or *Bacillus* and other dust and air borne pathogens, hence increasing the chances of infection at consumption dose.

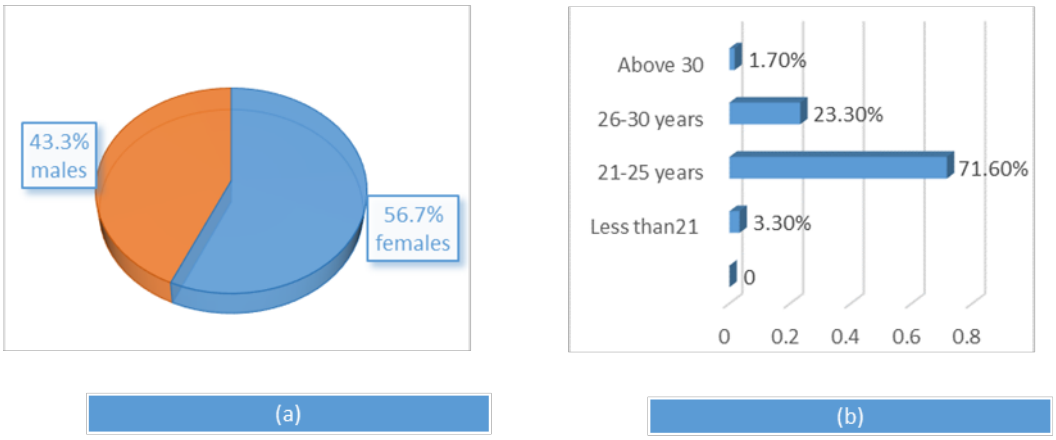


***Figure 13:*** *Demographic characteristics of survey respondents by (a) gender and (b) age in categories*

Furthermore, the use of personal clothing (51.67 %) other than the designated farm clothing exposes the raw milk to pathogen contamination e.g., *E. coli,* consistent with other studies (Nwankwo et al., 2015; Nyokabia et al., 2021). Just as the challenge of lack of dairy farm resources is reported elsewhere (Kale et al., 2013; Marichamy et al., 2014; Patil et al., 2009; Uddin et al., 2012), similar observations were made at the LUANAR-NRC dairy farm. This can be associated with opportunity for creating foodborne diseases (Schmidt et al., 2003; Taylor et al., 2001).

Despite majority of the respondents (73.3 %) not reporting their experience of disease symptoms due to consumption of fresh cow milk from LUANAR-NRC dairy farm, lack of serious sanitation at the farm still poses a threat to the lives of the consumers. Should the milking utensils still be reported clean at times (63.3 %), a high percentage of pathogens inside the milk (Figure 5) will continuously be reported. Sindani (2012) reported that a dumpy resting place for the cows heavily exposes them to a number of infectious agents from the environment and other secondary opportunistic microorganism. In addition, Bowen et al. (2005) reported that reduction in the contamination of fresh cow milk can be achieved by provision of infrastructure that facilitates hygiene. In this study, however, the survey results revealed cases of unsanitary kraals and poor unhygienic conditions at the dairy farm. These conditions point to a few cases where some survey participants felt clumpy stomachs (10 %), nausea and headaches (16.7 %) after consuming the milk (Figure 6). This could associated to either one or combination of intoxifications and

intoxications of *E. coli, Staphylococcal, Bacillus, Shigella* and other bacterial toxins identified (Figure 5) in the milk samples, consistent with other studies (Gume et al., 2023b; Marcotty et al., 2009; Pandey & Voskuil, 2011). Consistent with other studies (Mosalagae et al., 2011; Nyokabia et al., 2021; Shirima et al., 2003), in general, the current study survey outcomes reflected that the microbiological quality of fresh cow milk at LUANAR-NRC dairy farm was considerably unsatisfactory with respect to the identified pathogens.

