The effect of management practices on the hygienic quality of raw milk produced by some dairy Farms in Khartoum State

By

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بسم الله الرحمن الرحيم

قال تعالي

وإِنَّ لَكُمْ فِي الأَنْعَامِ لَعِبْرَةً نُّسْقِيكُم مِّمَّا فِي بُطُونِهِ
مِن بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا خَالِصًا سَآئِغًا لِلشَّارِبِين

صدق الله العظيم

الآية 66 – سورة النحل

DEDICATION

To my parent, Brothers,

Husband, Uncle

And

To all whom I love.

With my respect

Mahboba

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First of all, praise be to Allah the Almighty who gave me the health, strength and patience to complete this work.

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ABSTRACT

This study includes current dairy management and husbandry practices in 60 dairy farms at different locations in Khartoum, Khartoum North and Omdurman of Khartoum State (20 farms from each town), to evaluate their practices of dairy farming. It was also aimed to know the constrains and to identify the weakness that need correction. Information about dairy management was collected by questionnaire and direct interview with farms' owners. Moreover, this study was also aimed to evaluate the quality of raw milk produced by these farms. To achieve the objective of hygiene, 120 milk samples were collected from the selected 60 dairy farms and tested during summer and winter seasons. The study of chemical composition (total solids, lactose, protein, fat, ash, acidity, freezing point, temperature and pH) and some microbial hazards (*Brucella , E. coli, Staphylococcus aureus, Staphylococcus spp.* and *Salmonella spp.*), were carried out. Laboratory pasteurization counts, coliforms counts, Enterobacteriaceae counts and total bacteria counts associated with raw milk were estimated. Enumeration, isolation and identification of *S. aureus*, *E. coli*, *Salmonella spp.* and the presence of Brucella were also estimated.

The results indicated that there were significant differences in education levels of dairy farms' owners. Khartoum dairy farms' owners' exhibit higher illiteracy level (35%), Khartoum North (15%) and Omdurman (30%). Significant differences ($P < 0.05$) were obtained in herd size and herd structure. Moreover, dairy herd numbers were 170.25± 72.83, 123.10± 105.71 and 92.35± 29.23 in Khartoum North, Khartoum and Omdurman, respectively. Breed type revealed non-significant differences

between the three cities. However, 92% of the cattle in the studied farms were grade cattle and mainly they were of unknown foreign blood percentages.

Ideal building materials, health set up, design and different management practices rarely practiced in Khartoum dairy farms and they were only restricted to 9 farms (45%) in Khartoum and 6 farms (30%) in Khartoum North. Corrugated iron roof and concrete flour were reported to be very rare. The same was true for using machine milking and mastitis prevention practices such as strip cup dip and teat dipping as they were reported among three of the studied farms. Concerning the health services and preventive measures, the diseases control were not satisfactory, only 10% of the studied dairy farms had resident veterinarian. Vaccinations against diseases were rarely used in regular way. General hygiene and sanitation measures such as dung removal, disinfection, cleaning program and maintaining minimal contamination during milking process could not be observed in the majority of dairy farms; studied except for few farms in Khartoum (20%) and Khartoum North (10%). Also disposal of abnormal milk were applied directly in the pens in 83% of the farms. Moreover, testing, isolation and culling practices were not common. The highest numbers of aborted cows at late pregnancy were showed in 1-16 dairy farms in Khartoum. Similarly mastitis, which was found in 14 farms, in Khartoum and the cases ranged from 1–5 cases.

On the other hand laboratory tests were carried out for milk samples obtanied from the dairy farms. The results indicated that there were significant differences in temperature and freezing point ($P< 0.05$) between seasons. The result of fat, protein and ash content of milk samples revealed

non-significant differences (P> 0.05) between seasons and between cities. Lactose was found to be significant (P< 0.01) when comparing the interaction between seasons and cities. Total solids content of milk samples revealed significant differences (P< 0.01) between cities and non-significant differences (P< 0.05) between seasons and their interaction..

Brucella antibodies were detected in 104 out of 120 milk samples. Fifty (83.33%) and 54 (90%) of the milk samples were positive during summer season and winter, respectively.

Highly significant variations (P< 0.01) were reported for the milk samples collected from dairy farms in Khartoum for means of acidity, total bacterial counts, laboratory pasteurization counts, *Staphylococcus spp.* counts, Enterobacteriaceae counts and coliforms counts were compared for the three cities. However, highly significant differences (P< 0.05) were found in total solids of milk from Omdurman's dairy farms than other two cities.

بسم الله الرحمن الرحيم

خلاصة الأطروحة

تم مسح ستون مزرعة ألبان في مدن ولاية الخرطوم الثلاثة الخرطوم، الخرطوم بحرى وام درمان (20 مزرعة من كل مدينة) في محاولة لمعرفة نقاط الضعف في مزارع الألبان حتى تتم معالجتها وأعتمد في هذه الدراسة أسلوب الاستبيان المكتوب والمقابلات المباشرة خلال الزيارات الميدانية. أيضاً تمت دراسة لضبط الجودة الصحية للألبان الطازجة المنتجة بواسطة هذه المزارع إذ تم تجميع 120 عينة منها 60 في فصل الصيف والبقية خلال فصل الشتاء وتم فحصها معملياً لمعرفة التركيب الكيميائي والعدد البكتيري والتصنيف لبعض أنواع البكتريا الممرضة للإنسان والمؤدية للفساد و التي تشمل:ً

total bacterial counts, laboratory pasteurization counts, *Staphylococcus spp.* counts, *Salmonella spp.* counts, Enterobacteriaceae counts, coliform counts and *E. coli* counts.

تم عد وعزل والتعرف على بكتريا الاسترشيا القولونية والعنقودية الدهبية والسالمونيلا.كدلك تم الكشف على وجود البروسلا فى عينات اللبن. وشملت التحاليل الكيميائية للعينات إيجاد نسب الدهون، البروتين، اللاكتوز، الرماد، المواد الصلبة ودرجة الحموضة، الأس الهيدروجيني، نقطة التجمد ودرجة الحرارة.

لوحظ وجود فرق معنوي في المستوى التعليمي لأصحاب المزارع ($P< 0.01$) اد وجد ارتفاع فى نسبة الأمية لأصحاب المزارع التابعة لمدينة الخرطوم (35%) بينما بلغت في الخرطوم بحري وأم درمان 15% و 30%على التوالي .وقد لوحظ أيضاً عند مقارنة مزارع المدن الثلاث وجود فروقات معنوية ($P< 0.05$) في أعداد الأبقار الحلوب والجافة والعجلات البكر . وجد أن مزارع الخرطوم بحري تتميز بكبر حجم القطيع حيث وجد أن متوسط العدد الكلي للأبقار 72.83±170.25 بينما أقل عدد كان في مزارع أم درمان وهو يمثل 92.35± 29.35.

لم يتم رصد أي فرق معنوي في السلالات الموجودة في مزارع المدن الثلاثة وأتضح من الدراسة أن 92%

من العدد الكلي للأبقار هجين حيث لا تعرف نسبة الدم الأجنبي

التصميم المثالي والجيد للمباني ورعاية الأبقار والطرق المثلي لإدارة القطيع على أهميتها تمارس على نطاق ضيق للغاية بمزارع الألبان التي تمت دراستها وغالباً ما تمارس في قليل جداً من مزارع الخرطوم (45%) والخرطوم بحري (30%). أيضا تفتقر المباني إلي الأرضيات الثابتة والمبلطة والمظلات التي تعطي ظلاً شاملاً ومريحاً كالمصنوع من الزنك مثلاً. تستخدم ماكينات الحليب ووسائل الوقاية من التهاب الضرع في 3.3% مزارع الولاية.

أتضح من الدراسة أن غالبيه المزارع لا تخضع للإشراف البيطري المباشر وإن الوقاية والرعاية البيطرية ضعيفة جداً بينما يمارس الإشراف البيطري فى 10% من المزارع التي شملتها الدر اسه اذ تستخدم اللقاحات بطريقة غير منتظمة و لا يوجد برنامج للنظافة خصوصاً فيما يتعلق بمكان الحليب والأبقار والأبنية ويتم حلب الأبقار المصابة بالتهاب الضرع في أرضية الحظائر في 83% من المزارع موضع الدراسة. أيضاً هنالك عشرة مزارع أبقارها ا حالات إجهاض في اية مرحلة الحمل في مزارع ولاية الخرطوم حيث تتراوح أعدادها من حالة واحدة إلى ستة عشر حالة. أما التهاب الضرع فيتراوح من حالة إلى خمس حالات في المزرعة الواحدة في المزارع التابعة لمدينة الخرطوم.

بعد التحليل المعملي لعينات اللبن بلغت نسبة العينات الموجبة لاختبارات البروسيلا من جميع عينات اللبن 104 حالة .منها خمسون (83.33%) وجدت خلال فترة الصيف واربعه وخمسون (90%) في فصل الشتاء .

اختلفت نسبة درجة الحرارة ونقطة التجمد للعينات البن اختلافا معنوياً ($P< 0.01$) في الموسم والتداخل بين المواسم ، ولم يتم رصد أي فرق معنوي ($P> 0.05$) بين المدن الثلاثة وقد لوحظ أيضاً عند مقارنة الموسم والمدن الثلاث بأنه لا يوجد فرق معنوي ($P > 0.05$) في نسب كل من الدهن، البروتين والرماد .وجد فرق معنوي ($P < 0.01$) في نسبة اللاكتوز بسبب التداخل في الموسم واختلاف المدن بينما لم يرصد أي فروقات معنوية ($P > 0.05$) في الموسم والمدن. لوحظ وجود فروقات معنوية فى نسبة اللاكتوز لعينات البن ($P < 0.01$) فى نسب المواد الصلبة الكلية بين المدن الثلاث ولم يرصد أي فرق معنوي ($P>0.05$) باختلاف الموسم والتداخل في المواسم و المدن.

عند مقارنةالمتوسطات للألبان ا جمعة من مزارع المدن الثلاث وجدت فروقات معنويه ($P < 0.001$) لعدد البكتريا الكلى، laboratory pasteurization counts، *Staphylococcus spp.* counts, Enterobacteriaceae counts، coliform counts, ودرجة الحموضة في مزارع الابقار فى مدينة الخرطوم و كما لوحظ أيضاً ان وجود فرق معنوي ($P < 0.05$) في نسب المواد الصلبة في عينات البن التى تم تجميعها من مزارع أم درمان عند مقارنة المتوسطات.

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CHAPTER ONE

INTRODUCTION

The use of milk products as human food has got a very long history (Teuvo, 2000). Milk is the most complete single food for off spring of mammals and can be the sole source of food for nomads who live exclusively on milk for months (Kon, 1972). Milk is the only food that provides well balanced essential nutrients including proteins, fats, carbohydrates, vitamins and minerals in the form which is palatable, digestible and sanitary (Kordlylas, 1991). He also reported that milk thus deserves recognition as a whole meal and "perfect food" and man consumes various types of milk such as cow's, goat's, sheep's, mare's and reindeer's milk. In recent years the demand for liquid milk increased tremendously world wide due to the increase of the population growth.

Milk is synthesized in specialized cells of mammary gland and is virtually sterile when secreted into alveoli of the udder (Tolle, 1980). Beyond this stage of milk production microbial contamination generally occurs from three main sources, from within the udder, the exterior of the udder and from the milk handlers and equipment (Murphy and Boor, 2000). Milk as a perishable product, is also an ideal media for microorganisms and as it is a liquid is very easily contaminated and invaded by bacteria (IDF, 1994a). The health and hygiene of the cow, the environment in which the cow housed and milked and hygiene during milking and storage equipment, all influence microbial numbers in raw milk (Murphy and Boor, 2000). Thus milk can transmit diseases of microbial origin to the people from sick animals and/or people carrying certain diseases and contaminating the milk with pathogenic bacteria during its handling (Teuvo, 2000).

Assurance of the quality of dairy products begins at the farms and ends in the hands of the consumers (Murphy and Boor, 2000). They also reported that raw milk must meet other quality standards, including freedom from drugs residues, added water, sediments, contaminants and other abnormalities.

In Sudan most of important milk producing areas have no rigid systems of inspection on the farms and are not complying with sanitary standards, subsequently most of the products of these farms are sold through venders and groceries. Thus, there is a need for up- to- date sound information based on scientific data on the health, composition and safety measures of milk. Hence this study was conducted to evaluate raw milk produced by some big dairy cow's farms in Khartoum State with the following objectives:

1. To compare the management practices between the farms in Khartoum State.
2. To evaluate and compare the effect of season (summer and winter) on the quality (physical, chemical and microbial) of raw milk.
3. To study the hygienic quality (bacterial load and the presence of some pathogenic microorganism) of raw milk in Khartoum State.

CHAPTER TWO

LITERATURE REVIEW

2.1. Importance of milk

Milk is one of the oldest foods known to man and it is defined as the physiological secretion from the mammary gland of mammals (Nickerson, 1999). Fluid milk is unique among perishable foods in its combination of bulk water (87%) and nutrients (IDF, 1994a).

Milk is a complex mixture of fats, proteins, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water (Harding, 1999). Arora and Gupta (1969) reported that milk proteins furnish a favourable balance of essential amino acids and milk fat has a special significance in nutrition due to its content of essential fatty acids. They also added that there is a tendency to have more protein and solid not fat in milk.

Kordylas (1991) reported that milk protein consists mainly of casein with few other protein fractions such as lactalbumin and lactoglobulin. He reported that milk carbohydrate (lactose) is superior to other sugars especially for infant feeding because it prevents intestinal putrefaction by encouraging growth of acid- producing bacteria in the stomach, in addition sugar affects absorption of minerals such as calcium and phosphorus. He concluded that milk provides fat which contains high proportion of short-chain fatty acids. Milk and dairy products are outstanding sources of calcium, good source of phosphorous, potassium and many trace minerals and fresh whole milk is a valulable source of vitamin A, riboflavin, thiamin and other B vitamins (Payne, 1990). He also reported that milk is an important source of vitamin C in dry and arid areas where camel's milk is consumed and no vegetables are grown.

Kon (1972) stated that 500 ml of cow's milk contain 4.5% fat which provides 25% calories, 40% protein, 70% calcium and riboflavin and 30% vitamin A. Milk is fairly low- calorie food so it is a relatively expensive source of energy, however high- fat in milk of tropical breeds is a very important part in people's diet and it is very easily digested and is necessary for calcium absorption (Payne, 1990).

2. 2.Factors affecting milk composition

Synthetic and secretory tissues of the mammary gland, the initiation and establishment of lactation, the milk ejection reflex, the breeds and genetic factors, the nutrition, the environment and the milking management practices, all these factors have important effect on milk composition and quality (Nickerson, 1999).

White *et al.* (2001) found potentially important differences in fatty acids composition of milk from cows consuming a warm season pasture species compared with milk from cows consuming a total mixed ration, as well as differences between Holstein and Jersey breeds. Moreover, Auldist *et al.* (1996) reported that the effect of stage of lactation was magnified by an elevated bulk milk cell count and that many of the problems encountered when processing late season milk.

2.2.1. Season

Seasonal effects on milk composition are highly attributed to extremes in environmental temperatures (Nickerson, 1999). He also reported that the consumption of roughage is reduced during environmental heat stress, resulting in decreased milk production as well as on percentage of fat. Moreover, he reported that milk protein and lactose percentage are lower during warm season.

Auldist *et al.* (1998) reported increases in concentrations of many milk components (e.g. total protein, fat, casein, and whey protein) when

lactation progressed. Moreover they reported that the extent of these increases depend on the time of the year. However, Nickerson (1999) reported that the differences in milk composition between seasons may average 0.4% fat and 0.2% protein and cows that calved during the cooler season exhibits greater percentage of fat and solid not fat than cows that calved during warm season.

In Korea, Lee *et al.* (1985) reported that the contents of both fat and protein were highest in winter (3.59% and 3.54%, respectively) and were lowest in summer (3.28% and 3.34%, respectively). Also Hussein (1985) in Sudan working on the bulk milk supplied to Gezira Cooperative found that the fat content ranged from 4.63% to 5.26% (mean 5.06± 0.21). He also found that the fat content was 5.0% during January followed by a downwards trend to 4.75% during March. Then it showed temporary increase to 5.04% during April, then decreased to 4.63% during May and it increases to 5.26% during September, but showed a slight decrease to 5.24% during September. On the other hand Ballou *et al.* (1995) found that plasmin activities and psychotrophic bacteria counts were high from December to May.

2.2.2. Management

Milking interval, milking rate, frequency of milking, milking routine and cow preparation and residual milk were important factors that affected milking management practices (Nickerson, 1999).

Kalis *et al*. (2001) found that management practices were different between herds that were vaccinated and herds that were not. They also found that the owners of herds which were not vaccinated followed more preventive management procedures and practiced less feeding of raw milk to calves. They concluded that vaccination of calves with killed vaccine

does not prevent transmission of some diseases and therefore, hygienic practices remain essential in herd management.

To increase the protective properties of colostrums in dairy herd dry cow will be vaccinated (Roy, 1980) in order to reduce calf infection (Smith, 1977). Thomas *et al.* (1995) reported that the risk of Q fever on livestock farms is related to contact with the farm environment rather than any specific animal exposure; the absence of the increasing prevalence with age suggests that exposure may occur as clusters in space and time (outbreaks).

Wilson *et al*. (1993) mentioned that infected cows with paratuberculosis were culled from the herd at a faster rate than were paratuberculosis negative herd. Therefore, paratuberculosis was associated with financial loss attribute to reduce milk production and increased culling of infected cows. On the other hand Sischo *et al.* (1997) reported a lack of adequate treatment records as being the highest risk factor for antibiotics residues followed by deficiencies in understanding how to use antibiotics and poor relationships between veterinarians and their clients.

Tarabla and Dodd (1990) stressed the importance of the human factors in explaining variations in farm performance. According to Karib (1962) dairy farms must be under supervision of veterinarians and dairy herd throughout the country must be examined for diseases such as tuberculosis. It is also important that milkers and milkmen should be tested for health supervision for carrier of the diseases such as typhoid and tuberculosis (Ibrahim, 1969). Mastitis routine testing is very important because most of mastitis infection persist as subclinical, which will not be detected by stockmen (FAO, 1978 and Mohamed *et al*., 1993).

Testing is also superior to other diagnostic methods in cases where John's disease is suspected (Abu baker and Elsanousi, 1976). However,

due to the lack of diagnostic laboratories in most of tropical countries diagnosis was made depending on clinical signs (Pharo, 1987). On the other hand culling is practice due to low milk yield rather than to the specific diseases occurace (Allaire, 1981). Moreover, culling is practiced in order to reduce the opportunity of low yielder to stay in the herd' (Westel *et al*., 1982 and El amin and El Zubeir, 2002).

Goldberg *et al.* (1991) reported that the effect of improved management practices may be suppressed by insufficient hygiene prior to milking. However, they concluded that managing cows on pasture may help to reduce exposure to environmental pathogens.

2.3. Milk quality

High quality milk starts with a healthy cow in a clean sanitary environment, which demands overall good management programs (Murphy and Boor, 2000). Visible dirt from the udder must be removed before milking using clean water and disinfectant (sodium hypchlorite) and because cow's udder's external surfaces were usually contaminated even when they appear clean (Natzke, 1981). Similarly Nasri (1966) reported that to reduce or to minimize the chance of contact between the host and the infective agent, adequate cleaning and disinfection are the effective and cheapest way to prevent diseases and their spread. Buildings that could not be adequately cleaned or disinfected may cause many health problems (Sanisbury, 1970). For health supervision, the dairy farmer must build shade which should be easily cleaned and protected from winds and dust (Ibrahim, 1969). Daily removal of dung is known to be effective in prevention of nematode manifestation (Streat, 1979). Also other pathogens in urine and faeces may be reduced from animal environment through the removal of cattle excrete (Jone, 1980).

Hunderson (1971) stated that bacteria may get entry to the milk via the udder, other sources are the body of animal, utensils, milkers hands and milking machine, milk plant equipment and employees. Robert (1985) mentioned that in many countries specifications are made for all utensils that are used for milk production.

Murphy and Boor (2000) showed that pathogens present in raw milk originate from various sources which include infected udders of cows suffering from mastitis, cow faeces, urine and uterine secretions, equipment, contaminated water in addition to milkers hands and noses.

2.3.1. Mastitis and its effect on the quality of milk

Mastitis is one of the major problems of dairy industry not only because it causes loss of milk production and valuable cows, but is potentially dangerous to the health of human being (Mohamed *et al*., 1993; IDF, 1994a and Mohamed *et al.*, 1995).

Philpot (1972) showed that for every individual case of clinical mastitis in a herd, there is usually 25–50 subclinical cases which constitute reservoirs of organisms that may prompt new infection, reduce milk production and lower the quality of milk. Several agents are known to be incriminated in mastitis; these include *Staphylococcus aureus*, S. *epidermidis*, *Streptococcus spp*. and *Corynebacterium spp.* (Mohamed *et al.*, 1993). Moreover, Mohamed *et al.* (1997) found 174 isolates which identified as: 41 (23.56%) were *S. aureus*, 25 (14.34%) were *S. epidermids*, 15 (8.26%) were *Klebsiella aerogenes*, 11 (6.3%) were *Escherichia coli*, 17 (9.77%) were *Actinomyces pyogenes*, 12 (6.89%) were *Bacillus cereus*, 8 (4.59%) were *B. subtilis*, 4 (2.29%) were *Bacillus spp.*, 13 (7.47%) were *Acinetobacter antratus*, 22 (12.4%) were micrococci and 6 (3.4%) were yeast. Mastitis is well known to influence the composition of milk by varying the concentration of its constituents (Mernyi and Wagner, 1986;

Vianni and Nader, 1991 and Mohamed *et al.*, 1998). Mastitic milk is un suitable for human consumption, due to reduced nutritive value (Kitchen, 1981; Mohamed *et al.*, 1997 and Mohamed *et al.*, 1999).

2.3.2. Measurement of milk quality

2.3.2.1 Physical properties of milk

2.3.2.1.1 Freezing point

The average freezing point of normal milk is about -0.555º C below the freezing point of water (Harding, 1999). He also reported that the average freezing point of milk is -0.543. Similarly, Mohammedi (1988) mentioned that 90.3% of the samples collected from Khartoum North had freezing point of -0.5583± 0.0112º C. He found that the average of freezing point of samples collected from Elsagana, Burri and Omdurman were -0.5565± 0.0148, 0.556± 0.0111 and -0.558± 0.0123º C, respectively. He also reported that the freezing point of the milk of the herd of the University of Khartoum ranged from –0.541 to -0.569º C with average of –0.5525± 0.0071º C. However, Binder (1975) mentioned that the maximum freezing point of bulk milk was –0.529º C. Luck and Dresners (1975) found that the average freezing point depression of raw milk was –0.537± 0.0086º C. They also mentioned that evening milk appeared always to be of more freezing point (-0.539± 0.0093º C) than morning milk freezing point (-0532± 0.0079º C).

Bartsch and Wickes (1980) reported that the most significant correlation between the freezing point and the milk components was that between the freezing point and SNF content (r= 0.505). Ibrahim (1989) reported that the freezing point of milk from Dairy Land and University of Khartoum Farm were -0.539± 0.009 and -0.548± -0.0115 ºC, respectively. However, Rasmussen *et al.* (2002) reported that freezing point as –0.516º C.

2.3.2.1.2. pH– value

Hunderson (1971) reported that milk must bind to shift the pH from about 6.6 to 8.5. Similarly Kon and Cowie (1961) found that freshly drawn cow's milk has a pH in the mean of 6.6.

