(RESEARCH ARTICLE)

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Assessment and quantification of levels of microbial contamination in bovine milk from smallholder dairy farmers of Monze district in the Southern Zambia

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Abstract

Two types of bovine milk; processed milk (PM). and unprocessed milk (UM). each replicated five (5). times were tested for bacterial plate count (BPC). and microbial species (MS). Five replicates of each were randomly assigned for testing in a Time Series Experimental Design (TSED). at the Samora Machel School of Veterinary Medicine of the University of Zambia. The two types of milk samples were tested for quality and microbial load (ML). The two types of milk samples (PM and UM). did not differ significantly (p<0.05). in terms of ML. The coliforms values for PM samples were 45 x 10^4, 0.14 x 10^4, 1 x 10^4, and 0.1 x 10^4 while those for the UM samples were 38.6 x 10^4, 45.6 x 10^4, 83 x 10^4 , and 0.16 x 10^4. The bacterial Load (BL). in milk depends upon the hygienic practices and the method of processing used. Results of this study provide evidence that the processing method used at the plant is not effective in reducing BL at Monze Dairy Farmers Cooperative Society (MDFCS).

Keywords: Bovine; milk; Coliforms; Microbial; Small-scale; Farmers

1   Introduction

Monze Dairy Farmers Corporative Society is a private owned organization which is a bulking center for fresh milk from farmers. The cooperative society processes the milk for human consumption from farmers around Monze District and other surrounding areas. It was officially opened on 9th November, 2010. Its main objective is to improve on the dairy farming business in Monze district by offering market. Forester et al, (1). reported that most common vector-borne disease such as Lyme disease affected other areas after travel to high-incidence Lyme disease areas. Cases without travel-related exposure in low-incidence states differed epidemiologically. It works hand in hand with other milk processing companies such as Parmalat and Bonita where the surplus milk is sold for further processing into other milk by-products. Some of the milk is sold to consumers within Monze District after processing. The main idea for processing is to improve the quality by reducing bacterial load which are responsible for the spread zoonotic diseases. Raw milk and other dairy by-products are important components of a healthy diet. However, if consumed unpasteurized, it may present a health hazard due to possible contamination with pathogenic bacteria (2). Having been recognized that milk is a vehicle for the transmission of numerous bacteria species of both human and animal origin, it can be contaminated at any stage in the production-to-consumption continuum (3). These bacteria can originate even from clinically healthy animals from which milk is derived or from environmental sources during collection and storage (2). Yacine et al, (4). observed that microbial contamination of raw milk increases along the dairy supply chain.

The decreased frequency of bovine carriage of certain zoonotic pathogens and improved milking hygiene have contributed considerably to decreased contamination of milk but have not, and cannot, fully eliminate the risk of milk borne diseases (5). Pasteurization is one of the most effective methods of enhancing the microbiological safety of milk (6). The consumption of milk that is not pasteurized increases the risk of contracting diseases from a food stuff that is

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otherwise very nutritious and health (7). Despite concerns to the contrary, pasteurization does not change the nutritional value of milk (7). Work by Roberts et al (8). indicated that dairy farming involves frequent contact among animals, workers and farm environments. The diversity of species ranged from 9–15 *Staphylococcus* spp./farm with no difference between conventional and organic farms. *S. haemolyticus* [n = 60 isolates] was the most common species and was isolated from all farms and from cows, humans and environmental samples.

Advances in animal production, food processing and hygiene, and refrigeration have eliminated several foodborne diseases that plagued Americans in the past century (9). However, in the past 30 years, several previously unrecognized milk-borne bacterial infections, including infection with *Streptococcus aureus*, *Staphylococci bacilli*, Micrococcus, and *Escherichia coli* strains have emerged as significant causes of human morbidity and mortality (9). Milk borne illnesses have led to other infectious diseases once believed to be controlled to start reappearing and it is estimated that, each year, 76 million Americans become ill from eating contaminated food especially milk (10). The recent research also provided evidence that contamination of raw milk originates from milking, transportation and storage (11).

Zambia is not exempted from milk-borne diseases too. Poor pasteurization techniques give rise to food-borne, infectious diseases and lower shelf-life for milk (12). In a bid to assess and improve milk safety standards in some districts in Zambia, the University of Zambia conducted a series of platform tests for raw milk analysis where samples were sent to Food Science and Hygiene Laboratories as well as Microbiology Laboratories at the University (12). The study revealed that the bacteria plate count was high and concluded that the raw milk from farmers was contaminated and this poses a great risk to people who consume it in its raw state (12). The Ministry of Health reported that at least one third of the total population in Zambia suffers from milk borne diseases such as salmonellosis and others (13).

In Monze where this study was conducted, milk-borne diseases are registered every year. Statistical records indicate an increase in milk-borne diseases from 3.4% to 5% between January 2008 and December 2010 (14).

