

**A STUDY ON BACTERIAL CONTAMINANTS OF RAW
MILK IN SMALL DAIRY PRODUCING UNITS IN
OMDURMAN, KHARTOUM STATE**

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*In the name of Allah, the
compassionate and the Merciful*

PREFACE

This research was carried out at the Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum., under the supervision of Dr. Ahmed Zaki Saad and Co-supervision of Dr. Ibtam Elyas Elzubeir, Faculty of Animal Production.

Dedication

To my Fiancée,

Father,

Mother,

Brothers,

Lovely sister,

And other members of my family.

To all those who are on the line,

with best wishes and love.

Abstract

Sixty samples were collected from small dairy producing units in Omdurman, Khartoum state to study the bacteriological quality of milk.

Collected samples included thirty-six milk samples (30 from lactating cows, and 6 from bulk tanks), six swabs from milker's hands, six swabs from milk utensils, six samples from water which used in the farms, and six samples from the environment of the units.

All milk samples were investigated by total plate count and milk ring test. Results revealed that milk produced in these units was of good quality according to tropical standards, although 47% of samples were positive to milk ring test.

Many bacterial contaminants were isolated from different samples. *Bacillus cereus* was the most common (25% of the isolates). The environment of these producing units was the most probable source of this bacterium.

ملخص الأطروحة

تم جمع 60 عينة من وحدات إنتاج ألبان صغيرة في مدينة أمدرمان بغرض تحديد جودة اللبن بتقدير أعداد البكتيريا الحية فية ومعرفة مختلف أنواع البكتيريا الملوثة ومصادر التلوث وقد اشتملت هذه العينات على 36 عينة لبن (30 من الأبقار و 6 من وعاء التجميع)، 6 مسحات من اوانى الحليب، 6 مسحات من أيدي الحلابين بالإضافة إلى 6 عينات من مياة الشرب المستخدمة في هذه الوحدات و 6 عينات من البيئة في هذه المزارع.

أوضحت الدراسة أن اللبن المنتج في هذه الوحدات الصغيرة على الجودة مقارنة بمقاييس الجودة في المناطق المدارية بالرغم من وجود بعض أنواع الملوثات البكتيرية وقد تم إجراء اختبار حلقة اللبن للكشف عن البروسيلا و وجد أن 47% من العينات موجبة للاختبار.

تم عزل العديد من البكتيريا والتعرف عليها و وجد أن أكثر الأنواع الملوثة هي *Bacillus cereus* بنسبة 25% كما أثبتت الدراسة أن أكثر مصادر التلوث بهذة البكتريا هي بيئة هذه المزارع .

دلت الدراسة إلى أهمية الإرشاد والتوعية خاصة لصغار منتجي الألبان لأنهم من اكبر مصادر للألبان في الولاية.

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Introduction

Milk is the most complete food for all mammals especially new borns. It supplies the body with proteins, fats, carbohydrates, minerals and vitamins in a manner to suit the nutritional requirement.

Since milk is biological and public commodity, it must be produced and handled under hygienic condition. The Joint FAO/ WHO expert committee on milk hygiene (1970) recommended that milk should be produced under hygienic conditions to:

- Prevent animal diseases transmitted to man through milk and milk products such as bovine tuberculosis and brucellosis.
- Prevent human diseases which may result from consumption of milk such as septic sore throat.
- Ensure good nutritional status of human specially infants and elderly.
- Prevent milk from spoilage.

High quality milk can only be produced by healthy cows which are free from udder infection. Cows with mastitis or elevated somatic cell counts (SSC) are incapable of producing high quality milk until the inflammation and infection in the udder are brought under control.

Because the quality of milk can not be improved following extraction from the cow, the production of high quality milk requires an effective mastitis control program especially subclinical infection.

Once milk is produced, the retention or preservation of milk quality requires cleanliness, sanitation and careful handling. Maximum benefits are derived only when these traits are applied to all aspects of milk production system (cows, cow's environment, milking process, milking practices and milk storage or cooling system). A deficiency in any part

of the overall system will result in decreased milk quality by undesired growth of contaminating bacteria. Hence, regular bacteriological investigations should be carried out to ensure the provision of safe and nutritious milk to publics.

The present study was carried out in Omdurman, Khartoum state to:

- Determine the bacteriological quality of milk produced in small scale producing units.
- Isolate and identify bacterial contaminants of raw milk in these units.
- Detect the possible sources of bacterial contamination.

CHAPTER ONE

LITERATURE REVIEW

1.1 The milk:

Milk is a secretion of the mammary glands and is virtually sterile when secreted into the alveoli of the udder (Tolle, 1980). It is an excellent food especially for growing children (Hunderson, 1971). It is regarded as the only food that provides a well-balanced essential nutrients in a form which is palatable, digestible and sanitary (Kordylas, 1991). Hence, milk represents a sole source of nutrition for nomads who live exclusively on it for months (Kon, 1972).

Cow's milk is composed of water (87%), lactose (4.9%), fat (3.5-3.7%), protein (3.5%), and ash (0.7%) (Watt and Merrile, 1963).

Milk carbohydrates are sugars which are especially important for infant feeding because they prevent intestinal putrefaction by encouraging growth of acid-producing bacteria in the stomach. Sugars also affect the absorption of minerals such as calcium and phosphorus. Moreover milk proteins consist mainly of casein with few other protein fractions such as lactalbumin and lactoglobulin. It is an excellent source of proteins that contains all essential amino acids required by humans (Payne, 1990)

Milk fats contain high proportion of short-chain fatty acids especially butyric acid, and enzymes such as phosphatases and lipases that affect the flavour of milk. Moreover milk and dairy products are also outstanding sources of calcium, good sources of phosphorous, potassium and many trace minerals (Kordylas, 1991).

The salts of milk are considered to be the chlorides, phosphates and citrates of potassium, sodium, calcium, and magnesium (Verma, and Sommer, 1957).

Fresh whole milk is valuable source of vitamin A, riboflavin, thiamin and other B vitamins and is important source of vitamin C in dry areas (Payne, 1990)

1.2 Sources of contamination of raw milk:

Due to its high nutritional value, milk represents a good medium for bacteria and other microorganisms. The main sources of contamination in the farm are cow's udder and body, utensils, milking machines, stable and the transportation equipment (Hunderson, 1971). Generally, contamination of raw milk occurs from three main sources: within the udder, the exterior of the udder, and from the skin of the handlers and the surface of storage equipments (Bramley, and McKinnon, 1990).

1.2.1 The interior of the udder:

Milk as drawn from the normal udder is sterile but soon becomes contaminated by different bacteria.

Raw milk as it leaves the udder of healthy cows normally contains very low number of microorganisms and generally it contains less than 1000 total bacteria per ml. Sources of these bacteria are teat cistern, teat canal, and teat apex which may be colonized by a variety of microorganisms. However, the microbial contamination from within the udder of healthy animals is not considered to increase the total numbers of microorganisms in the milk or the bacterial numbers during refrigerated storage (Kurweil, 1973).

1.2.2 The exterior of the udder:

The exterior of the cow's udder and teats can contribute to contamination of raw milk by microorganisms. These microorganisms are either naturally associated with the skin of animal or the environment in which the cow is housed and milked (Brito *et al.*, 2000).

The teat skin is one of the main sources of the microbial contamination of raw milk as well as a source of mastitis infection (Brito *et al.*, 2000). It was found that the application of the different practices for preparing the udder including the use of calf suckling to stimulate the letdown of milk represents a major contamination source. However, rinsing of the teat with water and wiping dry reduces the number of microorganisms on the teat skin.

The contribution of microorganisms from teats soiled with manure, mud, feeds, or bedding is important. Teats and udder of cows inevitably becomes soiled when animals are held in muddy barnyards or when cows are lying in stalls. Soiled bedding can harbor large numbers of microorganisms, with counts exceeding $10^8 - 10^{10}$ cfu per gram, organisms associated with soiled bedding materials include *Streptococci*, *Staphylococci*, *Spore-formers*, *coliforms*, and other Gram-negative bacteria, both thermophilic and psychrotrophic strains of bacteria are commonly found on soiled teat surfaces (Bramley, 1990).

1.2.3 The handling and storage equipments:

Cleaning of milking system influences the total bacteria count in milk at least as much as any other factor, milk residues left on equipment contact surfaces supports the growth of a variety of microorganisms. Organisms considered to be natural inhabitants of the teat canal apex, and skin generally do not grow significantly on soiled milk contact surfaces or during refrigerated storage of milk. In general, environmental contaminations (i.e., from bedding, manure, feeds ...etc) are more likely to grow on soiled equipment surfaces than are organisms associated with mastitis (Olson *et al.*, 1980).

The farm water supply can also be a source of microorganisms (especially psychrotrophs) that can seed soiled equipment and/or the

milk (Bramley, 1990). Cleaning and sanitizing procedures that leave residual soil on equipment can dramatically increase the numbers and influence the types of microbes that grow on milk contact surfaces (Thomas, 1966). Effective use of chlorine or iodine sanitizers has been associated with reduced levels of psychrotrophic bacteria.

Psychrotrophic bacteria tend to be present in higher counts in milk and are often associated with occasional neglect of proper cleaning or sanitizing procedures (Olson, *et al.*, 1980) and /or poorly cleaned refrigerated bulk tanks (Mackenzie, 1973).

1.3 Bacteria in milk:

Bacterial contaminants of milk are either originate from diseased animal (systemic or local e.g. mastitis) or from the animal environment during milking process.

1.3.1 Mastitis:

Mastitis is the inflammation of the mammary glands caused by microbial infection (Cole, 1962). It may also be defined as inflammation of the udder irrespective of the cause (Blood *et al.*, 1986).

1.3.1.1 Types of mastitis:

Two forms of mastitis are known; clinical and subclinical mastitis (Blood *et al.*, 1986).

1.3.1.2 Clinical mastitis:

This form of mastitis is characterized by apparent change of both milk and mammary gland and it is further classified into peracute, acute, subacute and chronic mastitis.

This type of mastitis is easy to detect and hence the causative agent is suddenly contaminate milk in bulk tank.

1.3.1.3 Sub-clinical mastitis:

This is an invisible abnormality of milk or udder which is characterized by an increase in somatic cell and/or leukocyte count and it is a problem of the herd rather than individual animals. Early detection of this type of mastitis eliminates an important contamination source (Radostitis, Blood and Gat, 1994).

