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ASSESSMENT OF SAFETY STANDARDS OF MILK PRODUCED IN LARGE DAIRY FARMS IN ALTIBNA AREA, KHARTOUM NORTH, SUDAN

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ABSTRACT

This descriptive and experimental study was conducted between December 2019 and February 2020 in five large dairy farms all using milking parlors in Altibna area, Khartoum North, Sudan. The objectives of this study were to provide a perspective on the current status of management strategies set to minimize contamination of raw milk, assess the quality of bovine raw milk with an emphasis on levels of SCC, Standard Plate Count, and Total Coliform Count and to detect the presence of antibiotics residues in the investigated bovine raw milk. For the assessment of safety standards of milk quality, the sum of different parameters resembled the final grading of the investigated farms. For assessing farm hygiene and management strategies a structured audit list was implemented. Other parameters included microbial and antibiotic residues testing were also used. The result obtained from the audit list was used to attain the general classification of the dairy farms after deducting the values obtained from bacteriological testing and antibiotic residues from the audit list if they were found exceeding the limits. Accordingly, dairy farms were classified as class 'A' farm when it scored 90 to 100%, class 'B' from 80 to 89%, class 'C' from 70 t0 79%, and class 'D' <70%. The mean total bacterial count (TBC) in the five investigated farms was found to be $137\times10^5\pm666138.7$cfu/ml with significant difference between the standard mean and mean of TBC, with $p\leq0.05$. The mean of total coliform count (TCC) in the five investigated farms in bulk milk was found to be 11003.8±1.24cfu/ml, while there was a significant difference between the TCC in the five farms and standard permissible limits, with $p\leq0.05$. The mean total coliform count (TCC) in the investigated farms water was found to be 291.7±209.1cfu/ml with significant difference with standard mean and mean of TCC, with $p\leq0.05$. This study also revealed that the mean of bulk tank somatic cell count (BTSCC) in the investigated farms was found to be $348\times10^8\pm579784.4$ with highly significant difference between standard mean and mean of BTSCC, with $p\leq0.05$. The result of antibiotic residues in the investigated farms was 1.4000±0.24 with highly significant difference between the standard mean and mean of antibiotics residues with $p\leq0.05$. This study found that the mean of milk tank temperature was 10.4±2.31 ºC in the investigated farms with no significant difference between standard temperature and milk temperature, with $p\leq0.05$. The study suggests that the investigated farms need more management strategies to control and minimize the high raw milk contaminations and antimicrobial misuse.

KEYWORDS: Milk quality, Dairy management strategies, Antibiotics residues, Bulk tank somatic cell count.

INTRODUCTION

Raw milk which is a perishable product, is considered an ideal medium for the growth of a wide variety of bacteria.[1]

Many authors have called for concerned authorities to establish standards and grades of raw milk in order to ensure good safety and quality of raw milk.[2] In addition, factors affecting milk safety were studied by many authors. Murphy and Boor[3] reported that the health and hygiene of the cow house, milk storage, equipment, and hygiene during milking, all are factors that influence microbial contamination of raw milk.

Moreover, Oliver[4] reported that providing and maintaining a clean, dry and lactating cows, good environment will reduce the incidence of mastitis caused by environmental mastitis pathogens and minimize problem associated with environmental contaminations.

Both somatic cell count (SCC) and total bacterial counts (e.g., standard plate count, SPC), are raw milk quality measurements most often considered in regard to potential effect on processed product quality. These, at higher levels, are associated with increased activity of enzymes that damage milk components and potentially result in product defects.[5,6]

Food authorities in different countries designed standard permissible limits for milk in order to protect public health. For instance, FDA[7] reported that regulatory limits designed to protect public health under the US Pasteurized Milk Ordinance (PMO) for grade "A" producer milk are 750,000/mL bulk tank SCC (BTSCC) and 100,000 cfu/mL SPC. In most cases, meeting premium incentive requirements is based on meeting additional test criteria e.g., free from antibiotics.[8]

Milk-quality premiums are sometimes used as a competitive milk procurement tool to attract high-quality milk to a plant.[8] In this regard, Coliforms are often used as indicator microorganisms, so their presence in food implies poor hygiene and sanitary practices.[9,10] The European Union (EU) limit for coliforms in raw milk is >100 CFU/ml.[11]

MATERIAL AND METHODS

This cross-sectional study was conducted in five large dairy farms using milking parlors with machines in Altibna, Khartoum north, Sudan between December 2019 and February 2020.