Cow's milk is an unhealthy fluid from diseased animals that contains a wide range of dangerous and disease causing microorganisms such as *Streptococcus, Staphylococcus, Campylobacter, Escherichia coli and Micrococcus* that have cumulative negative effect on all who consume it (15). High percentages of the E. coli strains isolated from the water sources showed multiple resistances to most of the antibiotics commonly used by humans. Strains recovered from the stream and well water sources were most resistant and showed significantly higher minimum inhibitory concentration (MIC). and minimum bactericidal concentration (MBC).(P<0.05).than those from the tap water source (16).

1.1   Global perspective of milk-borne diseases

The World Health Organization (WHO). report (17). estimates that every year there are about 47.8 million milk-borne illnesses in the United States, (16,000 cases for 100,000 inhabitants)., 2 million in the United Kingdom (3,400 cases for 100,000 inhabitants). and 750,000 in France (1,220 cases for 100,000 inhabitants). According to American Public Health (10). the Centre for Disease Control Programme estimated 47.8 million milk-borne illnesses (16,000 cases for 100,000 inhabitants). Though difficult to give the exact figure of the global incidence of milk-borne diseases, it has been reported that in the year 2000 about 2.1 million people died from diarrhea diseases (10). Additionally, many of these cases have been attributed to contamination of food and drinking unpasteurized milk and diarrhea has been noted as one of the major causes of malnutrition in infants and young children (10).

Velazquez et al (18). reported that dairy products industry is going toward safe milk and its products in the food market. Milk quality and food safety concern in the consumers' health and nutrition in public health surveillance prevent food-borne diseases, food poisoning, and zoonosis risk by raw milk and fresh dairy products. These workers also observed that the microbial milk contamination source comes from herd hygiene and health status, mastitis prevalence, production environment, and milking parlor and milk conserving practices in dairy farms. Moreover, these facts are implicated in milk quality and milk spoilage and unsafe dairy products. The food hygiene protocols are fundament to reduce the microbial contamination of the raw milk and pasteurized milk, regarding the health risk by the microbial pathogens in the food borne diseases and bacterial spoilage, source of deteriorating dairy products and milk. The microbial quality of foods is required for the traceability in dairy products industry. Consumers' education programs and practices of good handling of foods could reduce the exposure to food borne pathogens and the consumption of unsafe food products. The traceability of milk and dairy products, from the production-distribution chain food and the consumption is a good policy for assurance of quality and to reduce the public health risks.

Bacteria multiply rapidly in milk due to its rich nutritional composition. According to Boycheva et al (19). studies on bacterial quality of milk show presence of different types of bacteria in milk like *Escherichia, Listeria, Staphylococcus, Streptococcus and Clostridium* species. *Listeria monocytogenes* is an organism which can cause serious and sometimes

fatal infections in young children, frail or elderly people, and others with weakened immune systems (20). The isolation of pathogenic and coli from milk indicates that milk may be contaminated from the udder of animals, utensils used for milking or water used (21). A strong increase in the total count (TC). of bacteria was observed during transport from the farm to the market (107 colony forming unit per ml (CFU ml–1).). The main indicators considered were TC, *Enterobacteriaceae* and *Staphylococcus aureus*. The milk containers of the farmer and the milk vendor played a major role in the increase in the milk flora that occurred during transport from the farm to the selling points (22). Microbial load in fresh milk is although very low i.e. less than 10^{-3} CFU/L may increase up to 100 fold if this milk is stored for many days at normal temperature (21).

Marwa and Lamiaa (21). further explains that consumption of raw milk has always been common among farm families, currently varying from 35% to 60% and most of these farm families report taste and convenience as the main reasons for raw milk consumption. The US Centers for Disease Control and Prevention's Food-Net Population Survey report (23). estimated that about 3.5% of respondents reported to have consumed unpasteurized milk in the past 7 days before the survey. Demand for raw milk has considerably increased in recent years, despite the fact that public health officials consider the benefits of milk pasteurization to be undisputable (19). According to American Public Health, (2010). [10], in the United States in 1938, milk-borne outbreaks constituted – 25% of all disease outbreaks due to contaminated food, milk and water. Between 1880 and 1907, averages of 29 outbreaks of milk-borne diseases were reported each year in the United States (24). Costard et al (25). reported 46 outbreaks of milk-borne disease in the 19-years period form 1973-1992; an average of 2.4 per year.