1.3.1.4 Bacterial causes of mastitis:

Healthy udder contributes very little to the total bacterial count of milk and a cow with mastitis has the potential to shed large numbers of microorganisms in milk (Bramley and Mckinnon., 1990). The influence of mastitis on the total bacterial count of milk depends on the strain of infecting microorganisms, the stage of infection, and the percentage of the herd infection. Infected cows have the potential to shed in excess of 10^7 bacterial cell per ml of milk (Bramley and Mckinnon, 1990).

Over 130 microorganisms have been isolated from bovine mastitic milk samples, but *Staphylococcus aureus*, *Streptococcus spp* and members of *Enterobacteriaceae* are among the most common aetiological agents in cows and in other animal species (Quinn *et al.*, 1999).

1.3.2 Bacterial contaminants of raw milk:

Milk in farm may become contaminated with different bacteria present on the cow and its environment including contaminated water used to clean the milking systems (Bramley and Mckinnon, 1990).

The most common spoilage microorganisms of milk and dairy products are Gram-positive spore forming bacteria and lactic acid producing bacteria [International Dairy Federation (IDF), 1994].

1.3.2.1 Gram - positive bacteria:

Lucheis *et al.*, (2000) collected 302 samples of cow milk directly from the teats. He found that 93 (30.9%) of the samples were negative and 209 (69.2%) were positive; The positive isolates include *Corynebacterium bovis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Actinomyces pyogenes*, *Micrococcus spp.*, *Enterococcus faecalis*, *Staphylococcus hyicus*, *Staphylococcus intermedius*, *Bacillus spp.* and *Morganella morganii*. In addition he found that *S. aureus* grows poorly in raw milk and is generally considered to be a poor competitor with other indigenous raw milk micro flora. Bell and Veils (1952) added that enterotoxigenic strains of *S. aureus* can be shed into milk by infected cattle. Clark and Nelson (1961) investigated raw milk samples and found that the average of coagulase-positive *Staphylococci* was 2.5×10^3 to 3.3×10^3 cfu / ml.

Lactic acid producing microorganisms (*Streptococcus spp.*, *Lactococcus spp.*, and *Leuconostoc spp.*) spoil milk by fermenting lactose to produce acid (International Dairy Federation, 1994). *Streptococcus agalactiae* and *streptococcus zooepidemicus* are well – recognized as etiologic agent of bovine mastitis and they may be shed in high numbers into milk of mastitic animals (Marth, 1985). They can also be carried by healthy cows (Barnham *et al.*, 1983).

The major sources of milk contamination by *Bacillus cereus* in farm were studied. It was found that high spore counts of toxic strains of *B. cereus* were detected in consumed grains, silage and faeces. These results indicated that *B. cereus* pass in the rumen and multiply in the digestive tract of the cow. *B. cereus* spores in the feed may also contaminate the environment directly. Moreover, indirect contamination

through the multiplication of the organism in the cow's digestive tract may also be possible (Torp *et al.*, 2001). *B. cereus* is a limiting factor for the self-life of pasteurized milk. The soil was the major contamination sources of *B. cereus* which can be reduced in milk by teat cleaning practice (Chrislinsson *et al.*, 1999).

1.3.2.2 Gram–Negative bacteria:

Gram – negative organisms associated with lowering of milk quality can be placed into two groups: coliforms and non coliforms.

Coliform bacteria are groups of Gram negative bacteria which ferment lactose. They include the genera *Escherichia*, *Citrobacter*, *Enterbacter*, and *Klebsiella* (Al– Ashmawy, 1990). The important source of these organisms is the intestinal tract of man and animals and they are also found in mastitic udder, soil, air, contaminated equipments feed and manure. Legal limits for coliform count, unlike for pasteurized milk; have not been established for bulk tank milk, it is generally accepted that counts >1000 cfu/ml of raw milk indicate that milk is produced under unhygienic condition (Bray *et al.*, 1996).

Gram–negative non coliform bacteria in bulk tank milk have been shown to belong to the genera *Acinetobacter*, *Aeromonass*, *Flavobacterium*, *Moraxella*, *Pseudomonas* and *Xanthobacter* (Bray *et al.*, 1996). Bacteria in these genera in particular, *Pseudomonas* were shown on several occasions to be responsible for defects in raw milk, pasteurized milk, and milk products (Suhren, 1989). *Pseudomonas spp.* are also the most important group of psychrotrophs associated with spoilage. They produced extra cellular enzymes (proteases and lipases) which were particularly destructive if high numbers of bacteria are present. These enzymes may produce flavors described as bitter, rancid, unclean, and fruity and yeast–like (International Dairy Federation, 1994).

Raw milk is an important source of *Salmonella* (Bryan, 1983). Dairy cattle may acquire *Salmonella* infection from various sources, including contaminated feed or water (Bryan, 1983). The most routinely recovered serotypes from raw milk are *S. typhimrium*, *S. enteritidis* and *S. Dublin*. The later is rare but particularly virulent serotypes are host adapted to cattle (Werner *et al.*, 1979). Wells *et al.* (2001) reported also that the serogroups *Salmonella montevideo*, *Salmonella cerro* and *Salmonella Kentucky* are adapted to cattle.

The main source of *Salmonella spp.* in dairy herds was cattle faeces. Carriage and faecal excretion of *Salmonella* were not systematically associated with post clinical salmonellosis in herd. Although dairy farms were exposed to environmental contamination, the occurrence of milk contamination with *Salmonella* was generally not frequent (Linda, *et al.*; 1995).

Brucella species exhibit pathogenicity towards a wide variety of animals, including dairy cattle. The genus contains many species but *Brucella abortus* is the only significant species with respect to animal and human health (Parry, 1966). It is localized in the uteri of the pregnant females and in the mammary glands of lactating ones, hence enabling the organism to be shed into milk for many years. Commercial pasteurization effectively kills *Br. abortus* with a large margin of safety (Faster *et al.*, 1953).

Coxiella burnetti is often isolated from domesticated animals including cattle. It can be shed in milk from infected cows and thereby be directly transmitted to humans presumably through raw milk consumption (Enright *et al.*, 1957).

Raw milk is often implicated as a source of *Campylobacter jejuni*; both the intestinal tract and the udder of the bovine are potential reservoirs of this bacterium, (Linder and Gill, 1980).

Listeria monocytogenes could cause mastitis in dairy cattle and can be shed in milk at a level of 2×10^3 to 2×10^4 cells per ml (Donker and Voelt, 1962).

1.3.2.3 Pathogenic bacteria:

Milk borne human infection and intoxication could be due to *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., *staphylococcus* spp., *Yersinia enterocolitica*, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringes*, *Clostridium botulinum* and *streptococcus zooepidemicus* (International Dairy Federation, 1994).

Giovannini (1998) reported that various zoonotic agents can be transmitted to human through milk. He reported *Brucella melitensis*, *Brucella abortus*, *Mycobacterium bovis*, *Salmonella* spp., *Listeria monocytogenes*, *Coxiella burnetti*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *E. coli* O157: H7 as important zoonotic organisms. He added also the toxins of *Clostridium perfringes*, *Clostridium botulinum* and *Corynebacterium diphtheriae* may cause food poisoning disease.

1.4 Bacteriological quality of raw milk:

There is no universal agreement as to what constitutes “bacteriological quality” and to overcome this difficulty the term “hygienic quality” has been proposed which include several items such as bacterial numbers, keeping quality, mastitis, visible dirt and temperature (Davis, 1950).

The bacteriological quality of raw milk is important for both producer and consumer, hence high bacterial count on the farm contribute to poor keeping quality and inferior product (Law, 1979).

Psychrotrophic bacteria were found to affect milk quality (Linda 1995). These bacteria survive optimally in low temperatures ($< 7^{\circ}\text{C}$) and can survive also the pasteurization process. Growth of these bacteria during refrigeration with the production of proteolytic enzymes results in biochemical alteration of milk.

Historically, bacteriological examination of milk began for the first time in 1900 to determine the incidence of pathogenic bacteria in raw milk supplies (Juffs, 1978). Dasai and Clancydon (1964) found that the average of initial total bacterial count of raw milk samples incubated at 35°C was 1.4×10^4 cfu / ml, while Bacic *et al.* (1968) found that the arithmetic mean of bacterial count of aseptically drawn milk from 79 cows was 3.4×10^3 cfu/ml. Randolph *et al.*, (1973) found that the mean standard plate count for grade A raw milk samples from 105 individual producers and 74 bulk tank trucks collected from different units in USA were 7.0×10^4 and 1.0×10^5 cfu/ml respectively.

1.4.1 Bacteriological quality of raw milk in Sudan:

Ibrahim (1973) found that the average total bacterial count in four dairy farms around Khartoum was 6.8×10^5 cfu/ml.

Mustafa and Idris (1975) tested 113 samples of milk collected from vendors in Khartoum. The average total bacterial count was found to be more than 10^6 cfu/ml.

Mohammed (1988) examined 290 samples of vendors' milk for total bacterial count and found that 54.4% had total bacterial count ranging between 5.0×10^5 and 5.0×10^6 cfu/ml

Ali (1988) collected five and eight milk samples from Kuku and Gezira dairy plant respectively. He found the mean bacterial counts were 3.4×10^6 cfu/ml and 4.4×10^5 cfu/ml and 1.99×10^4 cfu/ml for pasteurized milk in Kuku and Gezira dairy plants, respectively.

Nahid (2004) collected one hundred and twenty samples from supermarkets in Khartoum state. She found that there was high average of total bacterial count ($5.63 \times 10^9 \pm 2.87 \times 10^{10}$ cfu/ml) in milk samples. Moreover, during Summer season, the total bacterial count of milk ($1.04 \times 10^{10} \pm 4.01 \times 10^{10}$ cfu/ml) was higher than Winter ($9 \times 10^8 \pm 2.51 \times 10^9$ cfu/ml).

1.5 Grading of raw milk:

Raw milk under tropical condition was graded according to many factors which include the number of microorganisms present in milk, odor or flavor, a mount of sediment, appearance and temperature (Chandan *et al.*; 1979). They also reported that milk was graded as good when it had total bacterial count (TBC) of 5.0×10^5 cfu/ml or less, satisfactory when the (TBC) ranged between 5.0×10^5 to 5.0×10^6 cfu/ml and bad when the (TBC) was more than 5.0×10^6 cfu/ml.

