Original Research Article

Evaluation of boiled milk hygiene and safety in the markets of El-Obied City- North Kordofan State- Sudan

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ABSTRACT

Background: This study was conducted in the markets of Elobeid City- North Kordofan State-Sudan, to evaluate the hygiene and safety of the boiled milk vended in the markets of El-Obied City, Sudan, during the period from October 2012 to October 2015.
Methods: The study covered all the places for the boiled milk sale in the markets of El-Obeid City, which are 84 places. Data were collected from all milk handlers (87) by questionnaire and observation-check list about the health requirements for milk handling in those places. The data were analyzed manually and the results presented in tables and figures. Eighty-four milk samples were collected from the offering containers of boiled milk vended in that places, and examined for the total aerobic plate counts in the laboratory of veterinary research station in El-Obeid City. The microbial indicator (aerobic plates count for microbes) was used to evaluate the extent of boiled milk hygiene and safety.
Results: The results showed that the sound milk samples (free of microbes) concerning the aerobic plates count for microbes were only 9.52% and most milk samples (76.19%) were of low microbiological quality. Also this study demonstrated that the bubbling of milk without stirring contributed to the existence of microbial contamination of boiled milk by 41.67% and the use of dirty utensils and equipment contributed to the existence of microbial contamination by 15%.
Conclusions: This study concluded that the milk in the markets of El-Obeid City was marketed under unhygienic conditions and there is no assurance to its safety and wholesomeness in most places.

Keywords: Boiled, El-Obied, Hygiene, Milk, Safety

INTRODUCTION

Milk is one of the foodstuffs that need good handling to ensure the safety and the wholesomeness.[1]

According to the Center for Disease Control and Prevention (CDC), five food safety risk factors related to employee behaviors and preparation practices have been identified as the leading contributing factors to food borne illness. They are improper holding temperatures, poor personal hygiene, inadequate cooking, contaminated equipment and food from an unsafe source. Immediate correction is required whenever a risk factor violation occurs at a commercial establishment. Failure to take immediate corrective action increases the risk of food borne illness outbreak.[2] Foodborne disease takes a major toll on health. Thousands of millions of people fall ill and many die as a result of eating unsafe food.[3]

Many of people in El-Obeid city complain from clotting of the boiled milk purchased from the markets. And it has been noted that the milk in the markets of El-Obeid city is handled under unsanitary conditions and there is no microbiological surveillance for milk to ensure its safety and suitability. Therefore, it was decided to study this problem by subjecting it to scientific research.

According to Andrews, the total aerobic plate count was used to assess the microbial quality of milk, because the total aerobic plate count is useful for indicating the sanitary conditions under which the food was produced and/or processed.[4]

Also, the total aerobic plate count is useful for indicating the overall microbiological quality of a food and, thus, is useful for indicating potential spoilage in perishable products. In some cases general viable count may be used as indicator of standard of hygiene, or as indicators of a potential health hazard (incubation of 30- 37°C probably being best.[5]

The presence of indicator bacteria in ready-to-eat food, although not inherently a hazard, can be indicative of poor practice that may be one or more of the following:

Poor quality of raw materials or food components; undercooking; Cross-contamination; poor cleaning; poor temperature and time control.

Indicator bacteria may be associated with an increased likelihood of the presence of pathogens. Indicator organisms are useful in the assessment of food product safety because they tend to be present in higher numbers than most pathogens and are relatively quick and easy to identify.[6]