1.2 African perspective

According to Totera and Abebe (26). cattle-keeping in Africa is an aged-old tradition. Africans have historically been classified in two distinct groups, namely cattle keepers or pastoralists and farmers. Standard is something of value for others to look at and try to achieve. Milk safety standards are critical components to develop in any country's milk commodity (27). FAO and WHO (28)., adds that consumers need to learn how to distinguish between safe and unsafe milk and one way to achieve this is to impose a "quality standard" to help consumers choose safe products. The Mbarara district in south-western Uganda is home to many smallholders who collectively own more than 800 000 cows (29). The area mostly supplies milk to Kampala situated 280 km away and due to long distances covered by farmers, it gives a conducive temperature for microbial growth (29). Due to lack of pasteurizing equipment in some African countries, many people consume raw milk which puts them at a greater risk of contracting milk borne diseases (29).

1.3 Zambian perspective

The Ministry of Health food complaints statistical report (13). indicated many milk borne diseases among the Zambian community. Households that reported milk borne disease cases within the previous 12 months were approximately seven times more likely to be recorded if the situation is not controlled (odds ratio = 7.6; p=0.004). Milk in Zambia and other countries is contaminated indirectly through the milking system when milking equipment, utensils, containers or other milk contact surfaces that are not kept hygienically (30). Producers can prevent indirect milk contamination by using approved chemicals, following the label instructions, storing chemicals separately, and training employees to handle chemicals properly (30).

Monze Central records show an increase in prevalence of confirmed cases of milk-borne diseases among humans. Statistics records indicate that for the past three years (2008 to 2010)., there has been an increase from 3.4% to 5.1% of such cases (14).

1.4 Statement of the Problem

In spite of evidence of prevalence of milk-borne diseases in Monze District, levels of milk contamination at processing centres has not been evaluated. The efficacy and effectiveness of the milk processing methods at Monze Dairy Farmers Cooperative Society has not been documented.

1.5 Objective

To quantify microbial population in bovine milk from smallholder dairy farmers of Monze Central Dairy Farmers Corporative Society (MCDFCS).

2 Material and methods

To reduce microbial multiplication, milk samples and sterile syringes were carried in the cooler box where ice parks were put so as to maintain the milk temperature constant (within the range of 0°C to 4°C). This temperature had little

or no effect on microbial population growth in milk samples which (milk). were analyzed for microbial content and species within 48 hours after collection. This was done at the School of Veterinary Medicine of the University of Zambia.

2.1 Agar plating/colony counting

Two types of plate agars were made which were MacConkey and Blood agar in the sterilizer after which it was allowed to dry. The agars were to allow those microbes which could not grow on one agar; they may be seen on the other agar. After five minutes, streaking/inoculation was done followed by incubation. Incubation was done within 24hrs at 37°C after which microbes grew on those plates. For microscopic examination, a colony was scooped for Gram staining. This involved an addition of saline water, sticking using Bunsen burner and washing using running water. The type of microbes which were present was observed under the microscope. For plate counting, direct plate count was done. The researcher practically had to block some of possible confounding variables such as temperature for effective and quick statistical results.

2.2 Data collection

Data pertaining to types of microbes present and numbers of colonies were collected. These data were collected within 24 hours of sample collection. Each colony represented a single bacterium that was in the solution. The number was multiplied by the dilution factor which is a visible cells count method-meaning it only counts those bacteria which are alive, since dead bacteria cannot grow colonies.

Sampling formula

$$N = nfZ^2pq/d^2$$

Where:

Z^2 = 95% confidence level
d^2 = The error level
pq = The maximum outcome assumed.
Nf = The sample size with the target population in mind
N = The target population

2.3 Data analysis

Data was analyzed using the Statistical analysis System (SAS). and the treatment means were compared using F-test. Analysis of Variance (ANOVA). table was constructed to determine the reliability of the data.

3 Results

3.1 Percentage for microbes grown under blood agar and MacConkey agar

Percentage values varied for different species of microbes grown under two media (blood agar and MacConkey agar). as shown in Tables 1 and 2.

Table 1 Macroscopic Examination of Bacteria under Blood Agar

Bacterial species	Frequency	Percentage %
Bacillus	1	2.6
Bacillus	2	5.3
Bacillus-Staphilococcus	1	2.6
Bacillus-Staphilococcus	1	2.6
Staphilococcus H	1	2.6
Staphilococcus H	10	26.3
Staphilococcus H	2	5.3
Staphilococcus & bacillus	1	2.6
Staphilococcus & bacillus	2	5.3

Staphilococcus & streptococcus	10	26.3
Staphilococcus & streptococcus	1	2.6
Staphilococcus & streptococcus	1	2.6
Staphilococcus A	1	2.6
Streptococcus	1	2.6
Staphilococcus & bacillus	3	7.9
Total	38	100

Table 2 Macroscopic Examination of Bacteria under MacConkey Agar

Bacterial species	Frequency	Percentage %
Streptococcus.	1	2.6
Staphilococcus H	21	55.3
Bacillus	5	13.2
Escherichia Coli	11	28.9
Total	38	100.0

3.2 Colony Forming Units per ml

The mean values for colony forming units varied between unprocessed and processed milk with unprocessed samples having the highest value and processed milk having the lowest value (Table 3).