According to the US Department of Health Education and Welfare (1953), milk was graded as grade A when the bacterial count was less than 2.0×10^4 cfu/ml, grade B when the bacterial count ranged between 2.0×10^4 to 1.0×10^6 cfu/ml and grade C when the bacterial count was more than 1.0×10^6 cfu/ml.

1.6 Methods for detection of bacteria in milk:

There are many tools to detect bacteria in milk and are differentiated according to procedure used.

1.6.1 Traditional methods:

These methods include isolation of bacteria from samples followed by identification according to the procedure described by Elmer *et al.* (1997). Other indirect methods which are used normally to detect mastitis in milk include somatic cell count, California mastitis test.

1.6.2 Molecular methods:

These methods were used to detect bacteria and include for example portable real-time PCR which is useful for detection of *Salmonella* in raw milk. Results by this method could be obtained in 24 hours compared with 48 to 72 hours for traditional methods (Ven, *et al.*, 2003). Moreover DNA extraction and PCR techniques were evaluated using Enzyme-Link Immunosorbent Assay (ELISA) to detect *E. coli* DNA (Daly, *et al.*, 2002).

1.6.3 Serological methods:

1.6.3.2.1 Milk ring test (M. R. T.):

The test is used for screening and diagnosis of brucellosis. Morgan (1969) stated that three to four annual tests were found suitable to detect 85% of the infected herds containing 95% reactor animals. He also claimed that the possibility of obtaining positive M.R.T. on mixed milk of 25 cows with two reactors were 96% and the percentage increased to 99% when three cows were infected.

According to WHO (1992), the Milk Ring Test is not suitable for diagnosis of brucellosis and as a result, two or more tests are always needed to be used for diagnosis.

1.6.3.1.1 Factors affecting sensitivity of (MRT):

Hignott and Nagy (1967) stated that the excretion of antibody in the milk of infected cows is intermittent and the fat content and the size of the fat globules also affect the test. They also mentioned that blood antibody, which pass through the udder barrier during drying off period or in case of colostrums, were found to result in false positive reaction. Heating and violent agitation of milk samples will result in destruction of fat globules hence affected the test (Morgan *et al.*, 1978). They also mentioned that vaccination with Strain 19 vaccine gives a false positive

reaction to MRT for about three months after vaccination (Morgan *et al.*, 1969). Some environmental conditions such as hot and cold weather were found to affect the test (Roepke *et al.*, 1958).

1.7 Bacterial diseases transmitted in milk:

The presence of lactose, protein and fat together with vitamins and other growth factors with a suitable pH make milk a very suitable medium for growth of wide range of microorganisms that are capable of causing diseases to man and animals (Kotins, 1978). Different diseases could be transmitted through consumption of contaminated raw milk. These diseases include brucellosis, tuberculosis, scarlet fever, listeriosis, salmonellosis, candidiasis, and food poisoning caused by *Staphylococcus aureus*, *Clostridium perfringens*, *Colsteridium botulium*, *Bacillus cereus* and *Escherichia coli* (Tanwani and Yadava, 1983).

1.7.1 Brucellosis:

Brucellosis is one of the most important bacterial zoonosis worldwide (Young, 1995). It is a contagious bacterial disease of animals which is transmitted to man (anthropozoonosis) (Carpenter and Hubbert, 1963).

The etiological agents are gram–negative coccobacilli belonging to the genus *Brucella* (Kadohira *et al.*; 1997). The genus *Brucella* include *B. abortus*, *B. melitensis*, *B. suis*, and *B. canis* (Colmenero *et al.*; 1996).

B. abortus is one of four *Brucella* species associated with systemic disease in human (Corbel, 1997).

Nahid (2004) found that from one hundred and twenty milk samples collected from supermarkets in Khartoum state, 44.1% of the samples were positive for *brucella* by milk ring test. Moreover 54.4% and 45.6% of which were detected during winter and summer respectively.

The primary hosts acts as reservoirs of infection for each particular species, while the secondary ones usually play little part in the maintenance or spread of the disease (Corbel and Hendary, 1983).

Transmission from infected livestock to man can either be direct through contact with infected material, or indirect through consumption of animal products (Kadohira *et al*; 1997).

Buxton and Fraser (1977) stated that the disease is transmitted from infected animals to susceptible ones through mucous membrane of alimentary and respiratory tracts, conjunctiva, intact skin, artificial insemination and through the vagina in some species. Insects could also act as vehicles of infection (Corbel, 1989) and in man infection is by inhalation, ingestion through conjunctiva and skin.

Brucellosis in the Sudan was first reported in a dairy farm in Khartoum where *B. abortus* was isolated from an aborted cow (Bennett, 1943). It has been found that the occurrence of animal brucellosis has a direct impact on human health. Corbel (1989) stated that infection of human almost follows the same pattern as that in animals globally.

1.7.1.1 Epidemiology of brucellosis:

The epidemiology of brucellosis is complex. Important factors are contribute to the prevalence and spread of the disease in livestock. These factors include farming system and practices, farm sanitation, livestock movement, mixing and trading of animals and sharing of grazing ground. *Brucella* has a low infectious dose (10 organism of *B. melitensis* are sufficient to cause infection in man), making infection a genuine risk to those occupationally exposed such as farmers, veterinarians, and butchers and to the public through the consumption of contaminated unprocessed milk, milk product and meat (Kadohira *et al*; 1997).

Recently McDermott and Arimi (2002) summarized epidemiological findings for brucellosis in sub-Saharan Africa. Brucellosis is common in cattle but less well studied in small ruminants. Bovine brucellosis prevalence rates ranging from 3.3% for the Central Africa Republic to as high as 41% for Togo was reported (Doming, 2000; Nakoune *et al*; 2004). Values falling within this range were reported for Chad (Schelling *et al*; 2003), Sudan (El-Ansary *et al*; 2001), Eritrea (Omer *et al*; 2000), Tanzania (Weinhaupl *et al*; 2000), Burkina Faso (Coulibaly and Yemeogo, 2000), Ghana (Turkson and Boadu, 1992), Mali (Toukara *et al*; 1994), Nigeria (Ocholi, *et al*; 1996), and Zimbabwe (Mohan *et al*; 1996).

1.7.1.2 Diagnosis of brucellosis:

Definitive diagnosis of brucellosis is often difficult. Laboratory diagnosis of brucellosis in animals or man is achieved either through blood culture or serological testing (Maichomo *et al*; 1998).

1.7.2.1 Tuberculosis:

Tuberculosis (TB) is a chronic infectious disease of man and animals which is caused by the tubercle bacilli, *Mycobacterium tuberculosis*, an *Actinomycetes* that is characteristically acid alcohol fast. The disease occurs in all species including man, i.e. it is of public health importance as well as for its detrimental effect on animal production. The most commonly infected animals are cattle, pigs and chickens (Al-haji, 1976). In Sudanese cattle, TB was first reported in 1915 (Annual report of Sudan Veterinary Services, 1915).

1.7.2.2 Bovine tuberculosis:

Bovine TB (BTB) is classified by FAO and OIE as a disease of “List B”, this category includes all animal diseases which are considered important because of their socioeconomic and /or public health impact.

Grange (1994) mentioned that human TB due to *M. bovis* is still a public health problem of concern to both medical and veterinary professions and there is need to maintain careful bacteriological surveillances.

Mycobacterium bovis was first clearly distinguished from other types of tubercle bacilli by the Obald and Smith in 1898. It has a wider range of pathogenicity for different animal species than the other species of the genus. It causes TB in cattle, pigs, man, horses, sheep, goat, parrots, and other primate carnivores including doges and cats (Roberts *et al.*, 1991). *M. bovis* and *M. foruitum* are considered causative agent of mastitis in cattle (Nolte and Mitckock, 1995).

In the Sudan, bovine tuberculosis was thought to be a rare disease (Cummins, 1992).

1.7.2.6 Diagnosis of tuberculosis:

Tuberculosis is not an easy disease to diagnose. Direct microscopy with ziehl-neelsen staining of clinical specimen is the most commonly used and the cheapest method, but it lacks sensitivity and specificity (Roberts *et al.*, 1991). Although the intradermal test is a widely used method for the diagnosis of TB, there are clear data which indicated the unsatisfactory sensitivity of this test. Another inconvenience is that the test does interfere with the immune status of the animal and can not be reported in less than 60 day (Roberts *et al.*, 1991)

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of samples:

A total of sixty samples were collected from six small milk producing units in Omdurman (Gebal Touria, Elhatana and Elmarkhiat mountains) in Khartoum State Table (2).

Collected samples were milk (30 samples from lactating cows, 6 samples from bulk milk tanks), 6 swabs from milker's hands, 6 swabs from milk utensils, 6 water samples and 6 samples from the environment of these producing units.

For samples from cows, the whole udder was first washed with water to remove dust and then dried. The teat orifice was then thoroughly rubbed with 70% alcohol then 5 ml of milk were collected directly in sterile bottle. From Bulk tanks, 5 ml of milk was poured into sterile sample bottles.

Swabs were taken directly from the clean dry utensils and from hands of milkers immediately after milking.

Water samples were collected from the water sources, which were tap water brought from outside of the farm in tanks. For environmental samples, sterile blood agar plates were kept open for 10 minutes in farm then closed.

All samples were transported to the microbiology laboratory at the Faculty of Veterinary Medicine, University of Khartoum in a thermos flask on ice.

Table (1): Sources, types and number of samples used in the study:

| Place (Omdurman) | Milk producing units | Type of samples | | | | | | Total |
|---------------------|----------------------------|-----------------|----------------------|--------------------------------|-----------------------------------|-------|--------|-------|
| | | Cows milk | Bulk tank milk | Swabs from milkers hands | Swabs from milk utensils | Water | Envir. | |
| Gebal touria | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| | 2 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| Elhatana | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| | 2 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| Elmarkhiat | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| | 2 | 5 | 1 | 1 | 1 | 1 | 1 | 10 |
| Total | 6 | 30 | 6 | 6 | 6 | 6 | 6 | 60 |

2.2 Sterilization procedures:

Petri – dishes, test tubes, pipettes, and agglutination tubes were sterilized in hot air oven at 160° C for two hours. Screw-capped bottles were sterilized by autoclaving at 121° C for 15 minutes.