Data collection

To assess the milk quality, the sum of different parameters resembled the final grading of the investigated farms. Assessing farm hygiene and management strategies to minimize raw milk contamination a structured audit list was designed to collect variables such as the general environment, milking practices, such as udder cleanliness, equipment, water quality, milking facilities, udder hygiene. Other parameters included were microbial and antibiotic residues testing.

According to FDA[12] the standards limits of milk safety and quality for total bacterial count is $1{\times}10^5$c/ml, total coliform bacteria in milk <100cfu/ml, total coliform count in water 100cfu/ml, bulk tank somatic cell count $7{,}5{\times}10^5$, antibiotic present in milk is zero, and milk temperature 10 ºC.

Audit list preparation

The structured Audit list was designed with seven sections. Section 1: hygiene and food safety (eight variables and weight 54.1%); Section 2: housing and facilities (two variables and weight 11.7%); Section 3: plant and equipment (one variable and weight 5.9%); Section 4: feed (two variables and weight 10.7%); Section 5: herd health (sex variables and weight 11.1%); Section 6: stockmanship and training (two variables and weight 4.2%); Section 7: contingency procedures (only one variable and weight 2.3%).

Sampling

Five bulk tank milk samples of approximately 50 ml of raw milk for each of the investigated five dairy farms with the purpose of evaluating TBC, TCC, BTSCC and antibiotics residues were aseptically collected and then aseptically transferred into sterilized containers. The samples were then kept in an icebox and transported at 4°C to the laboratory of the College of Veterinary Medicine, University of Bahri. All the samples were tested in the same day of collection.

The five milk samples of antibiotic residues were preserved in icebox and sent the next day to the laboratory of the University of Sudan. Also five water samples were taken from the five farms for TCC count.

METHODS

In this study the approach for classification of milk and the dairy farms was based on the score attained from the field audit list in addition to the quality tests as described by Mustafa.[13]

The result obtained from the audit list was used to attain the general classification of the dairy farms after deducting the values obtained from bacteriological testing and antibiotic residues from the audit list if they were found exceeding the limits. For instance, if the TBC or TCC or BTSCC in milk were found above the standard limit 2 points each and 5 points for antibiotic residues in milk would be deducted. Also 2 points would deducted in case of milk temperature in bulk milk tank found more than 10 ºC.

Accordingly, dairy farms will be classified as class 'A' farm when it scored 90 to 100%; class 'B' farm 80 to 89%; class 'C' farm 70 to 79% and class 'D' farm <70%.

Procedure for evaluation of bacterial Load

Total bacterial count (TBC) was used as described by Marshall.[14] TBC was calculated by using spread plate method. One ml was taken from the sample and then added to 9ml of normal saline for the first dilution. Then, 1ml was taken from the first dilution and added to 9ml for the second dilution. Then, 1ml of the 5^{th} dilution that prepared previously was taken and added to labeled Petri dish with a bout 15ml plate count agar. Then, spread to facilitate absorption, incubated for 24 hours at 37 ˚C and bacterial colonies were counted.

TCC in milk and water was calculated by using the most probable number according to FDA.[15] 1 ml was taken from the samples (milk, water) and added to 9 ml of previously prepared Macconkey broth media for first dilution, then 1 ml was taken from the first dilution and added to second dilution and from second dilution 1 ml was taken to the third dilution, then incubated for 48 hours at 37 ˚C. Changes in media turbidity, change color from purple to yellow, production of gas were observed and the number of tubes with change was counted and the result was read. If all tubes showed growth, then the results would be noted as 333. If only one tube in each replicate showed growth it would be denoted as 111. The pattern of growth was then read from the table to provide the most probable number and 95% confidence interval.