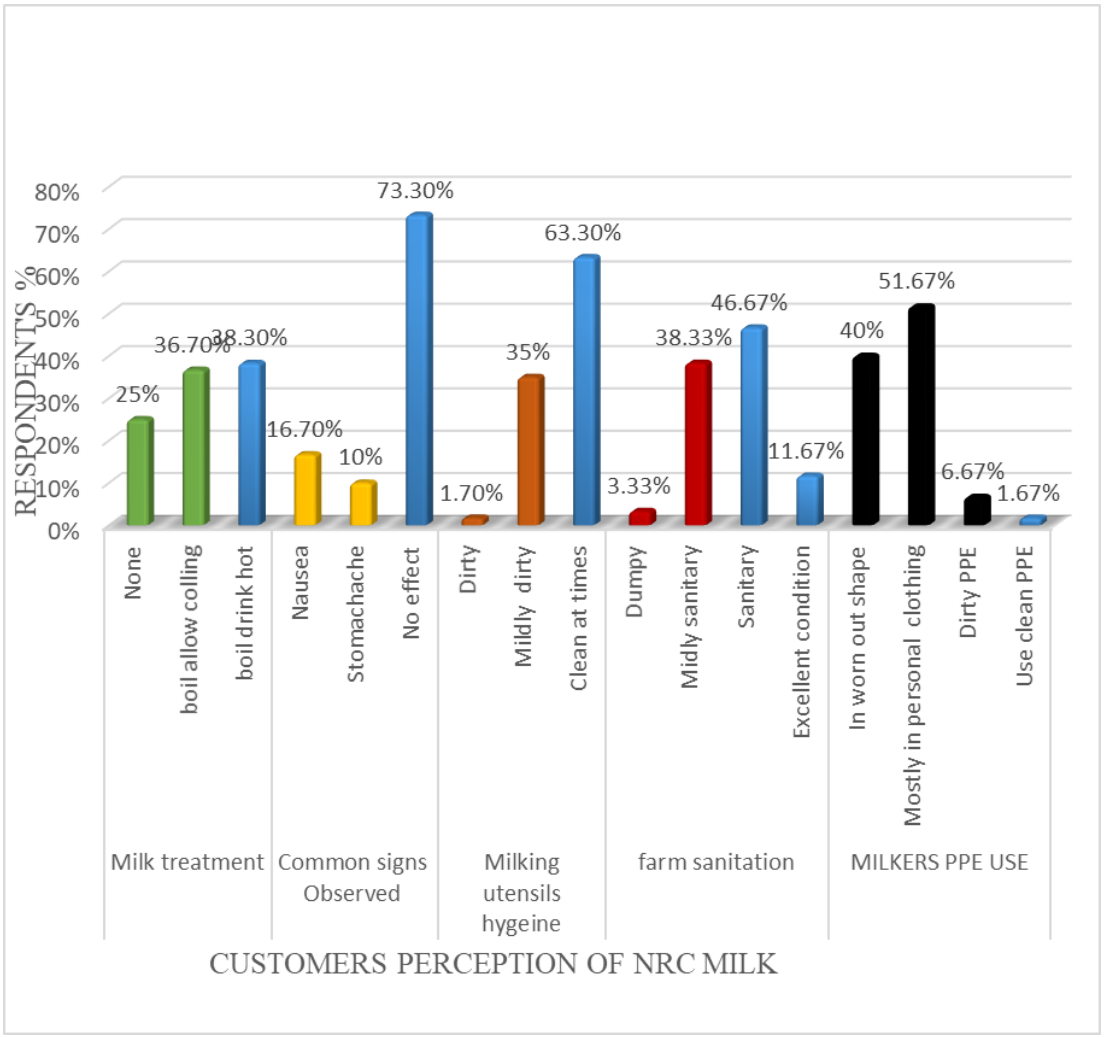


***Figure 14:*** *Raw fresh cow milk consuption and handling details of respondents*

## 4.0 CONCLUSION

Milk production in several LMICs including Malawi remains an important source of livelihood for both smallholder dairy farmers and the consumers. However, lack of regular monitoring and implementing hazard identification and critical control point principles along the dairy value chain poses several health risks to consumers. This study investigated the effect of milking time and handling technique on microbial quality and exposure assessment of fresh cow's milk consumption in Lilongwe, Malawi. In general, the study found that fresh cow milk at LUANAR-NRC dairy farm in Lilongwe, Malawi is contaminated with almost all types of bacterial hazards. The relative frequency of bacterial contaminants and the deterioration of raw milk at the farm were also demonstrated. Cumulatively, at least half of the total milk samples had some presence of *Staphylococcus Spp., E.coli, Streptococcus Spp., Bacillus Spp., Corynebacterium* and *Shigella* bacterial pathogens, an indication that fresh cow milk is sold to consumers whilst contaminated. In particular, the study has revealed that a combined effect of milking time and handling techniques significantly contributes to the presence of these bacterial pathogens. Therefore, our findings suggest the need for immediate actions along the dairy value chain to prevent the spread of foodborne diseases caused by bacterial hazards in fresh cow milk.