2.3 Bacteriological investigation of samples:

2.3.1 Receiving and treatment of samples in the laboratory:

After investigation with total plate count and Milk Ring Test (M.R.T), milk samples were incubated at 37° C overnight before culturing. Water samples were centrifuged at 5000 r/m for 15 minutes, the supernatant was discarded and the sediment was cultured. Swabs were immediately cultured. Environmental samples (on blood agar) were immediately incubated at 37° C over night before culturing.

2.3.2 Immediate bacteriological procedures:

In the laboratory, milk samples were immediately investigated using the total plate count and milk ring tests.

2.3.2.1 Total plate count:

2.3.2.1.1 Materials:

- 36 milk samples.
- Total plate count medium (2.4.1.1).
- The diluent:

Ringer solution was used to dilute the milk in total plate count. It was prepared by dissolving one tablet in 500 ml distilled water. The solution was distributed into 9ml amount into clean test tubes and sterilized by autoclaving at 121° C for 15 minutes.

2.3.2.1.2 Method:

The method was used as described by Richardson (1985). The milk sample was 10-fold serially diluted to the fourth dilution. A sterile one ml pipette was used to inoculate half ml amount of selected pretested dilutions (10^{-3} and 10^{-4}) on each of two plates which were rotated to ensure equal distribution of inoculums. The plates were then left for half an hour on the bench then incubated at 37° C and examined after 24 hours for bacterial growth. The colonies were counted and the total viable bacterial count was calculated by multiplying the number of colonies with the reciprocal of the dilution used. The mean and the standard deviation were calculated for all samples.

2.3.2.2 Milk ring test:

2.3.2.2.1 Materials:

- Samples: a total of thirty six fresh milk samples.
- Reagent: stained brucella antigen (Central Veterinary Research Laboratory, Soba).

2.3.2.2.2 Method:

This test was done according to Morgan *et al.* (1978). 0.03 ml of stained milk ring test antigen was added to one ml of milk in

agglutinating tubes, mixed well and incubated at 37° C for one hour. Development of a ring on the milk surface was regarded as positive result.

2.4 Cultivation of samples:

2.4.1 Culture media:

2.4.1.1 Total plate count medium (Oxoid):

| | |
|-----------------------------|-----------|
| Yeast extracts | 2.5 g /l. |
| Pancreatic digest of casein | 5.0 g /l. |
| Glucose | 1 g /l. |
| Agar | 15 g /l. |
| pH 7.0 (Approx.) | |

The medium was prepared according to manufacturer's instruction by suspending 23.5 g in one liter of distilled water and dissolved by heating. The medium was then sterilized by autoclaving at 121° C for 15 minutes and distributed aseptically in 15 ml amount into sterile Petri – dishes.

2.4.1.2 Nutrient broth (Oxoid):

| | |
|----------------------|---------|
| Lab – Lemco powder | 1 g /l. |
| Yeast extract powder | 2 g /l. |
| Peptone powder | 5 g /l. |
| Sodium chloride | 5 g /l. |
| pH 7.4 (approx.) | |

An amount of 13 grams was dissolved into one liter of distilled water by heating. The pH of the mixture was adjusted to 7.4, and then the medium was distributed in 5 ml amount into final containers and sterilized by autoclaving at 121° C for 15 minutes. For nutrient agar, 1.5% agar (w/v) were added. The prepared medium was distributed aseptically in 15 ml amount into sterile Petri - dishes.

2.4.1.3 Peptone water (Oxoid):

| | |
|------------------|--------|
| Peptone powder | 10 g/l |
| Sodium chloride | 5 g/l |
| PH 7.2 (approx.) | |

Fifteen grams were added to one liter of distilled water and dissolved by heating. The pH was then adjusted and the medium distributed aseptically into final containers then was sterilized by autoclaving at 121° C for 20 minutes and.

2.4.1.4 MacConkey agar (Oxoid):

| | |
|------------------|-----------|
| Peptone powder | 20 g/l |
| Lactose | 10 g/l |
| Bile salts | 5 g/l |
| Neutral red | 0.075 g/l |
| Agar No.3 | 15 g/l |
| pH 7.4 (approx.) | |

Fifty grams were dissolved in one liter of distilled water by boiling. The mixture was sterilized by autoclaving at 121° C for 15 minutes, and then dispensed in sterile Petri-dishes in 15 ml volume each.

2.4.1.5 Blood agar base No.2 (Oxoid):

| | |
|------------------|--------|
| Protease peptone | 15 g/l |
| Liver digest | 2.5g/l |
| Yeast extract | 5 g/l |
| Sodium chloride | 5 g/l |
| Agar No.3 | 12 g/l |
| pH 7.4 (Approx) | |

Forty grams of powder was suspended in one liter of distilled water and dissolved by boiling. The pH was adjusted to 7.4 and the

medium was sterilized by autoclaving at 121° C for 15 minutes. The medium was then cooled to (45°-50°) C and 7% defibrinated ovine blood was added aseptically, mixed gently and dispensed in sterile Petri-dishes in 15 ml volume.

2.4.1.6 Starch agar (Oxoid):

| | |
|-----------------|---------|
| Potato starch | 10 g/l |
| Distilled water | 50 ml |
| Nutrient agar | 100 g/l |

One hundred and fifty grams of starch was titrated with water to smooth cream, and then added to molten nutrient agar. The mixture was sterilized at 115° C for 15 minutes and distributed aseptically in 15 ml amount into sterile Petri-dishes.

2.4.1.7 Lecithovitellin (LV) agar:

| | |
|--|---------|
| Lecithovitellin solution (egg yolk saline) | |
| Hen eggs | 4 |
| NaCl (0.85%) solution | 1000 ml |

Egg yolk was separated from egg white and beaten in saline to form homogeneous mixture. Twenty five grams of kieselguhr (diatomite) was added, mixed and clarified by filtration through paper and sterilized by filtration (0.2 µm membrane filter, Sartorius).

| | |
|--------------------------|---------|
| Lecithovitellin Agar | (ml) |
| Lecithovitellin solution | 100ml |
| Nutrient Agar | 900 g/l |

Nutrient agar was melted and cooled to about 55°C and lecithovitellin solutions was then aseptically added, mixed and poured into sterile Petri–dishes in 15 ml volume each.

2.4.1.8 Motility medium (Oxoid):

| | |
|----------------------------------|--------|
| Dehydrated nutrient broth powder | 15 g/l |
| Agar No. 1 | 5 g/l |

An amount of 15g nutrient broth was added to 5 grams agar and dissolved in one liter of distilled water by boiling. The pH was adjusted to 7.2. The medium was then distributed in 5 ml volumes in test tubes, and sterilised by autoclaving at 115° C for 15 minutes.

2.4.1.9 Hugh and Leifson's (O/F) medium:

| | |
|--------------------------------|---------|
| Peptone powder | 2 g/l |
| Sodium chloride | 5 g/l |
| KHPO ₄ | 0.3 g/l |
| Agar | 3 g/l |
| Distilled water | 1000ml |
| Bromothymol blue 0.2% aq. Sol. | 15ml |

The ingredients were dissolved in distilled water in a boiling water bath. The pH was adjusted to 7.1. The indicator was added and the base medium was then sterilised by autoclaving at 115° C for 20 minutes. A sterile solution of glucose was aseptically added to give a final concentration of 1%. The medium was mixed and distributed aseptically in 10 ml volumes into sterile test tubes.

2.4.1.10 Peptone water sugars:

| | |
|---------------------------------------|-------|
| Peptone water | 900ml |
| Andrade's indicator (pH 7.1 – 7.3) | 10ml |

The pH was adjusted to 7.1- 7.3 and the Andrade's indicator was added bringing the pH to 7.5.

| | |
|-----------------|--------|
| Sugar | 10 g/l |
| Distilled water | 90ml |

The sugar was added to the mixture of peptone and the indicator, mixed thoroughly then distributed in 2 ml volume into sterile test tubes with an inverted inner Durham's tube. They were then sterilized by autoclaving at 115° C 10 minutes.

2.4.1.11 Nitrate broth:

| | |
|------------------|---------|
| KNO ₃ | 1 g/l |
| Nutrient broth | 1000 ml |

KNO₃ was dissolved in the broth, the pH was adjusted and the medium was distributed in 5 ml volumes into test tubes then sterilized by autoclaving at 115° C for 20 minutes.

2.4.1.12 VP, MR medium (Oxoid):

| | |
|---------------------------------|--------|
| Peptone powder | 5 g/l |
| K ₂ HPO ₄ | 5 g/l |
| Distilled water | 1000ml |
| pH 7.5 (Approx.) | |

Ten grams of solids were suspended in distilled water and dissolves by steaming then the pH was adjusted to 7.5. Five grams of glucose was added, the medium was then mixed and distributed into 5 ml volumes in test tubes and sterilised by autoclaving at 121° C for 15 minutes.

2.4.1.13 Nutrient gelatin (Oxoid):

| | |
|------------------|---------|
| Lab-Lemo powder | 3 g/l |
| Peptone powder | 5 g/l |
| Gelatin | 120 g/l |
| PH 6.8 (approx.) | |

An amount of 128 grams were suspended in one liter of distilled water, then boiled to dissolve completely, mixed well then poured into

sterile bijou bottles in portion of 2 ml volume. The medium was sterilized by autoclaving at 121° C for 15 minutes.

2.4.1.14 Simmon's citrate agar (Oxoid):

| | |
|-------------------------------|----------|
| Magnesium Sulphate | 0.2 g/l |
| Ammonium Dihydrogen Phosphate | 0.2 g/l |
| Sodium Ammonium Phosphate | 0.8 g/l |
| Sodium Citrate Ttribasic | 2 g/l |
| Sodium Chloride | 5 g/l |
| Bromo – Thymol | 0.08 g/l |
| Agar No. 3 | 5 g/l |

Twenty three grams was suspended in one liter of distilled water, boiled to dissolve completely, and then sterilized by autoclaving at 121° C for 15 minutes. It was aseptically poured in 10 ml amount into sterile McCartney bottles and allowed to set in slope position.