BTSCC was done as described by ISO 13366-1 and IDF 148-1.[16] Milk was mixed thoroughly before use. An amount of 0,01ml of milk was pipetted and spread on slide with an area of 1cm^2. After drying the slide was stained with freshly prepared stain (Newman Stain) for 3-5 minutes and dried in ambient temperature. The smear was dipped gently in tap water until all surplus dye washed away. Then the cells were counted using oil immersion lens. The somatic cells were counted in 10 fields throughout the area of the smear, and an average cell count per field was obtained as N Cell/ ml in a sample = N × 500,000 (microscope factor) ÷10 (number of the fields).

For antibiotic residues the method of Mahantesh *et al*.[17] was used. *Bacillus subtilis* culture was grown in Brain-Heart infusion liquid medium at 37˚C. After 6 h of growth, 0.1 ml of broth was spread on the surface of Mueller-Hinton agar plates. Sterile filter paper discs of 10 mm diameter were entirely dipped in 50 ml of milk sample using forceps until the discs were completely impregnated with the milk sample. Milk-wetted discs were air-dried and placed equidistantly around the margin of the inoculated plates. Plates were incubated at 37˚C for 24 h and inhibition zone of the bacterial growth was measured in mm using zone inhibition scale.

RESULTS

Table (1) displays the results of the bacterial quality of milk in the investigated farms. The total bacterial count of the five farms was found 300×10^{5}cfu/ml, 37×10^{5}cfu/ml, 18×10^{5}cfu/ml, 300×10^{5}cfu/ml, and 300×10^{5}cfu/ml, respectively.

The total coliform count in milk in the investigated farms was >1100cfu/ml.

The total coliform count in water in the five investigated farms was >1100cfu/ml, 290cfu/ml, 36cfu/ml, 3.6cfu/ml, and 27cfu/ml, respectively.

The somatic cell count in milk for the five investigated farms was 2.6×10^6, 3.3×10^6, 5.250×10^6, 4.250×10^6, and 2×10^6, respectively.

The presence of antibiotic in milk was detected in 3 (60%) farms.

The milk tank temperature for the five investigated farms was 7, 9, 7, 10, >10, respectively.

Table 1: The standards limits and laboratory test results of TBC, TCC in milk, TCC in water, Antibiotic residues and milk tank temperature in raw bovine milk in the investigated farms.

Laboratory tests	Standards limits	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
TBC	1×10^{5}c/ml	300×10^5	37×10^5	18×10^5	300×10^5	300×10^5
TCC in milk	<100cfu/ml	>1100	>1100	>1100	>1100	>1100
TCC in water	100cfu/ml	>1100	290	36	3.6	27
BTSCC	7.5×10^5	2.6×10^6	3.3×10^6	5.250×10^6	4.250×10^6	2×10^6
Antibiotic residues	Zero presence	No	Yes	yes	yes	No
Milk tank temp.ºC	10	7	9	7	10	>10

Table (2) shows that the mean of TBC in milk tank was 1370000.8±666138.1cfu with significant difference in count between standard mean and mean of TBC in milk with p≤0.05.

The mean of TCC in milk tank was 11003.8±1.2cfu with highly significant difference between standard mean and mean of TCC in milk with p≤0.05.

Also the table displayed that the mean of TCC in water was 291.7±209.1cfu with significant difference between the mean of TCC in water and standard mean with p≤0.05.

Table 2: The mean counts comparison of TBC, TCC in milk, and TCC in water with standard limits and their significance values.

