



DETECTION OF THE PRESENCE OF ANAEROBIC SPORE FORMING BACTERIA (ASFB) 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 in the markets of Elobeid City- North Kordofan State-Sudan, to detect the presence of anaerobic spore forming bacteria in boiled milk and to determine the factors that contribute to the presence of anaerobic spore forming bacteria in the milk during the period from October 2012 to October 2015. **Methodology:** The study covered all the places for the boiled milk sale found in the markets of Elobeid City which are 84 places. Data were collected from all milk handlers who were 87 by observation- check list.

The data were analyzed manually and the results presented in tables and figures showed for the percentages. Eighty four milk samples were collected from the offering containers of boiled milk vended in that places, and examined for the presence of anaerobic spore forming bacteria in boiled milk.

Results: The anaerobic spore forming bacteria were found in 30% of the boiled milk samples. The study detected that the holding of the boiled milk for extended periods of time at room temperatures contributed to the contamination of boiled milk by anaerobic spore forming bacteria by 24%.

Conclusion: This study concluded that the milk in the markets of Elobeid City was handled under unhygienic conditions and there is no assurance to its safety and wholesomeness. This study recommend the health authorities to take immediate measures for correction of violations of milk safety requirements, periodical surveillance, in addition to health education and training of milk handlers about hygienic handling of milk prior to licensing.



INTRODUCTION

Milk is one of the foodstuffs that need good handling to ensure the safety and the wholesomeness. Poor handling and undesirable practices introduce the bacteria or germs that cause the spoilage. Unhygienic handling can also affect human health (1). Food handlers should therefore receive suitable training in the basic principles of food safety. Examples of outline curricula for training courses for food handlers personnel are types of foodborne illness, Food preparation – cooking and serving, Survival of microorganisms because of insufficient heating, Procedures for preventing foodborne illness, Killing of bacteria, parasites and viruses (2). Training of food handlers regarding the hazards confronting their products, safe handling and preparation of food and good hygienic practice, as practicable under local street-vending conditions, is an essential part of any strategy to improve the safety and quality of street-vended food. This should, ideally, be done in conjunction with licensing, but ongoing education and training sessions at intervals are strongly suggested (3). 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, ...etc. Immediate correction is required whenever a risk factor violation occurs at a commercial establishment (4).

Clostridium perfringens

Clostridium perfringens are one of anaerobic bacteria which causes food poisoning and this cause severe stomach cramps and diarrhea. It is usually associated with food that has been allowed to stay warm for several hours. *Cl. perfringens* turns into another form called a spore. The spores of *C. perfringens* are very heat resistant and will withstand boiling for several hours (5). *Clostridium perfringens* is found in the gut and thus indicates faecal contamination although spores commonly occur in the environment. It is uncommon to detect this organism in properly handled ready-to-eat foods. Illness is caused by the ingestion of large numbers of viable vegetative bacteria, which sporulate in the lower small intestine and produces enterotoxin which causes diarrhoea. This enterotoxin is not produced in foods. Spores are common in the environment and may survive the cooking process such that low level contamination of the final product may occasionally occur. Control is achieved by preventing spore germination and growth in food and rapid cooling, adequate cold storage and adequate reheating of food are of paramount

importance. *Cl. perfringens* will grow between 15°C and 52°C with virtually no growth below 12°C. Not all *Cl. perfringens* produce enterotoxin and these non-toxicogenic isolates (irrespective of the numbers of bacteria present) will not produce foodborne disease. However, the presence of high numbers of non-toxicogenic *Cl. perfringens* in a ready-to-food is unsatisfactory and indicates poor processing, particularly during cooling (6). *C. perfringens* can be isolated from a variety of environments and foods. As the spores of *Cl. perfringens* can survive cooking, it is of most concern in cooked foods that have been temperature abused as this provides the opportunity for spores to germinate and grow. Potential temperature abuse scenarios include: Holding cooked foods at warm temperatures for extended periods of time, and cooling foods too slowly (7).