2.4.1.15 Urea agar base (Oxoid):

| | |
|--------------------------------|---------|
| Peptone powder | 1 g/l |
| Dextrose | 1 g/l |
| Sodium Chloride | 5 g/l |
| Disodium Phosphate | 1.2 g/l |
| Potassium Dihydrogen phosphate | 0.8 g/l |
| Phenol red | 0.012 |
| Agar No.3 | 15 g/l |
| pH 6.8 (approx.) | |

Twenty four grams was suspended in 95 ml of one liter of distilled water, boiled to dissolve completely, and sterilized by autoclaving at 115° C for 20 minutes. The preparation was cooled to 50° C and 5 ml sterile 40% urea solution was added aseptically, mixed well, and then the

medium was distributed in 10 ml volumes into sterile McCartney bottles and allowed to set in the slope position.

2.5 Isolation of bacteria:

For isolation of bacteria, incubated milk, swabs and water samples were streaked on blood agar plates which were incubated aerobically at 37⁰ C for 24 hours. Plates that showed no growth were further incubated for 48 hours before discarded as negative.

Isolates from environmental samples were separated each by culture in a new blood agar.

Bacterial isolates were purified by repeated subculture on Blood agar.

2.6 Preservation of purified cultures:

Pure isolates were cultivated onto sterile nutrient agar slant media. After incubation, purity of culture was checked by Gram's staining method. The cultures were then kept in the refrigerator at 4°C. Before investigation, isolates were streaked on nutrient agar plates and used as fresh culture for identification.

2.7 Identification of isolates:

All bacterial isolates were identified according to the procedure described in Barrow and Felthem (1993).

2.7.1 Primary tests:

2.7.1.1 Gram's method:

Smears were prepared from purified colonies. A part of colony was picked and dissolved into a drop of normal saline on clean slide glass, air dried, fixed by heating stained by Grams method (Barrow and Feltham, 1993) and examined microscopically under oil – emersion lens.

2.7.1.2 Motility test:

Test organism was inoculated into the Craigie tube and the medium was incubated at 37° C for 24 hours. Growth outside the Craigie tube indicated motility of the isolate.

2.7.1.3 The oxidation – fermentation test O/F:

The test culture was inoculated in duplicate test tubes of Hugh and Leifson's medium. A layer of sterile melted soft paraffin was used to cover one tube to the depth of 1cm and then they were incubated at 37° C for 5 - 7 days and examined.

Oxidative bacteria showed growth only in the open tube, fermentative bacteria showed growth in the closed tube and the bottom of the open one.

1.7.1.4 Oxidase test:

Strips of filter paper soaked in oxidase reagent (P- phenylene diamine dihydrochloride) and dried were used. The strips were placed on a clean slide using sterile forceps. A fresh colony on nutrient agar was picked with sterile glass rod and rubbed on the filter paper. A dark purple colour that developed within 5-10 seconds was considered positive reaction.

2.7.1.5 Catalase test:

On clean slide, a drop of 3% aqueous solution of hydrogen peroxide was placed. A colony of tested culture was put onto the hydrogen peroxide drop. Evolution of gas bubbles indicated a positive test.

2.7.2 Secondary tests:

2.7.2.1 Indole test:

Peptone water was inoculated with test culture and incubated at 37° C for 48 hours. One ml of xylol was added to the culture which was

shaken well and allowed to stand until the xylol was collected on the surface. Then 0.5ml of Kovac's reagent (p-dimethyl-aminobenzaldehyde) was poured down the side of tube. A pink ring which appeared on the xylol layer within a minute indicated positive reaction.

2.7.2.2 Vogues – Proskauer (VP) test:

The test culture was inoculated in glucose phosphate peptone water, and incubated at 37° C for 48 hours. 0.6 ml of 5% alcoholic solution of α – naphthol and 0.2 ml of 40% KOH were added to one ml of the culture. A positive reaction was indicated by development of bright pink color within 30 minutes.

2.7.2.3 Sugar fermentation test:

The ability of an isolate to ferment sugar was tested using peptone water containing 1% of desired sugar. The tubes of medium were inoculated with one to three colonies and then incubated at 37° C for 24 hours. Appearance of reddish color indicated positive test. The gas production was indicated by development of an empty space in Durham's tube.

2.7.2.4 Nitrate reaction:

Test culture was inoculated in nitrate broth and incubated at 37° C for two days. One ml of solution A (sulphanilic acid) was added to the test culture followed by one ml of solution B (α - naphthylamine). A positive reaction was indicated by development of red color. If the result was negative, zinc dust was added and the red colour indicated the presence of nitrate (Zobell, 1932).

2.7.2.5 Citrate utilization test:

This test was applied to test the ability of the organism to utilize citrate as sole source of carbon. A light suspension of organism in sterile

saline was inoculated in citrate medium with wire loop and incubated at 37° C for 48 hours. A positive test was indicated by the change of medium colour from green to blue.

2.7.2.6 Urease activity test:

The activity of the urease was shown by the alkali production (ammonia) from urea solutions. The test culture was streaked on urea agar slope and incubated at 37° C for two days. A positive reaction was indicated by changing of colour to pink.

2.7.2.7 Starch hydrolysis:

Starch agar plate was inoculated with test culture and incubated at 37° C for 24 hours. The plate was then flooded with Lugol's Iodine solution. Hydrolysis of starch was indicated by a clear colorless zone around growth. Starch which had not been hydrolyzed turned blue.

2.7.2.8 Coagulase test:

The test was done according to Gruickshank *et al*, (1975). Half ml of diluted citrated human plasma (1/10) was distributed in clean sterile agglutination tubes. 0.5 ml of young broth culture (18 – 20 hours at 37° C) of staphylococci isolates was added to each tube. Negative and positive controls and a tube of uninoculated plasma were also included in the test. Tubes were incubated in a water bath at 37° C and was read after 1, 2, 3, 6 and 24 hours. A positive reaction was shown by conversion of the plasma into a soft or stiff gel.

CHAPTER THREE

RESULTS

3.1 Total plate count:

Thirty six milk samples were investigated and results were shown in table (2).

The mean total count for samples collected from each farm was calculated together with the standard deviation. The mean total count for samples collected from all farms ranged between 0.55×10^5 to 0.36×10^5 cfu / ml.

According to tropical standards (Chandan *et al.*, 1979), all samples were classified as good, because the mean total bacterial count was less than 5.0×10^5 cfu/ml (table 3).

3.2 Milk ring test:

Thirty six milk samples were investigated by the milk ring test. Results are shown in table (4). Seventeen samples were positive (47.3%) and nineteen samples were negative (52.7%).

3.3 Isolation of bacteria:

3.3.1 Milk samples:

Results are shown in table (5). Fifty eight bacteria were isolated from thirty milk samples of lactating cows and 11 bacteria were isolated from six milk samples from bulk tanks.

The most frequently isolated bacteria were *Bacillus cereus* (14 isolates, 25%) (fig.1) and *Serratia plymuthica* (4 isolates, 6.9%). Three isolates of *Staphylococcus aureus* (fig. 4), *Bordetella pertusiss*, *Citrobacter koser*, *Vibro furnissi* and *E. coli* (fig. 2) were also isolated from lactating cow milk samples.

3.3.2 Swabs:

Fifteen bacterial isolates were obtained from milker's hand swabs (table 5). *Bacillus pumilus* was the most frequently isolated bacteria (4 isolates, 26.6%). Swabs from bulk milk containers revealed the isolation of 20 bacterial spp. (table 5). *Bacillus cereus* was the most frequently isolate (3 isolates, 15%).

3.3.3 Water samples:

Collected water samples (6) revealed the isolation of 18 different bacteria. The most frequently isolated bacteria belonged to the genus *Staphylococcus* table (5) fig. (5).

3.3.4 Environmental samples of farms:

Twenty six bacteria were isolated from the environment of the milk producing units (table 6). *Bacilli* were the most frequent isolates. They included *Bacillus cereus* (6 isolates, 23.2%) *Bacillus circulans* (4 isolates, 15%) and *Bacillus coagulans* (3 isolates, 11.6%).

Table (2): The frequency analysis of total bacterial count in small dairy producing units in Omdurman:

| Place | Farm | Mean \pm std. deviation | Maximum | Minimum |
|--------------|------|---|--------------------|-------------------|
| Gebal touria | 1 | $0.18 \times 10^5 \pm 0.28 \times 10^5$ | 0.75×10^5 | 0.2×10^4 |
| | 2 | $0.49 \times 10^5 \pm 0.33 \times 10^5$ | 1.00×10^5 | 1.8×10^4 |
| Elhatana | 3 | $0.55 \times 10^5 \pm 0.20 \times 10^5$ | 0.08×10^5 | 2.0×10^4 |
| | 4 | $0.46 \times 10^5 \pm 0.19 \times 10^5$ | 0.75×10^5 | 2.0×10^4 |
| Elmarkhiat | 5 | $0.45 \times 10^5 \pm 0.20 \times 10^5$ | 0.75×10^5 | 2.0×10^4 |
| | 6 | $0.36 \times 10^5 \pm 0.16 \times 10^5$ | 6.50×10^5 | 2.0×10^4 |
| Total | | $0.70 \times 10^5 \pm 1.20 \times 10^5$ | 7.50×10^5 | 1.8×10^4 |

Table (3): Grading of the milk samples according to tropical standard. (Chandan et al., 1979):

| Type of grade | No. samples | Percentage |
|------------------------|-------------|------------|
| Grade (1) good. | 36 | 100% |
| Grade (2) satisfactory | 0 | 0 |
| Grade (3) bad | 0 | 0 |
| Total | 36 | 100% |

Key ward:

Grade (1): the bacterial count $\leq 5.0 \times 10^5$ cfu /ml.

Grade (2): the bacterial count $> 5.0 \times 10^5$ to 5.0×10^6 cfu/ml.

Grade (3): the bacterial count $> 5.0 \times 10^6$ cfu /ml.