Milk as secreted by the udder cells of a healthy cow is probably sterile, i.e. it contains no microorganisms capable either of souring the milk or causing disease. But when milk reaches its reservoir within the udder, and particularly in passing through the teat of the udder and reaching the milking pail, the risk of picking up deterious microorganisms steadily increases. Raw milk must undergo heat treatment to prevent not only its rapid deterioration but also any risk of its conveying disease to the consumer. Heat treatment is generally most satisfactory because it causes the minimum of change in the composition of flavors and acceptability of the milk. Effective heat treatment does not necessarily entail the destruction of all micro-organisms originally present but it accomplishes the destruction of any pathogens in the milk. The most common method of treating raw milk is by applying heat. There are at least five methods of treating milk. These are boiling, pasteurization, cooling, sterilization and drying.[7] Boiling of milk is the easiest and most practicable method of making milk safe in every home. As soon as raw milk is produced or delivered it should be boiled. Boiling is raising the temperature of the milk to boiling point and maintaining the milk at this temperature for a few minutes. Then the milk should be immediately cooled. If it has to be stored the temperature should be maintained below 10°C. Since these may be impracticable in a home, every care should be taken to keep the milk as cool as possible. Preferably the milk should be consumed as soon as possible after cooling and not stored for an extended period of time after it has been boiled and cooled. Boiling of milk destroys all microorganisms except the spore formers but it changes the nutritive value of milk, its flavors and palatability and appearance. However, this disadvantage should be disregarded in favour of the safety of boiled milk i.e. its freedom from disease-causing microorganisms. Boiling is technically difficult to process on a large scale and is commercially uneconomical.[7] The boiling what it except an intense pasteurization, where the temperature arises to 100.17°C, and it a degree of milk boiling. The method followed in the houses for boiling the milk is putting it in container on flame and heating it until bubbling, then raised from the flame and let to cool spontaneously. And this method, unfortunately, not consider enough for heating all parts of milk. Whereas what observed of bubbling, completes usually before reaching the milk to the boiling temperature.[8]

Objectives of the study

The general objective was to evaluate the hygiene and safety of the boiled milk vended in the markets of El-Obied city. The specific objectives were to detect the extent of boiled milk contamination by indicator microbes; and to determine the factors that contribute to boiled milk contamination.

METHODS

This study was performed in the markets of El-Obeid city- North Kordofan State- Sudan, during the period from October 2012 to October 2015.

Study approach: Qualitative and quantitative approach.

Study type and design: Descriptive cross-sectional study.

Study variables

The variables of this study included various variables related to personal hygiene, surrounding environment, equipment, utensils of milk, presence of license and others as shown in the questionnaire.

Study area

The area of this study was the markets of El Obied City. El- Obeid is the capital of North Kordofan State, its area has been estimated by 81 km^2 and the distance from Khartoum is about 332 mile. There are about 30 markets in El-Obied city which are distributed in all parts of the City. El Obeid is supplied by milk from the surrounding villages by Lorries.

Study population

The populations for this study are vended boiled milk, the handlers of boiled milk and the offering containers of the boiled milk in the markets of El-Obeid city.

Sampling and milk sampling

Sampling

After the survey for all markets in El-Obeid city, it was found that there were only (84) places that handle in the boiled milk. The sale points of boiled milk for this study were chosen by total coverage for the 84 places. The milk handlers who were 87 individuals were chosen by total coverage in each place and the simple random sample was used to select 84 of them for the variables concerned to the relationships, also the boiled milk offering utensils were chosen by total coverage which are 84 utensils, then the milk samples were collected from each offering utensils of the boiled milk.

Milk sampling

The steps for milk sampling were according to the following orders as mentioned by Ministry of Agriculture, Food and Fisheries:[9]

Identifying the sample containers with waterproof markings. Avoiding the contact with the rim or inside of the container with fingers, as this will contaminate the inside of the container and alter the results. Taking the sample only after milk has been properly mixed. Taking the sample from an area free from foam. Filling the container away from the utensil opening. The container could be dropped into the milk. Filling the container two thirds full. Never fill the container completely, leave some space to permit mixing of the sample in the laboratory. Securely close the sample container and put on ice immediately. The samples were transported in cooler boxes with ice to the laboratory and analyzed (immediately).

Data collection

Data were collected by structured questionnaire and observations from all the places and handlers of boiled milk at the markets that handle boiled milk in El-Obeid city, and from the results of the laboratory analysis of collected milk samples. Boiled milk samples were collected in sterilized containers (100 ml) according to Harrigan from the offering utensils at the sale points in the markets of El-Obeid city, and were brought in ice box to the laboratory of veterinary research station in El-Obeid city to laboratory analysis.[5]
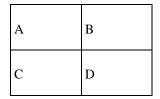
Data analysis

The data of questionnaire and observations were analyzed manually.

The results were presented in figures and tables showing the percentages. The relations between some variables were done according to El-Gassas and Le by McNemar's Chi-square (χ^2) for the correlative percentages in the table (2×2) by the formula:[10,11]

$$\chi^2 = \frac{(B - C)^2}{B + C}$$

Where:

B and C are cells in the table 2×2 (without the cells of the total) as in the following shape:

A	B
C	D

The value of χ^2 obtained from this formula compared with the value of χ^2 obtained from the table of χ^2. The result have statistical significance when the calculated value is larger than the tabulated value under significance level (0.05). The null hypothesis is rejected at the 0.05 level when $\chi^2 \geq 3.84$.