Payne (1990) mentioned that acidity is normal in the range of pH 6.5– 6.8. However, Bramley and McKinnon (1984) stated that if the milk tainted or having an acidity above specified limits, it will be down- graded or rejected.

2.3.2.1.3. Temperature

Haj Mahmoud (2002) found that the temperature of milk sold in Khartoum State ranged between 31–39º C. Also she reported that market milk temperature obtained from Elobeid (100 samples of milk) revealed that fifty four samples had a temperature ranging between 28–31º C, 12 samples had temperature ranging between 17–20º C and 34 samples had a range between 21 and 27º C.

Harding (1999) reported that when milk leaves the cow it is virtually free from bacteria and its temperature is about 37ºC. The temperature and duration of storage, the numbers and types of bacteria in the milk and natural inhibitory systems in milk all influence the increase in bacterial numbers which occurs in stored milk (Bramley and McKinnon, 1984). They also reported that temperature is the most important factor which affects milk of rather poor quality having an initial total count of 50000 cfu/ml and they stressed the importance of cooling, if milk is to keep for more than 12 hours and when bacterial count approaches 1×10^{7} cfu/ml adverse effects may results.

2.3.2.2. Chemical composition of milk

2.3.2.2.1. Fat

Fillipov *et al.* (1980) found that there was a seasonal variation in milk fat content (3.88% at autumn and 3.94% during spring). Mohammedi (1988) mentioned that 76.1% of milk samples collected from Khartoum North had fat % in the range of 4.1% to 5.5% and less than 15% showed reduced fat content that ranged from 3.1% to 4.0%.

Ibrahim (1989) reported that fat content of milk samples from Dairy Land and University of Khartoum farms were 3.14%± 0.134% and 4.763%± 0.196%, respectively. Moreover, Hamid (1994) found that the fat content of high and low grade cows in Sudan were 4.28± 0.80 and 4.45%± 0.79%. During summer Ibrahim and Samaha (1986) found that the average milk fat was 3.57%.

Webb *et al.* (1980) found that average milk fat percentage of temperate breeds was 3.7%. However, milk of local Zebu crossed with exotic breeds like Friesian has milk fat of 4.7% (Hussein, 1985). Harding (1999) found that milk fat was 3.9 %, while Ballou *et al.* (1995) mentioned that mean milk fat was 3.73 %.

Walstra (1992) reported that fat content range from 3.7% to 4.4 %. Also Casper *et al.* (1992) reported a milk fat of 2.99%, while Klungel *et al.* (2000) found that fat for the milk from farms using automatic milking systems were 4.46%± 0.28% and 4.0%± 0.32% before and after introduction of automatic milking, respectively.

2.3.2.2.2. Protein

Temperate breed's have an average milk protein of 3.5 % (Webb *et al.*, 1980). Korhn and Anderson (1980) reported an average protein percent of 3.3%. Moreover, Ballou *et al.* (1995) mentioned that milk protein for bulk tank milk collected from 200 farms for 12 months revealed 3.16%. Also Harding (1999) cited that milk protein was 3.2%, while Hamid (1994)

mentioned that mean milk protein percent of high and low grade cows were 3.06% ± 0.49% and 3.18%± 0.47%, respectively.

Elfaki (1988) mentioned that high grade dairy cows at the University of Khartoum farm revealed 3.4% protein and that low grade cow's revealed 3.3% protein. In the Netherland, determination of milk protein content for payment and milk- recording purposes was measured as 3.4% compared with the original 3.2% (Walstra, 1992).

Casper *et al.* (1992) reported that milk protein was 3.17%. Klungel et al. (2000) reported that protein for the milk from farms using automatic milking systems were 3.46%± 0.16% and 3.42%± 0.13% before and after introduction of automatic milking, respectively.

2.3.2.2.3. Lactose

Logacheva (1975) found that the lactose content of milk of different breeds was ranging from 4.5% to 4.6%. Similarly, Buchberger *et al*. (1976) mentioned that in 1209 milk samples the lactose content varies from 4.18% to 5.03%.

Ibrahim (1989) reported that mean milk lactose for Dairy Land and University of Khartoum farms were 4.5%± 0.013 % and 4.631± 0.125, respectively. Ballou *et al.* (1995) studies milk sample of 200 farms and reported that the mean lactose was 4.65%. Similarly, Harding (1999) mentioned that lactose content of milk was 4.6%.

2.3.2.2.4. Ash

Hussein (1985) reported that the ash content of cross dairy cows was 0.72%. Also he reported that in Sudan ash was almost varied from 0.68%± 0.10% at the start and peaked at the value of 0.78%± 0.10% at the third month, but the over all lactation yield of ash was 0.72%± 0.03%.

Hamid (1994) mentioned that ash content of two groups of high grade dairy cows was 0.30%± 0.04% and 0.32%± 0.04 %, respectively. However ash of milk varied from 0.6% to 0.8% (Hunderson, 1971).

2.3.2.2.5. Total content

Casper *et al*. (1992) mentioned that the total solid of raw milk was 11.735%, while Elfaki (1988) noticed that in Khartoum University farm the total solid content of milk was 12.9% for high grade dairy cows and 13.10% for low grade cows.

Ibrahim and Samaha (1986) reported that during summer total solid was averaged 12.35%. Moreover Strekozov and Eremina (1987) in Russia, showed that the total solids of three different breeds were 12.41%, 12.83% and 12.17%. Hussein (1985) found that average total solids were ranging between 12.91% and 14.78% with the minimum value being obtained 28 weeks post partum.

Harding (1999) reported total solid values of 12.6% while Abdalla (1987) found that the total solids of milk were 13.30, 13.60 and 14.30%. However, Hamid (1994) reported that the total solids of two groups of high grade cows were 13.0± 0.99 and 14.02± 1.43, respectively.

2.3.2.2.6. Titratable Acidity

According to Rumania Standards (1974) the acidity (T) of raw milk must be 15–20 T (0.15- 0.20%). Ibrahim (1989) reported that the titratable acidity of the milk collected from Dairy Land and University of Khartoum farms were 0.177± 0.022 and 0.172± 0.025.

Mohammedi (1988) found that the acidity of 82.9% of raw milk samples collected from Khartoum North were 0.18– 0.21%. The samples collected form Elsagana, Burri and Omdurman revealed 0.19%± 0.0117, 0.2% ± 0.014 and 0.21%± 0.013, respectively.

Ibrahim (1973) mentioned that mean titratable acidity was 0.18%± 0.06 for venders milk and 0.20% for dairy farms in the Sudan. However, Kon and Cowie (1961) found that fresh milk drawn from cow had acidity of 0.18%.

2.3.2.3. Microbial quality of milk

The cans temperature and duration of milk storage on the farms vary widely, so the numbers and the types of microorganisms present when the milk leaves the farm differ (Bramley and Mckinnon, 1984). Moreover, Murphy and Boor (2000) reported that the numbers and types of microorganisms in milk immediately after production, reflects directly microbial contamination during production.

Bramley and Mckinnon (1984) and Murphy and Boor (2000) mentioned that under any conditions, there are only three main sources of microbial contamination of milk: from within the udder; from the exterior of the teat and udder; and from the milking and storage equipment. They concluded that the measurement of bacterial numbers in raw milk is used to determine producer complian with regulatory standards as well as with milk quality in incentive programs.

2.3.2.3.1. Total bacterial count (TBC)

Total bacterial counts values were in the range of less than 1000 cfu/ml where contamination during production is minimal to more than 1×10^6 /ml of milk (Bramley and Mckinnon, 1984). Consequently high initial TBC values in milk, e.g. more than 1000/ml, are evidence of serious faults in production hygiene, whereas the production of milk having TBC value less than 2000/ml reflects good hygienic practices (IDF, 1974).

In Ethiopia, Godefay and Molla (2000) observed on milk taken from the bulk tank in and around Addis Ababa a high increase in the mean total

aerobic plate count in milk samples (1.1×10^5 cfu/ml), storage container before cooling (1.1×10^6cfu /ml) and upon arrival at the processing plant (1.9×10^8cfu/ml).

The bacteriological quality of pre-processed raw milk originating from all 16 milk collection centers in Trinidad was evaluated and the mean total bacterial counts per ml was generally high for all samples ranging from 5.8×10^5 $\pm3.1\times10^5$ to 5.7×10^8 $\pm5\times10^9$ as estimated by Adesiyun (1994). Raw milk quality in South Africa is poor and standard plate counts in the millions per ml are common which is largely due to insufficient cleaning and sanitizing of dairy equipment (Gilbert, 1982). However, the farms following cleaning programe are able to achieve standard plate counts of less than 10000 cfu/ml as he reported.

Klungel *et al*. (2000) studied the effect of automatic milking and showed that the TBCs for the milk from farms using automatic milking systems were 8000 cfu/ml and 19000 cfu/ml before and after introduction of automatic milking, respectively. Similarly Ravanis and Lewis (1994) claimed that standard plate count as initial quality measurement was very good and was 4790 cfu/ml.

Rasmussen *et al.* (2002) repoted that total bacterial count ranged from 7400/ml to 14000/ml for bulk milk of 98 Danish farms. Harding (1999) mentioned that many countries set penalties at 200000 organisms/ml or even 100000 organisms/ml. However, it was described that with good hygienic practices, average TBCs of 10000 organism/ml can be achieved as he reported.

In Sudan Barakat (1995) reported that total bacterial counts for good grade milk was 5.5×10^5 cfu/ml, for satisfactory grade it ranged between

5.5×10^5 and 5.0×10^6 cfu/ml and for bad grade was more than 5.0×10^6 cfu/ml. Ibrahim (1973) found that the average total bacterial counts for four dairy farms around Khartoum was 6.8×10^5cfu/ml. Mohammedi (1988) examined 290 samples of venders milk for total bacterial counts and found that 54.4 % had total bacterial counts ranging between 5.0×10^5 and 5.0×10^6 cfu/ml.

Mustafa and Idris (1975) tested 113 samples of milk collected from venders in Khartoum for total bacterial counts and found that 75.86% had total bacterial count more than 10^6cfu/ml. Ali (1988) examined milk samples from Kuku and Gezira dairy plants for total bacterial counts and found mean values of 3.4×10^6 cfu/ml and 4.3×10^6 cfu/ml, respectively.

In USSR, Golubeva (1984) found that the total bacterial counts of 102 milk samples taken from portable tanks immediately after milking ranged from 1.0×10^5 to 5.5×10^6 cfu/ml. In Germany, Suhren and Heeschen (1991) stated that since introduction of milk quality regulation in1981, the bacteriological quality of raw milk had improved markedly. They added that a survey in 1977-1979 established average bacterial counts of 6.3×10^5cfu/ml, while in 1989-1990 the average bacterial counts was 1.2×10^5 cfu/ml. In Croatio, Lukac (1990) conducted a study on bacteriological quality of raw milk collected daily from dairy farms and bulk milk tanks, the results indicated great variability of bacteriological quality of milk samples, which was attributed to ecological conditions and milk handling as well as husbandry practices and feeding conditions.

2.3.2.3.2. Coliform counts

Coliform bacteria are enteric group of bacteria commonly contaminate raw milk. They are Gram-negative rod shaped, ferment lactose and many strains are psychotropich (IDF, 1994a). Also they are responsible for spoilage, gas production from lactose and acid production (IDF, 1994a).

Murphy and Boor (2000) reported that the coliform counts procedure enumerates bacteria percent in milk that are most commonly associated with manure or environmental contamination. They also stated that although it was used as indicators of faecal contamination, some strains commonly exist in the environment. Moreover, they concluded that generally count above 50 cfu/ml indicate poor milking hygiene and high count most often result from dirty equipment and from milking of cows with coliform mastitis.

In Egypt, Ahmed and Sallam (1991) mentioned that all raw milk samples examined proved to be contamined with coilform organisms. They were isolated and identified as *E. coli, Enterobacter cloacae, Enterobacter aerogenes, Enterobacter gergoviae, Citrobacter amalonaticus, Citrobacter freundii, Klebsiella rhinoschleromalis, Klebsiella oxytocia, Klebsiella ozaenae* and *Klebsiella pneumonae.*

In Italy, Eposito *et al*. (1993) claimed that verocytotoxigenic *E. coli* isolated from milk and milk products, were found to associate with enteric infections, haemolytic uraemic syndrom and possibly thromobtic thrombocy to penic purpura in many countries in America and Europe. Bramley and Mckinnon (1984) reported that the incidence of coliforms and *Escherrichia coli* in raw milk has received considerable attention, on account of their associated with contamination of faecal origin and the consequent risk of more pathogenic faecal organisms being present.

In Ethiopia, Godefay and Molla (2000) studied raw milk at different sampling points from four dairy farms they found the mean coliform counts

ranged from 1.3×10^4 cfu/ml (storage container before cooling) to 7.1×10^4 cfu/ml (upon arrival at the processing plant). They also found that milk samples from the udder contained mainly staphylococci and micrococci as udder specific bacteria. Samples taken at later stages were additionally contamined with bacteria from environmental origin especially Enterobacteriaceae as they reported. Furthermore they concluded that lack of knowledge about clean milk production, use of unclean milking equipment and lack of potable water for cleaning purposes were some of the factors, which contributed to the poor hygienic quality of raw milk in the studied farms.

Jayarao and Wang (1999) studied the bulk tank milk from 131 dairy herds in eastern South Dakota and western Minnesota. They detected coliforms in 62.3% of bulk tank milk samples and the log counts ranged from 0 to 4.7 cfu/ml with mean log count of 3.4 cfu/ml. They reported that 42.2% of the milk samples from farmers cans were found to be free of coliforms.

Ombui *et al.* (1994) reported that 89.5% of the samples could be considered to be of good quality with no more than 50000 coliforms/ml of milk. Moreover, they isolated 42% of *E. coli* strains from milk samples, five of which were found to be enterpathogenic, while none was found to be of serogroup O157:H7. They indicated that a good number of farmers draw milk under satisfactory conditions, but awareness campaigns on clean milk, milk handling and storage practices should be stepped up in order to reach farmers who may not be informed.

Gilbert (1982) suggested that the use of detergents sanitizers and all cleaning programs follow the same fundamental steps in the farms are able to achieve coliforms counts of less than 10/ml for raw milk.

Bramley and Mckinnon (1984) reported that coliforms counts regularly in excess of 100 cfu/ml are considered by some authorities as evidence of unsatisfactory production hygiene.

2.3.2.3.3. Laboratory pasteurization count (LPC)

Murphy and Boor (2000) reported that pasteurization not only kills pathogenic organisms in raw milk but also destroys a wide range of other bacteria and bacteria which survive laboratory pasteurization at 63º C for 30 minutes are thermoduric. Furthermore IDF (1994a) classified the group of bacteria which survive heat treatment at 80º C for 10 minutes as "spores". Robinson (1990) showed that isolates found in milk subjected to heating to 80º C for 10 minutes were 61 *Bacillus spp.,* 37 coliform group and two other Gram positive organisms. Six concentrate feed samples from five different farms were analyzed, high levels of spores (up to 16) were founds (Vaerewijck *et al*., 2001).

The main classes of spoilage bacteria which survive pasteurization are belonging to the spore– forming bacteria (IDF, 1994a). The vegetative cells of such bacteria are destroyed by pasteurization but the spores are heat resistant, after heat treatment the spores germinate in the product and eventually cause spoilage via their degradative enzymes (IDF, 1994a). The most important spoilage bacteria type which produce heat resistant spores, are, *Bacillus* and *Clostridium spp*., which may be frequently present in raw milk and large numbers are often related to poor farming practices (for example feeding cows with poor quality silage) and poor cleaning programs (IDF, 1994a). However, they concluded that *Bacillus cereus* is of greatest significance of the *Bacillus species* because it can grow quickly. Vaerewijck *et al.* (2001) mentioned that feed concentrate can be a source of spores contamination, including those of *Bacillus spp.* for raw milk at the farm level. Sutherland and Murdoch (1994) reported that the incidence of

mesophilic isolates was highest in the winter and lowest in summer/autumn, while pschrotrophic incidence was conversely lowest in winter and highest in late summer/autumn.. Also they noticed that spores of *Baciluus spp.* were isolated from raw milk taken from farms milking machines and bulk tanks, milk tankers, dairy silos and pasteurized milk. They concluded that consistent seasonal fluctuation in incidences throughout these samples suggested that spores of *Bacilluse spp*. drive from the farm environment survive as important contaminant right through the milk chain to the pasteurized products. Laboratory pasteurized counts are generally much lower than standard plate count (Murphy and Boor, 2000). Moreover, they reported that LPCs higher than 200 cfu/ml suggested that the milk was not properly protected from bacterial contamination. However, they concluded that the high LPCs are generally associated with a chronic or persistent cleaning failure in some area of the system or with significant levels of milk contamination from soiled cows. Other common causes of high LPCs are old piplines gaskets inflation and other rubber parts, milk stone deposits, and leaky pumps as they stated.

In Norway, Granum *et al*. (1993) stated that 59% of *Bacillus cereus* isolated from raw milk was enterotoxin producer. Lukasova *et al.* (2001) reported the occurrence of the genus Bacillus in bulk tank milk and environment of the farm. *Bacillus licheniformis* was the most commonly isolated from milk followed by *Bacillus cereus*.

IDF (1994a) the spores of *Clostridium botulinum* are widely distributed in animal faeces and soil and often contaminate milk and milk products and *Clostridium perfringes* may produce a peracute gangrenous mastitis.

Bramley and Mckinnon (1984) stated that bedding materials used in houses during winter season have very high bacterial count (10^8-10^{10} cfu/g)

of mesophilic bacteria, although the bedding may appear relatively clean and dry. So teats of cows kept in straw yards can become heavily and visibly soiled and bacterial contamination is correspondingly high unless teats are thoroughly washed, while during summer when cows are turned out to pasture, a marked decline in the level of contamination of teat occurs, as they stated. They concluded that thermoduric and /or laboratory pasteurization counts has been proposed and indeed, used for hygienic quality control of milk.

2.3.3. Grading of raw milk

Raw milk under tropical conditions was graded according to microorganisms present in milk, odour or flavour, amount of sediment, appearance and temperature (Chandan and Hedrick, 1979). They reported milk was graded as good when it had total bacterial counts (TBC) of 5.0×10^5 cfu/ml or less, satisfactory when TBC ranged between 5.0×10^5 to 5.0×10^6 cfu/ml and bad when TBC was more than 5.0×10^6 cfu/ml.

According to the US Department of Health 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.

In many countries a standard for grade A or grade one raw milk has a total bacteria counts $< 1\times10^5$ cfu/ml, and this may be obligatory for raw milk intended for heat treatment before liquid consumption (Bramley and Mckinnon, 1984). They also reported that in North America TBC values of $< 3\times10^6$ cfu/ml or equivalent are acceptable for manufacturing grade milk.

In UK for example, no distinction is made between raw milk for manufacture and that for liquid consumption as they stated. However, they suggested that for milk that is to be consumed raw, a more stringent standard adopted on where milk is refrigerated. Murphy and Boor (2000) reported that especially collected milk from clean healthy cows has an standard plate count less than 1000 cfu/ml, higher standard plate counts suggested that bacteria are entering the milk from variety of possible sources. They also reported that SPCs less than 5000 cfu/ml are common, and count less than 10000 cfu/ml should be achievable by most farms.

2.4. Microorganism of milk

Milk in addition to be a nutritious media, presents a favourable physical environment for multiplication of microorganism (Gilmour and Rowe, 1990).

Murphy and Boor (2000) reported that as bacteria can enter milk production systems from multiple and various sources, determining the causes of high bacteria numbers is not always straight forward. Moreover, they suggested that high bacterial counts can result from a combination of factors. These include mastitic cows, animal skin, the environment in which cow is housed and milked, dirty equipment, old cracked rubber hoses and marginal cooling.

2.4.1. Spoilage microorganisms

The most common spoilage microorganism of milk and milk products are the Gram– negative rod shaped bacterial (coliforms and *Pseudomonas spp*.), Gram- positive, spore forming bacteria, lactic acid producing bacteria, yeast and mould (IDF, 1994a).

Bramley and Mckinnon (1984) mentioned that the spoilage organisms that become predominant at 24-30º C are mainly coliforms and streptococci and other types, which increase the acidity of milk. Moreover,

they concluded that Gram– negative rods other than coliforms and micrococci including staphylococci will also multiply unless or until any developed acidity become inhibitory to them. A number of Gram– negative rod shaped bacteria also grow rapidly at refrigeration temperature and the possible source for spoilage of dairy products include *Acinetobacter*, *Moraxella* and *Flovobacterium* (IDF, 1994a). However, Bramley and Mckinnon (1984) reported that *Pseudomonas spp*. is the most important group of Psychrotrophs associate with spoilage, they grow rapidly at refrigeration temperature and often dominate the microbial population. They concluded that these are common in the environment particularly in water. As stated by IDF (1994a) lactic acid producing microorganisms including *Streptococcus*, *Lactobacillus, Lactococcus* and *Leuconostoc* spoil milk by the fermentation of lactose to produce acid.

Jayarao and Wang (1999) reported that coliforms and non coliforms were detected in 62.3% and 76.3% of bulk tank milk samples from 131 dairy herds, respectively. They concluded that the results provide an indication of current and potential problems associated with bacterial counts and milk quality.

2.4.2. Pathogenic Microorganisms

Harding (1999) stated that milk is good medium for bacteria including pathogenic organisms and if it is produced and processed under unhygienic conditions; frequently outbreaks of diseases may result. Milk serves as an excellent culture and protective media for certain microorganism, particularly bacterial pathogens whose multiplication depends mainly on temperature and competing microorganisms and their metabolic products (Heeschen, 1987). Furthermore he concluded that several important pathogenic bacteria are present in milk like *Mycobacterium tuberculosis* and *Brucella spp.*. Pathogenic microorganism

of current concern, include *Salmonella spp.*, *Compylobacter spp.*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Yersinia enterocolltica* (IDF, 1994a). Outbreak of listeriosis have been related to the consumption of dairy products, such as Swiss soft cheese, Mexican style soft cheese, chocolate milk and butter (Dalton *et al*., 1997 and Lyytikainen *et al*., 1999). Moreover Klausner and Donnelly (1991) mentioned that *Listeria spp.* has been found in different places in the environment of dairy plants and the bacteria may also survive for along time in a dairy. Some bacteria may cause diseases in frequently or which cause diseases only occasionally e.g *Escherichia coli*, *Bacillus cereus, Clostridium perfringens, Clostridium botulinum* and *Streptococcus zooepidermicus* (IDF, 1994a).

In Sudan many researchers have mentioned the importance of milk in public health and stated that communicable diseases of milk such as tuberculosis, brucellosis and outbreaks of poisoning attached more attention than physical and chemical studies (Mustafa and Idris, 1975; Ibrahim and Habiballa, 1978; Barakat, 1995; Adam, 1997 and Haj Mahmoud, 2002). In France, Buyser *et al.* (2001) found that milk was implicated in 1-5% of total bacterial diseases outbreaks.

2.4.2.1. Major diseases transmitted through milk

Several diseases of man and animals may be transmitted through consumption of raw milk (Haj Mahmoud, 2002). Those reported by Hunderson (1971) include human and bovine tuberculosis, typhoid and paratyphoid fever, scarlet fever, septic sorethroat, diphtheria, staphylococcus infection, bacillary dysentery, brucellosis, foot and mouth disease, milk sickness, anthrax and Q-fever. Similarly Ibrahim and Habiballa (1978) and Tanwani and Yadava (1983) mentioned that outbreaks of food poisoning, T.B, brucellosis, staphylococcus poisoning, septic sore throat, tonsillitis, *E. coli infections, Clotridium perfringenes,*

Clotridium botulinum infections and *Bacillus cereus* infections are the common diseases that are caused through consumption of infected milk.