Table (4): The incidence of *Brucella* antibodies (milk ring test) in the milk samples from Omdurman small dairy producing units:

| Results | No. samples | Percentage |
|----------|-------------|------------|
| Positive | 17 | 47.3% |
| Negative | 19 | 52.7% |
| Total | 36 | 100% |

Table (5): Isolates of G+ve bacteria from different samples collected from small milk producing unites in Omdurman.

| Isolated bacteria | Lactating cow's milk | Bulk tank milk | milkers Hand's | Bulk tank swab | Water | Environment |
|--------------------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|--------------|--------------------|
| <i>Bacillus pummels</i> | 2 (3.5 %) | - | 4 (26.6 %) | 1 (5%) | 1 (5.3%) | 1 (3.8%) |
| <i>Bacillus cereus</i> | 14 (25.0 %) | 1 (9.1 %) | 1 (6.6%) | 3 (15%) | 1 (5.3%) | 6 (23.2%) |
| <i>Bacillus coagulans</i> | | | 1 (6.6%) | - | - | 3 (11.6%) |
| <i>Bacillus circulans</i> | 1 (1.7 %) | - | - | 1 (5%) | - | 4 (15%) |
| <i>Bacillus subtilis</i> | 1 (1.7 %) | - | - | - | - | 1 (3.8%) |
| <i>Bacillus megaterium</i> | - | - | - | - | - | 2 (7.7%) |
| <i>Bacillus sphaericus</i> | - | - | - | - | - | 1 (3.8%) |
| <i>Bacillus mycoidis</i> | - | - | - | - | 1 (5.3%) | 1 (3.8%) |
| <i>Staphylococcus capitis</i> | 1 (1.7 %) | - | 3 (20%) | - | - | - |
| <i>Staphylococcus cohnii</i> | - | - | 1 (6.6%) | 1 (5%) | 2 (10.4%) | - |
| <i>Staphylococcus intermedius</i> | 1 (1.7 %) | - | 1 (6.6%) | 1 (5%) | - | - |
| <i>Staphylococcus kloosii</i> | 1 (1.7 %) | - | - | 1 (5%) | 1 (5.3%) | 1 (3.8%) |
| <i>Staphylococcus lentus</i> | - | - | - | - | 1 (5.3%) | - |
| <i>Staphylococcus caseolyticus</i> | - | - | - | 1 (5%) | 1 (5.3%) | 1 (3.8%) |
| <i>Staphylococcus warner</i> | - | - | - | - | 1 (5.3%) | - |
| <i>Staphylococcus smian</i> | - | - | - | - | 1 (5.3%) | - |
| <i>Staphylococcus haemolyticus</i> | - | - | - | 1 (5%) | - | - |
| <i>Staphylococcus epidermidis</i> | - | - | - | 1 (5%) | 1 (5.3%) | - |
| <i>Staphylococcus sacchorolyticu</i> | - | - | - | 1 (5%) | - | - |

Table (5) Cont.: Isolates of G+ve bacteria from different samples collected from small milk producing unites in Omdurman:

| Isolated bacteria | Lactating cow's milk | Bulk tank milk | milkers Hand's | Bulk tank swab | Water | Environment |
|-----------------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|--------------|--------------------|
| <i>Staphylococcus caprae</i> | 1 (1.7 %) | 1 (9.1 %) | - | - | 1 (5.3%) | - |
| <i>Staphylococcus paratyphi A</i> | 1 (1.7 %) | - | - | - | - | - |
| <i>Staphylococcus aureus</i> | 3 (5.0 %) | - | - | - | - | - |
| <i>Staphylococcus auriculanu</i> | 2 (3.5 %) | - | - | - | - | - |
| <i>Micrococcus roseus</i> | 1 (1.7 %) | | 1 (6.6%) | 1 (5%) | - | - |
| <i>Micrococcus varians</i> | 1 (1.7 %) | 1 (9.1 %) | - | 1 (5%) | - | 1 (3.8%) |
| <i>Micrococcus luteus</i> | - | - | - | 2 (10%) | 1 (5.3%) | - |
| <i>Micrococcus kristinae</i> | - | - | - | - | - | 1 (3.8%) |
| <i>Corynebacterum dipheriae</i> | 1 (1.7 %) | - | - | 1 (5%) | - | - |
| <i>Aerococcus pediococcus</i> | - | - | - | 1 (5%) | - | 2 (7.7%) |

Table (6): Isolates of G–ve bacteria from different samples collected from small milk producing unites in Omdurman:

| Isolated bacteria | Lactating cow's milk | Bulk tank milk | Bulk tank milk | Bulk tank swab | Water | Environment |
|--|-----------------------------|-----------------------|-----------------------|-----------------------|--------------|--------------------|
| <i>Vibrio furnissii</i> | 3 (5.0 %) | - | - | - | - | - |
| <i>Bordetella parapertussis</i> | 1 (1.7 %) | - | - | 1 (5%) | - | - |
| <i>Bordetella pertussis</i> | 3 (5.0 %) | 1 (9.1 %) | 1 (9.1 %) | - | 1 (5.3%) | 1 (3.8%) |
| <i>Providencia sturattii</i> | - | - | - | 1 (5%) | 1 (5.3%) | - |
| <i>Providencia denciaalcalifaciens</i> | - | - | - | - | 1 (5.3%) | - |
| <i>Kingella kinga</i> | - | - | - | - | 1 (5.3%) | - |
| <i>Citrobacter koseri</i> | 3 (5.0 %) | 3 (27.2 %) | 3 (27.2 %) | - | - | - |
| <i>Citrobacter freundii</i> | 2 (3.5 %) | - | - | - | 1 (5.3%) | - |
| <i>Serratia plymuthica</i> | 4 (6.9 %) | 1 (9.1 %) | 1 (9.1 %) | - | - | - |
| <i>Escherichia coli</i> | 3 (5.0 %) | 1 (9.1 %) | 1 (9.1 %) | - | - | - |
| <i>Proteus mirabilis</i> | 2 (3.5 %) | - | - | - | - | - |
| <i>Shewanella putrefaciens</i> | 2 (3.5 %) | - | - | - | - | - |
| <i>Klebsiella pneumoniae</i> | 2 (3.5 %) | 1 (9.1 %) | 1 (9.1 %) | - | - | - |
| <i>Haemophylus haemolyticus</i> | 1 (1.7 %) | 1 (9.1 %) | 1 (9.1 %) | - | - | - |
| <i>Salmonella arizona</i> | 1 (1.7 %) | - | - | - | - | - |



Figure: (1) *B. cereus* on blood agar after 24 hours incubation.

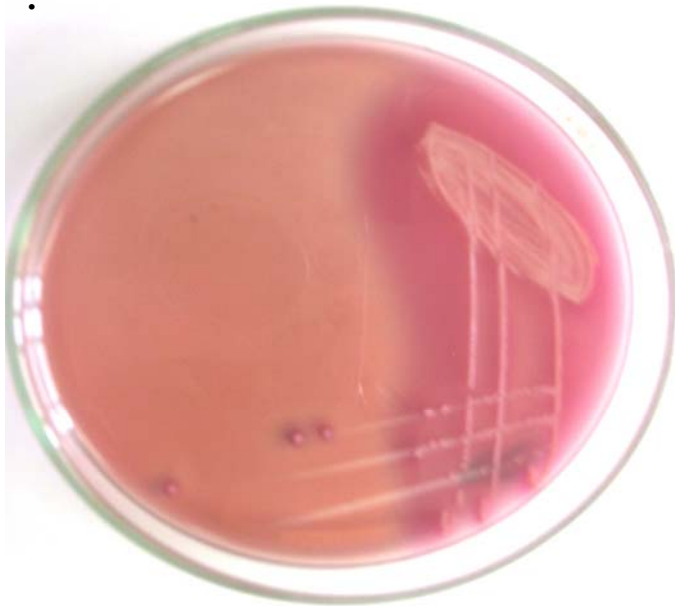


Figure: (2) *E. coli* on MacConkey agar after 24 hours incubation.



Figure: (3) *Klebsiella pneumoniae* on MacConkey agar after 24 hours incubation.



Figure: (4) *S. aureus* on blood agar after 24 hours incubation.



Figure: (5) *S. caprae* on blood agar after 24 hours incubation.