Procedures of aerobic plate count

According to Murshidy and Harigan and Cain et al the procedures was as follows:[5,12]

One ml of milk sample transferred to tube containing 9 ml diluent to obtain dilution of 10^{-1} using separate sterile pipette. After agitation, the previous tube well, then using another sterile pipette, transferred 1 ml to the second tube containing 9 ml diluent to obtain dilution of 10^{-2}. The previous step was repeated to obtain dilutions of 10^{-3}, 10^{-4}. By using sterile pipet, transferred 1 ml from each of the dilutions 10^{-3}, 10^{-4} after agitating to a sterile petri dishes (two dishes for each dilution), then poured 15 ml of plate count agar media (plate count agar allows the growth of more types than does nutrient agar), then the plate was turned like the number (8). The plate was left to solidify, then incubated in upside down position, at temperature 37°C for 48 hour (incubation temperature of 37°C was chosen because it probably being best as indicators of a potential health hazard as mentioned by Harigan.[5] At the end of incubation period according to Spencer and Cain et al select two plates corresponding to one dilution and showing between 30 and 300 colonies per plate (to ensure statistically significant data).[12,13] Count all colonies on each plate, using the colony counter and tally register. Take the average of the two counts and multiply by the dilution factor. Then express the aerobic plate count as number of organisms per ml of milk. When plates from all dilutions have no colonies, the result was expressed as less than 1×10^1 CFU/ml.[14]

Ethical consideration (clearance)

Ethical permission for the study was obtained prior to the beginning of the study, by contacting the environmental health manager of the locality of Shekan, in El-Obied city; as well as from milk handlers.

RESULTS

A total of 84 milk samples were chosen by total coverage. A quality check of the collected milk is mainly performed at the milk collection centers (markets), of 84 samples 76 (90.48%) were contaminated by microbes (Table 1).

Table 1: The microbial growth on petri dishes for the samples of boiled milk vended in El-Obeid markets- 2015.

The microbial growth	Frequency	Percentage
Present	76	90.48
Absent	8	9.52
Total	84	100

Table 2: The aerobic plates counts (APC) of boiled milk vended in El-Obeid markets- 2015.

Aerobic plate counts of microbes per ml of milk samples	Frequency of samples	Percentage
Ranged from 7×10^2 to 1.8×10^7 CFU/ml	54	64.29
Too numerous to count (TNTC)	10	11.90
Too few to count (TFTC)	12	14.29
Less than 1×10^1 CFU/ml (no growth)	8	9.52
Total	84	100

In this current study the aerobic plates counts of the boiled milk samples, where 64.29% of sample was ranged from 7×10^2 to 1.8×107 CFU/ml, 11.90% of sample was too numerous to count, 14.29% of sample was too few to count, and 9.52% of sample was less than 1×101 CFU/ml (Table 2).

Table 3A: The relation between milk boiling methods and the presence of microbial growth on Petri dishes in El-Obeid markets- 2015 (N=84).

Milk boiling methods		Bubbling of milk then moved down without stirring (%)	Stirring milk continually till boils then moved down (%)	Stirring milk after bubbling then moved down (%)	Total (%)
The microbial growth on petri dishes	Present (%)	41.67	17.86	30.95	90.48
	Not present (%)	1.19	7.14	1.19	9.52
	Total (%)	42.86	25	32.14	100

The presence of microbial growth in the case of stirring of milk was 17.86% and in the case of absence of stirring of milk was 72.62 % (Table 3B).

Table 3B: The relation between the presence of stirring /turning of the milk continually till boiling and the presence of microbial growth on Petri dishes for milk samples from El-Obeid markets.

Stirring of the milk		Present %	Absent %	Total %
Presence of microbial growth	Present (%)	17.86	72.62	90.48
	Not present (%)	7.14	2.38	9.52
	Total (%)	25	75	100

N=84. χ^2: calculated=53.8 and tabulated=3.84, Significant level =0.05

The presence of microbial contamination in case of the presence of dirty utensils and equipments was 15% and in the case of clean utensils and equipments was 75% (Table 4).

As illustrated in Table 5, there was 42.86% of milk handlers said they look to milk until it bubble then down it without turning, 25% of them said they turn the milk continually until it boils then down it and 32.14% of them mentioned the turning of the milk after bubbling then down it.