Collins *et al*. (1988) reported that microorganism enter the udder through the duct at the teat tip. Furthermore Bramley (1987) stated that certain species of bacteria, most notably *Staphylococcus aureus* readily colonize the teat duct, particularly in the region of the teat orifice. This colonization may persist for many weeks without the bacteria penetrating to the teat sinus and producing mastitis.

2.4.2.1.1. *Staphylococcus aureus*

The species belongs to the family Micrococcaceae that consist of twenty three species and four sub species (Barrow and Feltham, 1993). They are Gram- positive cocci, aerobic, catalyse positive, oxidase– negative, non motile. The colonies are smooth, raised, glistening, circular and entire (Asperger, 1994). Also he reported that colonial pigments are variable from grey or grey white with a yellowish tint through yellow-orange to orange. The natural habitat of *S. aureus* is worm blooded animals including human and can exist in the carrier state at a variety of body sites including the nose, pharynx, axilla, umbilicus, perineum, gastrointestinal and urogenital tracts and different areas of the skin (Noble, 1981). Moreover, he reported that nose appears to be the principal site for multiplication. Adesiyun *et al*. (1997) reported that enterotoxin producing Staphylococcus species; *Staphylococcus aureus*; in particular; are the leading cause of foodborne illness throughout the world. Similarly Asperger (1994) reported that milk and milk products can become contaminated unless good hygiene (including mastitis control) occurs on farms. The bacterial content of raw milk may be increased by the presence of mastitis among the producing animals (Bramley and Mckinnon, 1984 and Mohamed *et al.,* 1998). Moreover Doyle (1989) reported that some

strains produce toxins in foods and when consumed they cause symptoms, which includes vomiting, diarrhea, severe abdominal cramps and sometimes collapse. He also mentioned that although the organisms themselves are readily destroyed at pasteurizing temperature, the toxin appear to be heat–resistant and is inactivated by boiling or refrigeration for long periods. Mladenow *et al*. (1984) studied 649 batches of raw cow milk produced on 42 farms for the presence of pathogenic staphylococci. They found that 18.03% of the batches contained coagulase positive staphylococci.

Umoh *et al*. (1990) showed that staphylococci isolates from 135 raw milk samples, forty two of them obtained from settled herds (SH) and samples from nomadic herds (NH), were characterized and assayed for enterotoxin production. They found that of the 42 samples (SH), 13 (31%) were California Mastitis Test (CMT) positive, while all samples contained staphylococci. Only 3 (3.2%) of nomadic herds were CMT- positive but 58 (62.4%) contained staphylococci. Furthermore they concluded that 13 isolates from CMT- positive milk obtained from settled herds, one produced enterotoxin A, while amongst the 29.4 % elaborated enterotoxin A and 3 produced type D and none of the isolated from milk obtained from nomadic herds was enterotoxigenic. However Wilson *et al*. (1997) mentioned that the important factors associated with bulk tank milk somatic cell counts were prevalence of *Streptococcus agalactiae and S. aureus* mastitis.

Lengauer and Stumtner (1994) examined bulk milk from various producers, they found 37% of the samples contained coagulase positive staphylococci and in more than 90% of the positive sample the counts were not higher than 500 cfu/ml. Similarly Vautor *et al*. (2003) investigated the genetic diversity of 179 *Staphylococcus aureus* isolates recovered from

various sites in 10 farms producing cheese manufactured with raw ewe's milk. Their results showed that a single clone of *S. aureu*s is widely distributed both in infected mammary glands and in cheese produced from raw milk. Moreover they concluded that this study confirms that infected mammary glands are the main source of the contamination of dairy products in sheep. On the other hand Bagadi (1970); Kapur and Singh (1978); Mohamed *et al*. (1993) and Mohamed *et al.* (1998) found that staphylococcus infection was the commonest cause of both clinical and subclinical mastitis in cows. Several surveys in Sudan have shown that *S. aureu*s is the most frequently isolated udder pathogen and the economic losses are tremendous (Adlan *et al*., 1980; Mohamed *et al*., 1993 and Mohamed *et al.*, 1995). Similarly, Haj Mahmoud (2002) reported that one hundred and fifty isolates of 218 Gram positive isolates (68.8%) were identified as Staphylococci; forty–four of them (29.3%) were coagulase positive and *Staphylococcus aureus* were 12.2%. On the other hand, Bystron *et al*. (2001) in Poland found that 66% of *S. aureus* strains isolated from raw milk have the ability to produce entrotoxins. However APHA (1960) reported that because of stability of staphylococcus entrotoxin; it may remain in food products after the organism which produced it has been killed by heat.

2.4.2.1.2. Other staphylococci in raw milk

Diveries (1979) isolated *Staphylococcus xylosus*, *S. epidermidis*, *S. scuiri, S. haemolyticus, S. hyicus, S. chromogenes, S. simulans* and *S. cohnii* from the teat and milk of cows. Similarly Verma (1977) and Mohamed *et al*. (1993) found that *S. epidermidis* was invariably isolated from clinical and subclinical mastitis cases and that all strains were pathogenic for the udder. In Sudan, Haj Mahmoud (2002) found that 106 (70.7%) of the identified isolates were coagulase negative staphylococci

and in all test samples there was 1, 2 or 3 *Staphylococcus spp.* present. They are identified as: *Staphylococcus epidermidis* (5.6%), *S. choromogenes* (4.4%), *S. caseolyticus* (4.1%), *S. xylosus* (3.8%), *S. haemolyticus* (3.1%), *S. cohnii* (2.5%), *S. simulans* (2.2%), *S. hominis* (2.2%), *S. saprophyticus* (1.9%), *S. delphini* (0.6%), *S. sciuri* (0.9%) and *S. carnosus* (0.9%). In Poland, Bystron *et al.* (2001) found that none of the coagulase negative strains of *S. intermedius* have any ability to produce entrotoxins.

2.4.2.1.3. *Brucella*

Animal brucellosis is a disease of world wide importance (Fensterbank, 1994). It is also called “Malta fever”, "Mediterranean fever" or “undulant fever”. Brucella was isolated from milk sample of dairy cows (Tadayon, 1968).

Garin and Verger (1994) found that *Br. abortus* and *Br. melitensis* are the most wide spread brucella species isolated from domestic ruminants. The disease can naturally affects numerous species of wild and domestic animals (Fensterbank, 1994). Furthermore Young (1994) and Roux (1994) reported that humans are accidental and almost always dead end hosts of brucella infections. They also reported that the disease is primarily an occupational risk and occurs mainly in exposed professions, which are veterinarians, farmers, laboratory technicians, abattoir workers and others who work with animals and their products. Memish and Balkhy (2004) clamied that the disease is mainly transmitted to human through the ingestion of raw milk or non pasteurized cheeses contaminated with one of the four *Brucella spp.*, which are pathogenic to human. Similarly Talukder *et al*. (1984) studied the prevalence of brucellosis in farming community of Saudi Arabia. They suggested that farm workers and those who drink raw milk are more likely to contacts brucellosis than are the general population.

Kasimoglu (2002) found that *Br. melitensis* was isolated from 5 (14.2%) of 35 ewe's milk cheeses samples, while *Brucella spp*. were not detected in any of raw milk and cows cheeses samples. He concluded that ewe's cheese is an important source of *Brucella spp*. and they have been risk for public health. Similarly Mohamed (1989) investigated twenty– nine patients with symptoms and signs suggestive of brucellosis in Gezira area, central Sudan. The majority of the patients (76%) were found to be infected with both *Br. abortus* and *Br. melitensis* with titer of 1/160 and above.

The epidemiology of human brucellosis and disease distribution in the world is mainly influenced by the disease in infected animals (Young and Gorbed, 1989). Many workers reported the incidence of brucellosis in the Sudan (Abdalla, 1966; Mustafa and Hassan, 1969 and Ibrahim and Habiballa, 1975). Their findings indicated that bovine brucellosis leads to major problems in some areas. Moreover, Haj Mahmoud (2002) found that out of 98 milk samples tested, 53 samples were negative for the brucella test and 45 samples were positive for Milk Ring Test in Sudan.

In Turkey Iihan *et al*. (1999) described *Brucella abortus* outbreak in a commercial farm and he stated that 58% of the milk samples were positive for Milk Ring Test and *Brucella abortus* was isolated from milk samples and vaginal swab. In India, Hussain *et al*. (2000) reported 25 cases of brucellosis in cows and 3 cases in men who were involved in animal husbandry.

24.2.1.4. *Escherichia coli (E. coli)*

Escherichia coli is classified as being a member of the family Entrobacteriaceae, the bacteria are Gram- negative, non spore forming straight rods (Barrow and Feltham, 1993). They also reported that it can grow in media with glucose as the sole organic constituent, it ferment lactose producing acid and gas. Infantile diarrhea was caused by several

serologicaly identifiable strains of *E. coli* (Rea and Fleming, 1994). They also added that four pathogenic categories of diarrheagic *E. coli* are recognized: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enterohaemoragic *E. coli* (EHEC). *Escherichia coli* is the normal inhabitant of the intestinal tract of man and animals and some strains can cause acute enteritis in infants and to some extent in adults (Doyle, 1989). Moreover he reported that the incubation period is 12- 72 hours and symptoms include dysentry which may associate with diarrhea or may appear as a separate symptom and the disease may last up to one week or more.

Ombui *et al*. (1994) reported that raw milk can get contaminated with enteropathogenic strains of *E. coli* that can pose a potential risk to humans. They call for extra care when preparing milk and milk products to be consumed by human beings. Moreover, Huber *et al*. (1998) mentioned that with using a report system of the Bavarian Public Health services, 300 cases of enterohaemarrhagic *E. coli* (EHEC) infection were registered within one year (April 1996 to March 1997) in Bavaria. The infections registered in relevant frequencies were: raw milk (18%), farm animal associated contact (43%) and contact with patients suffering from diarrhea (36%).

Baljer and Wieler (1999) showed that enterohaemarrhagic *E. coli* (EHEC) bacteria are known only since 1982 where it recognized as zoontic pathogens which differ from *E. coli* intestinal common sales because lysogenic infecteion with bacteriophage which carry the genetic information for the production of shiga-toxin. They also reported that EHEC are one of the world wide important causes of food borne infections and incidences in Germmany is about 13 cases per 100000 inhabitants. Furthermore, they suggested that EHEC were isolated from feacal samples

world wide from large numbers of cattle and it was also isolated sporadically from fecal samples of other animals and healthy human. Ombui *et al*. (1994) investigated the incidence of *E. coli* in raw milk supplied by farmers to dairy cooperative societies in Kiambu District, Kenya. They found that 42 strains of *E. coli* were isolated from milk samples, five of which were found to be entropathogenic, while none were found to be of sero group O157: H7.

Mckee *et al*. (2003) found nine (2.14%) of the 420 dairy samples were positive for vercytotoxin- producing *Escherichia coli* (VTEC). On the other hand Allerberger *et al*. (2003) mentioned that two Austrlian cases of haemolytic uremic syndrome (HUS) which caused by entrohaemarrhagic *Escherichia coli* (EHEC) O26: H affecting an 11- months old boy and a 28– months old girl, where transmission through unpasteurized cow's milk was positively identified. Moreover, Matthews *et al*. (1997) investigated the ability of verocytotoxin producing *Escherichia coli* (VTEC) O157–H7 to enter selected human and bovine epithelial cell lines. However they suggested that ability of VTEC to invade bovine mammary epithelial cells may be important in pathogenesis in the bovine, which may indicate the route by which raw milk may potentially become contaminated and may provide reservoir of bacteria for the contamination of workers, equipment and carcass at time of slaughter.

Argentina has one of the highest records of hemorrhagic uraemic syndrome (HUS) in human (300-400 cases/ year; 22/100000 in children under 4 year of age), which is haemorrhagic colitis (HC), thrombotic thrombocytopenic purpura which caused by EHEC (Padola *et al*., 2002). They also showed that EHEC was isolated from cattle in an Argentinian feedlot. Similarly Allerberger *et al*. (2001) detected *Escherichia coli* O157 infection in two children, one of whom developed haemolytic uraermic

syndrome (HUS), both had drunk raw cows or goat's milk in the week before their illness. Shiga–toxin producing *Escherichia coli* were examined in bulk tank milk from 131 dairy herds in eastern Dakota and western Minnesota (Jayarao and Henning, 2001). Moreover four out of the five isolates of *E. coli* encoded for the shiga-toxin Z gene, while one strain encoded for the shiga toxin I gene. However *E. coli* was not isolated from those bulk tank milk samples. Similarly, Boer and Heuvelink (2000) reported that shiga toxin producing *Escherichia coli* (STEC) are an important cause of haemorrhagic colitis and the diarrhea associated form of haemolytic uraemic syndrome, of the numerous serotypes of *E. coli* that have been shown to produce shiga toxin (stx), *E. coli* O157: H7 and *E. coli* O157: NM (non motile) are most frequently implicated in human disease.

Adam (1997) isolated *Escherichia coli* from 30 samples of different sources of contamination from dairy farms were: 2 from milkers hands, 2 from teats and 7 from utensil's. Furthermore, the similarity between the bacterial species isolated from sources of contamination with those from milk samples may reflect the origin of bacteria and indicated the poorness of hygienic practices.

2.4.2.1.5. Salmonella

It is a member of the family Entrobacteriaceae, the genus salmonella subdivided into seven subgenera; they are Gram- negative, short rods non-sporeforming bacteria (Vlaemynck, 1994). Thornton (1973) reported that salmonella comprise more than 2000 serotypes and all are known to be potential causative agents of food poisoning. Moreover the food poisoning of salmonella needs 3-36 hours for the onset of symptoms.

Bulk tank milk samples from 131 dairy herds from eastern south Dacota and western Minnesota were examined for the presence of *Salmonella spp*. (Jayarao and Henning, 2001). They found that 6.1% of

bulk tank milk samples were positive and the isolates belonged to group D, B, C and E serogroups.

Reed and Grivettit (2000) investigated outbreaks in February and April of 1997, where more than 150 people, mostly very young Hispanic children, became ill in California by *Salmonella serotype, typhimurium* DT104; and an incidence of similar etiology occurred in Washington State between January and May of the same year. They found that resistant salmonella was linked directly to a product made from raw milk.

2.5. Factors associated with improving raw milk quality

International Dairy Federation (1994a) stated that milk is a magnificent medium for growth of microorganisms and, therefore risk of quick microbiological deterioration of quality is present. So the raw milk must be of a high microbiological quality, the microbiological quality of milk must not deteriorate from the time of milking to the manufacturing at the dairy.

Harding (1999) mentioned that in order to achieve a high bacteriological quality of milk at farm level, it is important for farmers to be aware to the sources of contamination and to understand how they can be controlled. He concluded that to produce milk of a high hygienic standard the milkers must prepare the cow thoroughly before milking, adopts a good milking technique and use a good post milking routine. Similarly Reed and Grivettit (2000) reported that on-farm programs similar to hazards analysis critical control point, which target pathogen reduction and screening, can provide assurance to processors and consumers that on-farm safety is a high priority. Moreover they mentioned that additional voluntary oversight of farm practices including monitoring and controlling access to raw milk supplies on the farm could further contribute to public food safety. Badini *et al*. (1996) studied sixty commercialized raw milk

samples, the results showed unsatisfactory hygienic and sanitary conditions of the raw milk, which suggested the existence of great risk to the health of the consumers, especially when the product is taken without being boiled. Furthermore Schukken *et al*. (2003) reported that monitoring tools are required to find the area of risk in the herd. Hence they concluded that more complete udder health programs and monitoring systems are to be developed and implemented. Furthermore they recommended that implementation of complete udder health programs should be a accompanied by research effort to complete udder health control and monitoring programs.

Parkpian *et al*. (2003) investigated lead (Pb) and cadmium (Cd) contamination in grazing land located near a high way and they noted that improvement of farm management give significant reduction in elevated levels of lead (Pb**)** and cadmium (Cd**)** in soil and plant, which resulted in minimizing amount of Pb and Cd in consumed milk. Vanschaik *et al*. (2002) examined milk quality data on monthly basis from March 1999 to December 2000 and their results illustrated that farms with high somatic cell counts (SCC) had higher plate loop count (PLC) and more antibiotic violation. Moreover they reported that measurable improvements in overall quality of the milk in New York State would most likely occurred by targeting incentives, education and training programs for any farm with very high SCC and for larger farms with SCC between 400000 and 700000 cell/ml.

According to Creamer *et al*. (2002) recent advances in the chemical, physical and information sciences and technologies will be utilized to gain greater understanding of increasingly complex food systems and to support consumer objectives. The lack of knowledge about clean milk production, use of unclean milking equipment and lack of potable water for cleaning

purpose were some of the factors which contributed to the poor hygienic quality of raw cow's milk at farms and at collection centres, in and around Addis Ababa (Godefay and Molla, 2000). However, Sischo (1996) reported that ultimately, the milk testing programs should become a component of the quality process that is centered on the farm and that measures the success of the industry in producing high quality milk rather than being a regulatory program.

Jayarao and Henning (2001) observed that 26.6% dairy producers who consumed raw milk had one or more pathogenic bacteria in their bulk tank milk. Their findings warrant the need for educational programs for dairy producer about the risk associated with consumption of raw milk. Moreover Shiferaw *et al*. (2000) described risk factors for foodborne diseases including consumption of raw milk (1.5%) by surveyes done in California, Connecticut, Georgia, Minnesota and Oregon. Also Allerberger *et al*. (2003) reported the hazards associated with the consumption of raw milk and underline the importance of microbiological diagnostic approaches. However, the Danish automatic milking system self-monitoring program was set up to reduce the impact of clinically infected cows on bulk milk somatic cell count and to help farmers in the transition period going from conventional to automatic milking (Rasmussen *et al.*, 2002).

Milking machine has to be cleaned after each milking. Because of the complexity of milking machines and some of their components makes cleaning and in particular disinfection not fully effective so that milk residues and associated bacteria are not completely removed from the equipment and tend to accumulate daily (Bramley and Mckinnon, 1984). Moreover they stated that when cows are hand milked, the actions of milkers may add microorganisms to milk by increasing dust and dirt

particles contamination of the udder or by contact with hands. They concluded that risks of contamination from the milkers are much less with machine milking but there is an increased possibility of infection of milk with pathogens. Similarly Harding (1999) reported that dirt and dung harbour large numbers of bacteria, hence, the first action in cleaning the plant is to remove all foreign matter from teat cups, by using water and/or detergents. Many countries have regulations by which persons known to be suffering from certain diseases are not permitted to part in the production of milk (Bramley and Mckinnon, 1984).

Ministry of Agricultre and Forestry (1999) recommended that all water that comes into contact with raw milk intended for the manufacture of dairy products should be of suitable quality to ensure that the raw milk is safe from microbiological and physical contaminations. Moreover, the NZDWS (2000) provides measures of potability of water by two tests as follows:

1- No more than three faecal coliforms per 100 ml.
2- No greater than 5 NTU (Nephelometric Turbidity Unit) for turbidity.

Many farms rely on untreated water supplies from bore holes, wells, lakes, springs and rivers; some of these may be contaminated with microorganisms of faecal origin, e.g. coliforms, faecal strpetococci and Closatrida (Bramley and Mckinnon, 1984). Also they stated that warm water for hose washing of udders and teats is supplied from a warm tank controlled at 37º C, the risk of such udder infection can be reduced by entering a disinfectant, e.g. hypochlorite, or iodophor into the hose water. It also helps to reduce the numbers of bacteria left on teat after udder washing.

International Dairy Federation (1994a) recommended that hygiene control in raw milk handling as follows:

1- Cow:

The hazards are that milk is obtained from unhealthy animals, and that antibiotic or other veterinary drugs are present. The preventative measure is health control applied by farmer and supervising veterinarian using strip cup control.

2- Milking:

The hazards are that milking routines may damage tissues leading to infection of the udder, contamination of milk by stable environment; milking equipment and others. The preventive measure is cleaning the udder before and after milking with appropriate antiseptics.

Harding (1999) reported that milk temperature when milk leaves cow udder is 37º C, although careful milking conditions will result in low numbers of bacteria, the lower the storage temperature decreases it and whilst bacteria numbers increase rapidly at 10º C and above. However, Bramley and Mckinnon (1984) reported that refrigeration, by delaying bacteria multiplication, masks the effects of unhygienic production conditions and when is poorly cooled in warm weather, the result is souring and other obvious forms of spoilage of milk. Similarly IDF (1994a) recommended that hygienic control of raw milk handling include cool storage of milk. However, Bramley and Mckinnon (1984) stated that the initial total viable count of raw milk is of little value for predicting its count after refrigerated storage.

The general appearance, cleanness, colour, smell, taint (taste) and laboratory pasteurization count were checked as hygienic quality of raw milk; moreover, bacteriological spoilage can be minimized by keeping milk cool preferably at temperature below 4º C and if milk is to be stored for long periods deep cooling to 2º C is recommended (Harding, 1999).

CHAPTER THREE

MATERIAL AND METHODS

3.1. Source of milk samples

A total of 120 raw bulk milk samples were collected from 60 dairy farms in Khartoum State, during summer and winter seasons between August 2003- January 2004. Five dairy farms were selected from each region in Khartoum State as mentioned bellow:

1- Khartoum: Tayba Elhasanab, Soba, Algerif and Albageir.

2- Khartoum North: Shambat, Alailafoon, Kuku and Aid Babiker.

3- Omdurman: Gabel Torya, Alsarha, Almarkhiat and Almakaweir.

3.2. Data collection

A questionnaire was filled at each selected dairy farm before collection of milk samples. The data collected were in Appendix 1.

3.3. Collection of milk samples

Raw bulk milk samples (100 ml each) were collected in the afternoon under aseptic conditions in clean sterile bottles. They were kept in an ice box, then brought to the laboratory for analysis which were carried out imediately for microbial analysis and acidity.

3.4. Laboratory examination of milk samples

The raw milk samples were subjected to physical, chemical and microbiological tests at the laboratory of the Deparment of Dairy Production, Faculty of Animal Production, University of Khartoum.

3.4.1. Physical test

3.4.1.1. Temperature

The bulk milk samples were stired, then temperature was taken at the farms using Chem. Thermometer Milch glass Kalla according to Marshall (1992).

3.4.1.2. Freezing point

The freezing point was determined as described by Harding (1999), using a Fiske Ms Cryoscope, which was manufactured by Fiske Med. Sc. Inc. (USA). After setting the machine the accuracy and calibration was done using the standard solutions. Two ml of the milk samples were put in samples tubes and immersed in a bath at –7º C. The temperatures of the samples were measured using a probe, centered in the body of the samples, which stirred, by a vibrating stirrer wire. The results immediately displayed in the digital screen.

3.4.1.3. pH

The pH of the milk samples was determined by a pH– meter model L as described by Marshall (1992). The instrument was callabrated with pH 4 and pH 7 solutions. About 20 ml of the milk sample (at 20 ºC) was taken in a beaker (volume 50 ml), and the pH of milk sample was measured. Two pH determinations were made from each sample, and the average was calculated. The electrodes were rinsed properly between samples.