Table 3 Average Numbers of Coliforms in Processed and Unprocessed Milk Samples

Samples	Total(y).	Mean($\bar{y}$).
Processed	46.36×10^4	9.272×10^4
Unprocessed	189.96×10^4	37.992×10^4
	$\sum y = 236.32 \times 10^4$	$\bar{\bar{y}} = 23.632 \times 10^4$

Table 4 Analysis of Milk Microbial Content

	Processed	Unprocessed
Mean	9.272×10^4	37.992×10^4
Standard Deviation	$\pm 19.98 \times 10^4$	$\pm 30.63 \times 10^4$
Sample Size	5	5
Standard Error	8.92×10^4	13.67×10^4
WHO Permissible Values		
Grade A Pasteurized	Not to exceed 10/mL coliforms Not to exceed 20,000/mL total bacteria	
Pre-pasteurized Commingled	not to exceed 300,000/mL	
Raw milk	not to exceed 30,000/mL	

Commingled milk is the farm and has other individual tank, either during processing plant

t-calculated	0.0000108	p>0.01
t-tabulated($t_{0.01}$).	3.355	p>0.05
t-tabulated($t_{0.05}$).	2.306	

milk that has left been mixed with producer milk in a shipment or at the

Table 5 Anova for Coliform Numbers in Processed and Unprocessed Milk Samples

Source of variation	Degrees of freedom	Sum of squares	Mean square	Fcal	Ftab
Total	9	$12,939.30246 \times 10^8$			
Treatment	1	$2,062.96 \times 10^8$	$2,062.096 \times 10^8$	1.52	5.32
Error	8	$10,877.20646 \times 10^8$	$1,359.650808 \times 10^8$		

CV=12%

4 Discussion

Losses incurred in milk quality are due to use of unclean storage utensils, which are prone to high microbial contamination. There was no significant ($p>0.05$). difference in the microbial contamination between the processed and unprocessed milk at Monze Dairy Farmers Cooperative Society. This study indicated that farmers at Monze Dairy Farmers Cooperative Society produce good quality and safe milk through observance of good hygiene practices. The findings of this study are in consonance with those of Theodore et al, (31). who observed that smallholder dairy farmers in Western Zambia produce milk of good initial quality. Contamination is due to lack of storage or refrigeration during transit. Results of this study are at variance with the findings of Meranga et al, (32). who observed higher TAPC and coliform counts in raw milk samples from producer and collector bulk milk samples from smallholder dairy farms around Addis Ababa in Ethiopia.

The presence of bacteria in tables 1 and 2 indicates contamination of milk. The results show that all milk samples were contaminated with hemolytic Staphylococci. Bacteria present in cows or on equipment may have been the most likely source of milk contamination in milk from farmers in Monze Dairy Farmers Cooperative Society (MDFCS).

The mean values for microbial contamination in processed milk ranged between 0.1×10^4 and 45×10^4 (Table 1). Similarly, mean values for microbial contamination in the unprocessed milk were observed to lie between 0.16×10^4 and 83×10^4. Even though the magnitude of microbial content in unprocessed milk were higher than that of the processed milk, the levels of content did not differ significantly ($p>0.05$).

When evaluated across samples, values of bacteria species content varied from 1(2.6%). to 10(26.3%). (Table 1). when using blood agar. Similarly, bacteria species content varied from one sample to another when analyzed using MacConkey agar. The lowest being 1(2.6%). and the highest being 21(55.3%). (Table 2).

5 Conclusion

The findings of this study show that the two samples (processed and unprocessed). had no significant ($p>0.05$). difference in terms of microbial content/load. This could be attributed to the method used to destroy the microbes in milk at MDFCS, the temperature at which the milk is heated, the duration and the response of microbes to the temperature. This is because different microbes respond differently to temperature levels. The other contributing factor could be the equipment used to store milk before and after processing. The kind of disinfectants used to clean to equipment and how often the disinfection is done can be a contributing factor for many microbes to grow since milk is a good media for many microbes. The current status of the MDFCS is that they used a detergent paste for cleaning instead of antibacterial which may allow some microbes to grow on the equipment.

Improving of microbial quality of raw milk requires the establishment of a quality policy with training of farmers on good hygienic practices. With the upcoming technological advances, the research recommends that mycobacterium TB could be researched form bovine milk at the same Corporative since it is one of the world's most problematic diseases. It is also recommended that the research can be replicated during the rainy season.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares that he has no competing interests. There is no conflict of interest regarding the publication of this article.

Statement of informed consent

The research sought the consent and permission of the smallholder farmers and the management of Monze Central Dairy Farmers Cooperative Society.

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