Table 4: The relation between the presence of dirty utensils and equipment, and the presence of microbial contamination for boiled milk samples in El-Obeid markets.

Presence of dirty utensils and equipments		Present (%)	Absent (%)	Total (%)
Microbial contamination (APC)	Present (%)	15	75	90
	Absent (%)	6	4	10
	Total (%)	21	79	100

N=84; χ^2: calculated=58.8 and tabulated=3.84, Significant level=0.05

As shown in Table 6, the methods of cleaning for utensils that is done by the milk handlers are manually with water only (2.3%), manual with water and soap (36,8%), manual with hot water and soap (32.2%), manual with water, soap and Silica (18.4%), other (6.9%), and 3.4% of them were not answered.

Table 5: What done for milk when it was put on the fire in El-Obeid markets.

What done for milk	Frequency	Percentage
Looking to milk until it bubble then down it (without turning/stirring)	36	42.86
Turning/stirring the milk continually until it boils then down it	21	25
Turning/stirring the milk after bubbling then down it	27	32.14
Total	84	100

Table 6: The method of cleaning for utensils that is done by the milk handlers in El-Obeid markets.

Methods	Frequency	Percentage
Manual with water only	2	2.3
Manual with water and soap	32	36.8
Manual with hot water and soap	28	32.2
Manual with water, soap and silica	16	18.4
Other	6	6.9
Not answered	3	3.4
Total	87	100

It was found that in 21% of the milk sale points the equipment and utensils were dirty, and in 79% of them was clean as shown in Table 7.

Table 7: The general cleanliness of equipment and utensils of boiled milk vended at the sale points in El-Obeid markets (N=84).

The general cleanliness	Frequency	Percentage
Clean	66	79
Dirty	18	21
Total	84	100

Table 8: The period of time for milk on the fire in El-Obeid markets.

The period of time	Frequency	Percentage
Ranged from 35 minutes to 2 hour	37	42.5
¼ -½ hour	34	39.1
Unlimited	16	18.4
Total	87	100

Table 8 shows the period of time for milk on the fire, where 42.5% of milk handlers put the milk on the fire for period ranged from 35 minutes to 2 hour, 39.1% of them put the milk on the fire for ¼-½ hour, and 18.4% of them put the milk on the fire for unlimited period of time

DISCUSSION

The present study was performed to evaluate the hygiene and safety of boiled milk marketed in El-Obeid city, north Kordofan state, Sudan, during the period October 2012 to October 2015.

Discussion of the aerobic plate counts (APC)

The total aerobic plate counts were used as indicators to assess milk safety and hygiene in El-Obied markets according to Harigan "in some cases general viable count may be used as indicator of standard of hygiene, or as indicators of a potential health hazard (incubation of 37°C probably being best)".[5] In this study the total aerobic plate counts were used as indicators to evaluate the microbiological quality of milk, and this agrees with Harigan "the total aerobic plate count is useful for indicating the overall microbiological quality of a food and, thus, is useful for indicating potential spoilage in perishable products".[5] Forsythe stated that "microbial indicators are more often employed to assess food safety and hygiene than quality", and according to Health Protection Agency "indicator organisms are useful in the assessment of food product safety because they tend to be present in higher numbers than most pathogens".[6,15] In this study the aerobic plate count was used to assess the microbial quality of boiled milk sold in El-Obied markets, and this according to Andrews "the total aerobic plate count is used to assess the microbial quality of milk, because the total aerobic plate count is useful for indicating the sanitary conditions under which the food was produced and/or processed".[4]

In this study, the samples of boiled milk vended in El-Obeid markets were classified into four categories (Table 2) concerning the aerobic plate counts, these are:

Fifty four samples (64.29%) have aerobic plates counts ranged from (7×102 to 1.8×10^7) CFU. per ml. This was obtained from the plates whose numbers fall within the range (30-300 colonies), in order to be statistically accurate according to Cain et al.[12] The number of microorganisms was high, in spite of the period of time for milk on the fire was long and enough in most cases (ranged from ¼ hour to 2 hours) to kill the microorganisms, as it noted in Table 8. The high counts of the aerobic plate counts in boiled milk samples does not agree with what mentioned by Woldecherkos and Yitayal "Boiling of milk destroys all microorganisms except the spore formers".[7]

Ten samples (11.90%) were found "too numerous to count", because the plates were more than 300 colonies per ml of milk.