3.4.2. Chemical composition

3.4.2.1. Determination of titratable acidity

The acidity of the milk samples was determined according to AOAC (1990). Ten ml of milk was measured into a conical flask and 5 drops of phenolphthalein indicator (Product of BDH Chemical Ltd., England) were then added. This mixture was titrated against N/9 NaOH (sodium hydroxide) until a faint pink color lasting for not less than 30 seconds was obtained. The titration figure was divided by 10 to give the acidity of the sample expressed as percent of lactic acid.

3.4.2.2. Determination of protein content

Total protein was determined using Kjeldahl method according to Bradley *et al.* (1992). Ten ml of milk samples were weighed into a Kjeldahl digestion flask. Twenty five ml of sulphuric acid (sp.g. 1.84, nitrogen free)

was added to the flask. Two tablets of Kjeldahl catalyst (each tablet contain 1 gm of Na_2SO_4 and equivalent of 1 gm Hg) was also added to the flask. The flask was placed on a Kjeldahl digestion heater for three hours or until the solution become clear. The flask was then cooled to room temperature and the solution was diluted to 100 ml into volumetric flask (100 ml) using distilled water. Five ml of the sample was transferred to distillator and then ten ml of 40% sodium hydroxide were added in Markham distillator. The distillate was received in a conical flask, containing 25 ml boric acid (2%) and methylene blue plus bromocresol green indicator (3 drops); until the volume reach 75 ml. The sample was then titrated with N/10 HCl (0.1N) and the acid consumed was then read. The protein content was calculated as follows:

$$\text{Nitrogen (\%)} = \frac{T \times 0.1 \times 0.014 \times 20}{w} \times 100$$

Protein (%) = (N %) × 6.38

Where:

T = titration reading.

w = weight of sample.

N% = nitrogen content

3.4.2.3. Determination of fat content

Duplicates samples were determined by Gerber method according to Bradley *et al.* (1992). Ten ml of sulphuric acid (specific gravity 1.820 at 15.5º C) was measured into a Gerber butyrometer and from a well mixed milk sample at 24º C; a sample of milk (10.94 ml) was with drawn using milk pipette. Milk was allowed to drain into the butyrometer slowly to prevent any violent reaction with the acid, then the pipette was permitted to drain normally. One ml amyl alcohol (sp.g. 0.814 at 15.5º C) was added and the lock stopper was inserted securely. With the stopper end up, the

butyrometer was grasped at the graduated column and shaken until the contents were thoroughly mixed. Holding the butyrometer (hot) at the stopper and neck, it was inverted four times to mix the acid remaining in the bulb with the contents. The butyrometers were then placed in racks and centrifuged at 1100 rpm for 5 minutes. The butyrometers were placed in a water bath (65º C), leaving only the bulb exposed for 5 minutes. Then the straight line at the bottom of the fat column was pushed gently upwords so that it coincided with the nearest whole percentage graduation mark. The scale at the bottom of the meniscus at the top of column was read promptly to the nearest 0.05% graduation. The lower reading was subtracted from the upper reading and the difference was recorded as the fat content.

3.4.2.4. Determination of total solids

The analysis was carried by the IDF gravimetric method described by Foley *et al.* (1974). Porcelain dishes were dried for 15 minutes in the oven (100º C) and transferred to a desiccator to cool and weighed. Three milliliters of milk samples were weighed in the dishes, placed on a boiling water bath for 15 minutes, then the bottom of the dishes were wiped and transferred into the oven at 100º C. After 3 hours the dishes were transferred to desiccator for 2–3 minutes and weighed. The heating and cooling were repeated until the differences between successive weighings did not exceed 0.2 mg, the percent of the total solids was calculated as follows:

$$\text{Total solids} = \frac{\text{Weight of dry sample}}{\text{weight of sample}} \times 100$$

3.4.2.5. Determination of ash

The method described by AOAC (1990) was used. Five ml of milk sample was weighed in a suitable crucible and evaporated to dryness on a steam bath. Then the sample was placed in a muffle furance (550º C) for

1.5 – 2 hours, then cooled in a desiccator and weighed. The ash content was calculated using the following equation:

$$\text{Ash \%} = \frac{\text{Weight of ashed sample}}{\text{weight of sample}} \times 100$$

3.4.2.6. Determination of lactose

Lactose was determined by anthrone method (Richards, 1959). The standard solution was prepared by dissolving 5 grams of lactose into 95 ml distilled water to give 5% (w/v) solution of monohydrate. The anthrone reagent was prepared by dissolving 150 mg of anthrone into 100 ml of 70% (v/v) sulphuric acid. The solution was then cooled and aged overnight. One ml of the standard solution was diluted with dislled water to 500 ml in a volumetric flask. The solution was mixed well. One ml of milk was pipetted in a 500 ml volumetric flask and diluted to 500 ml with distilled water. The solution was mixed thoroughly, then 0.5 ml was transferred to a boiling tubes in a duplicate. The standard stock solution (0.5 ml) was transferred to second boiling tubes (standard). Similarly distilled water (0.5 ml) was transferred to a third boiling tube (blank). Then all tubes (samples, standard and blank) were put in ice bath. To each tube, 10 ml of ice cooled anthrone reagent was added. Then the tubes were stoppered and the contents were well mixed. The tubes were held in the boiling water bath for six minutes. The tubes were then transferred back to the ice bath for 30 minutes. The optical density (OD) was read at 625 nm. Lactose content (in gm/ 100 m) was calculated as follows:

$$\text{Lactose content} = \frac{\text{OD of sample} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 4.75$$

3.4.3. Microbiological examination

The samples were enumerated for total bacterial counts, Enterobacteriacae counts, laboratory pasteurization counts, *Staphylococcus*

aureus and *Staphylococcus spp*. counts, coliforms counts, *Escherichia coli* counts and *Salmonella spp*. counts.

3.4.3.1. Preparation of the media

All media were obtained in dehydrated form and prepared according to manufacture's instructions.

3.4.3.2. Types of culture media

3.4.3.2.1. Solid media

3.4.3.2.1.1. Plate count agar (Oxoid CM463)

The standard plate count agar was used to determine total bacterial counts and labarotory pasteurization counts (Houghtby *et al.*, 1992). The media was prepared by suspending 23.5 grams of powder in one litre of distilled water and dissolving by heat. The pH was adjusted to approximately 7.0± 0.2 then it was sterilized.

3.4.3.2.1.2. Mannitol salt agar (Oxoid CM85)

The media was used as selective medium for Staphylococci according to Davis (1959). The medium was prepared by suspending 111 grams in one litre of distilled water then brings to the boiling to dissolve completely. The pH was adjusted to approximately 7.4± 0.2 then it was sterilized.

3.4.3.2.1.3. MacConkey agar (Hi media M081)

It was used as a differential selective medium for the detection, isolation and enumeration of coliforms and intestinal pathogens. It was recommended by the World Health Organization (1963). Fifty two grams of this medium were suspending in one litre of distilled water. After boiling to dissolve completely, the pH was adjusted to 7.2± 0.2 then it was sterilized.

3.4.3.2.1.4. Eosin methylene blue agar (Oxoid CM69)

The media is recommended by the APHA (1958) for the differentiation of *Escherichia coli*. It was prepared by dissolving 37 grams in one litre of distilled water. It was boiled to dissolve completely. The final pH was adjusted to 6.8 ± 0.2 and it was then sterilized.

3.4.3.2.1.5. Salmonella – Shigella agar (Hi media, M108)

It was used as a differential selective medium for the isolation of salmonella and shigella species. The medium was prepared by suspending 63 grams in one litre of distilled water then boiled until dissolved completely and it was sterilized by water bath at 100º C for 30 minutes (the final pH was adjusted to 7.0± 0.2).

3.4.3.2.1.6. Nutrient agar

This medium was prepared by dissolving 28 grams in one litre of distilled water, then boiled until dissolved completely. Final pH was adjusted to 7.4± 0.2 then it was sterilized.

3.4.3.2.1.7. Deoxyribonucleic acid agar (DNAse agar DM132**)**

This medium produced by MAST Laboratories Merseyside, UK. It was prepared by dissolving 40 grams in one litre of distilled water and dissolved by boiling. The pH was adjusted to 7.3 and it was sterilized.

3.4.3.2.1.8. Urea agar media (Himedia M112)

The medium was prepared by dissolving 2.4 grams of powder in 95 ml of distilled water and it was boiled to dissolve completely. The pH was adjusted to 6.8 ± 0.2 and then it was sterilized. Then cooled to 50º C, a septically 5 ml of sterilize 4% urea solution were added and mixed well. The medium was then distributed (12 ml amounts) into a sterilize test tubes and allowed to solidify in a slope position.

3.4.3.2.1.9. Kiliger iron agar (Himedia MO78)

It was prepared by suspending 57.5 grams in one litre of distilled water. After boiling the medium was distributed into sterile test tubes and then were put in a slope position to solidify.

3.4.3.2.2. Liquid media

3.4.3.2.2.1. Nutrient broth (Hi media M002)

This medium obtained in dehydrated form. The broth was prepared by dissolving 13 grams into one litre of distilled water. It was mixed well by boiling then distributed into final containers and sterilized (the pH was adjusted to 7.4 ± 0.2).

3.4.3.2.2.2. Peptone water (Himedia M028)

This medium was prepared by dissolving 15 grams in one litre of distilled water. It was mixed well by boiling, then distributed into 5 ml amount in screw–capped bottles (pH= 7.2± 0.2) and it was sterilized.

3.4.3.2.2.3. MR-VP medium

This medium was prepared by dissolving 15 grams in one litre of distilled water and distributed into 2 ml volume into test tubes and it was sterilized.

3.4.3.2.2.4. Koser citrate (Himedia M069)

It was prepared by dissolving 5.7 grams in one litre of distilled water, then it was distributed into 10 ml volume into MacCartaney bottles (the pH 6.7 ± 0.2) and it was sterilized.

3.4.3.2.2.5. Peptone water sugars

These were prepared according to Barrow and Feltham (1993). Sterile peptone water (900 ml) and bromocresol purple indicator (10 ml) were prepared and sterilized. Ten grams of appropriate sugar (lactose, mannitol and glucose ect..) in 90 ml of distilled water were added to the sterile peptone water with indicator. It was then distributed into 2 ml volumes in sterile test tubes and steamed.

3.4.3.3. Sterilization

3.4.3.3.1. Sterilization of culture media

Plate count agar, mannitol salt agar, eosin methylene blue agar, MacConkey agar, nutrient broth, peptone water, MR-VP medium and Koser citrate medium were sterilized by autoclaving at 15 pound pressure for 15 minutes at 121º C. Sugars were sterilized by steaming for 10 minutes at 10 pound at 105º C. Salmonella- Shigella agar was heated only.

3.4.3.3.2. Sterilization of equipment

Glass wares such as Petri-dishes and test tubes, pipettes, flasks and large bottles were sterilized in a hot oven at 160º C for one hour (Barraw and Feltham, 1993). Whereas, MacCartaney, universal bottles, distilled water, tips and cotton were sterilized in the autoclave for 15 minutes at 121º C.

3.4.3.4. Culture method

3.4.3.4.1. Dilution method

One millilitre of milk sample was transferred with a sterile 1.0 ml automatic pipette to 9.0 ml sterile Ringer's solution in test tubes. It was mixed thoroughly. Using another sterile pipette, 1 ml of the first dilution was transferred to a second diluents tube. Then the process was repeated to make ten fold dilutions from 10^{-1} to 10^{-9} (Richardson, 1985). Furthermore, the labarotory pasteurization count was done according to Harrigan and McCance (1976). The milk sample was first heated to 80º C for 10 minutes. Then one ml milk sample was transferred with sterile 1 ml automatic pipette to 9 ml sterile Ringer's solution in a test tube. Using another sterile pipette the dilution was mixed thoroughly and 1 ml of the dilution was transferred to a second dilution test tube. This process was repeated to make ten fold dilutions from 10^{-1} to 10^{-9}.

3.4.3.4.2. Enumeration technique for bacteria

Solid agar method was followed for (15-18 ml) sterile melted and cooled plate count agar, mannitol salt agar, MacConkey agar, eosin methylene blue agar, Salmonella Shigella agar and nutrient agar were poured in sterile Petri deshes (Banwart, 1981). From each selected dilution, 0.2 ml was taken and added to the dry surface of duplicate plates with the use of automatic pipette (Jay, 1986). The added aliquot was then uniformly spread using a sterile glass rod, bent in the shape of a hockey stick (Banwart, 1981). The plates were then inverted and incubated at 37º C for 24 hours. However, in case of total and laboratory pasteurization count the incubation was at 32º C for 48 hours (Harrigan and McCance, 1976). Then the plates were examined and the counts were made from plates containing 30–300 (Harrigan and McCance, 1976). After counting, the number obtained was multiplied by 5 then by the dilution factor and referred to as colony forming units per ml of sample (cfu/ml) as stated by Banwart (1981).

3.4.3.4.3. Examination of culture

Growth on solid media was examined visually with naked eye for colony appearance and changes in the media. Growth on liquid media was examined for colour, turbidity and sediment formation.

3.4.3.4.4. Isolation of bacteria

After the counts were recorded, the smears were then made from representative colonies and stained by Gram's method in order to determine the morphology and stain reactions of the organisms.

3.4.3.4.5. Purification of isolates

It was made by subculturing part of a single, typical and well isolated colony on a nutrient agar (Barrow and Feltham, 1993). The purified cultures were stored on nutrient agar slants and incubated at 37º C for 24 hours.

3.4.3.4.6. Storage of prepared culture media

All cultures were kept at 4º C in the refrigerator until used.

3.4.3.5. Reagents

3.4.3.5.1. Hydrogen peroxide (S.d. Fine–CHEM. Ltd 74077)

Hydrogen peroxide was prepared as 3% solution for the catalase test.

3.4.3.5.2. Methyl red solution (BDH laboratory Ltd 20087)

It was prepared as 5% solution for the methyl red test (Barrow and Feltham, 1993).

3.4.3.5.3. Potassium hydroxide (Hopkin and Williams Ltd 70074)

It was prepared as 40% solution according to Barrow and Feltham (1993).

3.4.3.5.4. Alpha-Naphthol

This was obtained from BDH Laboratory reagents (British Drugs Houses). It was prepared as 5% solution.

Both potassium hydroxide and alpha-naphthol were used for Voges Proskaur test (VP).

3.4.3.5.5. Kovacs' reagent

3.4.3.5.5.1. Para-dimethyl-aminobenzalodehyde (5 grams)

3.4.3.5.5.2. Amyl alcohol (75 ml)

The reagents were heated in a water bath at 50º C until dissolved. When cooled, 25 ml of concentrated HCl were slowly added. The reagent was then stored in a brown stoppered bottle in the dark until used.

3.4.3.5.6. Rabbit plasma

Citrated rabbit blood plasma was collected under aseptic conditions into 10 ml sterile bottles. It was used for coagulase test (Barrow and Feltham, 1993).

3.4.3.6. Indicator

3.4.3.6.1. Bromocresol purple (S.d. Fine CHEM-Ltd 80014)

This was prepared according to Barrow and Feltham (1993). Two percent solutions were prepared by dissolving one gram of the indicator in 50% ethanol and then 32 ml of N NaOH solution were added to it. The final pH was 5.2 to 6.8. It was used as indicator for sugar fermentation.

3.4.3.7. Milk ring test reagent (Brucella antigen)

The tetrazolium stained antigen which was used in this test was obtained from the Central Veterinary Research Laboratory (Soba). The method was described by Cruickshank *et al.* (1975). After thorough mixing of the bulk milk sample, 0.03 ml brucella stained antigen was added to one ml milk sample in an agglutination tube. Then the tube was shaken gently and the mixture was incubated at 37º C for one hour.

The result was recorded as positive if the intensity of the colour in the cream layer was deeper than in the milk column and negative result when the intensity of colour in the cream layer was equal to or less than that of the milk column.

3.4.3.8. Identification of bacteria

The purified bacteria were identified according to criteria described by Barrow and Feltham (1993) which include:

1- Morphological appearance (shape of the organism).
2- Grams reaction (shape of cell).
3- Biochemical tests.
4- Colonial characteristics on different media.
5- Aerobic growth.

3.4.3.8. Biochemical methods for identification of bacteria

3.4.3.8.1. Primary tests

3.4.3.8.1.1. Morphological appearance (culture appearance):

Colonies of *Satphylococcus aureus* are typically circular, smooth, and convex and they are yellow in colour in mannitol salt agar media.

Escherichia coli were a greenish metallic sheen by reflected light and dark purple centre by transmitted light in eosin methylene blue agar. Colonies of *Salmonella spp.* were yellow with black centres in salmonella shigella agar.

3.4.3.8.1.2. Gram's techniques

3.4.3.8.1.2.1. Preparation of smears

Part of a colony was emulsified by a drop of sterile water and spread on a clean slide. Then allowed to dry and fixed by gentle heating.

3.4.3.8.1.2.2. Staining method

Gram's stain was performed as described by Barrow and Feltham (1993). Crystal violet was added to smears on slide for one minute, followed by washing with distilled water. Lugol's iodine was added for one minute then removed by washing with distilled water. The slide was decolorized by alcohol for ten seconds and the residue was removed by distilled water. The slide was counter-strained with bacteriological Gram Saffranin for 30– 60 seconds and washed with distilled water. The slide was then dried with a filter paper. After fixation a drop of immersion oil was added followed by examination under the microscope. Gram positive organism appeared purple, while Gram negative ones appeared pink.

3.4.3.8.1.3. Catalase test

The isolate to be identified was grown on nutrient agar at 37º C for 24 hours. Then it was put onto a sterile slide. One ml of 3% hydrogen peroxide (H_2O_2) was added to the colony and emulsified. An immediate production of gas bubbles indicated a positive reaction.

3.4.3.8.1.4. Acid production from glucose

Glucose media were inoculated with a 24 hours growth bacteria in peptone water medium. The change of colour to yellow indicated a positive reaction. Gas was accumulated in the Durham's tubes, when produced and the cultures were examined daily for up to 7 days.

3.4.3.8.2. Secondary biochemical reaction

According to their types, the isolates were subjected to further identification tests as described by Barrow and Feltham (1993).

3.4.3.8.2.1. Fermentation of sugar

Test tubes containing the differential peptone water sugars (mannitol; aerobic and anaerobic, lactose and glucose, ect..) were inoculated with the colonies to be tested. Examination was done after 24 hours for up to 7 days. Positive reactions were indicated by change in colour from purple to yellow due to acid production. Durham's tubes were also examined for presence of gas.

3.4.3.8.2.2. Coagulase test

It was performed as described by Barrow and Feltham (1993). Half millilitre of undiluted rabbit blood plasma was mixed with an equal volume of an 18 – 24 hours culture of the tested organism (colony suspected for *Staphylococcus aureus*), then incubated at 37º C. They were examined after one hour and after 4 hours for the coagulum. Negative tubes were left at 37º C over night and reexamined.

3.4.3.8.2.3. DNAse test

DNAse agar was inoculated and incubated at 37º C for 24 hours. Then flooded with HCl (Barrow and Feltham, 1993). Positive reaction was indicated by clear zone around the streaks of the tested organisms (*Staphylococcus aureus*).

3.4.3.8.2.4. and 3.4.3.8.2.5. Voges-Proskaur (VP) and Methyl red (MR) tests

The MR test was performed as described by Barrow and Feltham (1993). Glucose phosphate medium was inoculated with the test organisms (colonies suspected for *Staphylococcus aureus* and *E. coli*) and incubated at 37º C for 2 days. About 2 drops of methyl red solution were added to it,

well shaken and examined. The red colour indicated a positive result; yellow colour indicated negative, while orange was a doubtful. The same culture was then used for VP test after MR test result was taken. It was done by the addition of 0.6 ml of 5% α-naphthol solution and 0.2 ml of 40% KOH aqueous solution. It was shaken well, slope the tube and was examined after 15 minutes and one hour. A positive reaction was indicated by production of a strong red colour.

3.4.3.8.2.6. Indol test

To 24- hours culture (colony suspected for *E. coli*) in nutrient broth, 0.5 ml Kovacs' reagent was added. It was shaken well and examined after one minute. Production of red colour was indicative of a positive result.

3.4.3.8.2.7. Citrate utilization

Using a sterile straight wire (to avoid carry over of the medium), small inoculums of the tested organism (colony suspected for *E. coli*) was taken from suspension and incubated at 37º C on Koser citrate medium, that was in chemically clean tubes. It was examined daily for up to 7 days for either turbidity or production of blue colour which was indicative of a positive reaction.

3.4.3.8.2.8. Urease test

It was done as described by Barrow and Feltham (1993). Urease activity is shown by alkali production (ammonia) from urea splitting by the tested organism. Inoculum of the tested organisms were cultured on the surface of urea slope and incubated at 37º C and examined daily for 7 days. A positive test was indicated by pink or red colour.

3.4.3.8.2.9. Kiliger suger iron test (Hydrogen sulphide test)

The medium was used to test the ability of the organisms to produce hydrogen sulphide (H_2S). Inoculum was cultured on the surface of Kiliger iron slope. It was incubated at 37º C and examined daily for 7 days. A

positive reaction was indicated by black colour. Yellow or pink colour were considered as negative results for H_2S.

Statistical analyses

The analysis was carried out using SPSS program (Statistical Package for Social Sciences). All the data of this experiment were analyzed statistically by using complete randomized design and least significant difference test. One way anova was used to determine the effect of management, season, city, interaction between season and city and the area on milk samples.

CHAPTER FOUR

RESULTS

1. Management practices in the studied farms

1.1. Farm owners education level

Table 1 shows the education level of farm's owners. It is clear that only 3 (5%) of the farm's owners have B.V.Sc. level of education. One of them was in Khartoum North (5%) and the other 2 (10%) were in Khartoum. Six farms were found to be managed by other university graduats, one (5%) was found in each of Khartoum and Omdurman, while 4 (20%) were at Khartoum North. Furthermore, the farm's owners that completed their seconadry education were found to be 2 (10%), 7 (35%) and 1 (5%) in Khartoum, Khartoum North and Omdurman, respectively.

Owners of the farms with intermediate and primary school level of education in Khartoum, Khartoum North and Omdurman were reported to be 5 (25%) and 3 (15%), 1 (5%) and 4 (20%), 1 (5%) and 11 (55%) , respectively. However, illiteracy among the owners of farms studied was found to be 7 (35%), 3 (15%) and 6 (30%) in Khartoum, Khartoum North and Omdurman, respectively. Moreover, this study revealed significant differences ($P < 0.01$) in the education levels of the farm's owners in the three cities.

1.2. Types of breed

Sixty herds were studied for the distribution of the types of the cattle breeds during the present survey, in the 3 big cities of Khartoum State (Table 2). Khartoum North was found to contain the highest number of cross cows in all farms that included in this study (100%). In Omdurman 18 farms (90%) out of 20 were composed of cross cows. The rest 2 farms (10%) were composed of Kenana breed. The least percent of cross cows were reported in Khartoum (17 farms, 85%). One farm (5%) was found to be composed of either Kenana, Fresian and Jersy, the values indicated non-

significant differences ($P < 0.05$) between the three cities for the type of breed.

