



RESEARCH ARTICLE

DETECTION OF INDICATORS ORGANISMS IN BOILED MILK VENDED IN THE MARKETS OF EL-OBIED CITY-NORTH KORDOFAN STATE - SUDAN

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Abstract

Background: This study was conducted within the markets of Elobeid City- North Kordofan State-Sudan, to detect the presence of indicator organisms (coliform and E.coli bacteria) in boiled milk, during the amount from October 2012 to October 2015.

Methodology: The study covered all the places for the boiled milk sale found within the markets of Elobeid City, which are 84 places. Data were collected from all milk handlers who were 87 by observation-checklist. The info were analyzed manually and therefore the results presented in tables and figures showing for the odds. 84 samples of boiled milk were collected from the offering containers of boiled milk, and examined for the presence of coliform bacteria.

Results: The coliforms bacteria contaminated most boiled milk samples (77.38%) and 43% of positive milk samples for coliforms was found contaminated by E.coli bacteria. The factors that contributed to the microbial contamination for boiled milk by coliform bacteria are lack of the health licenses for the milk handling (57.14%), and lack of milk covering (47.6%).

Conclusion: This study concluded that the milk was marketed under unhygienic conditions and there's no assurance to its safety and wholesomeness. This study recommend the health authorities to require immediate measures for correction of the health violations of milk safety requirements, additionally to the health education for milk handlers about the diseases that are transmitted by contaminated milk, and training about hygienic handling of milk before licensing.

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Introduction:-

Food hygiene: Means all measures necessary to make sure the security and therefore the wholesomeness of food during preparation, processing, manufacture, transportation, storage, distribution, handling, and offering purchasable or supply to the buyer (1,2). Food Safety: Assurance that food won't cause harm to the buyer when it's prepared and/or consumed consistent with its intended use (2). The key sources of milk contamination are; faecal contamination from soiled animals, especially teats, udders and tails, bacterial contamination from soiled hands,