## ACKNOWLEDGEMENTS

The authors would like to thank the directorate of LUANAR-NRC farm and technicians of Central Veterinary Laboratory (CVL), for the ethical approval and technical support rendered during the process of data collection. Many thanks also to Aubrey John Chirwa for the pre-publication support offered in the process of production of this article.

## AUTHOR CONTRIBUTIONS

Albert Ngolombe conceived and designed the study, prepared material and collected the data, and performed experimental analysis. Elias Mwakilama provided guidance in the study design, data handling and analysis and managed the manuscript writing process. Lizzie Saka supervised Albert Ngolombe, reshaped and re-organised some arguments and participated in the finalisation of the manuscript. All authors proof-read and approved the final manuscript.

### Conflict of 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.

**Availability of data and materials**

All data and materials are available for this work and can be accessed from the corresponding author.

**REFERENCES**

Achchuthan, S., & Kajananthan, R. (2012). A Study on Value Chain Analysis in Dairy Sector Kilinochchi District, Sri Lanka. *Global Journal of Management and Business Research*, *12*(21): 1–15. https://globaljournals.org/GJMBR_Volume12/1-A-Study-on-Value-Chain-Analysis.pdf

Adugna, M., Seifu, E., Kebeded, A., & Doluschitz, R. (2013). Quality and Safety of Camel Milk along the Value Chain in Eastern Ethiopia. *International Journal of Food Studies* **2**(2): 150–157. https://doi.org/https://doi.org/10.7455/ijfs/2.2.2013.a2.

Ansary, M. A. (2014). Assessment of microbial quality of Yoghurt. *Alexandria*.

Bacigale, S. B., Basengere, A. R., Bwana, M. V., Yannick, M., Zirhumana, M. J., Zabron, N., Mamadou, F., Patchimaporn, U., & Jacob, M. (2023). Assessing milk products quality, safety, and influencing factors along the dairy value chain in eastern Democratic Republic of the Congo. *Frontiers in Sustainable Food Systems* **7**. https://doi.org/10.3389/fsufs.2023.1105515

Baur, I., Tabin, L., Banda, M., Chiumia, D. & Lips, M. (2017). Improving dairy production in Malawi: a literature review. *Tropical Animal Health and Production* **49:**1–8. https://doi.org/10.1007/s11250-016-1184-5

Black, D. G. & Barach, J. T. (2015). *Canned Foods: Principles of Thermal Process Control, Acidification and Container Closure Evaluation* (8th ed.). GMA Science and Education Foundation.

Bowen, G. J., Wassenear, L.,. & Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* **143:**337–338. https://doi.org/https://doi.org/10.1007/s00442-004-1813-y

Brown, H. (2015). *Assessment of Microbiological Quality of Raw Milk and Antimicrobial Susceptibility of Selected Milk-Borne Bacterial in Kilosa and Mvomero Districts Tanzania* [Sokoine University of Agriculture]. https://cgspace.cgiar.org/bitstream/handle/10568/52233/RawMilkQuality-TZ.pdf;sequence=1

Chekabab, S. M., Paquin-Veillette, J., Dozois, C. M., & Harel, J. (2013). The ecological habitat and transmission of Escherichia coli O157:H7. *FEMS Microbiology Letters* **341**(1):1–12. https://doi.org/https://doi.org/10.1111/1574-6968.12078.

Clarence, S. Y., Obinna, C. N., & Shalom, N. C. (2009). Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research*, ***3***(7), 390–395. https://doi.org/https://doi.org/10.5897/AJMR.9000103.

FAO. (1990). The technology of traditional milk products in developing countries. *Animal Production and Health Paper* ***85***(85): 25–64.