1.3. Herds size and milk yield

Table 3 shows the herd composition in relation to the city. The highest mean of cows number were reported as 170.25± 72.83 cows in Khartoum North with maximum and minimum of 386 and 75 cows, respectively. In Khartoum the mean of the herd was 123.10± 105.74 cows, while the maximum and the minimum were 560 and 66 cows, respectively. The mean of the herds in Omdurman was 92.35± 29.23 cows and the maximum and minimum were 147 and 52 cows, respectively (Table 3). The same Table also showed significant variation ($P < 0.01$) of the herd's numbers between the three cities. Also the mean of lactating cows were found to be higher in Khartoum North (76.45± 24.31 cows; 45.82± 8.49%), the maximum and the minimum were found as 133 and 30 cows; 57.857 and 22.45%, respectively. Lactating cows in Khartoum and Omdurman revealed 51.8±21.615 and 45.90± 15.83 cows; 46.68± 9.925 and 50.07± 6.89%, respectively. The maximum and minimum numbers of the lactating cows were 125 and 28 and 75 and 24 (69.767 and 25.99 and 66.07 and 36.67%, respectively).

The data showed highly significant variations ($P < 0.001$) in the number of lactating cows and non significant variation in their percentages. Similarly, the dry cows showed mean values of 31.10± 15.20 (18.86± 9.152), maximum and minimum values of 75 and 11 cows (47.059 and 9.375%), respectively. Among farms in Khartoum, the mean number of cows was 24.3± 34.72 (17.179± 6.71%), the maximum and the minimum numbers of the dry cows were 168 and 3 (34.927 and 3.093), respectively. Dry cows mean number in Omdurman was found to be 13.65± 5.715 cows; 15.806± 6.717%, the maximum and the minimum were 24.0 and 3 cows (27.69 and 3.33%), respectively. The results showed significant variations ($P < 0.05$) in the means and non significant variations in the percentages.

The distributions of heifers among cities were as follows: 20.8± 12.71 heifers (18.66± 8.931%), 38.25± 24.32 heifers (21.424± 6.76%) and 22.85± 11.636 heifers (23.7± 8.686) for Khartoum, Khartoum North and Omdurman, respectively. The highest number of heifers was found in Khartoum North (119 heifers; 34.759%), which the minimum number of 8.0 heifers (7.5%). Khartoum farms, revealed a maximum number for heifer of 55 (33.537%) and minimum of 5 heifers (4.366%), while Omdurman farms revealed a mean of 39 heifers (40.21%) and a minimum of 4 heifers (7.143%). Significant variations ($P < 0.01$) were reported among heifer's means number and non significant values ($P > 0.05$) were reported for their percentages. Khartoum North farms showed the highest values for calves means, which reach 27.3± 26.28 calves (13.9± 6.73%), the maximum and minimum of 127 and 4 calves (33.159 and 4.651%), respectively. In Khartoum farms, the calf mean value was 23.5± 34.96 calves (17.48± 10.797%), while the maximum and the minimum values were 167 and 5 calves (39.175 and 4.268%), respectively. The farms in Omdurman revealed mean of 9.35± 5.99 calves (10.43± 5.76%) and a maximum and a minimum of 25 and 2 calves (23.81 and 2.381%), respectively. Non significant differences were obtained for calf mean numbers, while significantvariations($P<0.05$)wer e found for their percentages. However the highest numbers of calves were found in the farm that raised foreign breed (167 calves, 39.18%).

The average milk production in the farms of the different cities showed high significant variations ($P<0.001$) and were found as 581.25± 248.08, 885.15± 261.67 and 517.15± 203.14 litre for Khartoum, Khartoum North and Omdurman, respectively (Table 3).

Table 1: Educational level of the dairy farms owners at Khartoum State

City	Level of Education					
	Illiterate	**Primary school**	**Intermediate school**	**Secondary school**	**Other university graduate**	**Veterinary graduate**
Khartoum	7 35%	3 15%	5 25%	2 10%	1 5%	2 10%
Khartoum North	3 15%	4 20%	1 5%	7 35%	4 20%	1 5%
Omdurman	6 30%	11 55%	1 5%	1 5%	1 5%	0 0.0%
Total	16 26.67%	18 30%	7 11.67%	10 16.67%	6 10%	3 5%
P=0.008**						

**** : Significant (P < 0.01).**

Table 2: Types of dairy cattle breed dairy farms at Khartoum State

Breed / City	Cross cows	Kenana	Friesian	Jersey
Khartoum	17 85%	1 5%	1 5%	1 5%
Khartoum North	20 100%	0 00%	0 00%	0 00%
Omdurman	18 90%	2 10%	0 00%	0 00%
Total	55 91.67%	3 5%	1 1.67%	1 1.67%
P=0.509^{ns}				

ns=nonsignificant.

Table 3: Comparison of dairy herds size, herds structure and milk yield in the dairy farms in the three towns of Khartoum State

Cities / Measurements	Khartoum			Khartoum North			Omdurman			Level of significant
	Means ± Sd.	Max	Min	Means ± Sd.	Max	Min	Means ± Sd.	Max	Min	
Herd number	123.1±105.74	560.0	66.0	170.25±72.83	386.0	75.00	92.35±29.23	147.0	52.0	0.0076^{**}
Lactating cows	51.8±21.615	125.0	28.0	76.45±24.31	133.0	30.0	45.90±15.83	75.0	24.0	0.00004^{***}
% lactating cows	46.68±9.925	69.767	25.99	45.82±8.49	57.857	22.45	50.07±6.89	66.071	36.67	0.259^{ns}
Dry cows	24.3±34.72	168.0	3.00	31.10±15.20	75.0	11.00	13.65±5.715	24.00	3.00	0.049^{**}
% dry cows	17.179±6.71	34.927	3.093	18.86±9.152	47.059	9.375	15.806±6.717	27.69	3.33	0.45^{ns}
Heifers	20.8±12.71	55.0	5.0	38.25±24.32	119.0	8.0	22.85±11.636	39.00	4.00	0.0031^{**}
% Heifers	18.66±8.931	33.537	4.366	21.242±6.76	34.759	7.5	23.7±8.686	40.21	7.143	0.158^{ns}
Calves	23.5±34.96	167.0	5.0	27.3±26.28	127.00	4.00	9.35±5.99	25.00	2.0	0.072^{ns}
% calves	17.48±10.797	39.175	4.268	13.9±6.73	33.159	4.651	10.43±5.76	23.81	2.381	0.027^{**}
Milk yield (litres)	581.25±248.08	1446.00	302.0	885.15±261.67	1530.0	385.0	517.15±203.14	880.0	286	0.00006^{***}
Total herd number	123.10^{ab}			170.250^{a}			92.53^{b}			
Lactating cows	51.80^{b}			76.45^{a}			45.90^{b}			
Dry cows	24.30^{ab}			31.10^{a}			13.650^{b}			
Heifers	20.80^{b}			38.250^{a}			22.850^{b}			

[ns: not significant(P>0.05)]

***: (P < 0.05)**

****: (P < 0.01)**

*****: (P < 0.001)**

a and b = means in the same row with different superscript letters are significantly different(P<0.05)]

Table 3 also presents the herd structure and composition of the dairy farms in the three cities. The results indicated that the total herd number, dry cows and claves in Khartoum and Khartoum North, which were 123.10, 24.3 and 23.5 cows, and 170.25, 31.10 and 27.3 cows, respectively. They were 123.10, 24.3 and 23.5 cows and 92.35, 13.65 and 9.35 cows in Khartoum and Omdurman, respectively and they were not significantly different. However, the total number, dry cows and calves of dairy farms in Khartoum North were significantly higher ($P < 0.05$) than that of Omdurman. Furthermore the lactating cows and heifers of the farms in Khartoum North which were 76.45 and 38.25 were significantly higher ($P < 0.05$) than those of Khartoum and Omdurman (51.80 and 20.80, and 45.90 and 22.85 cows, respectively) as shown in Table 3.

1.4. Building materials

Table 4 shows the numbers and the percentages of farms that use building material for cattle housing. The studied farms in Khartoum, Khartoum North and Omdurman were found to use pipes for their fences in 9 farms (45%), 6 farms (30%) and zero, respectively. One, zero and zero farm were found to use metal iron and one (5%), 8 (40%) and 5 (25%) farms were found using stone for their fences, respectively. The broken cars fence, thorn and mud fences were found to be 5 (25%), 0 (0.0%) and 5 (25%); 4 (20%), 1 (5%) and 0 (0.0%); and 0 (0.0%), 5 (25%) and 10 (50%) of the total numbers of farms at the three cities, respectively (Table 4). These values were found to show highly significant variations ($P < 0.001$). Only six farms (10%) out of 60 farms were reported for using concrete floor. Three of these farms (15%) were found in Khartoum and the other three farms were found in Khartoum North. Corrugated iron roof was found only in 2 farms (10%) in each of Khartoum and Khartoum North, while 39 farms were reported to use hasir roof; 7 (35%) in Khartoum, 15

(75%) in Khartoum North and 17 (85%) in Omdurman. Two farms (10%) were using rakoba and one farm was using trees in Khartoum North (Table 4). However, 14 farms were reported not to have a roof, 11 farms (55%) of these were distributed in Khartoum and the rest three (15%) were in Omdurman. These results showed significant differences ($P > 0.01$).

1.5. Farms facilities

Water supply to the farms collected by donkeys was found in 5 (25%), 1 (5%) and 10 (50%) of the farms in Khartoum, Khartoum North and Omdurman, respectively. Furthermore water pipes were found in 15 (75%), 19 (95%) and 10 (50%) of the farms, respectively (Table 5). The data showed significant differences ($P < 0.01$). The presence of veterinary clinic in the farms and farms records were found in 1 (5%) and 2 (10%) of the farms at each of Khartoum and Khartoum North, respectively.

Store rooms for feed and the milking containers were found in 11 (55%), 7 (35%) and 10 (50%) of the studied farms at Khartoum, Khartoum North and Omdurman, respectively. At Khartoum, Khartoum North and Omdurman plastic, aluminum and stainless steel containers for collection of milk were used in 16 (80%), 11 (55%) and 15 (75%); 3 (15%), 7 (35%) and 4 (20%); and 1 (5%), 2 (10%) and 1 (5%) of the farms, respectively (Table 5).

1.6. Hygiene program in the farms studied

As shown in Table 6 only one farm (5%) in Khartoum was found to use milking parlour. Also one farm 1 (5%) in Khartoum North was reported using milking machine. Hence hand milking was found to be the common practice of milking the cows. From the survey it was also noticed that clean cow's udder program was practiced only in 3 farms (15%), one of them in Khartoum and the other two in Khartoum North. The results indicated significant difference ($P < 0.05$) between the three cities for clean udder

program. Similarly clean milkers were found only in 4 farms (20%) in Khartoum and 2 farms (10%) in Khartoum North. However, sanitation and cleaning of the milk utensils were clearly observed in 6 farms (30%) in Khartoum, 11 (55%) in Khartoum North and 3 farms (15%) in Omdurman (Table 6). Moreover, there were significant differences ($P < 0.05$) between the farms in the three cities for cleaning of milk utensils (Table 6). All farms except one farm in Khartoum have no cooling facilities. From the survey it was obviously clear that 4 (20%) of the farms in Khartoum North and 2 (10%) farms in Khartoum were under veterinary supervision. Their visits to the farm were reported either daily in 2 (10%) and 3 (15%) in Khartoum and Khartoum North or weekly as in one farm in Khartoum North. The rest of the farms were not under veterinarian supervision.

1.8. Dung removal

Daily dung removal was found to be practiced in one farm (5%) in each of Khartoum and Khartoum North (Table 7). Dung removal each 2 to 7 days was found in 10 (50%) farms in Khartoum, 10 (50%) farms in Khartoum North and 12 (60%) in Omdurman. Weekly dung removal was practiced in 8 (40%) farms in Khartoum, 9 (45%) farms in Khartoum North and 8 (40%) farms in Omdurman. One farm was found to practice dung removal once each 7-10 days in Khartoum. The data indicated highly significant differences ($P < 0.001$) for dung removal in the dairy farms at Khartoum State.

1.8. Diseases in the farms studied and vaccine programs

The numbers and percentages of the farms that suffer from abortion at late pregnancy and weither they test for the presence of the diseases were presented in Table 8. Similarly the same Table shows the rate of

Table 4: Comparison of the building material in some dairy farms at Khartoum State

Cities	Building materials												
	Fence						Floor		Roof				
	Pipes	Metal	Stone	Broken cars	Thorn	Mud	Sand	Concrete	No roof	Hasir	Corrugated iron	Rakoba	Trees
Khartoum	9 45%	1 5%	1 5%	5 25%	4 20%	0 0.0%	17 85%	3 15%	11 55%	7 35%	2 %	0 0.0%	0 0.0%
Khartoum North	6 30%	0 0.0%	8 40%	0 0.0%	1 5%	5 25%	17 85%	3 15%	0 0.0%	15 75%	2 10%	2 10%	1 5%
Omdurman	0 0.0%	0 0.0%	5 25%	5 25%	0 0.0%	10 50%	20 100%	0 0.0%	3 15%	17 85%	0 0.0%	0 0.0%	0 0.0%
Total	15 25%	1 1.67%	14 23.33%	10 17.1%	5 8.33%	15 25%	54 90%	6 10%	14 23.3 %	39 65%	4 6.66%	2 3.33%	1 1.67%
Level of significant	0.001^{***}						0.189^{ns}		0.004^{**}				

*****: highly Significant (P<0.001), ns= non significant**

**** = Significant (P<0.01) .**

Table 5: Comparison of farms building facilities, water supply, record keeping and milk containers in the dairy farms at Khartoum State

Cities	Water supply		Clinic in the farm		Record keeping		Store		Milk containers		
	Water Pipes	Donkey	No	Yes	No	Yes	No	Yes	Plastic	Aluminum	Stainless steel
Khartoum	15 75%	5 25%	19 95%	1 5%	19 95%	1 5%	11 55%	9 45%	16 80%	3 15%	1 5%
Khartoum North	19 95%	1 25%	18 95%	2 10%	18 90%	2 10%	7 35%	13 65%	11 55%	7 35%	2 10%
Omdurman	10 50%	10 50%	20 100%	0 0.0%	20 100%	0 0.0%	10 50%	10 50%	15 75%	4 20%	1 5%
Total	44 73.3 %	16 26.67 %	57 95 %	3 5 %	57 95 %	3 5 %	28 46.67 %	32 53.33 %	42 70 %	14 23.33 %	4 6.67 %
Level of significant	0.0055^{**}		0.35^{ns}		0.35^{ns}		0.418^{ns}		0.380^{ns}		

****: significant difference (P< 0.01), ns: non significant.**

Table 6: General hygiene and milking process in some dairy farms at Khartoum State

Cities	Type of milking			Cleaning the udder		Hygiene of milkers		Cleaning of milk utensils		Cooling facilities		Veterinary visits		
	Milking parlour	Milking machine	Hand milking	No	Yes	No	Yes	No	Yes	No	Yes	No visit	Yes daily	Yes weekly
Khartoum	1 50%	0 0.0%	19 95%	19 95%	1 5%	16 80%	4 20%	14 70%	6 30%	19 95%	1 5%	18 90%	2 10%	0 0.0%
Khartoum North	0 0.0%	1 5%	19 95%	18 90%	2 10%	18 90%	2 10%	9 45%	11 55%	20 100%	0 0.0%	16 80%	3 15%	1 5%
Omdurman	0 0.0%	0 0.0%	20 100%	20 100%	0 0.0%	20 100%	0 0.0%	17 85%	3 15%	20 100%	0 0.0%	20 100%	0 0.0%	0 0.0%
Total	1 1.67%	1 1.67%	58 96.67%	57 95%	3 5%	54 90%	6 10%	40 66.67%	20 33.33%	59 98.33%	1 1.67%	54 90%	5 8.33%	1 1.67%
Level of significant	0.596^{ns}			0.042^{*}		0.105^{ns}		0.025^{*}		0.36^{ns}		0.20^{ns}		

***: significant different at (P < 0.05)**

ns= non significant

Table 7: Dung removal programs in dairy farms at Khartoum State

Cities	Dung removal										
	Daily	**Every 2-4 days**	**3 days**	**3 – 4 days**	**4 days**	**4-6 days**	**4-7 days**	**5 days**	**5-7 days**	**Every week**	**Every 7-10 days**
Khartoum	1 5%	0 0.0%	0 0.0%	1 5%	5 25%	0 0.0%	4 20%	0 0.0%	0 0.0%	8 40%	1 5%
Khartoum North	1 5%	0 0.0%	1 5%	4 20%	0 0.0%	0 0.0%	4 20%	1 5%	0 0.0%	9 45%	0 0.0%
Omdurman	0 0.0%	3 15%	0 0.0%	0 0.0%	1 5%	4 20%	0 0.0%	0 0.0%	4 20%	8 40%	0 0.0%
Total	2 3.33%	3 5%	1 1.67%	5 8.33%	6 10%	4 6.67%	8 13.33%	1 1.67%	4 6.67%	25 41.67%	1 1.67%
Level of significant	0.0007^{***}										

*****: significant different at (P < 0.001)**

isolation and culling for the diseased cows in the studies farms. The occurrence of abortion at late pregnancy was found to be higher in Khartoum farms. These are 1, 2, 4, 10, 15 and 16 cases in one farm, while 3 and 5 cases were reported; each in 2 farms. The rest of the farms (10) recorded the absence of the abortion. At Khartoum North 1 case was reported in 4 farms, 2 cases were recorded in one farm, 3 cases were reported in 3 farms and no cases were recorded in 12 farms (Table 8). However, the lower numbers and percentages of abortion were recorded in Omdurman (one case in one farm, 2 cases in one farm and no cases in 18 farms). The values indicated non significant differences ($P > 0.05$) between the three cities. Only one farm in Khartoum was reported to practice testing for diseases with non significant differences ($P > 0.05$) from other farms. However the culling practices were found to be as one cow was culled in 2 farms (10%) in Khartoum, 2 farms (10%) in Khartoum North and 1 farm (5%) in Omdurman. Three cows (15%) were culled in 1 farm (5%) in each of three city. Four cows were culled in 1 farm (5%) in each of Khartoum and Khartoum North and five cows were culled in 2 farms (10%) in Khartoum and 1 farm (5%) in Khartoum North. Eight cows were culled in 1 farm (5%) in Khartoum North and 15 cows were culled in 1 farm (5%) in Khartoum.

The present data showed that culling was not practiced in 13 (65%), 14 (70%) and 18 (90%) of the dairy farms in Khartoum, Khartoum North and Omdurman, respectively. The data also revealed non significant differences ($P > 0.05$) concerning culling practices in the three cities of Khartoum State (Table 8).

It is clear from Table 9 that with exception of vaccines provided by veterinary authorities for control of contagious disease, other vaccinations for management and preventive measures were not common. The latest

vaccines administrated during May 2003 was done in 7 (35%) farms in Khartoum, 5 (25%) farms in Khartoum North and 5 (25%) farms in Omdurman (Table 9). During March 2002, it was done in 5 (25%), 6 (30%) and 1 (5%) farms respectively, while at September 2001, only 1 (5%), 1 (5%) and 3 (15%) farms respectively, were able to vaccinate their herds. However 7 (35%), 8 (40%) and 11 (55%) farms respectively, were found not to vaccinate their herds (Table 9).

Table 10 shows the results of mastitis cases in the studied farms. Also it explains the preventive measures applied in the farms and the ways of disposing the abnormal milk. The incidences of mastitis were reported as one case in each of 7 farms (35%) in Khartoum, 4 farms (20%) in Khartoum North and 4 farms (20%) in Omdurman. Also two cases were found in 5 (25%), 3 (15%) and 4 (20%) farms in Khartoum, Khartoum North and Omdurman, respectively. Similarly 3 cases were found in 1 farm (5%) in Khartoum and 4 farms (20%) in Khartoum North. Furthermore 5 cases were reported in 1 (5%), 1 (5%) and 2 (10%) farms at Khartoum, Khartoum North and Omdurman, respectively. However, 6 (30%), 8 (40%) and 10 (50%) farms at Khartoum, Khartoum North and Omdurman were reported not to claim cases of mastitis. These values showed non significant ($P > 0.05$) variations (Table 10). Only one farm in Khartoum was found to use strip cup and iodine before milking, after washing the udder by water. Most of the farms were found not to use any of preventive measures like isolation, diagnosis and treatment of diseased cows. Moreover, they used some drugs without veterinary advices or inspections. These drugs were found to be both "udderiod" and "neomastipra" in 2 (10%) farms in Khartoum. Terrexine (tetracycline) was used in 2 farms (10%) in Khartoum and 1 farm (5%) in Khartoum North.

Table 8: Incidences of brucella and culling practices of diseased cows in the dairy farms at Khartoum State

	No of abortion at late pregnancy									Brucella test		Number of brucella culled cows						
	0	1	2	3	4	5	10	15	16	No	Yes	0	1	3	4	5	8	15
Khartoum	10 50%	1 5%	1 5%	2 10%	1 5%	2 10%	1 5%	1 5%	1 5%	19 95%	1 5%	13 65%	2 10%	1 5%	1 5%	2 10%	0 0.0%	1 5%
Khartoum North	12 60%	4 20%	1 5%	3 15%	0 0.0	0 0.0%	0 0.0%	0 0.0%	0 0.0%	20 100%	0 0.0%	14 70%	2 10%	1 5%	1 5%	1 5%	1 5%	0 0.0%
Omdurman	18 90%	1 5%	1 5%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	20 100%	0 0.0%	18 90%	1 5%	1 5%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
Total	40 66.6 %	6 10%	3 5%	5 8..33%	1 1.67%	2 3..33%	1 1.67%	1 1.67%	1 1.67%	59 98.33%	1 1.67%	45 75%	5 8.33%	3 5%	2 3..33%	3 5%	1 1.67%	1 1.67%
Level of significant	0.202^{ns}									0.361^{ns}		0.758^{ns}						

ns : non significant

Table 9: Comparison of the latest vaccines programs in the dairy farms at Khartoum State

Cities	Brucella and other vaccines* adminsteration in the dairy farms under studied			
	No vaccine administration	September 2001	March 2002	May 2003
Khartoum	7 35%	1 5%	5 25%	7 35%
Khartoum North	8 40%	1 5%	6 30%	5 25%
Omdurman	11 55%	3 15%	1 5%	5 25%
Total	26 43.33%	5 8.33%	12 20%	17 28.33%
Level of significant	0.36^{ns}			

ns= non significant.

* Foot and mouth disese, brucella, Haemorrahgic septisemea, Anthrax, Rinder pest ect….

Udderiod was used in 3 (15%), 9 (45%) and 4 (20%) of the farms in Khartoum, Khartoum North and Omdurman, respectively. Similarly systemic antibiotic and udderiod were found to be used in 1 (5%) farm in each of Khartoum and Omdurman and both udderiod and terrexine (tetracycline) in 1 farm (5%) in Khartoum and 3 (15%) farms in Omdurman. However, one farm in Khartoum North was used systemic antibiotic only. The result also showed non significant differences ($P > 0.05$) for the preventive measure applied in the farms in the three cities at Khartoum State.

Disposal of abnormal milk in pots that were latter thrroughen in the floor were found to be applied in 2 (10%) and 4 (20%) farms in Khartoum and Khartoum North, respectively, while isolating mastitic cows and milking them out of the pens was reported in 4 (20%) farms at Khartoum only (Table 10). However, 14 (70%), 16 (80%) and 20 (100%) farms in Khartoum, Khartoum North and Omdurman were found to milk the diseased cows directly in the floor of the pens. Moreover, the data indicated significant differences ($P < 0.05$) between the three cities (Table 10).