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physical contamination, especially from, dust, bedding materials, dung, insects and animal hair, the equipment and utensils utilized in the preparation of food [milk] also can act as sources of contamination, and flies and other insects can transfer organisms from sources contaminated with pathogens to foods [milk] respect as they're related to both food handling areas and contaminated areas like toilets and refuse heaps (3). Pests pose a serious threat to the security and suitability of food. Pest infestations can arise wherein there are breeding place and a availability of food. Good hygiene practices must be used to keep away from developing surroundings conducive to pests. Good sanitation, inspection of incoming materials and good monitoring can minimize the likelihood of infestation and thereby limit the necessity for pesticides (4). Food handlers may introduce biological hazards when affected by specified diseases, from organisms on the food handlers' skin or in their intestine and faeces, when tract organisms contaminate foods or food contact surfaces and by cross-contamination after handling raw materials, physical hazards can be introduced by food handlers wearing jewelry, bandages or by careless food-handling practices (5). Milk borne diseases a spread of microorganisms may gain access in to exploit and milk products from different sources and causes differing types of food borne illnesses. Milk and milk products may carry organisms intrinsically or their toxic metabolites (poisons) called toxins to sensitize consumers. Ingestion of poisons already synthesized within the food (pre-formed) brings about poisoning syndrome and called 'food intoxication' and therefore the toxins affecting alimentary canal are called enterotoxins. On the opposite hand, the ingestion of viable pathogenic bacteria alongside the food results in their lodgments and are termed as 'Food infections', the opposite organisms infect intestines when ingested alongside food and produce toxins in place to cause symptoms of poisoning and called Toxic-infection (6). As reported by Park (7), milk borne diseases includes; Tuberculosis, Brucellosis, Streptococcal infections, Q fever, enterotoxin poisoning, Salmonellosis, Cow box, Food and mouth diseases, Anthrax, Leptospirosis, Tick-borne encephalitis, Typhoid and paratyphoid fevers, Shigellosis, Cholera, Enteropathogenic Escherichi coli, Non-diarrheal diseases (Streptococcal infections, Staphylococcal gastrointestinal disorder, diphtheria, tuberculosis, enteroviruses and viral hepatitis). WHO records imply that handiest a bit quantity of matters related to meals dealing with are responsible for an oversized percentage of foodborne disorder episodes everywhere. Common errors include; preparation of food several hours before consumption, combined with its storage at temperatures which favour the expansion of pathogenic bacteria or the formation of toxins, inadequate cooking or reheating of meals to cut back or put off pathogens, cross- contamination and those with improper personal hygiene coping with the food (3). Indicator organisms: The term 'indicator organisms' are often applied to any taxonomic, physiological or ecological group of organisms whose presence or absence provides circumstantial evidence concerning a specific feature within the past history of the sample. it's often related to organisms of intestinal origin. for instance, the presence of members of 'all Gram-negative bacteria' in heat treated foodstuffs is indicative of inadequate heat treatment (relative to the initial numbers of those organisms) or of contamination after heating. Microbial indicators are greater frequently used to investigate food hygiene than quality (2). Hygiene Indicator Organisms refers to the chosen surrogate markers. The most objective of using bacteria as indicators is to reflect the hygienic quality of food (8). Indicator organisms are bacteria that are wont to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods (9) The presence of indicator bacteria in ready-to-eat food, will 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 could also be related to an increased likelihood of the presence of pathogens. Indicator organisms are useful within the assessment of foodstuff safety because they have a tendency to be present in higher numbers than most pathogens and are relatively quick and straightforward to spot (10).The common indicators are Coliforms and E.coli (2). The coliform group includes Escherichia, Klebsiella, Enterobacter and Citrobacter, and includes Escherichia coli (2). The existence of the Coliforms has been considered as resulting in the very fact that the merchandise was subject to process under inefficient hygienic conditions (12). E. coli is an organism that's a part of the traditional microflora of the intestinal tract of humans and warm-blooded animals. As such, their presence in ready-to-eat foods (fully cooked) are often a sign of poor hygiene and sanitation or inadequate heat treatment (13). As mentioned by Centre for Food Safety (8), E. coli may be a commonly used faecal indicator organism. The presence E.coli in food generally indicates direct or indirect faecal contamination. Substantial number of E. coli in food indicates lack of cleanliness in managing and incorrect storage (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 presence of indicator organisms (coliform and E.coli bacteria) in boiled milk and to determine the factors that contribute to boiled milk contamination.

Materials & methods:-**Study approach:**

Qualitative and quantitative approach.

Study type and design:

Descriptive cross-sectional study.

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 km² 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 in the markets of El-Obied City.

Sampling and milk sampling:**Sampling:**

After the survey for all markets in Elobeid 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 (14):

1. Identifying the sample containers with waterproof markings.
2. 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.
3. Taking the sample only after milk has been properly mixed.
4. Taking the sample from an area free from foam.
5. Filling the container away from the utensil opening. The container could be dropped into the milk.
6. Filling the container two thirds full. Never fill the container completely, leave some space to permit mixing of the sample in the laboratory.
7. Securely close the sample container and put on ice immediately.
8. The samples were transported in cooler boxes with ice to the laboratory and analyzed [immediately].

Data collection:

Data were collected by structured questionnaire observations from all the handlers of boiled milk at the markets that handle boiled milk in El-Obied 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 (11) from the offering utensils at the sale points in the markets of El-Obied City, and were brought in ice box to the laboratory of veterinary research station in Elobeid City to Laboratoryanalysis.

Data analysis:

The data of questionnaire was analyzed manually.

The results were presented in tables showing the percentages. The relations between variables were done according to El-Gassas (15) and Le (16) by McNemar's Chi- square (X^2) for the correlative percentages in the table (2x2) by the formula:

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

Where:

B and **C** are cells in the table 2x2 (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 (appendix B). 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 $\chi^2 \geq 3.84$.