Flint, J., Duynhoven, Y., Angulo, F., Delong, S., Braun, P., Kirk, M., Scallan, E., Fitzgerald, M., Adak, G., Socket, P., Elias, A., Hall, G., Gargour, N., Wale, H., & Braam, P. (2005). Estimating the burden of acute gastroenteritis, food-borne diseases and pathogens commonly transmitted by food. *Journal of Clinical Infectious Diseases* **41:**698–704.

Garantjang, S., Rusdy, M., Hatta, M., Nohong, B. & Sema. (2020). Effect of milking time on milk production and milk quality of dairy cow fed with fermented corn cob. *IOP Conference Series: Earth and Environmental Science* 492(1):14–19. https://doi.org/10.1088/1755-1315/492/1/012054.

Gume, B., Berhanu, L., Kassa, T., Bediru, H., Fikre, A. G., Dadi, L. S. & Mereta, S., T. (2023a). Bacterial hazard identification and exposure assessment of raw milk consumption in Jimma zone, South West Ethiopia. *BMC Microbiology* **23**(166). https://doi.org/10.1186/s12866-023-02910-0.

Gume, B., Berhanu, L., Kassa, T., Bediru, H., Fikre, A. G., Dadi, L. S. & Mereta, S., T. (2023b). Bacterial hazard identification and exposure assessment of raw milk consumption in Jimma zone, South West Ethiopia. *BMC Microbiology* 23(1):1–10. https://doi.org/10.1186/s12866-023-02910-0.

Gwandu, S. H., Nonga, H. E., Mdegela, R. H., Katakweba, A. S., Suleiman, T. S. & Ryoba, R. (2018). Assessment of Raw Cow Milk Quality in Smallholder Dairy Farms in Pemba Island Zanzibar, Tanzania. *Veterinary Medicine International* 9. https://doi.org/10.1155/2018/1031726.

Harding, F. (1995). *Milk Quality* (F. Harding (ed.); 1st ed.). Chapman and Hall. https://doi.org/https://doi.org/10.1007/978-1-4615-2195-2.

Hussain, K. I. S. (2010). *Microorganisms involved in traditional fermented milk in Sudan*. University of Khartoum.

Hussein, H. S. (2007). Prevalence and pathogenicity of Shiga toxin-producing

Escherichia coli in beef cattle and their products. *Journal of Animal Science* **85**(13):E63-72. https://doi.org/https://doi.org/10.2527/jas.2006-421

Igumbor, E. O., Obi, C. L., & Milingo, T. (2000). Bacteriological examination of milk and milk products sold in Harare. *African Journal of Health Sciences* **7**(3–4):126–131. https://pubmed.ncbi.nlm.nih.gov/17650038/

Kale, R. A., Tekale, V. S., & Gaikwad, J. H. (2013). Constraints faced by farm women in dairy farming. *Research Journal of Animal Husbandry and Dairy Science* **4(**2):58–60. http://researchjournal.co.in/upload/assignments/4_58-60-33.pdf

Kivaria, F. M., Noordhuizen, J. P. T. M., & Kapaga, A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es Salaam region, Tanzania. *Tropical Animal Health Production* **38**(3):185 – 94. https://doi.org/10.1007/s11250-006-4339-y

Kleinbaum, D. G., Kupper, L. L., Nizam, A., & Muller, K. E. (2007). *Applied Regression Analysis and Other Multivariable Methods* (4th ed.). Duxbury Press.

Kon, S. K. (1972). Milk and milk products in human nutrition. *FAO Nutritional Studies*, *27*, 1–80.

Kvoger, M., Kurman, J. A. & Rasic, J. L., (1989). Fermented milk based and soya based yoghurt. *J. Food Sci.* **55(**2):502–536.

Lagier, J. C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev.* **28**(1):208–236. https://doi.org/10.1128/CMR.00110-14.

Mahari, A. T. & Yemane, H. (2016). Cow Milk Handling Practices and Factors Contributing to Quality Deterioration in Ethiopia. *Food Science and Quality Management* **48:**14–17. https://core.ac.uk/download/pdf/234684182.pdf.