Test	Sample site	Mean	Std .deviation	Standard error	Significance
TBC	Milk tank	1370000.0000	14895302.36685	6661381,72754	.129
TCC	Milk tank	11003.8000	2.77489	1.24097	.000
TCC	Farm water	291.7200	467.71764	209.16969	.028

As appears in table (3) the mean of BTSCC in milk tank was found to be 3480000.0000±579784.4 with highly significant difference between the standard mean and mean of somatic cell count with p≤0.05.

The result of antibiotics residues in milk was 1.4000±.24 with highly significant difference between antibiotics residues in milk and the standard permissible limits with $p \leq 0.05$.

Also the mean of bulk tank temperature in the investigated farms was 10.4±2.31, with no significant difference between standard temperature and temperature of milk sample with $p \leq 0.05$.

Table 3: The mean count of different tests on milk compared to standard limits and their significant values.

Test	Sample site	Mean	Std .dev	Standard error	Sig.
BTSCC	Milk tank	3480000.0000	1296437.42618	579784.44270	.009
Antibiotic residues	Milk tank	1.4000	.54772	.24495	.005
Bulk tank temp.	Milk tank	10.4000	5.17687	2.31517	.871

Table (4) contains the results of the field audit list. The five dairy farms scored 35.2%, 39.4%, 30.9%, 35.8%, 14.3% in section one, respectively. In section two they scored 9.4%, 11.0%, 9.1%, 11.0%, 9.1%, respectively and in section three 5.2%, 3.6%, 5,2%, 4,9%,1.6%, respectively. In section four they scored 8.7%, 8.5%, 8.1%, 6.5%, 8.1%, respectively. In section five they scored 5.9%, 7.2%, 7.8%, 5.9%, 3.6%, respectively. In section sex they scored 3.9%, 3.9%, 3.3%, 3.9%, 3.3, respectively and in section seven they scored 0.6%, 1.6%, 0.7%, 0.7%, 0.7%, respectively.

The final evaluation based on field audit list was found 69.0%, 75.2%, 65.1%, 68.7%, and 40.7% for the five investigated farms, respectively.

Table 4: Assessment of farm hygiene and management strategies for the investigated dairy farms using field audit list.

Standards	Hygiene and food safety	Housing and facilities	Plant and equipment	Feed	Herd health	Stockman ship and training	Contingency procedures	Total evaluation
%	54.1	11.7	5.9	10.7	11.1	4.2	2.3	100
Farm evaluation								
1	35.2	9.4	5.2	8.7	5.9	3.9	0.6	69.0
2	39.4	11.0	3.6	8.5	7.2	3.9	1.6	75.2
3	30.9	9.1	5.2	8.1	7.8	3.3	0.7	65.1
4	35.8	11.0	4.9	6.5	5.9	3.9	0.7	68.7
5	14.3	9.1	1.6	8.0	3.6	3.3	0.7	40.7

Table (5) explains the final classification of the five investigated dairy farms based on the score attained from the field audit list in addition to the quality tests.

Table 5: Final classification of dairy farms in the investigated area.

Farm No.	Field audit	Total bacterial count	Total coliform in milk	Total coliform in water	Somatic cell count	Antibiotic residues	Temperature of milk	Final evaluation	Classification
1.	69.0	-2	-2	-2	-2	ND	AL	61%	D
2.	75.2	-2	-2	-2	-2	-5	AL	62.2%	D
3.	65.1	-2	-2	AL	-2	-5	AL	54.1%	D
4.	68.7	-2	-2	AL	-2	-5	AL	57.7%	D
5.	40.7	-2	-2	AL	-2	ND	-2	32.7%	D

AL= Accepted Limit
ND= Not Detected

DISCUSSION

The aim of this study was to provide a perspective on the current status of management strategies set to minimize contamination of raw milk in five large dairy farms equipped with milking machines.

This is first study in Sudan about classification of large dairy milking parlors based on the assessment of safety and quality standards of raw bovine milk.

In the current study the field audit list evaluation revealed low values (between 40.7% and 75.2%) for the investigated farms. This may be due to low scores attained in assessing the hygiene and food safety section of the audit list.