Source of *Cl. perfringens*:

According to Park (8), source of *Cl. perfringens*: the organism has been found in the faeces of the humans and animals, and in soil, water and air. The usual story (of outbreaks) is that food has been prepared and cooked 24 hours or more before consumption, and allowed to cool slowly at room temperature and then heated immediately prior to serving. Prevention consists either by cooking food just prior to its consumption or, if it has to be stored, by rapid and adequate cooling.

The existence of anaerobic spore forming bacteria like (*clostridium perfringens*) in milk considers as indicator and guide upon its contamination by feces, and it is distributed in the earth and water beside its existence in the human and animal intestine. As that the existence of these microbes in milk forms a seriousness upon the public health for consumer, and the detection of these microbes can be by stormy fermentation test (9). According to Harrigan (10): "This method can be used to demonstrate the presence of *clostridium perfringens* in milk by the 'stormy-clot' reaction".

According to Forsythe (11) the food safety defined as: "assurance that food will not cause harm to the consumer when it is prepared and/or consumed according to its intended use".

Objectives of the study:

The general objective: To evaluate the safety of the boiled milk vended in the markets of El-Obied City.

The specific objectives: To detect the presence of anaerobic spore forming bacteria (ASFB) in boiled milk, and to determine the factors that contribute to the presence of anaerobic spore forming bacteria in the milk.



Materials & Methods

Study approach: Qualitative and quantitative approach.

Study type and design: Descriptive cross-sectional study.

Study variables: The variables of this study are holding of the boiled milk at room temperatures , observations from all the handlers of boiled milk at the slowly cooling of milk at room temperature and the markets that handle boiled milk in El-Obied City, and from covering of boiled milk.

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 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 were chosen by total coverage in each place who were 87 individuals, 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 were 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 (12):

- 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 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

containers (100 ml) according to Harrigan (10) from 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 Laboratory analysis.

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 (13) and Le (14) by McNemar's Chi- square (X²) 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 x² obtained from this formula compared with the value of x² obtained from the table of x² . 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 X² ≥ 3.84.

Methods of detection of anaerobic spore forming bacteria in milk samples

According to El-kholy (9) the method is:

- In sterile test tube with stopper,10 ml of well mixed milk sample was put.
- Put in the tube on the surface of the milk 1-2 cm of melted paraffin wax. Or sterile melted vaseline according to Harrigan (10).
- The tube was put in water bath at 80° C for 30 minutes to kill the vegetative microorganisms.
- Then the tube was put in the incubator at 37° C for 3-5 days.

In the case of formation of the clot and gas production in the tube, the test was considered positive for



anaerobic spore forming bacteria (See picture of stormy fermentation test in the section of the results at page 5).

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.

THE RESULTS

Table 1: The presence of anaerobic spore forming bacteria in boiled milk vended in Elobeid markets - 2015

The presence of anaerobic spore forming bacteria	Frequency	Percentage
Present	25	30
Not present	59	70
Total	84	100

In above table the **presence of anaerobic spore forming bacteria in boiled milk was found in 30%**.