Marcotty, T., Matthys, F., Godfroid, J., Rigouts, L., Ameni, G., Pittius, N. G. van, Kazwala, R., Muma, J., Helden, P. van, Walravens, K., de Klerk, L. M., Geoghegan, C. D. & Mbotha, M. (2009). Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcomes of a multi-disciplinary workshop. *Annals of Tropical Medicine and Parasitology* **103**(5):401–411. https://doi.org/10.1179/136485909X451771.

Marichamy, K., Aananthi, N. & Lakshmanamoorthy, S. (2014). Constraints Faced by Rural Women Dairy Farmers in Virudhunagar District. *Golden Research*

*Thoughts* **4(**2):GRT-4336. http://aygrt.isrj.org/.../4336.pdf.

Markey, B., Leonard, F., Archambault, M., Cullianane, D. & Maguire, D. (2013). *Clinical veterinary Microbiology* (2nd ed.). Elsevier Ltd.

MBS. (2022). *Catalogue of Malawi Standards*.

McCullagh, P. (1989). *Generalized Linear Models* (2nd ed.). Routledge.

McGee, H. (2004). *On Food and Cooking: The Science and Lore of the Kitchen* (2nd ed.). Hodder & Stoughton.

Mosalagae, D., Pfukenyi, D. M. & Matope, G. (2011). Milk producer's awareness of milk- borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Tropical Animal Health and Production* 43:733–739. https://doi.org/https://doi.org/10.1007/s11250-010-9761-5;

Munthali, J. K. (2000). *Smallholder Dairy Development in Malawi*.

Nargunde, A. S. (2013). Role of Dairy Industry In Rural Development. *International Journal of Advanced Research In Engineering & Technology*, *4*(2), 8–16. https://www.researchgate.net/publication/362517997_ROLE_OF_DAIRY_INDUSTRY_IN_RURAL_DEVELOPMENT;

Nell, A. J., Schiere, H., & Bol, S. (2014). *Quick Scan Dairy Sector Tanzania*. https://books.google.co.ke/books?id=0UWMrgEACAAJ

NSO. (2019). *2018 Malawi Population and Housing Census: Main Report*. https://malawi.unfpa.org/sites/default/files/resource-pdf/2018 Malawi Population and Housing Census Main Report %281%29.pdf

Nwankwo, A. I. U., Adiele, N. & Winnie, A., (2015). Microbial Evaluation of Raw Milk from Dairy Farms in Udi. *Journal of Agriculture and Veterinary Science* **8**(3):60–65. https://doi.org/10.9790/2380-08336065.

Nyokabia, S., Luning, P. A., de Boera, I. J. M., Korird, L., Muundad, E., Bebec, B. O., Lindahle, J., Bettd, B. & Oostinga, S. J. (2021). Milk quality and hygiene: Knowledge, attitudes and practices of smallholder dairy farmers in central Kenya. *Food Control* ***130:***108303. https://doi.org/https://doi.org/10.1016/j.foodcont.2021.108303.

Ogodo, A. C., Agwaranze, D. I., Daji, M. & Aso, R. E. (2022). Microbial techniques and methods: basic techniques and microscopy. In C. Egbuna, K. C. Patrick-Iwuanyanwu, M. A. Shah, J. C. Ifemeje & A. Rasul (Eds.), *Analytical Techniques in Biosciences From Basics to Applications* (pp. 201–2020). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-822654-4.00003-8.

Pandey, G. S. & Voskuil, G. C. S. (2011). *Manual on Milk safety, quality and hygiene*. https://doczz.net/doc/8280182/manual-on-milk-safety--quality-and-hygiene.

Parekh, T. S. & Subhash, R. (2008). Molecular and Bacteriological Examination of Milk from Different Milch Animals with Special Reference to Coliforms. *Current Research in Bacteriology* **1**:56–63. https://doi.org/10.3923/crb.2008.56.63.

Patil, A. P., Gawande, S. H., Nande, M. P. & Gobade, M. R. (2009). Constraints Faced by the Dairy Farmers in Nagpur District while Adopting Animal Managenment Practices. *Veterinary World* **2**(3):111–112. https://www.ejmcr.com/fulltext/2-1273840048.pdf.