Table 11 shows the estimated means and standard deviations of aborted cows in Khartoum, Khartoum North and Omdurman. They were found to be 3.2± 4.948, 0.75± 1.118 and 0.15± 0.489 cows respectively, which revealed significantly different ($P < 0.05$). The number of culled cows in Khartoum was 1.7± 3.585 cows, in Khartoum North was 1.10± 2.198 cows and in Omdurman was 0.20± 0.696 cows, with non significant differences ($P > 0.05$). Abortion at late pregnancy in Khartoum North and Omdurman farm's revealed non significant variations ($P > 0.05$), while there was significant differences ($P < 0.05$) between farms in Khartoum and Omdurman and between farms in Khartoum and Khartoum North (Table 11).

Table 10: Incidences of mastitis and its treatment in some dairy farms at Khartoum State

Cities	Mastitis															
	Number of cases reported					Preventive measure								Disposal of abnormal milk		
	0	1	2	3	5	No preventive measure applied	Udderiod± neomastipra	Terrexin (Tetracycline)	Udderiod	Antibiotic + udderiod	Udderiod + Terrexin	Strip + iodine	Antibiotic only	In floor of pens	In pot	Cow isolated and milked out of pen
Khartoum	6 30%	7 35%	5 25%	1 5%	1 5%	10 50%	2 10%	2 10%	3 15%	1 5%	1 5%	1 5%	0 0.0%	14 70%	2 10%	4 20%
Khartoum North	8 40%	4 20%	3 15%	4 20%	1 5%	9 45%	0 0.0%	1 5%	9 45%	0 0.0%	0 0.0%	0 0.0%	1 5%	16 80%	4 20%	0 0.0%
Omdurman	10 50%	4 20%	4 20%	0 0.0%	2 10%	12 60%	0 0.0%	0 0.0%	4 20%	1 5%	3 15%	0 0.0%	0 0.0%	20 100%	0 0.0%	0 0.0%
Level of significant	0.395^{NS}					0.172^{NS}								0.011^{*}		

ns:non significant ,*:significant (P<0.05)

Table 11: Comparison of aborted cows and culling practiced in Khartoum State dairy farms

Cities	Khartoum	Khartoum North	Omdurman	Level of significant
Abortion at late pregnancy	3.20±4.948	0.75±1.118	0.15±0.489	0.004^{**}
Number of culled cows	1.70±3.585	1.10±2.198	0.20±0.696	0.16^{NS}
Abortion at late pregnancy by Duncan test	3.200^{a}	0.8333^{b}	0.1500^{b}	0.004^{**}

****: significant (P<0.05)**

a and b = means in the same rows with different superscript letters are significant different (P < 0.05)

2. The effect of interaction of season and cities on milk contents

The standard plate counts of the milk samples collected from Khartoum, Khartoum North and Omdurman during summer and winter were $1.6 \times 10^{11} \pm 1.5 \times 10^{11}$ and $5.6 \times 10^{7} \pm 1.4 \times 10^{8}$, $1.1 \times 10^{8} \pm 1.0 \times 10^{8}$ and $1.6 \times 10^{8} \pm 4.3 \times 10^{8}$ and $7.6 \times 10^{8} \pm 1.5 \times 10^{9}$ and $7.9 \times 10^{6} \pm 9.76 \times 10^{6}$, respectively (Table 12 and Table 13). There were highly significant differences ($P < 0.001$) due to variations of seasons, cities and their interactions (Table 16). The laboratory pasteurization mean counts for milk samples collected from Khartoum, Khartoum North and Omdurman recorded during summer and winter were $1.4 \times 10^{9} \pm 1.5 \times 10^{9}$ and $4.6 \times 10^{6} \pm 1.2 \times 10^{7}$, $2.4 \times 10^{6} \pm 3.2 \times 10^{6}$ and $1.5 \times 10^{6} \pm 2.7 \times 10^{6}$ and $4.6 \times 10^{7} \pm 1.1 \times 10^{8}$ and $2.7 \times 10^{5} \pm 6.5 \times 10^{5}$, respectively (Table 12 and Table 13). The results also indicated highly significant differences ($P < 0.001$) for season, cities and their interactions (Table 16). The staphylococcus counts of the milk collected during summer and winter from Khartoum, Khartoum North and Omdurman were $4.6 \times 10^{8} \pm 6 \times 10^{8}$, $1.3 \times 10^{5} \pm 3.2 \times 10^{5}$, $5.6 \times 10^{7} \pm 2.5 \times 10^{8}$ and $5.8 \times 10^{4} \pm 9.2 \times 10^{4}$ and $4.9 \times 10^{5} \pm 8.6 \times 10^{5}$ and $3.0 \times 10^{5} \pm 7.0 \times 10^{5}$, respectively (Table 12 and Table 13). Also those values showed highly significant differences ($P < 0.001$) as shown in Table (16). The Enterobacteriaceae mean counts were estimated as $7.1 \times 10^{9} \pm 6.7 \times 10^{9}$ and $7.5 \times 10^{4} \pm 1.6 \times 10^{5}$, $1.9 \times 10^{5} \pm 3.0 \times 10^{5}$ and $2.0 \times 10^{6} \pm 3.2 \times 10^{6}$ and $9.6 \times 10^{6} \pm 2.3 \times 10^{7}$ and $4.3 \times 10^{5} \pm 1.2 \times 10^{6}$, for milk samples collected

from Khartoum, Khartoum North and Omdurman during summer and winter respectively, as shown in Table 12 and Table 13. These values were found to be highly significantly different ($P < 0.001$) as shown in Table 16. The coliforms mean counts of milk samples collected from Khartoum, Khartoum North and Omdurman during summer and winter were $7.2 \times 10^9 \pm 7.1 \times 10^9$ and $2.3 \times 10^5 \pm 4.3 \times 10^5$, $1.6 \times 10^5 \pm 2.7 \times 10^5$ and $1.7 \times 10^6 \pm 2.9 \times 10^6$ and $2.9 \times 10^7 \pm 1.1 \times 10^8$ and $4.3 \times 10^5 \pm 1.2 \times 10^6$, respectively (Table 12 and Table 13). The results also indicated highly significant differences ($P < 0.001$) as shown in Table 15.

The mean of temperature of milk from the farms in Khartoum, Khartoum North and Omdurman were found to be 35.9± 7.64, 37.7± 2.3 and 33.15± 2.76º C respectively, during summer. They were 33.8±5.26, 32.6± 2.87 and 33.25± 2.45ºC during winter, respectively (Table 14 and Table 15). The result indicated significant differences ($P < 0.01$) due to season and interaction between seasons and cites (Table 16). Also the means of the freezing point of milk were estimated as -525.85± 22.05, -511.5± 22.59 and -518.35 ± 30.61 during summer and -526.60± 14.12, -537.25 ± 13.92 and -534.9 ± 10.26% during winter for the milk collected from Khartoum, Khartoum North and Omdurman, respectively (Table 14 and 15). The values for freezing point were found to show significant differences ($P < 0.001$) due to the variations of season and their interaction with cities. The means of pH of the milk samples collected from Khartoum, Khartoum North and Omdurman were estimated as 6.35± 0.177, 6.232± 0.080 and 6.44± 0.28 during summer and 6.74 ± 0.049, 6.65 ± 0.095 and 6.82± 0.079 during winter, respectively (Table 14 and 15). These values were found to show non significant variations ($P >0.05$) for interaction of seasons and cities as shown in Table 16. The means of acidity of the milk

collected during summer and winter were 0.2± 0.026%, 0.186± 0.012% and 0.194± 0.02%7 and 0.185± 0.025%, 0.160± 0.013% and 0.148± 0.0088%, respectively. Those values indicated highly significant differences due to seasonal variations and of cities ($P > 0.001$) and due to interactions of seasons and cities ($P < 0.01$).

The means of the fat content of the milk were 4.57± 0.516% in Khartoum, 4.44± 0.46% in Khartoum North and 4.5± 0.412% in Omdurman during summer. Similarly they were 4.54± 0.67% in Khartoum, 4.45± 0.501% in Khartoum North and 4.64 ± 0.59% in Omdurman during winter season. The values indicated non significant differences ($P > 0.05$) as shown in Table 16. Moreover the protein content of the milk samples collected from Khartoum, Khartoum North and Omdurman were 3.8± 0.69%, 3.82± 0.76% and 3.68± 0.7% during summer and 3.73± 0.399%, 3.69± 0.422% and 3.67%± 0.4% during winter season, respectively (Tables 14 and Table 15). The results indicated non significant differences ($P > 0.05$). The results showed that ash content of the milk samples collected during summer and winter. The milk collected from Khartoum revealed 0.58 %± 0.133% and 0.59%± 0.095%, from Khartoum North revealed 0.62%± 0.079 and 0.6%± 0.105% and from Omdurman revealed 0.68%± 0.1 and 0.591± 0.07, respectively.

The means of lactose of milk samples collected from Khartoum, Khartoum North and Omdurman were found to be 3.77± 0.416%, 4.2± 0.521% and 3.89± 0.66% during summer, respectively. They were 3.98± 0.814%, 3.88± 0.516% and 4.32± 0.39% during winter, respectively (Table 14 and 15). The results indicated significant differences ($P < 0.01$) for the interactions of seasons and cities.

The total solids content of milk samples collected from Khartoum, Khartoum North and Omdurman were reported as 12.24± 1.251%, 12.29±

1.1% and 12.87± 1.74% during summer season and 12.6± 0.874%, 12.51± 0.94% and 13.21± 0.82% during winter season, respectively (Table 14 and Table 15). The total solids content revealed non significant differences ($P > 0.05$) due to the interaction of seasons and cities.

3. The effect of season on milk contents

Table 17 give the microbial content of the raw milk samples collected during summer, winter and both seasons. The results showed that the mean of the standard plate counts of milk samples were $5.3\times 10^{10}\pm 1.1 \times 10^{11}$ during summer, $7.5 \times 10^{7}\pm 2.6 \times 10^{8}$ during winter and $2.6\times 10^{10}\pm 8.4 \times 10^{10}$, for both seasons, respectively. The results indicated highly significant differences ($P< 0.001$) in standard plate counts between summer and winter season (Table 16). The estimated laboratory pasteurization counts, staphylococcus counts, Entrobacteriaceae counts and coliforms counts of milk samples collected during summer were assessed as $4.7\times 10^{8}\pm 1.1\times 10^{9}$, $1.7 \times 10^{8}\pm 4.2 \times 10^{8}$, $2.4 \times 10^{9}\pm 5.1 \times 10^{9}$ and $2.4\times 10^{9}\pm 5.3 \times 10^{9}$,respectively. However, those collected during winter revealed $2.1\times 10^{6} \pm 7.1\times 10^{6}$, $1.6 \times 10^{5}\pm 4.5 \times 10^{5}$, $8.4 \times 10^{5}\pm 2.1 \times 10^{6}$ and $7.8\times 10^{5}\pm 1.9 \times 10^{6}$. The total bacterial counts in both seasons were $2.4\times 10^{8}\pm 7.9\times 10^{8,}$ $8.6 \times 10^{7}\pm 3.1\times 10^{8}$, $1.2 \times 10^{*}\pm 3.8\times 10^{9}$ and $1.2\times 10^{9}\pm 3.9 \times 10^{9}$, respectively (Table 17). The laboratory pasteurization counts, staphylococcus counts, Enterobacteraceae counts and coliforms counts as viewed in Table 16 were highly significantly different ($P < 0.001$) due to variation of seasons.

Table 12: Variation in microbial counts of raw milk of somee dairy farms in Khartoum State during summer season

Cities / Measurement	Khartoum				Khartoum North				Omdurman			
	Mean	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**
Standard plate count (SPC cfu/ml)	1.6×10^{11}	1.5×10^{11}	3.3×10^{11}	1.1×10^{6}	1.1×10^{8}	1.0×10^{8}	2.9×10^{8}	1.8×10^{6}	7.6×10^{8}	1.5×10^{9}	5.2×10^{9}	7.7×10^{5}
Laboratory pasteurization count (LPC cfu/ml)	1.4×10^{9}	1.5×10^{9}	3.1×10^{9}	4.0×10^{4}	2.4×10^{6}	3.2×10^{6}	1.2×10^{7}	6.0×10^{3}	4.6×10^{7}	1.1×10^{8}	4.8×10^{8}	4.0×10^{5}
Staphylococcus count (cfu/ml)	4.6×10^{8}	6.0×10^{8}	2.1×10^{9}	1.3×10^{5}	5.6×10^{7}	2.5×10^{8}	1.1×10^{9}	0	4.9×10^{5}	8.6×10^{5}	2.8×10^{6}	0
Enterobacteriaceae count (cfu/ml)	7.1×10^{9}	6.7×10^{9}	1.5×10^{10}	0	1.9×10^{5}	3.0×10^{5}	1.1×10^{6}	0	9.6×10^{6}	2.3×10^{7}	8.3×10^{7}	0
Coliforms counts (cfu/ml)	7.2×10^{9}	7.1×10^{9}	1.5×10^{10}	1.4×10^{6}	1.6×10^{5}	2.7×10^{5}	1.1×10^{6}	0	2.9×10^{7}	1.1×10^{8}	4.9×10^{8}	0

SD: standard deviation

Table 13: Variation in microbial counts of raw milk of some dairy farms in Khartoum State during winter season

Cities / Measurement	Khartoum				Khartoum North				Omdurman			
	Mean	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**
Standard plate count (SPC) (cfu/ml)	5.6×10^7	1.4×10^8	6.1×10^8	4.0×10^5	1.6×10^8	4.3×10^8	1.4×10^9	2.5×10^6	7.9×10^6	9.6×10^6	4.5×10^7	5.0×10^5
Laboratory pasteurization count (LPC cfu/ml)	4.6×10^6	1.2×10^7	5.3×10^7	$6..5 \times 10^4$	1.5×10^6	2.7×10^6	1.0×10^7	0	2.7×10^5	$6..5 \times 10^5$	2.4×10^6	0
Staphylococcus count (cfu/ml)	1.3×10^5	3.2×10^5	1.4×10^6	2.0×10^3	5.8×10^4	9.2×10^4	3.4×10^5	1.1×10^3	3.0×10^5	7.0×10^5	3.2×10^6	0
Enterobacteriaceae count (cfu/ml)	7.5×10^4	1.6×10^5	6.3×10^5	0	2.0×10^6	3.2×10^6	9.9×10^6	0	4.3×10^5	1.2×10^6	5.3×10^6	0
Coliforms counts (cfu/ml)	2.3×10^5	4.3×10^5	1.5×10^6	0	1.7×10^6	2.9×10^6	1.1×10^7	1.5×10^4	4.3×10^5	1.2×10^6	5.5×10^6	5.0×10^2

SD: standard deviation

Table 14: Variations in some physial and chemical properties of raw milk of some dairy farms in Khartoum State during summer season

Cities / Measurment	Khartoum				Khartoum North				Omdurman			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Temperature (º C)	35.9	7.64	39	4	37.7	2.3	40	31	33.15	2.76	38	30
Freezing point	-525.85	22.05	-450	-568	-511.5	22.59	-448	-540	-518.35	30.61	-440	-577
pH	6.35	0.177	6.67	6.15	6.232	0.080	6.33	6.04	6.44	0.28	6.90	6.01
Acidity (%)	0.20	0.026	0.25	0.17	0.186	0.012	0.22	0.17	0.194	0.027	0.26	0.16
Fat (%)	4.57	0.516	5.30	3.40	4.44	0.46	5.30	3.60	4.5	0.412	5.5	3.95
Protein (%)	3.8	0.69	5.9	2.9	3.82	0.76	5.70	3.0	3.68	0.7	5.9	2.5
Ash (%)	0.58	0.133	0.78	0.33	0.62	0.079	0.77	0.50	0.68	0.1	0.82	0.46
Lactose (%)	3.77	0.416	4.8	3.0	4.2	0.521	4.90	3.50	3.89	0.66	4.9	3.0
Total solids (%)	12.24	1.251	13.6	8.25	12.29	1.1	13.75	10.10	12.87	1.74	15.20	9.55

SD =standard deviation

Table 15: Variations in some physical and chemical properties of raw milk of somee dairy farms in Khartoum State during winter season

Cities / Measurment	Khartoum				Khartoum North				Omdurman			
	Mean	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**	**Mean**	**SD**	**Max**	**Min**
Temperature (º C)	33.8	5.26	38	15	32.60	2.87	39	26	33.25	2.45	36	26
Freezing point	-526.60	14.12	-491	-546	-537.25	13.92	-520	-578	-534.9	10.26	-518	-564
pH	6.74	0.049	6.80	6.70	6.65	0.095	6.80	6.50	6.82	0.079	6.90	6.60
Acidity (%)	0.185	0.025	0.23	0.14	0.1602	0.013	0.19	0.14	0.148	0.0088	0.17	0.14
Fat (%)	4.54	0.67	5.9	3.1	4.45	0.501	5.35	3.5	4.64	0.59	5.4	3.10
Protein (%)	3.73	0.339	4.3	2.9	3.69	0.422	4.9	3.10	3.67	0.40	4.10	2.7
Ash (%)	0.59	0.095	0.74	0.42	0.60	0.105	0.78	0.14	0.591	0.07	0.73	0.48
Lactose (%)	3.98	0.814	6.6	3.00	3.88	0.516	4.7	3.0	4.32	0.39	4.8	3.4
Total solids (%)	12.6	0.874	14.25	11.10	12.51	0.940	14.55	10.30	13.21	0.82	14.35	11.01

SD = standard deviation

Table 16: The level of significant for physio-chemical and microbial properties of raw milk of some dairy farms at Khartoum State

	Level of significant		
Measurements	**Season**	**Cities**	**Interaction between seasons and cities**
Temperature	0.01**	(P > 0.05)	0.01**
Freezing point	0.001***	(P > 0.05)	0.001***
PH	0.001***	0.001***	(P > 0.05)
Acidity	0.001***	0.001***	0.01**
Fat	(P > 0.05)	(P > 0.05)	(P > 0.05)
Protein	(P > 0.05)	(P > 0.05)	(P > 0.05)
Ash	(P > 0.05)	(P > 0.05)	(P > 0.05)
Lactose	(P > 0.05)	(P > 0.05)	0.01**
Total solids	(P > 0.05)	0.01**	(P > 0.05)
Standard plate count	0.001***	0.001***	0.001***
Laboratory pasteurization count	0.001***	0.001***	0.001***
Staphylococcus count	0.001***	0.001***	0.001***
Entrobacteriacaeae count	0.001***	0.001***	0.001***
Coliforms counts	0.001***	0.001***	0.001***

0.01 = level of significant (P < 0.01).

0.001 = level of significant (P < 0.001).

(P > 0.05) = Non significant

The mean value of temperature of the milk collected from dairy farms at Khartoum State during summer, winter and both seasons were 35.58±5.15, 33.22±3.71 and 34.4±4.62º C, respectively (Table 18). The values of temperature of milk samples collected during summer and winter were found to be significantly different ($P < 0.01$) as shown in Table 16. The mean values of the freezing point of milk samples were -518.57±25.65, -532.92±13.48 and -525.74±21.64º C during summer, winter and both seasons, respectively. The results indicated highly significant differences ($P< 0.001$) in freezing point between the two seasons (Table 16). The estimated pH of milk samples during summer, winter and both seasons were reported as 6.34±0.211, 6.73±0.10 and 6.54±0.257 respectively, with highly significant differences ($P< 0.001$). During summer, winter and both seasons the mean values of acidity of milk samples were 0.193±0.023%, 0.164±0.023% and 0.179±0.027%, respectively. There were high significant differences ($P< 0.001$) as shown in Table 16. Furthermore, the mean values of fat, protein, ash, lactose and total solids of milk samples were 4.5±0.46%, 3.77±0.71%, 0.63±0.11%, 3.95±0.561% and 12.47±1.39% during summer season. They were 4.54±0.586%, 3.69±.3997%, 0.593±0.0896%, 4.06±0.618% and 12.77±0.919% during winter. Their averages were found to be 4.52±0.525%, 3.73±0.575%, 0.61±0.103%, 4.01±0.59% and 12.62±1.19% for both seasons, respectively (Table 18). However, the values of fat, protein, ash, lactose and total solids of milk samples collected during summer and winter revealed non significant differences ($P > 0.05$) as viewed in Table 16.

4. Variation of microbial counts and physicochemical properties of raw milk in dairy farms

The mean of the total bacterial counts and the laboratory pasteurization counts (cfu/ml) were $7.9\times 10^{10} \pm 1.3\times 10^{11}$ and 6.9×10^{8}±1.3

× 10^9 in the milk samples collected from Khartoum. They were 1.4 × 10^8± 3.1 × 10^8 and 2× 10^6± 3× 10^6 in the milk samples collected from Khartoum North and 3.8× 10^8± 1.1× 10^9 and 2.3× 10^7± 8.3× 10^7 in the milk samples collected from Omdurman, respectively (Table 19). These values were found to be highly significantly different (P< 0.001) as shown in Table 16. The mean of staphylococcus counts, Enterobacteriaceae counts and coliforms counts of the milk samples collected from Khartoum were assessed as 2.3× 10^8± 4.8× 10^8, 3.6× 10^9± 5.9× 10^9 and 3.6× 10^9± 6.2 × 10^9. Those collected from Khartoum North revealed means of 2.3× 10^7± 1.7× 10^8, 1.1 × 10^6± 2.4× 10^6 and 9.2× 10^5± 2.2× 10^6 cfu/ml. Similarly the means counts of the milk samples collected from Omdurman were 3.9× 10^5± 7.8× 10^5, 5.0 × 10^6± 1.7× 10^7 and 1.5× 10^7± 7.8× 10^7 cfu/ml, respectively (Table 19). The values were found to be highly significantly different (P< 0.001) as shown in Table 16. Furthermore, the acidity, total bacterial counts, laboratory pasteurization counts, Staphylococcus counts, Enterobacteriaceae counts and coliforms counts, of the raw milk samples collected from the dairy farms in Khartoum were 0.193, 7.9 × 10^{11}, 6.9 × 10^9, 2.3 × 10^8, 3.6 × 10^{10} and 3.6 × 10^9 cfu/ml. They were significantly higher (P< 0.05) than those of Khartoum North and Omdurman (0.173, 1.4 × 10^9, 2 × 10^6, 2.8 × 10^7, 1.1 × 10^6 and 9.2 × 10^5 and 0.171, 3.8 × 10^9, 2.3 × 10^8, 4.0 × 10^5, 5 × 10^6, and 1.5 × 10^7 cfu/ml, respectively) as shown in Table 19.

The mean values of temperature and freezing point of milk samples were 34.85± 6.56 and -526.22±18.28 in Khartoum dairy farm's, 35.15± 3.64 and -524.38± 22.65 in Khartoum North dairy farm's and 33.2±2.57 and -526.63±24.04 in Omdurman dairy farm's, respectively (Table 20). However, the values of the temperature and freezing point showed non significant variations (P> 0.05) due to variations of cities. The mean of pH and acidity of milk samples collected from Khartoum, Khartoum North and Omdurman revealed 6.544± 0.232 and 0.193± 0.026%, 6.441± 0.2289 and 0.173± 0.018% and 6.627± 0.2789 and 0.171± 0.031%, respectively (Table 20). The results indicated that there were highly significant differences (P< 0.001) between the three locations. The estimated means of fat, protein, ash and lactose of the milk samples which were collected from Khartoum were 4.56±0.5904%, 3.76±0.559%, 0.586±0.115% and 3.876±0.647%. Those from Khartoum North revealed 4.45±0.476%, 3.75±0.612%, 0.608±0.092% and 4.038±0.536%. However, the milk samples which were collected from Omdurman revealed 4.569±0.507%, 3.67±0.563%, 0.635±0.097% and 4.103±0.575%, respectively (Table 20). The results showed that the means for total solids of the milk samples collected from Khartoum, Khartoum North and Omdurman were found to be 12.42±1.081%, 12.399±1.012% and 13.04±1.35%, respectively. They revealed significant differences (P < 0.01) due to variations of locations (Table 16).