Detection and enumeration of total coliforms:

Multiple tube system was used for samples examination, which is described as following:

1. Ten fold serial dilutions of samples were made up to 10^{-4} in sterile distilled water.
2. 1 ml were added to each of the three tubes containing 10 ml of MacConkey broth culture medium with a Durham's tube.
3. Put all tubes and control tube in incubator at 37°C for 24 - 48 h.
4. Positive tubes was registered after 48 h.
5. Presumptive positive results (acid and gas produced) were confirmed by streaking across MacConkey's agar plates.
6. Numbers of bacteria was looked out from statistically calculated, MPN prepared table used for Most Probable Number (11).

Most Probable Number (MPN):

For calculating the MPN, used the dilution factor of the middle set of the three dilutions selected according to the table of MPN and the following rule as mentioned by El-kholy (17):

Most Probable Number (MPN) for each ml of milk:

Rules of selecting the three dilutions for table reference:

Select the highest dilution (last dilution, i.e. dilution with the smallest quantity of product) that gives positive results in all tubes and the next two higher dilutions (17), Oblinger and koburger, (18)

In the case of no any dilution contain 3 positive tubes, select the tubes of highest 3 dilutions that contain positive result (17).

If there were not enough higher dilutions behind the 3 positive tubes select the last 3 dilutions used in the test (17).

If there are positive results in higher unselected dilutions, add those higher-dilution positive results to the results for the highest selected dilution (18, 19).

If only one volume set gives a positive reaction, refer to this set and the one higher and the one lower (20).

Detection of E. coli:

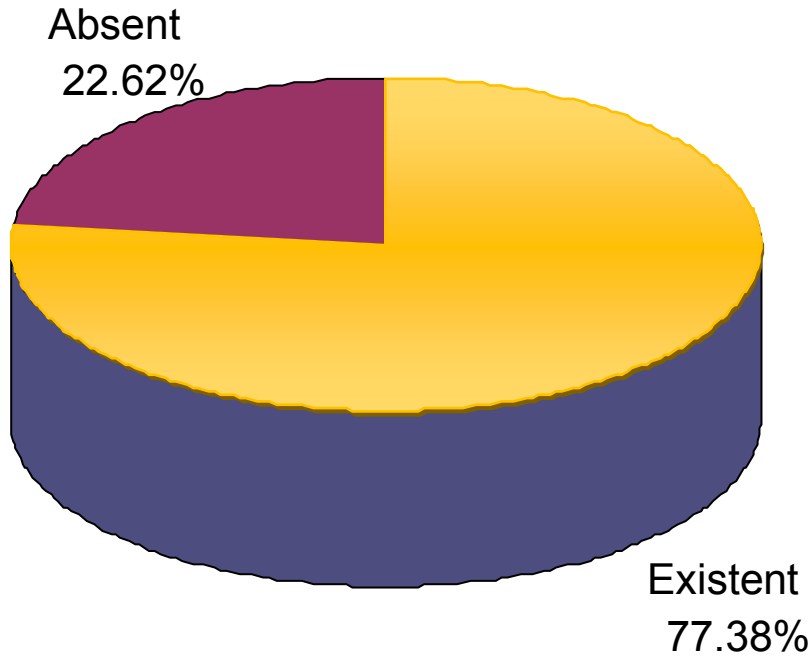
Each positive tube of above macconkey broth (for coliforms) was inoculated into tow tubes, one containing peptone water and the other containing macconkey broth and incubated at 44°C for 24, 48 h. Positive tubes appear acid and gas on MacConkey broth and indole on peptone water.

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.

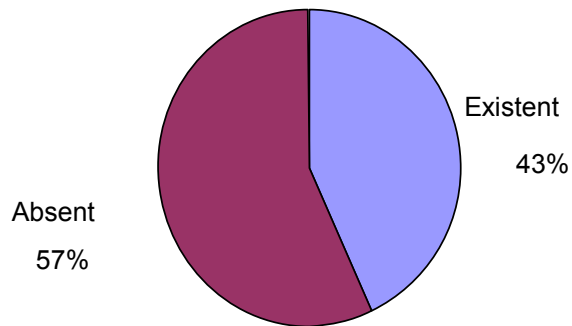
Results:-

The existence of coliforms bacteria in boiled milk was found in 77.38% (Fig. 1), the existence of *E.coli* bacteria in positive samples of coliforms was found in 43% (Fig. 2), the existence of the health licence was found in 27.38% of the places of milk handling (Fig. 3) and 58.3% of boiled milk found not covered (Fig.4).