Picture of stormy fermentation test

For the detection of anaerobic spore forming bacteria (ASFB) in the milk samples

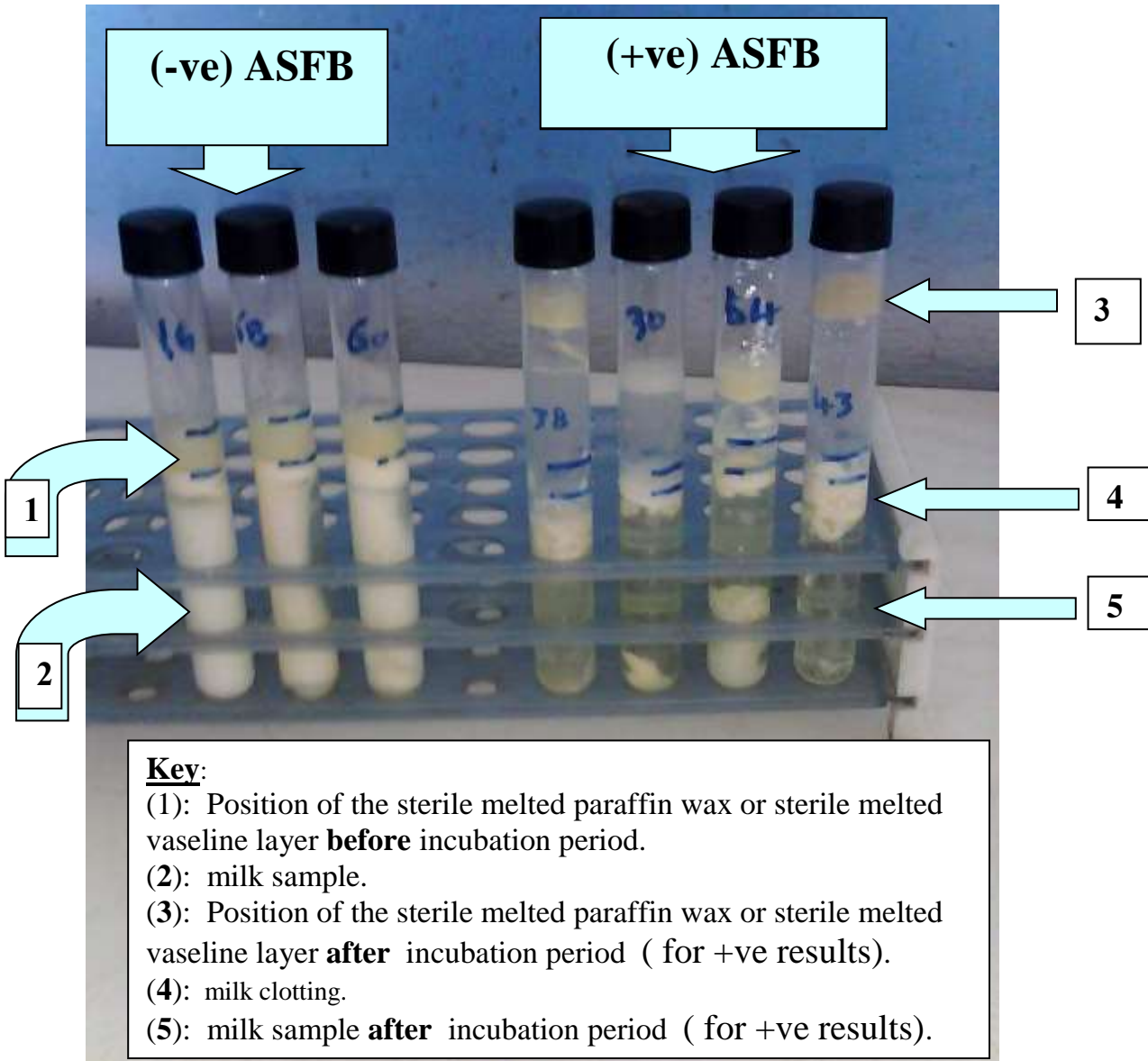


Table 2: Holding of the boiled milk at room temperatures for extended periods of time in Elobeid markets - 2015