Twelve samples (14.29%) were "too few to count", because the plates had fewer than 30 colonies per ml of milk, therefore this was ignored to count, where this not

represent the bacterial count for sample according to El-kholy.[8]

Eight samples (9.52%) had less than 1×10^1 CFU/ml (have no colonies in all dilutions). This classification was according to (FSSAI, 2012). And this was considered the sound samples concerning the microbial growth on aerobic plates. According to above results of aerobic plate counts there was 76.19% of milk samples (64.29% + 11.90% (too numerous to count) as in Table 2 have low microbiological quality. This attributed mainly to inadequate boiling method and several factors leading to contamination that occur after boiling, because boiling of milk destroys all microorganisms except the spore formers as above-mentioned by Woldecherkos and Yitayal.[7] Remember that the spore formers needs to grow for anaerobic condition.

Discussion of the factors that contribute to the low microbiological quality of boiled milk in the markets of El-Obeid city

Inadequate cooking (under heat treatment): whereas 42.86% of milk were seen until bubbling then moved down as in Table 5. This method does not kill or reduce the microbes in all parts of milk because it is not considered enough for heating all parts of milk as mentioned by El-kholy "This method, unfortunately, is not considered enough for heating all parts of milk, whereas what observed of bubbling, completes usually before reaching the milk boiling temperature".[8] As it demonstrated in Table 3A, the results show that the high percentage (41.67%) of microbial growth belongs to samples taken from the bubbling without stirring. The minimum percentage (17.86%) of microbial growth belongs to samples taken from those stirring continually till boils. Those results confirm that the continual stirring of the milk until it boils reduces the microbial existence on milk when it was put on the fire. As it demonstrated in Table 3B, this results were have statistical significance, because the calculated value of χ^2 was larger than the tabulated value (53.8>3.84) under the significance level 0.05. Note that the Table 3B is summarization of Table 3A for two categories (presence and absence) of milk stirring continually till boils, where the number 72.62 in Table 3B resultant of bubbling milk without stirring + stirring milk after bubbling in Table 3A.

Also in this study (Table 6) there is only 32.2% of the milk handlers were use the hot water in washing the utensils. The lack of using the hot water to sterilizing the utensils by most milk handlers is consider another factor for the presence of microbes in boiled milk, because the hot water kills a large number of the microbes. This agree with what mentioned by Vishweshwar and Krishnaiah about the hot water "it is one of the most effective germicidal agents as it can contact all clean surfaces of the equipment. It is used in sufficient quantities and it kills a large percentage of the bacteria".[16]

In addition to the presence of the dirty utensils as in Table 7 which showed that (21%) of utensils of milk at the sale points were dirty. The dirty utensils are consider one of the factors that contribute to the low microbiological quality of boiled milk in the markets of El-Obeid city. This confirms what was mentioned by Adams, and Motarjemi "the equipment and utensils used in the preparation of food can also act as sources of contamination".[7] And according to Table 4 it was noted that the presence of dirty utensils and equipment contributes to the microbial contamination for boiled milk by 15%, and 75% referred to other factors. This results were have statistical significance, because the calculated value of χ^2 was larger than the tabulated value (58.8>3.84) under the significance level 0.05.

Limitation of the study was that sample size not cover the surrounding villages supplied El-Obeid city by milk. Some references used in this study were old due to difficulties to find new editions. The instruments and equipment used in the laboratory were not advanced. The study was limited in the microbial indicator (aerobic plates count for microbes) to evaluate the extent of boiled milk hygiene and safety, there for the bacteriological isolation for the types of organisms in milk vended at the markets of El-Obeid city is necessary. There is a little number of similar study used in this study.

CONCLUSION

This study concluded that the boiled milk was handled under unhygienic conditions and there is no assurance to the safety and wholesomeness of the boiled milk offered in the markets of El-Obeid city. This evaluation based mainly on the presence of low microbiological quality for most milk samples where (64%) of milk samples have (APC) ranged from 7×10^2 to 1.8×10^7 CFU per ml of sample. This study detected an existence for the milk safety risk factors leading to food borne illness, which include; Inadequate cooking or inadequate heat treatment for milk (the bubbling of milk without turning) which was 42.86% and this factor contributed to the presence of contamination of boiled milk in 41.67%. Contaminated equipment and utensils, which was 21%, and this factor contributed to the presence of contamination of boiled milk in 15%.

ACKNOWLEDGEMENTS

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