Ridwana, G. A. B., Ahmad, A., Rasool, U., & Fayaz1, S. (2017). Prevalence of Staphylococcus aureus and Methicillin Resistant Staphylococcus aureus from Milk and Milk Products in and around Srinagar Area of Kashmir, India. *International Journal of Current Microbiology and Applied Sciences* **6**(9):690–696. https://doi.org/https://doi.org/10.20546/ijcmas.2017.609.084.

Schmidt, R. H., Goodrich, R. M., Douglas, L., Archer, D. L., & Schneider, K. R. (2003). *General overview of the causative agents of foodborne illness*. https://edis.ifas.ufl.edu/pdf%5CFS%5CFS099%5CFS099-9208954.pdf.

Shirima, G. M., Fitzpatrick, J., Cleave-land, S., Kambarage, D. M., Kazwala, R. R., Kunda, J. & French, N. P. (2003). Participatory survey on zoonotic diseases affecting livestock keeping communities in Tanzania. *Journal of Animal and Veterinary Advances* **2**(4):253–258. https://medwelljournals.com/abstract/?doi=javaa.2003.253.258

Sindani, W. G. (2012). *The dairy industry in Malawi: A description of the Milk bulking groups in Malawi* [North Carolina State]. http://www.lib.ncsu.edu/resolver/1840.16/7881

Singh, P., & Prakash, A. (2008). Isolation of Escherichia coli, Staphylococcus aureus and Listeria monocytogenes from milk products sold under market conditions at Agra Region. *Acta Agricultural Slovenica* **1**(1): 83–88.

Sivapalasingams, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States. *Journal of Food Protection* **67**(10): 2342–2353. https://doi.org/https://doi.org/10.4315/0362-028X-67.10.2342.

Swai, E. S., & Schoolman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pacific Journal of Tropical Biomedicine* **1**(3): 217–222. https://doi.org/10.1016/S2221-

1691(11)60030-0.

Tadesse, A., Ulfina, G. G., & Bekuma, A. (2020). Milk Handling , Processing Practices and Quality Evaluation. *Global Journal of Animal Scientific Rsearch* 8(1):56–74.

Tamine, A. Y., & Robinson, R. K. (2007). *Yoghurt: Science and Technology* (3rd ed.). Woodhead Publishing Limited.

Tanganyika, J., Mfitilodze, J. W., Mtimuni, J. P. & Phoya, R. K. D. (2017). Microbial quality of goat carcasses in Lilongwe, Malawi. *Chemical and Biological Technologies in Agriculture* 4(27): https://doi.org/10.1186/s40538-017-0109-5.

Taylor, L. H., Latham, S. M., & Wool-house, M. E. J. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society B: Biological Sciences* **356**(1411): 983 – 989. https://doi.org/https://doi.org/10.1098/rstb.2001.0888.

Uddin, M. N., Uddin, M. B., Al Mamun, M. H., Hassan, M. M. & Khan, M. M. (2012). Small scale dairy Farming for livelihoods of rural farmers: Constraint and prospect in Bangladesh. *J Anim Sci Adv* **2**(6):543–550. http://www.grjournals.com/portals/grjournals/JASA/Vol2 Issue6/JASA-2012-06-543-550.pdf.

Vaclavik, V. A. & Christian, E. W., (2014). *Essentials of food science* (4th ed.). Springer Science & Business Media. https://doi.org/https://doi.org/10.1007/978-1-4614-9138-5.

Walliman, N. (2021). *Research Methods, the basics* (3rd ed.). Routledge Taylor and Francis group.

Walstra, P. R. (2006). *Dairy Chemistry and Physics*. John Wiley & Sons.

Wang, H., Wang, T., Pometto, A. L., & Johnson, L. A. (2009). A Laboratory Decanting Procedure to Simulate Whole Stillage Separation in Dry-Grind Corn Ethanol Process. *Journal of the American Oil Chemists' Society* **86(**12): 1241–1250. https://doi.org/10.1007/s11746-009-1451-y

Yilmaz, T., Moyer, B., MacDonell, R. E., Cordero-Coma, M., & Gallagher, M. J. (2009). Outbreaks associated with unpasteurized milk and soft cheese: An overview of consumer Safety. *Food Protection Trends* **29**(4): 211–222. https://openagricola.nal.usda.gov/catalog/IND44385912