N=84

Fig. (1):-The existence of coliforms bacteria in boiled milk vended in Elobeid markets – 2015



n = 65

Fig. (2):-The existence of *E.coli* bacteria in positive samples of coliforms in boiled milk vended in Elobeid markets - 2015

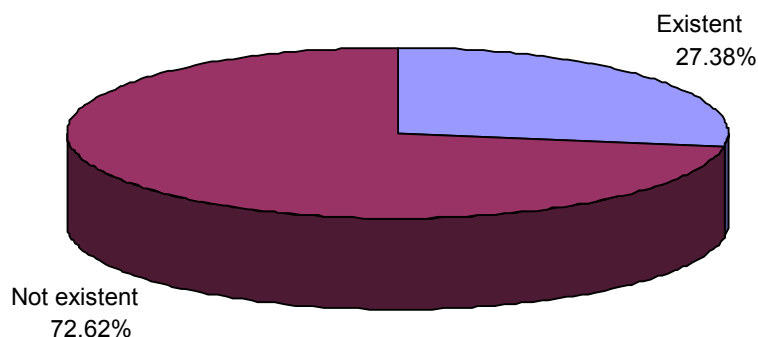


Fig. (3):- The existence of the health licence in the places of milk handling in Elobeid markets – 2015.

Table (1):-The relation between the presence of health licenses and the presence of coliforms bacteria in the samples of boiled milk vended in Elobeid markets – 2015

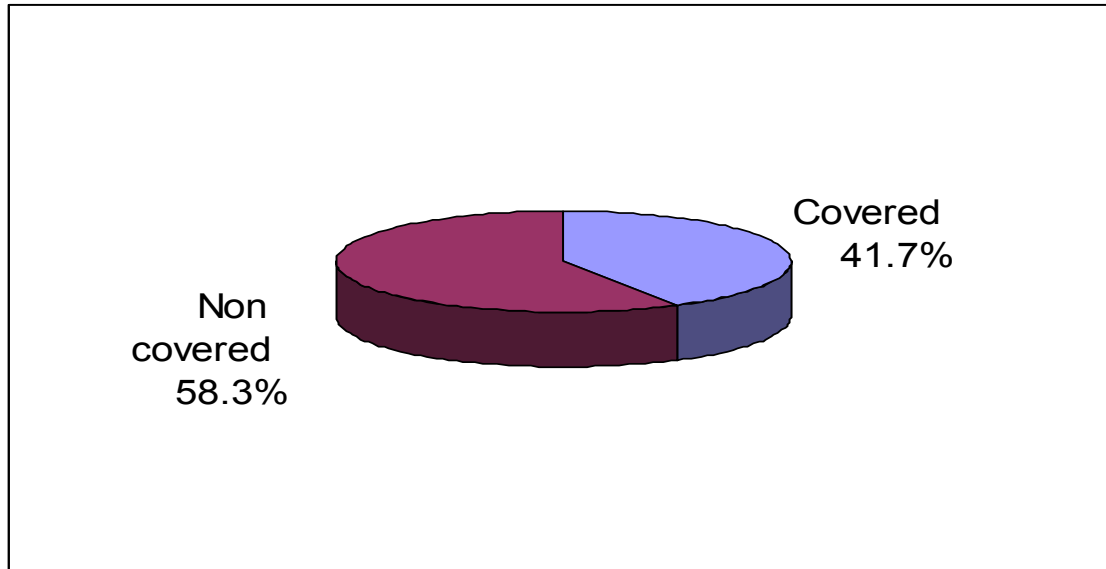
Presence of coliforms bacteria Health License	Present(%)	Absent(%)	Total
	Existent	20.24	7.14
Not existent	57.14	15.48	72.62
Total	77.38	22.62	100%

$N = 84$, X^2 : calculated = **38.89** and tabulated = **3.84**, Significant level = **0.05**.