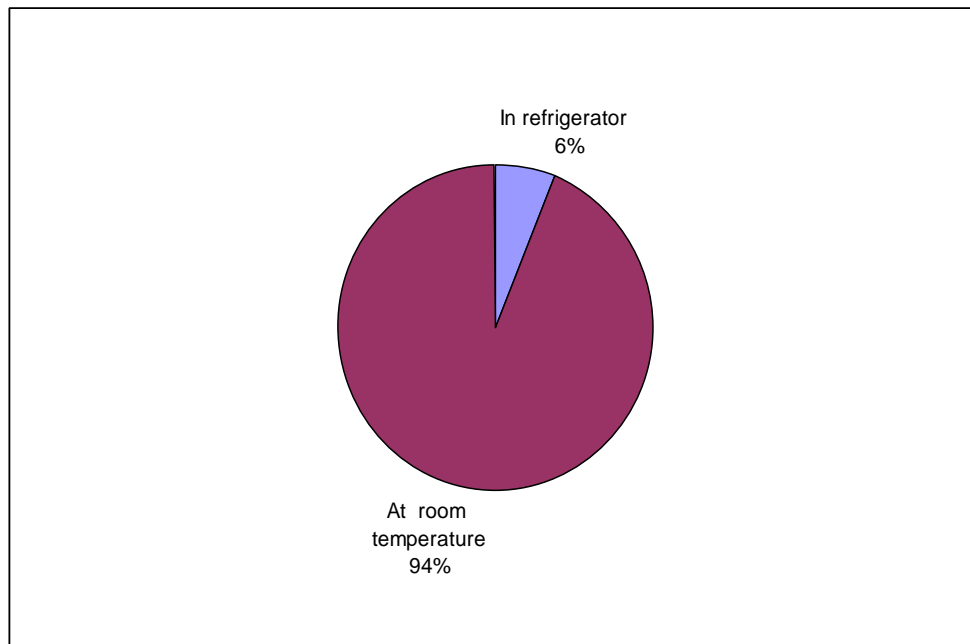
Holding of the boiled milk at room temperatures	Frequency	Percentage
Hold	56	67
Not hold	28	33
	84	100

As shown in table (2) the holding of the boiled milk at room temperatures for extended periods of time was found in 67% of milk places and not found in 33%.

Table 3 : What done for milk after completing the boiling or bubbling in Elobeid markets - 2015

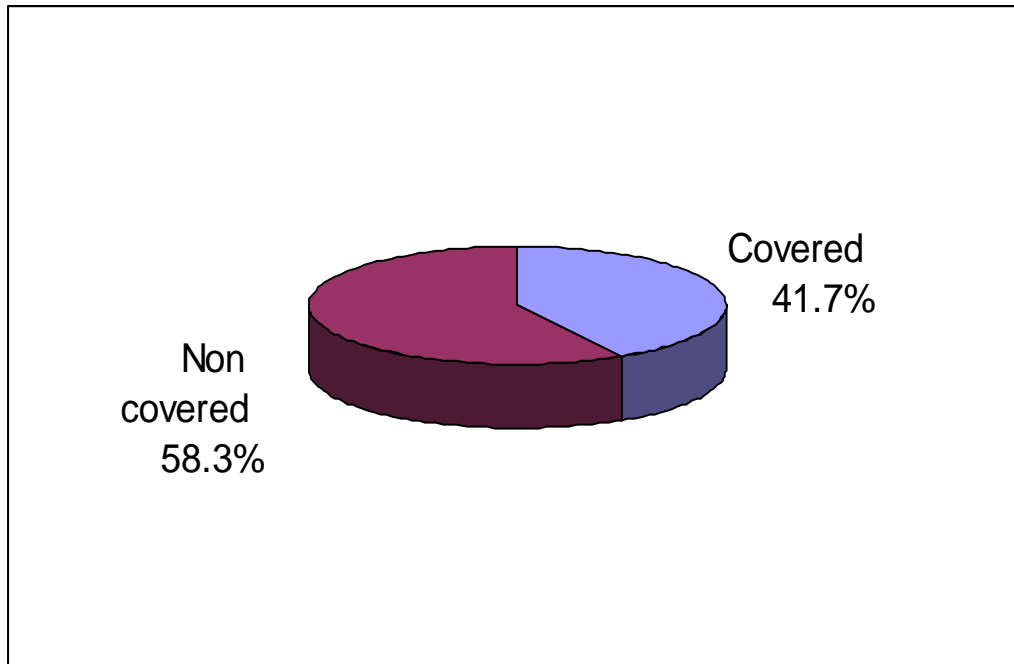
What done to milk	Frequency	Percentage
Immediate and quick cooling on ice water	6	7.14%
Slowly cooling at room temperature (on the land, table or bunsen)	78	92.86%
Total	84	100%

In above table (3) the immediate and quick cooling of milk on ice water was only (7.14 %) and slowly cooling on the land, table or bunsen at room temperature was (92.86 %).