The results indicated that the pH of milk samples collected from Khartoum and Khartoum North, which was 6.54 and 6.44 revealed non significant differences (P> 0.05). The pH of the milk samples were 6.54 and 6.62 in Khartoum and Omdurman, respectively. They were not significantly different. However, the pH of milk samples collected from dairy farms in Omdurman were significantly higher (P < 0.05) than that collected from Khartoum North. Also the results indicated that the means of total solids content of raw milk samples, which collected from

Omdurman was 13.04% and was found to be significantly higher (P< 0.05) than those of Khartoum and Khartoum North (12.42 and 12.39%, respectively) as shown in Table 20.

5. Seasonal distribuation of some pathogenic microorganisms in raw milk

Bacteriological finding in this study were based on the isolation of some pathogenic bacteria as shown in Table 21. Brucella milk ring test revealed that out of 120 samples 104 were positive for brucella. They were found to be 50 (85.33%) during summer and 54 (90.0%) during winter season. Those values revealed non significant differences (P> 0.05). Furthermore, fifty three isolates were identified as *Staphylococcus aureus*, 23 (38.33%) were found during summer, while 30 (50%) were isolated during winter season. Also their values were non significantly different (P > 0.05). No case was reported for *Escherichia coli* during summer but 13 isolates (21.67%) were found during winter season. Moreover, a highly significant difference (P< 0.001) was recorded for variation between seasons for the presence of *E. coli*. However one isolate of *Salmonella spp.* was recorded during summer season in the milk collected from Soba at Khartoum (Table 21).

Milk samples for brucella, *Staphylococcus aureus, Escherichia coli* and *Salmonella spp*. were tested and identified (Table 22 and 23). The positive results of isolates were recorded as numbers and percentages at summer and winter for the different selected places in Khartoum State as shown in Table 22. For brucella incidences were 17 (85%), 17 (85%) and 16 (80%) in the milk samples collected from Khartoum, Khartoum North and Omdurman, respectively at summer season. The distribution were

Table 17: Frequency analysis of microbial contents of raw milk of some dairy farms in Khartoum State during summer and winter seasons

Season / Microbial measurement (cfu/ml)	Summer				Winter				Total			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Standard plate count	5..3 $\times 10^{10}$	1.1 $\times 10^{11}$	3..3 $\times 10^{11}$	7.7 $\times 10^{5}$	7.5 $\times 10^{7}$	2..6 $\times 10^{8}$	1.4 $\times 10^{9}$	4.0 $\times 10^{5}$	2..6 $\times 10^{10}$	8.4 $\times 10^{10}$	3.3 $\times 10^{11}$	4..0 $\times 10^{5}$
Laboratory pasteurization count	4.7 $\times 10^{8}$	1.1 $\times 10^{9}$	3.1 $\times 10^{9}$	6..0 $\times 10^{3}$	2.1 $\times 10^{6}$	7.1 $\times 10^{6}$	5..3 $\times 10^{7}$	0	2.4 $\times 10^{8}$	7.9 $\times 10^{8}$	3.1 $\times 10^{9}$	0
Staphylococcus count	1.7 $\times 10^{8}$	4..2 $\times 10^{8}$	2.1 $\times 10^{9}$	0	1..6 $\times 10^{5}$	4.5 $\times 10^{5}$	3..2 $\times 10^{6}$	0	8..6 $\times 10^{7}$	3.1 $\times 10^{8}$	2.1 $\times 10^{9}$	0
Enterobacteriaceae count	2.4$\times 10^{9}$	5.1 $\times 10^{9}$	1..5 $\times 10^{10}$	0	8.4 $\times 10^{5}$	2.1 $\times 10^{6}$	9..9 $\times 10^{6}$	0	1..2 $\times 10^{9}$	3.8 $\times 10^{9}$	1..5 $\times 10^{10}$	0
Coliforms counts	2.4 $\times 10^{9}$	5..3 $\times 10^{9}$	1..5 $\times 10^{10}$	0	7..8 $\times 10^{5}$	1..9 $\times 10^{6}$	1.1 $\times 10^{7}$	0	1.2 $\times 10^{9}$	3.9 $\times 10^{9}$	1.5 $\times 10^{10}$	0

SD=Standard deviation

Table 18: Frequency analysis of some physical and chemical properties of raw milk of some dairy farms in Khartoum State during summer and winter season

Season / Measurement	Summer				Winter				Total			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Temperature °C	35.58	5.15	40	4	33.22	3.71	39	15	34.40	4.62	40	4
Freezing point	-518.57	25.65	-440	-577	-532.92	13.48	-491	-578	-525.74	21.64	-440	-578
pH	6.34	0.211	6.9	6.01	6.73	0.10	6.90	6.50	6.54	0.257	6.90	6.01
Acidity (%)	0.193	0.023	0.26	0.160	0.164	0.023	0.23	0.14	0.179	0.027	0.26	0.14
Fat (%)	4.50	0.46	5.5	3.4	4.54	0.586	5.90	3.10	4.52	0.525	5.90	3.10
Protein (%)	3.77	0.71	5.90	2.50	3.69	0.3997	4.9	2.7	3.73	0.575	5.90	2.50
Ash (%)	0.63	0.11	0.82	0.33	0.593	0.0896	0.78	0.41	0.61	0.103	0.82	0.33
Lactose (%)	3.95	0.561	4.90	3.00	4.06	0.618	6.60	3.00	4.01	0.59	6.60	3.00
Total solids (%)	12.47	1.39	15.20	8.25	12.77	0.919	14.55	10.30	12.62	1.19	15.20	8.25

SD=Standard deviation

Table 19: Frequency analysis of some microbial content of raw milk of the dairy farms of the three cities of Khartoum State

Cities / Microbial measurement (cfu/ml)	Khartoum				Khartoum North				Omdurman			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Standard plate count	7..9 ×10^{10}	1..3 ×10^{11}	3..3 ×10^{11}	4..0 ×10^{5}	1.4 ×10^{8}	3.1 ×10^{8}	1.4 ×10^{9}	1..8 ×10^{6}	3..8 ×10^{8}	1.1 ×10^{9}	5..2 ×10^{9}	5..0 ×10^{5}
Laboratory pasteurization count	6.9 ×10^{8}	1..3 ×10^{9}	3.1 ×10^{9}	4..0 ×10^{4}	2.0 ×10^{6}	3..0 ×10^{6}	1..2 ×10^{7}	0	2..3 ×10^{7}	8..3 ×10^{7}	4..8 ×10^{8}	0
Staphylococcus count	2..3 ×10^{8}	4..8 ×10^{8}	2.1 ×10^{9}	2..0 ×10^{3}	2..3 ×10^{7}	1.7 ×10^{8}	1.1 ×10^{9}	0	3..9 ×10^{5}	7..8 ×10^{5}	3..2 ×10^{6}	0
Enterobacteriaceae count	3.6 ×10^{9}	5..9 ×10^{9}	1..5 ×10^{10}	0	1.1 ×10^{6}	2.4 ×10^{6}	9..9 ×10^{6}	0	5..0 ×10^{6}	1.7 ×10^{7}	8..3 ×10^{7}	0
Coliforms counts	3.6 ×10^{9}	6..2 ×10^{9}	1..5 ×10^{10}	0	9..2 ×10^{5}	2..2 ×10^{6}	1.1 ×10^{7}	0	1..5 ×10^{7}	7..8 ×10^{7}	4..9 ×10^{8}	0
Standard plate count cfu/ml	7.9 × 10^{11a}				1.4 × 10^{9b}				3.8 × 10^{9b}			
Laboratory pasteurization count cfu/ml	6.9 × 10^{9a}				2.0 × 10^{6b}				2.3 × 10^{8b}			
Staphylococcus count cfu/ml	2.3 × 10^{8a}				2.8 × 10^{7b}				4.0 × 10^{5b}			
Enterobacteriaceae count cfu/ml	3.6 × 10^{10a}				1.1 × 10^{6b}				5.0 × 10^{6b}			
Coliforms counts cfu/ml	3.6 × 10^{9a}				9.2 × 10^{5b}				1.5 × 10^{7b}			

SD=Standard deviation

a and b: means in the same raw with different superscript letters are significantly different (P < 0.05)

Table 20: Frequency analysis of some physical and chemical properties of raw milk of the dairy farms of the three cities of Khartoum State

Measurement \ Cities	Khartoum				Khartoum North				Omdurman			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Temperature ºC	34.85	6.56	39	4	35.15	3.64	40	26	33.20	2.57	38	26
Freezing point	-526.22	18.28	-450	-568	-524.38	22.65	-448	-578	-526.63	24.04	-440	-577
pH	6.544	0.232	6.8	6.15	6.441	0.2289	6.8	6.04	6.627	0.279	6.9	6.01
Acidity (%)	0.193	0.026	0.25	0.14	0.173	0.018	0.22	0.14	0.171	0.031	0.26	0.14
Fat (%)	4.56	0.5904	5.9	3.10	4.45	0.476	5.35	3.5	4.569	0.507	5.5	3.10
Protein (%)	3.76	0.559	5.90	2.90	3.75	0.612	5.7	3.0	3.67	0.563	5.9	2.5
Ash (%)	0.586	0.115	0.78	0.33	0.608	0.092	0.78	0.41	0.635	0.097	0.82	0.46
Lactose (%)	3.876	0.647	6.60	3.00	4.038	0.536	4.90	3.00	4.103	0.575	4.9	3.0
Total solids (%)	12.42	1.081	14.25	8.250	12.399	1.012	14.55	10.10	13.04	1.35	15.20	9.55
pH	6.54^{ab}				6.44^{b}				6.62^{a}			
Acidity	0.193^{a}				0.173^{b}				0.171^{b}			
Total solids	12.42^{b}				12.39^{b}				13.04^{a}			

SD=Standard deviation

a and b: means in the same raw with different superscript letters are significantly different ($P < 0.05$)

reported as 5 (100%) in each of Tayba Elhasanab, Aid Babiker and Almarkhiat, 4 (80%) in Soba, Elgerif, Albageir, Shambat, Alailafoon, Kuku, Jabal Torya and Alsarha, while 3 (60%) positive milk samples were found in Almakaweir at summer season. During winter season at Khartoum, Khartoum North and Omdurman the incidences were 19 (95%), 17 (85%) and 18 (90%), respectively. The incidences of brucella were reported in all milk samples collected from Tayba Elhasanab, Elgerif, Albageir, Kuku, Aid babiker, Jabal Torya and Almarkhiat. Positive milk samples for brucella were found in 4 (80%) samples collected from Soba, Alailafoon, Alsarha and Almakaweir, while 3 (60%) brucella incidences were reported in Shambat (Table 22).

The distribution of *Staphylococcus aureus* isolated was differing widely (Table 22). The isolates were reported as 9 (45%) isolates in Khartoum, 6 (30%) isolates in Khartoum North and 8 (40%) isolates in Omdurman during summer. The highest numbers and percentages (4; 80%) were reported in Alsarha, followed by 3 isolated (60%) which were reported in Soba, Kuku and Almarkhiat. Also 2 isolates (40%) of *S. aureus* were found in the milk samples that collected from Tayba Elhassanab, Elgerif, Albageir and Shambat, while one isolate (20%) was detected in Alailafoon and Almakaweir. However, no isolate of *S. aureus* could be identified in the milk samples that collected from Aid babiker and Jabal Torya, at summer season. Similarly during winter season the incidences of *S. aureus* in the milk samples collected from Khartoum, Khartoum North and Omdurman revealed 7 (35%), 12 (60%) and 11 (55%) isolates, respectively. Moreover, the results indicated that 5 isolates (100%) were found in the milk samples collected from Aid babiker, 4 (80%) were from Elgerif, Shambat, Jabal Torya and Almarkhiat. Three isolates (60%) were found in the milk samples that collected from Soba, Kuku and Almakawier.

However negative isolation of *S. aureus* was recorded in Tayba Elhasanab, Albageir, Alailafoon and Alsarha. Also the results revealed negative isolation of *Escherichia coli* during summer season, while during winter season the incidence of *E. coli* were 3 (15%) in Khartoum, 9 (45%) in Khartoum North and 1 (5%) in Omdurman, respectively (Table 22). The distribution of these isolated were reported as 5 (100%) in Alailafoon, 4 (80%) in Kuku and 1 (20%) in Soba, Elgerif, Albageir and Almarkhiat. On the other hand there was only one isolate of *Salmonella spp.* in one of the milk samples, which was collected from Soba (Khartoum) during summer season.

6. Biochemical reactions of the isolated bacteria

Results of the primary and the secondary biochemical test to which the potential pathogenic isolated bacteria were subjected were shown in Table 23.

7. The correlation coefficient of milk constituents

Table 24 shows the obtained correlations coefficient between physical, chemical and microbial properties of the raw milk collected from Khartoum State. The results showed significant positive correlation ($P<0.05$) of temperature with lactose ($r = 0.184$), total solids ($r = 0.186$), lab pasteurization counts ($r = 0.224$) and staphylococcus counts ($r = 0.181$). Similarly highly significant positive correlation ($P < 0.01$) were obtained for acidity ($r = 0.255$) total bacterial counts ($r = 0.254$) and coliforms counts ($r = 0.242$), while the pH ($r = -0.363$) showed a highly significant negative correlation ($P < 0.01$) with temperature. There were correlation of freezing point with the pH ($r = -0.251$, $P < 0.01$) and with total solids (-0.255, $P< 0.01$). There were highly significant negative correlation ($P < 0.01$) for pH with acidity ($r = -0.438$), total bacterial counts ($r = -0.385$), laboratory pasteurization counts ($r = -0.357$), staphylococcus counts ($r = -0.324$), Entertobacteriaceae counts ($r = -0.369$) and coliforms counts ($r = -$

0.377). However, protein showed significant negative correlation ($P < 0.05$) with pH ($r = -0.189$). The result indicated highly significant positive correlation ($P< 0.01$) between acidity and each of total bacterial counts ($r= 0.459$), laboratory pasteurization counts ($r= 0.437$), staphylococcus count ($r = 0.355$), Enterobacteriaceae ($r= 0.474$) and coliforms counts ($r= 0.487$). The fat was found to show highly significant positive correlation ($P< 0.01$) with total solids ($r = 0.350$). The protein and lactose were found to show highly significant positive correlation ($P < 0.01$) with total solids ($r = 0.413$ and $r = 0.406$, respectively). Furthermore the correlation of the standard plate counts showed highly significant positive correlation ($P< 0.01$) with laboratory pasteurization counts ($r= 0.902$), staphylococcus count ($r= 0.729$), Entrobacteriaceae counts ($r= 0.944$) and coliforms counts ($r= 0.945$). Similarly the laboratory pasteurization counts showed highly significant positive correlation ($P< 0.01$) with staphylococcus counts ($r = 0.810$), Enterobacteraiceae counts (0.952) and cloiforms counts ($r = 0.960$). Also staphylococcus counts revealed highly significant positive correlation ($P< 0.01$) with Enterobacteriaceae counts ($r = 0.780$) and coliforms counts ($r = 0.799$). Similarly the results showed a highly significant positive correlation ($P< 0.01$) between Enterobacteriaceae counts and coliforms counts ($r = 0.987$).

Table 21: Comparison of distribution of some pathogenic microorganisms in raw milk of some dairy farms at Khartoum State during summer and winter season

Measurement / Season	*Brucella test positive*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella positive*
Summer	50 (83.33%)	23 (38.33%)	0.000	1 (1.67)
Winter	54 (90.00%)	30 (50.0%)	13*(21.67*)	0 (0.0)
Total	104	53	13	1
P	NS	NS	***	NS

Ns = not significant (P > 0.05)

*****= highly significant (P < 0.001)**

P: level of significant

Table 22: Frequencies and incidences of distribution of some pathogenic microorganisms in raw milk in some dairy farms in Khartoum State

Organisms / Place	*Brucella test positive*		*Staphylococcus aureus*		*Escherichia coli*		*Salmonella positive*	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Tayba Elhasanab	5 (100%)	5 (100%)	2 (40%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Soba	4 (80%)	4 (80%)	3 (60%)	3 (60%)	0 (0.0)	1 (20%)	1 (20%)	0 (0.0)
Elgerif	4 (80%)	5 (100%)	2 (40%)	4 (80%)	0 (0.0)	1 (20%)	0 (0.0)	0 (0.0)
Albageir	4 (80%)	5 (100%)	2 (40%)	0 (0.0)	0 (0.0)	1 (20%)	0 (0.0)	0 (0.0)
Khartoum	17 (85%)	19 (95%)	9 (45%)	7 (35%)	0 (0.0)	3 (15%)	1(20%)	0 (0.0)
Shambat	4 (80%)	3 (60%)	2 (40%)	4 (80%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Alailafoon	4 (80%)	4 (80%)	1 (20%)	0 (0.0)	0 (0.0)	5 (100%	0 (0.0)	0 (0.0)
Kuku	4 (80%)	5 (100%)	3 (60%)	3 (60%)	0 (0.0)	4 (80%)	0 (0.0)	0 (0.0)
Aid babiker	5 (100%)	5 (100%)	0 (0.0)	5 (100%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Khartoum North	17 (85%)	17 (85%)	6 (30%)	12 (60%)	0 (0.0)	9 (45%)	0 (0.0)	0 (0.0)
Jabal Torya	4 (80%)	5 (100%)	0 (0.0)	4 (80%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Alsarha	4 (80%)	4 (80%)	4 (80%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Almarkhiat	5 (100%)	5 (100%)	3 (60%)	4(80%)	0 (0.0)	1 (20%)	0 (0.0)	0 (0.0)
Almakaweir	3 (60%)	4 (80%)	1 (20%)	3 (60%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Omdurman	16 (80%)	18 (90%)	8 (40%)	11 (55%)	0 (0.0)	1 (5%)	0 (0.0)	0 (0.0)
Total	50 (83.33%)	54 (90.00%)	23 (38.33%)	30 (50.0%)	0.00	13 (21.67%)	1 (1.67%)	0.00

Table 23: Primary and secondary biochemical tests carried out for the identification of pathogenic bacteria isolated from milk of some dairy farms at Khartoum State

Organism / Test	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella ssp.*
Gram reaction	+	-	-
Shape	Cocci cluster	Short rod	Rod
Catalse	+	+	+
Coagulase	+	Nd	Nd
DNase	+ (Clear zone)	Nd	Nd
Acid and gas from glucose	+	+	+
Lactose	+	+	-
Mannitol (aerobic)	+	+	+
Mannitol anaerobes	+	+	
MR	-	+	+
VP	+	-	-
Urease	-	-	-
Citrate as C source	-	-	+
Indol	-	+	-

Nd : not done

+ : positive reaction

- : negative reaction

MR: Methyl red

VP: voges-proskaur

Table 24: Correlations between some physical, chemical and microbial properties of the raw milk samples collected from some dairy farms at Khartoum State

Measurements	Temp	Freezing point	pH	Acidity	Fat	Protein	Ash	Lactose	Total solids	SPC	LPC	Staph	Entro.	Coliforms count
Temperature (Temp)	1.0	-0.33	-0.363**	0.255**	-0.012	0.099	-0.053	0.184*	0.186*	0.254**	0.224*	0.181*	0.160	0.242**
Freezing point		1.0	-0.251**	0.120	-0.174	-0.168	-0.157	-0.08	-0.255**	-0.088	-0.038	-0.003	-0.03	-0.078
pH			1.0	-0.438**	0.041	-0.189*	-0.021	-0.045	-0.053	-0.385**	-0.357**	-0.324**	-0.369**	-0.377**
Acidity				1.0	0.032	0.059	-0.035	-0.124	-0.010	0.459**	0.437**	0.355**	0.474**	0.487**
Fat					1.0	0.087	0.014	-0.022	0.350**	0.044	0.057	0.082	0.027	0.060
Protein						1.0	-0.049	0.101	0.413**	0.013	0.019	-0.075	-0.014	0.002
Ash							1.0	-0.016	-0.008	-0.10	-0.068	-0.09	-0.128	-0.114
Lactose								1.0	0.406**	-0.086	-0.082	-0.093	-0.090	-0.091
Total solids									1.0	-0.049	-0.044	-0.111	-0.113	-0.066
Standard plate count(SPC)										1.0	0.902**	0.729**	0.944**	0.945**
Lab pasteurization (LPC)											1.0	0.810**	0.952**	0.960**
Staphylococcus count (Staph)												1.0	0.780**	0.799**
Enterobacteriaceaea count (Entro)													1.0	0.987**
Coliforms counts														1.0

****: correlation is significant at the 0.01 level.**

***: correlation is significant at the 0.05 level**.

CHAPTER FIVE

DISCUSSION

In this study, most of the producers of milk in the dairy farms were found to be unaware of the effect of animal health and environmental conditions on producing safe milk. Since the percentage of illiteracy was high among the dairy farms owners in Khartoum (7, 35%) and Omdurman (6, 30%). Moreover, primary, higher and university graduates in Khartoum and Omdurman were found to be fewer between the dairy farms owners than those of Khartoum North (Table 1). This might be due to absence of full certification, untrained employees and absence of technical and training staff. This was in agreement with the reports of Payne *et al.* (1999) who reported that training is an important and useful component of California dairy quality assurance programs.

Cross breed cows represent the highest number (20, 100%) among the breeds in the farms located at Khartoum North (Table 2). This indicated that cross breed cows were best adapted and predominated in the farms of Khartoum State (Mohamed, 1995). Moreover, this supported Tibin *et al*. (1990) findings when studying herd in Kuku dairy project farms and found 67.2% of the herd were grade cattle, 27.8% were local type and 4.9% constituted other types.

The number of total herds, lactating cows, dry cows, heifers and calves showed higher numbers for farms in Khartoum North than those in Khartoum and Omdurman (Table 3). This might be due to Artificial Insemination Center in Kuku Project area provided some services. Moreover, the old relatively big cooperative dairy sector, which contributes to milk production in Khartoum State, was another reason. Since it started to modernize husbandry practices and improve local breed by cross

breeding with foreign blood in order to increase milk production to narrow the gab between the production and consumption (Tibin *et al.,* 1990). Furthermore, these are one of the largest areas for the planting green fodders mainly Abu70 and facilities of water supply are found (Mohamed. 1995).

Results from this survey demonstrated clear advantages for larger herds in Khartoum North, which provides large amount of milk production (Table 3). Moreover, as stated by Bewley *et al*. (2001) milk production increased as herd size increased. Also Table 3 shows the highest numbers of calves were found in the farms that raise the foreign breed (Dairy Land Farm). These variations might be due to veterinary supervision, level of education and farms management (Table 1 and Table 6). Moreover, the other herds suffer from the lack of planning for herd replacement in addition to the high mortality due to improper management. Paton (1989) found that dairy farms with resident veterinarians showed to achieve performance goals for production and disease control. Ideal building material was seldom used in dairy farms in this study (Table 4). Only 10% of the studied farms used concrete floor, corrugated iron roof is used by 6.67% of farms as in Table 4. This was the same situation for the herd studied by Badi (1988) in Gezira Scheme, where he reported that most of animals were kept in open areas or zaribas (made from Acacia branches) without shade. Most of the farms were found to use local building materials. Moreover, they were similar to the type of building which was described by A/Magid (1988). He also mentioned that at Kuku Dairy Project, the farms buildings were poorly designed with no space requirement for cows. Tjokrohoesodo and Grossman (1975) reported that in Indonesia the dairy cattle housing depend mainly on the purpose and the wealth of the owner. However, the building design helps to reduce

environmental stress and provides safe and hygienic conditions to raise the level of production and to cover the additional cost (Mohamed, 1995).