The presence of coliforms bacteria in milk samples in the cases of the existent of health license was found in **20.24%** of milk samples, and the presence of coliforms bacteria in the case of the absence of health license was found in **57.14%** of milk samples (table 1).

Table (2):- The relation between the milk covering and the presence of coliforms bacteria in the samples of boiled milk vended in Elobeid markets – 2015.

Coliforms bacteria milk covering	Present (%)	Absent (%)	Total (%)
	Covered(%)	29.8	11.9
Not covered(%)	47.6	10.7	58.3
Total(%)	77.4	22.6	100



N=84

Fig. (4):- The covering of boiled milk when the samples collected from the places at the sale points in Elobeid markets – 2015

N = 84,

X^2 : calculated = 21.42 and tabulated = 3.84, Significant level = 0.05 .

The presence of coliforms bacteria in milk samples in the cases of **milk covering** was found in 29.8% of milk samples and the presence of coliforms bacteria in the case of the lack of milk covering was found in 47.6% of milk samples (table 1).

Discussion:-

The existence of coliforms bacteria in milk samples is used as indicator to evaluate the milk hygiene and safety in Elobeid markets, and this according to what mentioned Ekici, Bozkurt and Isleyici (13): "The Coliform organisms are good indicators of the standard of hygiene and handling". In addition, it is used to assess the milk safety according to Health Protection Agency (11): "Indicator organisms are useful in the assessment of food product safety because they tend to be present in higher numbers than most pathogens".

As shown in **Fig. (1)**, the existence of coliforms bacteria in boiled milk vended in El-Obeid markets was found in (77.38%) of samples. This considers as indicator for contamination after heat treatment, or as indicator to under heat treatment for milk. This agrees with Forsythe (2): "the presence of members of 'all Gram-negative bacteria' in heat treated foodstuffs is indicative of inadequate heat treatment (relative to the initial numbers of these organisms) or of contamination subsequent to heating". Lack of covering the boiled milk, is considered one of the factors that contribute to the existence of indicator bacteria (coliforms) in boiled milk, whereas (58%) of milk observed none covered as in **Fig. (4)**. The results of table (2) indicate that the high percentage (47.6%) for the presence of coliforms bacteria belongs to the lack of milk covering, and the minimum percentage (29.8%) for the presence of coliforms bacteria belongs to the milk covering. Those results confirm that the lack of milk covering increases the presence of coliforms on milk to 47.6%. This results were have statistical significance, because the calculated value of X^2 was larger than the tabulated value ($21.42 > 3.84$) under the significance level 0.05.

As presented in Fig. (3), the health licences in the places of milk handling was found in (27.38%) of places. It is noted that there was (72.62%) of milk sale points in Elobeid markets that have no health licences to work in milk handling. This consider disagreement for the legislation about food handling, and this indicates the lack of the safety and wholesomeness of the offered milk, because the health licences did not gave to any one except after ensuring the health requirements. The results of table (1) indicate that the high percentage (57.14%) for the presence of coliforms bacteria in milk samples belongs to the lack of health licenses , and the minimum percentage (20.24%)

for the presence of coliforms bacteria belongs to the existence of the health licenses. Those results confirm that the lack of the health license for milk handling contributes the presence of coliforms on milk in 57.14%. This results were have statistical significance, because the calculated value of X^2 was larger than the tabulated value (38.39 > 3.84) under the significance level 0.05.

Conclusion:-

This study concluded that the boiled milk in the markets of Elobeid City was handled under unhygienic conditions and there is no assurance to its safety and wholesomeness. This evaluation based mainly on the following findings: The existence of coliforms bacteria in 77.38% of milk samples and the existence of *E.coli* bacteria in 43% of milk samples (positive for coliform). This study also concluded that the factors contributed to the contamination of boiled milk by coliform bacteria are lack of the health licenses for the milk handling (57.14%), and lack of milk covering (47.6%).

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