N=84

Fig. (1): The keeping of boiled milk at the sale points in Elobeid markets - 2015



N=84

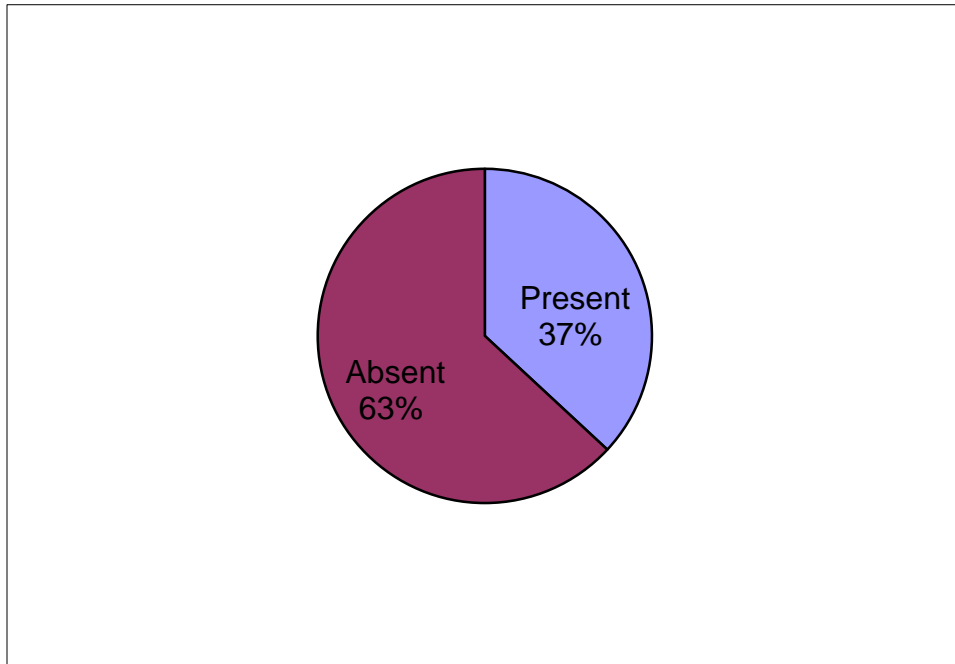
Fig. (2): The covering of boiled milk when the samples collected from the places at the sale points in Elobeid markets - 2015.

Table (4): Making (leave) the milk open after it down from the fire by milk handlers in Elobeid markets - 2015

Making the milk open	Frequency	Percentage
Yes	84	96.6%
No	1	1.1%
Not answered	2	2.3%
Total	87	100%

It was found that in 96.6% of milk handlers, the milk was leaved open after it down from the fire, at 1.1%

of them it was covered, and 2.3% of them not answered as presented in above table (4).



N=84

Fig. 3: The presence of the dust around the milk sale points in Elobeid markets – 2015

As it shown in above Fig. (3). the presence of dust around the milk sale points, was found in 37% of milk sale points.