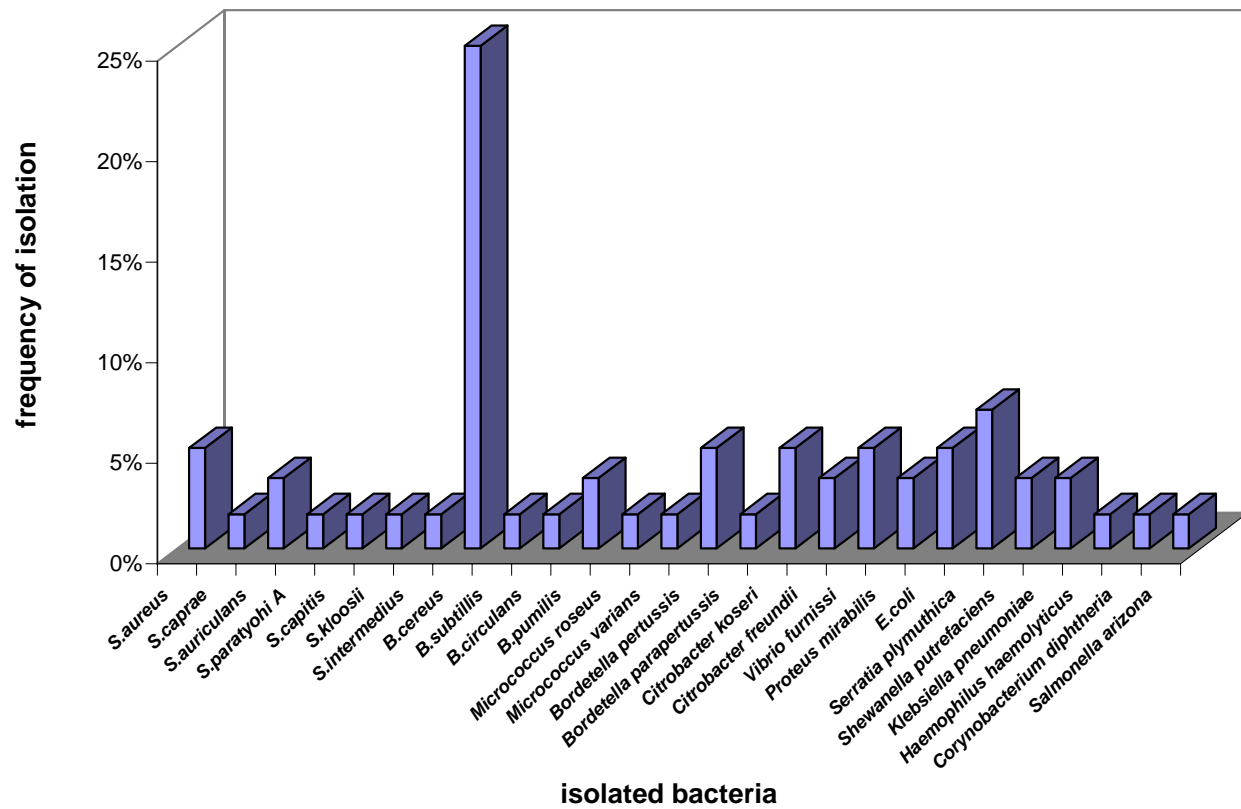


Fig (6) Frequency of bacterial isolates from cow's milk samples

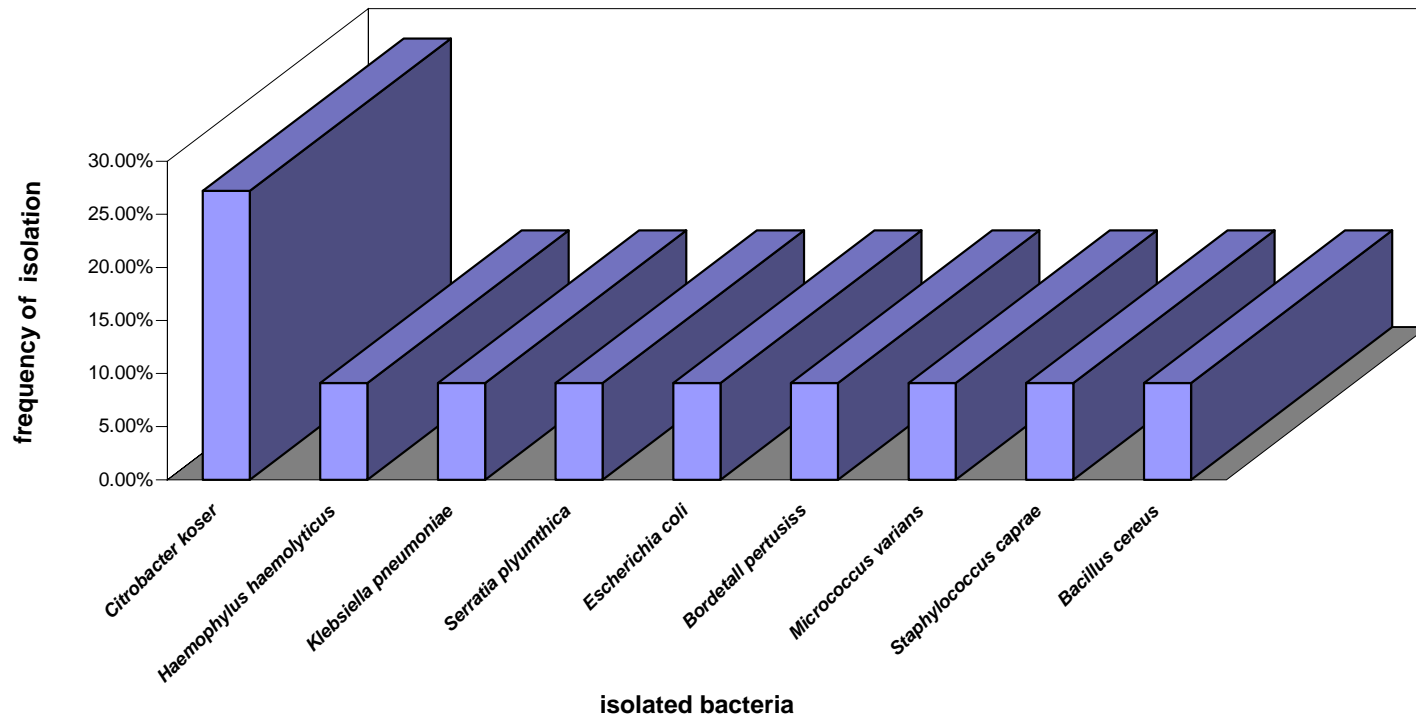


Fig (7) Frequency of bacterial isolates from bulk tank milk samples

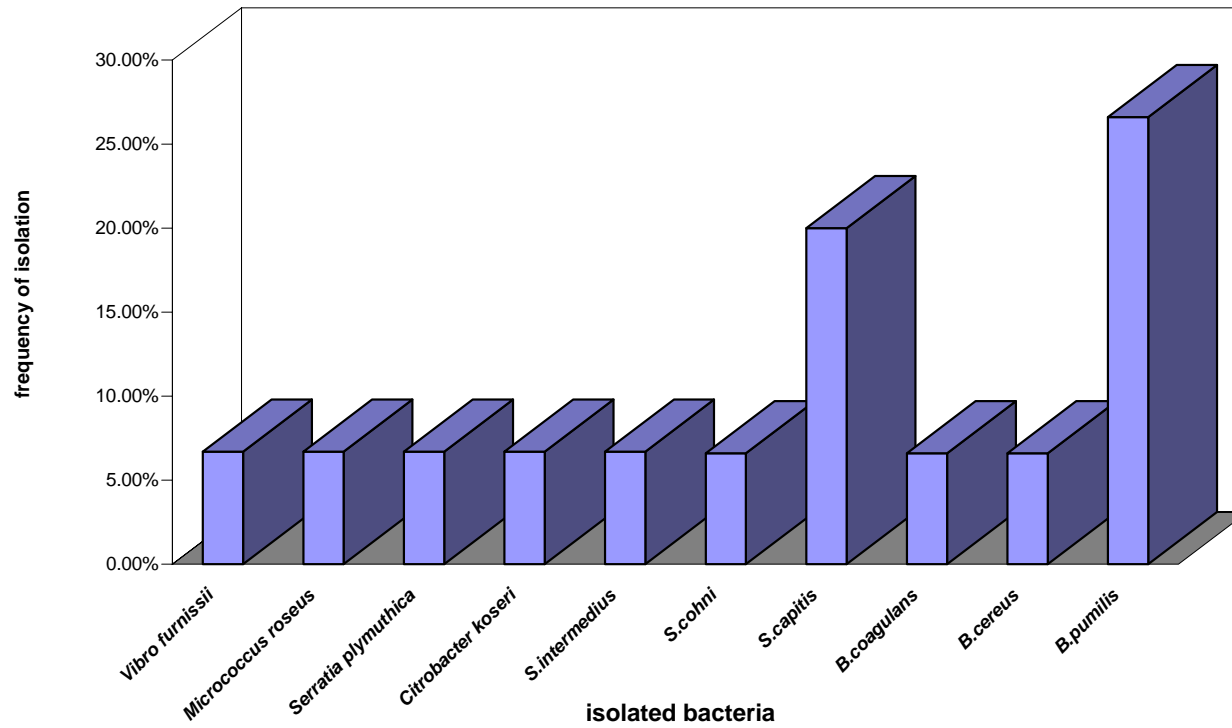


Fig (8) Frequency of bacterial isolates from milkers hand's samples

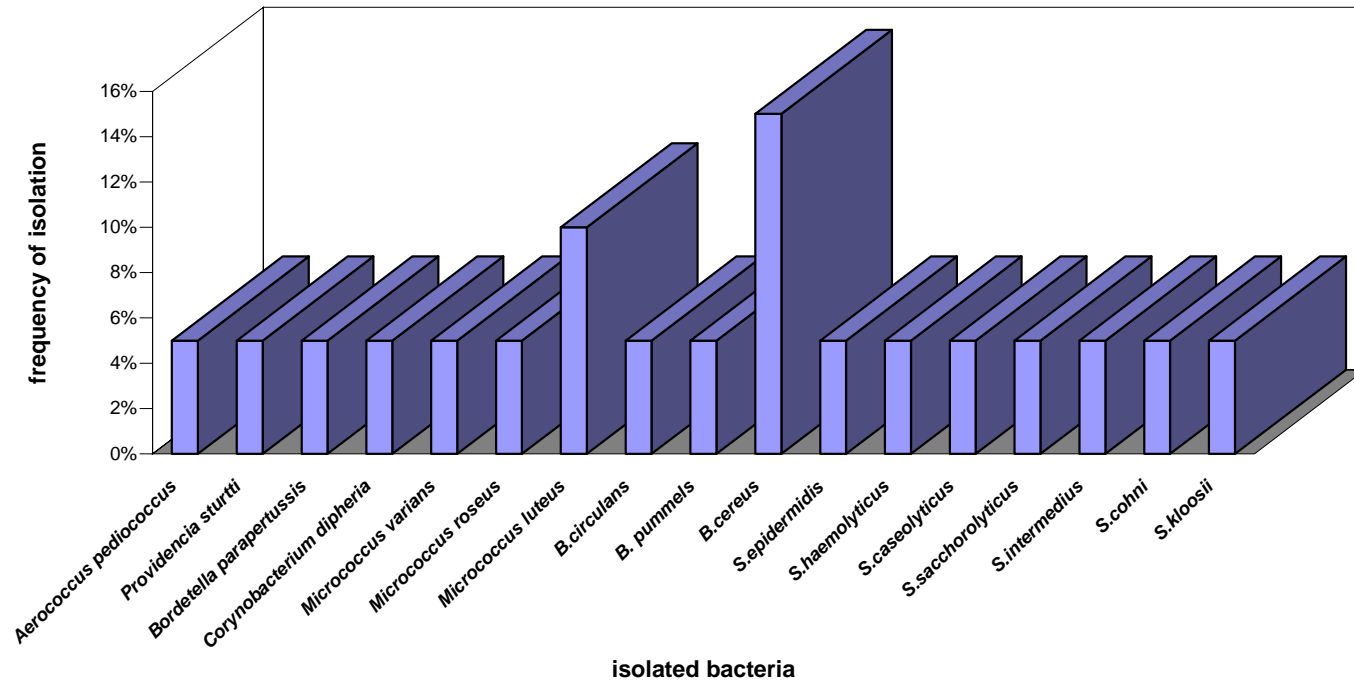


Fig (9) Frequency of bacterial isolates from bulk tank swab samples

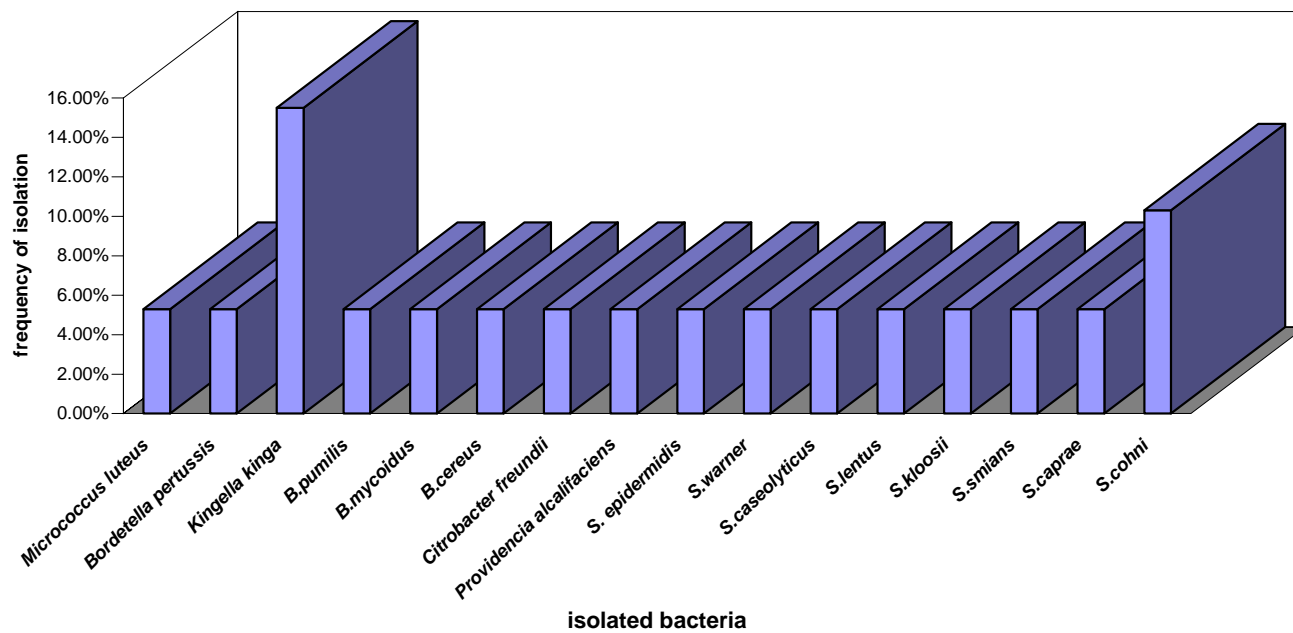


Fig (10) Frequency of bacterial isolates from water samples

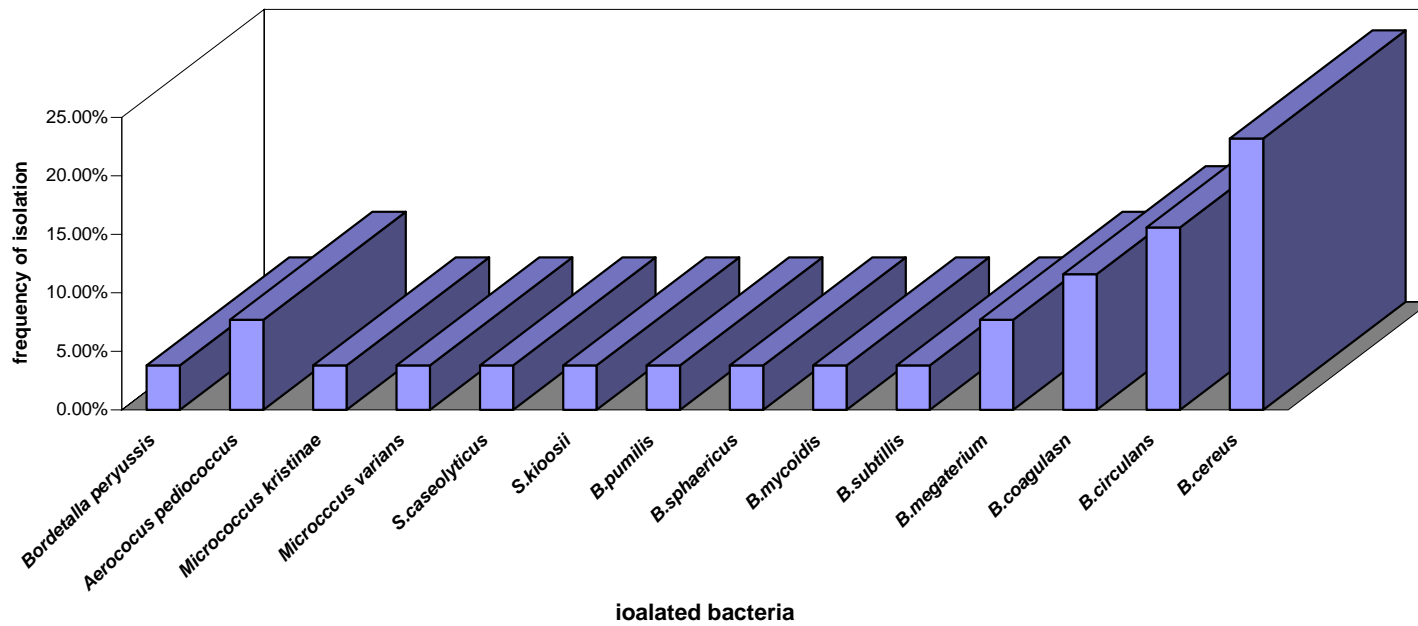


Fig (11) Frequency of bacterial isolates from environment samples

DISCUSSION

Milk is an excellent food that provides the public with nutrients in palatable and digestible form. Bacterial contamination of such food may result in the transmission of life-threatening diseases including tuberculosis, Brucellosis and enteric fevers. In addition, bacterial contamination results in the loss of valuable nutrients in milk, hence downgrading its nutritive value.

The present study was carried out in small milk-producing units in Omdurman, Khartoum state as these units provide milk to a large percentage of people.

The results of total plate count were good compared to those of previous studies [Ibrahim (1973); Mustafa and Idris (1975); Mohammed (1988); Ali (1988) and Nahid (2004)].

The mean total bacterial count of all milk samples ranged between 0.49×10^5 cfu/ml to 0.36×10^5 cfu/ml. This was in contrast to the finding of Mohammed (1988) who examined 290 samples and found that 54.4% of samples contained between 5.0×10^5 to 5.0×10^6 cfu/ml. Our finding was also disagreed with Ali (1988) who investigated milk samples from KuKu and Gezira dairy plant who found that the mean bacterial count was 3.4×10^6 cfu/ml and 4.4×10^5 cfu/ml respectively.

As Sudan is a tropical country, the mean total count of all milk samples were assessed for quality using the tropical standard. Accordingly, the investigated milk samples were classified as good (containing mean total bacterial count less than 5.0×10^5 cfu/ml). When we graded the investigated milk samples according to U. S. A. standard (Welfare, 1953), 19.4% of milk samples fall in grade A ($\leq 2.0 \times 10^4$ cfu/ml.), 80.4% of milk samples fall in grade B (between 2.0×10^4 cfu/ml to

1.0 ×10⁶ cfu /ml.) and no milk samples were graded in grade C (≥1.0 ×10⁶ cfu /ml).

The milk ring test results revealed that 47.3% of the samples were positive for brucella antibodies. This result may need more investigation by other confirmatory tests. However, the risk of transmitting Brucellosis to human will be much reduced when milk is properly pasteurized.

Most isolated Gram positive bacteria belonged to the genera *Staphylococcus* and *Bacillus*. In general, farm environments represent the most possible source of contamination with the members of the genus *Bacillus* especially *Bacillus cereus* being the most frequent (table 5). The most possible source of contamination of milk with *Staphylococcus spp.* were principally water and milkers hands table (2). The later represent the only source of milk contamination with *Staphylococcus capitis*. Although bulk tanks contained different *Staphylococcus spp.* but the possible original source may be water which was used for cleaning of these tanks.