This result supports the finding of[18] who reported that in Sudan there is no rigid system of inspection and that dairy farms are not complying with sanitary standards.

According to the final classification of dairy farms in this study, all farms attained 'D' score. This poor result might be due to the low values scored in milk bacterial quality and antibiotic residues. This finding supports that stated by Silva[19] who reported TBC and TCC may pose health hazards when present at high levels and may include pathogenic microorganisms that may lead to a short shelf life of food products**.**

This study revealed significant differences between the TBC in milk and the standard mean with p value$\leq$0.05. Similar result was obtained by Ye *et al.*[20] who found that the TBC revealed high contaminated level in milk (2.55×10^7cfu/ml). The high level of TBC in this study may be due to poor cleaning in place (CIP) system and that there were no special programs designed for control of mastitis in the investigated farms. This finding also supports the result of Oliver**[4]** who reported that the most frequent cause of high SPCs is poor cleaning systems, milk residues on equipment surface, cows with mastitis, and failure of water heater. Coliforms as a reliable indicator of fecal contamination can sometimes be present in contaminated equipment and utensils, as well as in foods.[21] The five dairy farms in this study showed high level in TCC and TBC in milk. This may be attributed to the similar hygiene practices adopted in farms and parlors, the general culture of milking labors, and lack of information of people in this area about hazard of contaminated milk to public health.

The difference between the TCC of milk in the five milking parlors in this study was significantly higher than the permissible TCC level. Similar result was obtained by Ye *et al.*[20] who found that the TCC in milk was1.59×10^5 cfu/ml. The high level of TCC in milk in this study may be due to ineffectiveness of sanitization of milking equipment, as low water temperature used for CIP was observed during auditing. Also, high levels of TCC may be attributed to poor cleanliness of cows' environment and ineffectiveness of teat preparation before milking.[22] This was also evidenced by the low scores of the investigated farms attained during auditing.

This study detected that the mean of TCC in water was higher than the permissible level showing significant difference with p$\leq$0.05. This may be due to insufficient sanitation of stored water containers. The finding also supports that of[23,24] who reported water used in sanitation and milking environment are considered as one of critical sources for coliform in raw milk.

Schallibaum[25] stated that somatic cell count (SCC) is used by milk quality laboratories to determine quality and acceptability of milk.

This study revealed highly significant difference between the BTSCC in milk and standard permissible limits with p$\leq$0.05. The high level of BTSCC in this study may be due to the unhealthy animals, poor hygiene, and absence of mastitis control program. This is also evidenced by the low level of the hygiene and food safety attained during auditing.

Raw milk can be contaminated with residue of anti-microbial agents which are used to treat a variety of diseases or added intentionally to increase the usable life of the product. This study revealed significant difference between antibiotics residues in milk and standard limits which recommend zero presence of antibiotics in milk with p$\leq$0.05. The presence of antibiotics in milk in this study may be due to excessive use of antibiotics for treatment purposes, or poor monitoring procedures to withdrawal periods of antibiotics in treated cows or neglecting separation of animals under treatment from healthy ones. This finding supports the results of[26,27] who reported that 80% of conventional dairy herds use antibiotics for treatment of mastitis which was the first disease and remains the most common reason for administration of antibiotics in cattle. It also supports the finding of Hind[28] who stated that the use of penicillin and other intra mammary antibiotics, lack of information about withdrawal periods of drugs, handling of animals under treatment, workers insufficient experience, are among the major predisposing factors for the presence of antibiotic residues in milk. This observation also was coincided with the previously published work of imprudent usage of antibiotics in dairy farms in Khartoum State.[29,30]

CONCLOUSION AND RECOMMENDATIONS

All dairy farms in this study were classified as 'D' class which means poor score. The competent authority must set up an inspection and monitoring program for dairy farms with incentives to encourage owners to produce high safety and quality milk.

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Competing Interest: The authors declare that they have no competing interests**.**

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