Table (5): The relation between holding of the boiled milk at room temperatures for extended periods of time and the presence of anaerobic spore forming bacteria (ASFB) in the samples of milk vended in Elobeid markets – 2015

Presence of ASFB Holding of the milk	Presence of ASFB		Total (%)
	Present (%)	Absent (%)	
Holding(%)	24	43	67
Not holding(%)	6	27	33
Total(%)	30	70	%100

N = 84 χ^2 : calculated = 27.9 and tabulated = 3.84 Significant level = 0.05



DISCUSSION

As illustrated in table (1) the presence or existence of anaerobic spore forming bacteria in the samples of boiled milk was 30% , and this existence was obtained by stormy fermentation test, which is used to demonstrate the existence of *Clostridium perfringens* in milk, as mentioned by Harrigan (10) "This method can be used to demonstrate the presence of *clostridium perfringens* in milk by the 'stormy-clot' reaction".

The existence of anaerobic spore forming bacteria (*Clostridium perfringens*) in milk samples is considered an indicator of its contamination by feces and an indicator for seriousness the consumption for that milk. This agrees El-kholy (9) "The existence of anaerobic spore forming bacteria like *Clostridium perfringens* in milk considers as indicator and guide over its contamination by feces, and ... as that the existence of these microbes in milk forms seriousness over the public health for consumer and the detection of these microbes can be by stormy fermentation test".

Also the positive samples of boiled milk were considered containing *Cl. perfringens* organisms according to what was mentioned by Ramanathan (5): "*Cl. perfringens* turns into another in most milk form called a spore, it is usually associated with food that has been allowed to stay warm for several hours", because it was found in most milk places (67%) the boiled milk was **hold** at room temperatures for extended periods of time, as shown in table (2), and most milk handlers (92.86%) let the milk to **cool slowly** at room temperature (on the land, table or bunsen) after completing the boiling or bubbling as shown in table (3).

The factors that contribute to the presence of anaerobic spore forming bacteria in the milk are:

- Holding of milk handlers for the boiled milk at room temperatures for extended periods of time, and slowly cooling of milk at room temperature as illustrated in tables (2 , 3). This conforms with what was mentioned by NSW Food Authority (7): " as the spores of *Cl. perfringens* can survive cooking, it is of most concern in cooked foods that have been temperature abused as this provides the opportunity for spores to germinate and grow. Potential temperature abuse scenarios include: holding cooked foods at warm temperatures for extended periods of time, and cooling foods too slowly".

Holding of the boiled milk at room temperatures by milk handlers is considered one of the factors that lead to food borne illness as mentioned by CDC (4): " 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 , ... etc "

The keeping of boiled milk at room temperature was found in (94%) of sales points (Fig.1) and this provides favorite temperature for *Cl. perfringens* to grow as mentioned NSW Food Authority (7): "*Cl. perfringens* will grow between 15°C and 52°C with virtually no growth below 12°C."

The presence of dust around the milk sale points was found in 37% (Fig. 3) , and making the milk open after it down from the fire was 96.6% (table 4), this considers another factors leading to milk contamination by anaerobic spore forming bacteria like *Cl. perfringens* because it found in the air as mentioned by Park (8): " the organism has been found in the ... **soil**, ... and **air** ".

The results of table (5) indicate that the high percentage (24%) for the presence of anaerobic spore forming bacteria (ASFB) belongs to holding of the boiled milk at room temperatures for extended periods of time. Those results confirm that the holding of the boiled milk at room temperatures for extended periods of time increases the presence of anaerobic spore forming bacteria on milk to 24%. This result was statistically significant, because the calculated value of X^2 was larger than the tabulated value (27.9 > 3.84) under the significance level 0.05.

CONCLUSION

- The presence of anaerobic spore forming bacteria was found in 30% of milk samples.
- The factors that contribute to the presence of anaerobic spore forming bacteria in the milk are: Slowly cooling of milk at room temperature (92.86%).
- Also the holding of the boiled milk for extended periods of time at improper temperatures (at room temperatures) contributed to the presence of anaerobic spore forming bacteria in the boiled milk in 24%.
- The study recommends that health authority of Shekan locality is strongly recommended by doing health education and necessary training to the milk handlers about milk hygiene and safety (or hygienic handling) prior authorizing.

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