Other isolated Gram positive bacteria (*Micrococcus*, *Corynebacterium* and *Aerococcus*) appeared to originate from different milk contaminating sources investigated in this study.

Different Gram negative bacteria were isolated from milk samples. The most frequently isolated bacteria belonged to the members of the family *Enterobacteriaceae*. This finding agreed with that of Well's *et al.* (2001). Milkers hands and water represent the most possible source of contaminant of milk with Gram negative bacteria. The environments of these farms appear to play a minor role as a source of contamination of milk with Gram negative bacteria.

Many isolated Gram negative bacteria were isolated from milk samples and not from sources of contamination investigated in this

study. This finding does not neglect the role of the previous investigated sources in contamination of milk due to the fact that few samples were taken from these sources in this study. In addition most of the isolated bacteria belonged to *entrobacteria* group which originate principally from animals manure which was abundant on animal bedding.

Conclusions and Recommendations

Conclusions:

- Although the hygienic measures were not properly established in small milk producing units, the produced milk is of good quality according to the tropical standard.
- Initial results revealed the high percentage of *Brucella* antibodies (47.3% of samples).
- *Bacillus spp.* especially *Bacillus cereus*, were the most frequent bacteria which contaminate milk in small milk producing units followed by *Staphylococcus spp.* in Omduram, Khartoum state.
- The most possible source which contaminates milk with *Bacillus spp.* was the environment whereas the most possible source which contaminate milk with *Staphylococcus spp.* were water and milkers hands respectively.

Recommendations:

The present study draws the following recommendations:

- 1- Milk must be produced, distributed, handled and marketed under the control of public health authority which must have a sanitary inspector and dairy specialist to enforce its methods and standards.
- 2- Employees in farm should be inspected at periodical intervals and they must be free from communicable diseases.
- 3- Since brucellosis is an important zoonotic diseases, control program must be established in farms to eliminate positive reactors.

- 4- Milk should be cooled immediately after milking, during transportation and storage to eliminate growth and multiplication of contamination microorganisms.
- 5- Sanitary standard in particular should be established in Sudan to control milk production and marketing. Cooling and storage equipment should be properly sterilized and guarded against contamination from air, water and human contact.

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