The present survey indicated that modern technologies for milking cows were rarely used in the dairy farms under the present study. Practices such as milking parlour, dry cow therapy and iodine dip were rarely used (only one out of 60 surveyed farms) (Table 6). Also machine milking was used in one farm in Khartoum North, while the rest of the farms used hand milking. This agreed with the findings of Mohamed (1995) who found that milking machine and strip cup were used in 3 farms (3.5%) and teat dipping was practiced in 5.9% of that farms. Also it was agreed with Berrett and Larkin (1974) who reported that machine milking was not widely used in the tropics and often cows dry off themselves. Similarly Williamson and Payne (1978) reported that the use of modern technologies as milking machines in small holder's dairy farms are completely in economical, and indeed undesirable because of surplus labours.

Cleaning of cow's udder was only practiced in one farm in Khartoum and 2 farms in Khartoum North, also insufficient milkers cleaning and equipment cleaning were noticed and reported during the present survey among the farms in Khartoum State (Table 6). The same findings were noticed by Bramley and Mckinnon (1984) who reported that in addition to contamination of milk with hands, high risk of milkers might occur when suffering from certain diseases. It was also observed that detergents were not used and no cleaning program and udder wash in all farms under study except Arab Dairy Farm (Dairy Land).

Many farms included in this study showed the lack of knowledge about water quality laws. Since it was clear that about half of the farms in Omdurman and 25% of farms in Khartoum used untreated water supplies from bore holes and others sources by donkeys (Table 5). Some of these

might be sources of contamination for milk with faecal organisms. NZDWS (2000) reported that water should not conatin more than three faecal coliforms per 100 ml and not greater that 5 NTU (Nephelometric Turbidity Unit) for turbidity. However, Bramley and Mckinnon (1984) reported that the farm water supply can be a source of microorganisms (especially psychrotrophs) that can seed soiled equipment and or the milk.

The present data also showed that storage room for forage were absent in most of the farms in Khartoum State (Table 5). The present of feeding beside pens might be the cause and source of contamination of raw milk with thermoduric bacteria or heat resistant bacteria as stated by Vaerewijck *et al.* (2001). Various illegal milk containers for milking and transporting the milk were found to be commonly used among most of the farms (Table 5). However, Bramley and Mckinnon (1984) reported that if cans are not effectively cleaned and still moist when the lids are put on them, high bacterial multiplication on the interior surfaces can result in very high bacterial counts.

As shown in Table 9 vaccination against contagious diseases such as rinderpest, anthrax, contagious bovine pleuro pneumonia, hemorrhagic septicemia, black quarter and brucella were rarely used in the farms under study. Similarly, Mohamed (1995) reported that brucella and foot and mouth disease vaccines were rarely used in the farms in Khartoum State. Moreover, the vaccination provided by veterinary authorities as governmental services available to help owners to maintain animal health was the only dominant vaccine practiced in the farms under study. This because of national interest to preserve livestock wealth of the country (Mohamed 1995). This indicates improvements in veterinary services as reported by Baasher (1969) that since 1900, when the first time veterinary effort was made, disease prevention passed certain developmental changes.

The less brucella vaccines and foot and mouth disease vaccine were rarely used and that might be due to their higher cost as stated by Williamson and Payne (1978). They reported that in herd kept in subsistence level in tropics, brucella vaccination is not practiced or possible so farmers have to tolerate the disease in their herds. Moreover, the incidence of abortion at late pregnancy was higher at the farms in Khartoum, while those of Khartoum North and Omdurman were relatively lower (Table 8). However testing, isolation and culling were practiced by few of the dairy farms included in the present survey. In some farms culling was practiced to some extend (Table 8). The same was noticed by Lwajock (1992) who reported that in some dairy farms in Khartoum State, farmers continue to maintain cows with only one functional teat without culling. Also Mohamed (1995) noticed that culling was not common practice in Khartoum dairy farms. Also FAO (1982) reported that in tropical countries management practices and preventive measures are not adequate and practices such as isolation and quarantine facilities were not common. Brucella disease incidences were correlated positively with testing and culling of the disease cows (Mohamed, 1995). Furthermore, Alkhalaf *et al.* (1992) reported that effective brucella control is by testing and culling or slaughter of the infected animals and vaccinate the clean ones. However, Gameel (1983) reported that on management of brucellosis, immunological problems arise because infected cows with titers below reactors level were usually left in the herd and constitutes continuous reservoir for brucella transmission.

Mastitis was found in the farms of Khartoum, Khartoum North and Omdurman as 14 (70%), 12 (60%) and 10 (50%) for the farms under study and the number of cases were range from 1 to 5 cows (Table 10). Also the same table indicated that no control programs were used for disposable of

abnormal milk. Moreover, in most of the farms, the labours milked the mastitic cows in the floor of the pens (Table 10).

Unhygienic condition and low standard of management practices in the dairy farms under study supported Bagadi (1966) who reported that unhygienic conditions were the reasons for increasing udder infection. The same result was found by Rodrigues (1988) that in 10 dairy herds in Savannah, 47% cows were affected by mastitis, where treatment and preventive measures were poorly applied. Similarly Mustafa *et al.* (1977) studying mastitis in Atbara dairy herd and found that 20 out of 35 cows were infected with mastitis. Moreover, Mohamed (1995) found high correlation between mastitis and milking place ($r = 0.75$), isolation ($r = 0.62$) and veterinary supervision ($r = 0.72$).

In this survey, it was noticed that many farms owners were used traditional treatment like fire for mastitis and other diseases. Moreover, most of the farms applied drugs without veterinary instructions or inspections. Also from the survey it was noticed that in most of the farms, diseases control and management were not satisfactory. These facts explained the types of preventive measures that applied for eradication of some diseases like mastitis in the farms surveyed. So many health problems that might arise in those farms are due to the complete absent of veterinary supervision (Table 6). Since higher correlation was noticed between mastitis and veterinary supervision which supported Mohamed (1995).

Poor records were most commonly observed (Table 5). The absence of records in the farms were considered as serious problems especially for diseased and treated cows. Since the treatment with drugs and milking the diseased cow with other cows might create the risk hazard of antibiotic in raw milk (Sischo *et al.,* 1997). They also reported that lack of adequate on-farm treatment records for antibiotic residues are the highest facter

followed by deficiencies in understanding how to use antibiotic. Similarly, Harding (1999) stated that it is essential that milkers should know exactly which cows have been treated and they should take care with the milk. Also he reported that all treatments of cows should be recorded including identity of the cow, the person giving the antibiotic treatment, type of treatment, dosage given, date and time (or milking) and when cows brought into the herd should be checked before their milk is included for sale.

Most of the pens appeared heavily contaminated with dung especially those in Khartoum (Table 7). Also in this survey, it was noticed that cows in the pens appeard with teat heavily soiled with dung. Although the cows were heavily visibly soiled, most of the farms owners stated that they removed dung between 3-7 days. These might be due to small size of the pens and the large number of herds enclosed as stated by A/Magid (1988) that the space requirement for cows was not considered. Heavily contaminated animal environment might be the cause of risk hazard for milk production. This supported Jone (1980) findings who reported that pathogens in urine and faeces can be reduced through the removal of cattle excrete. Also Streat (1979) reported that daily removal of dung is known to be effective in prevention of nematode manifestation.

In this survey, it was observed that one farm in Khartoum North produced milk for cheese – making (near the farm). So milk was used without pasteurization or any heat treatment by traditional method for cheese–making and that milk might cause risk hazard. This supported Harding (1999) findings, since he considered pathogenic organisms in raw milk, which produced and processed under unhygienic conditions, were frequent causes of outbreaks of diseases.

The farms visits revealed that no cooling was applied for milk at production areas in the farms under study during summer and winter

season, except Arab Dairy Farm in Khartoum. Their milk temperature were 4 and 15º C during summer and winter season, respectively as shown in Tables 14, 15, 18 and 20. Milk was produced and therefore handled in temperature ranges between 30-40º C during summer and 26-39º C during winter season (Table 14 and 15). These temperature ranges, facilitated the growth and multiplication of pathogenic and non-pathogenic bacteria during production, transportation and distribution (Bramley and Mckinnon, 1984).

In Table 24 high correlation coefficient was noticed between temperature and acidity (r = 0.255), total bacteria count (r = 0.224), coliforms counts (r = 0.242), laboratory pasteurization count (r = 0.224) and staphylococcus count (r = 0.181). This might be due to the fact that bacteria grow in milk held at temperature greater than15º C and the types of bacteria that grow and become significant will depend on the initial microflora of the milk (Murphy and Boor, 2000). Moreover, when raw milk is allowed to remain at room temperature for some time, it is soured by lactic acid bacteria as stated by Edwin *et al.* (1958). Similarly Bramley and Mckinnon (1984) reported that degree of temperature, numbers and types of bacteria in milk influence the increase in bacterial numbers. However, Ahmed (1995) observed that the keeping quality of the raw, pasteurized and sterilized milk samples at refrigerator temperature were 70, 124 and 188 hours, respectively. This indicated that refrigeration has better effect on keeping quality.

Higher total bacterial counts were estimated during the present study ($2.6 \times 10^{10} \pm 8.4 \times 10^{10}$ cfu/ml) as shown in Table 18. Also higher total bacterial count were observed for the samples collected during summer than those collected during winter season ($5.3 \times 10^{10} \pm 1.1 \times 10^{10}$ and 7.5

$\times 10^7 \pm 2.6 \times 10^8$ cfu/ml, respectively). Higher significant differences ($P < 0.001$) were found to be between seasons (Table 16). Higher significant correlation was also noticed between total bacterial counts and laboratory pasteurization count ($r = 0.902$), staphylococcus count ($r = 0.729$), Enterobacteriaceae ($r = 0.944$), coliforms count ($r = 0.945$) and acidity ($r = 0.459$). These results were very high compared to the acceptable standard of grade A raw milk reported by Randolph *et al.* (1973). The standard plate count for grade A raw milk is 7×10^4 to 1.0×10^5 cfu/ml, while that for bulk milk ranges from 1.0×10^5 to 2.4×10^6 (Pankey and Philpot, 1971). The high bacterial count of the farms which exceeded the international standard for grade A raw milk indicated that the hygienic and sanitary control measures and the health supervision applied in those farms were poor. This might be due to either the milk producing animals and/or milkers hygiene were weak or completely lacking (Haj Mahmoud, 2002). Also the results of this work were higher than that reported by Ibrahim (1973) who found that the mean total bacterial count for four dairy farms around Khartoum was 6.8×10^5 cfu/ml. Also they were disagreed with the findings of Golubeva (1984) who found that bacterial counts from portable tanks immediately after milking in USSR ranged from 1.0×10^5 to 5.5×10^6 cfu/ml. Similarly Barakat (1995) cited that total bacterial count for good grade milk was 5.5×10^5 cfu/ml, for satisfactory grade it ranged between 5.5×10^5 and 5.0×10^6 cfu/ml and for bad grade milk it was more than 5.0×10^6 cfu/ml. The higher bacterial counts are expected under tropical condition like Sudan due to the fact that high temperature enhances growth and multiplication of

bacteria (Barakat, 1995). This supported the findings of Dirar (1975) who stated that under tropical condition many factors such as high temperature, absence of sanitary conditions for production of milk in the farms and unavailability of cooling during handling and transportation of milk affected the quality of milk. Similarly Bramley and Mckinnon (1984) reported that microflora of milk when it leaves the farms is determined by the temperature to which the milk has bean cooled, the storage temperature, the time elapsing before collection and the initial microflora. Also the absence of udder cleaning programs in the majority of the farms studied (Table 6) might cause increase in bacterial count. This was in support to Bramley and Mckinnon (1984) findings who considered unwashed teats, which soiled with dung, have bacteria count of 100000 cfu/ml. However, all farms under study except Arab Dairy farm were found not to use hot water so insufficient cleaning of equipment might be the cause of risk hazard. This was the same as reported by Murphy and Boor (2000) who found that ineffective cleaning, insufficient hot water temperatures and/or the absence of sanitizers tends to select for faster growing of less heat resistant organisms. Also Harding (1999) reported that circulation in place (CIP) cleaning system are recommended and achieve a high level of hygiene. Poor bacteriological quality was also observed by Godefay and Molla (2000) and Adesiyun (1994). Furthermore, Gilbert (1982) reported that poor quality of raw milk and standard plate counts in millions per ml are common in South Africa. This is largely due to inefficient cleaning and sanitizing of dairy equipment.

The present result revealed that 83.33% and 90.00% of the samples showed brucella milk ring test positive milk samplesduring summer and winter (Table 21 and 22). These result indicated non significant differences ($P > 0.05$) between seasons (Table 21). This might suggest that nearly all of

the herds, which produce milk, could either have been or actually infected with brucella. This was a supported by Dafalla (1962) findings who considered that the highest percentage was found among dairy farms. Also it is observed that the detection of brucellosis in the dairy farms was low despite the poor hygienic conditions and the weak veterinary supervision as presented in Table 6. This is in accordance with Mohamed (1995) who found that the incidence of brucella which was found to be positive for brucella test especially where there was no brucella control program. He attributed that to be due because farms owners considered the disease unimportant as well as testing and disposal of diseased animals as economically unfeasible. However, Dafalla (1962) reported that if cattle kept intensively and the animals were in close contact, high infection rates would be expected. The rate of incidences of brucella during the present study was found to be higher than other previous reports. Abdalla (1966) found 3% of cattle were serologically positive in Wadi Halfa, while Habiballa (1977) studied dairy farms in Gezira and found brucella incidences ranged from 6.7% to 28.5% in Gezira livestock center and in Gezira province. He found the incidence was up to 36%, while Shallali *et al.* (1982) studying dairy herd in Sinnar, he found that 9.9% of herd were brucella positive. Moreover Memish and Balkhy (2004) stated that brucellasis is a zoonotic disease of world wide distribution that mainly affect persons working with domestic animals and animals products. They concluded that the disease is mainly transmitted to human through the ingestion of raw milk or non-pasteurized cheese contaminated with brucella species pathogenic to humans. This should pay attention to brucellosis since the milk is marketed raw in Sudan, which might cerate health hazards to consumers and handlers especially during processing of products without boiling of milk.

During the present study a number of potentially pathogenic bacteria (*Staphylococcus aureus, Escherichia coli* and *Salmonella spp.*) were isolated from bulk milk (Table 21 and Table 22). It is well known that poor milk hygiene reflects risk to public health. Since a lot of diseases and conditions might originated from milk sources (Adam, 1997). Moreover, higher counts were estimated from those selected organisms. The occurrence of coagulase positive staphylococci and coagulase negative staphylococci are the most numerous microorganisms present in the teat and udder skin (Cullin and Herbert, 1967). Moreover, it is observed that the rate of isolation of *Staphylococcus aureus* is relatively high in the milk of the dairy farms collected during winter season (30, 50%) compared to the isolation during summer seasons (23, 38.33%). Furthermore high rate of the isolation of *Staphylococcus aureus* on those farms could be attributed to unsanitary milking environments and poor hygienic conditions in these farms (Tables 21 and 22). Similarly Asperger (1994) stated that milk and milk products can become contaminated unless good hygiene (including mastitis control) occurs on the farms. The count of *Staphylococcus aureus* estimated during the present study was high compared to the regulatory standard. Since Harrigan and MacCance (1976) reported that the food should not contain more than 10 *S. aureus* organisms/gm. It is probable that the high incidence of *Staphylococcus aureus* was favoured by lack of hygiene during milking and also due to lack of attention to the udder, which enables many microorganisms present in the environment to enter the udder (Table 21 and 22). Also the high incidence of *S. aureus* might be attributed to udder infection as it is the first microorganism's incremented in mastitis. This is in accordance with the findings of Mohamed *et al.* (1993) and Mohamed *et al.* (1997) who reported that *S. aureus was* isolated (23.56%) as the predominant cause of mastitis in Khartoum State.

Moreover, Bystron *et al.* (2001) found that 66% of *S. aureus* strains isolated from raw milk have the ability to produce entrotoxins because of the stability of staphylococcus entrotoxins, it may remain in food products after the organisms which produced it has been killed by heat. In the present study the count of coagulase negative staphylococci was higher as shown in Table 12, 13, 17, and Table 19 with significant differences ($P< 0.001$) between seasons and cities. The presence of these organisms in milk could also be attributed to mastitis. Since they are regarded as udder pathogens (Verma, 1977 and Mohamed *et al.,* 1993). Moreover mastitis is well known to influence the composition of milk by varying the concentration of its constituent (Mernyi and Wagner, 1986; Viaanni and Nader, 1991; Mohamed *et al.,* 1997 and Mohamed *et al.,* 1999).

The seasonal variation was observed during the present work for the isolation of *Escherichia coli.* Since *Escherichia coli* was isolated during winter season only (Table 21 and 22). These bacteria might originate from contamination due to mismanagement practices such as poor milking system, absence of equipment maintanance or poor environmental conditions upon milking (Adam, 1997) or from udder infection (Gilmour and Rowe, 1990 and Mohamed *et al.*, 1997). These results were in agreement with findings of Adam (1997) who reported that the presence of *Escherichia coli* in faeces and that it might be recovered from water, dust in the air and milk or urine. Furthermore he isolated 9 *S. aureus* and 5 *Escherichia coli*. Similarly Nyaga *et al*. (1982) isolated *Escherichia coli* from unhygienic milk. However, Padhye and Doyle (1992) stated that *Escherichia coli* in milk might cause illness to human, this illness ranged from self limited watery diarrhea to the life threatening manifestations.

The incidences of coliforms in raw milk were $1.2\times10^{9}\pm 3.9\times10^{9}$ cfu/ml (Table 17). Higher significant differences ($P< 0.001$) were found

between seasons and cities. The presence of these organisms in milk indicated faecal contamination origin and the consequent risk of more pathogenic faecal organisms being present (Bramley and Mckinnon, 1984). They also reported that their growth in milk at ambient temperatures can produce spoilage of milk. Coliforms bacteria are also found to be in per-acute and acute mastitis (McDonald *et al.,* 1970; Bagadi, 1970; Mohamed *et al*., 1993 and Mohamed *et al*., 1997). Moreover, this study indicated that milk in Khartoum State was produced under poor sanitary conditions and was highly contaminated with coliform organism.

The milk samples were found positive for the presence of coliforms bacteria and Enterobacteriaceae, which were an indication of lack of sanitary measures during production and handling of milk. Moreover, our findings were in agreement with those reported by Adam (1997) who isolated Corynebacteria, *Entrobacteria aerogens* and *Klebsiella spp*. from milk. Also William *et al.* (1988) stated that these microorganisms are found in the external environment and commonly gain entrance to the bulk tank milk from none milk sources. Similarly Murphy and Boor (2000) stated that coliforms may enter the milk supply as a consequence of milking soiled cows or of dropping equipment into manure during milking.

Highly significant differences ($P< 0.001$) were found due to the incidences of Enterobacteriaceae in raw milk samples between seasons and cities (Table 16). These might be due to contamination from outside or around milking area. This supported Godefay and Molla (2000). Similarly the findings supported Gilmour and Rowe (1990) who stated that Enterobacteriaceae occur in the natural environment, water, soil, sewage, etc, and they gain access to milk from several of these sources.

The result revealed that the laboratory pasteurization count of milk samples collected during summer were higher than those collected during

winter season that range from 6.0×10^3- 3.1×10^9 and zero to 5.3×10^7cfu/ml, respectively (Table 17). However, highly significant differences ($P< 0.001$) were found between seasons and cities (Table 16). These findings were disagreed with those reported by Sutherland and Murdoch (1994) who stated that incidence of mesophilic isolates was highest in the winter and lowest in summer/autumn, while pyschrotroph incidence was conversely lower in the winter and higher in the late summer/autumn. They illustrated that as might be due to bedding materials on which cows are housed in winter. For the farms under study, the cows were enclosed almost all times in pens. High incidence and values of laboratory pasteurization count found during this study might be due to contamination of feed, equipment by soil. These findings were supported Bramley and Mickinnon (1984) who reported that spores are derived almost exclusively from milking equipment and silage used for feeding. Also it supported Murphy and Boor (2000) who stated that highest laboratory pasteurization counts are associated with a chronic or persistent cleaning failure in some area of the system or with significant levels of milk contamination from soiled cows and milking machine. Similarly Bramley and Mckinnon (1984) stated that most thermoduric organisms do not multiply appreciably in raw milk even at ambient temperature, and thus a high thermoduric count in milk up to 24 hold is reliable evidence of cross contamination from milking equipment.

The result obtained during the present work revealed that there was only one case of *Salmonella spp.* during summer season in milk sample collected from Soba at Khartoum (Table 21 and 22). This might be due to direct or indirect (for example via udders, hide, etc.) faecal contamination. Contamination may also occur during milking and subsequent practices such as poor hygiene (IDF, 1994a). Reed and Grivettit (2000) found that resistant salmonella was linked directly to product made from raw milk.

The means of freezing point of milk samples collected during summer, winter and both seasons were -518.57± 25.65 ^{o}C, -532.92± 13.48 ^{o}C and -525.74± 21.64^{o} C, respectively (Table 18). These findings were disagreed with those reported by Mohammedi (1988) who obtained lower freezing point (-0.558± 0.0112 ^{o}C). The difference might be attributed to nutrition effect (Bartsch and Wickes, 1980 and IDF, 1983), difference of cow's breeds and impact of stress during summer season resulting in high water intake (IDF, 1983). The findings were in agreement with results of Luck and Dresners (1975), Ibrahim (1989) and Rasmussen *et al.* (2002).

Higher pH was obtained during winter season (6.73± 0.10) than those during summer season (6.34± 0.211). The reductions of pH during summer were disagreed with the findings of Hunderson (1971), Kon and Cowie (1961) and Payne (1990) who mentioned that acidity is normal in the range of pH 6.5-6.8. The reduction in pH might be due to increased in acidity during summer and so decreased the pH level. However, the results during winter season were in agreement with those of Kon and Cowie (1961), Hunderson (1971) and Payne (1990).

The higher level of acidity was found during summer season (0.193±0.023%), while that during winter was 0.164± 0.023%. However, Ibrahim (1989) and Mohammedi (1988) stated that fresh milk have acidity of 0.17%. The findings during winter were agreed with Rumania Standards (1974) which indicated that the acidity of raw milk must be 0.15 -0.20%.

The fat content obtained during this study (Table 18) was higher during winter than those during summer (4.54%± 0.59 and 4.50%± 0.46, respectively). The fat content during the present work was higher than those obtained by Ibrahim (1989), Ibrahim and Samaha (1986), Webb *et al.* (1980) and Harding (1999). This might be attributed to genetic (60 % of the variation), plane of nutrition and yield of cows as reported by Ibrahim

(1989). The findings were in accordance with Hamid (1994) and Klungel *et al.* (2000).

Higher protein content was estimated during the present study (3.73± 0.58%). These findings were conflicting with the findings of Korhn and Anderson (1980), Elfaki (1988) Walstra (1992) and Ballou *et al.* (1995). These differences might be due to planning of nutrition (Osman, 1996), foreign blood percentages and stage of lactation (Hussein, 1985).

Ash content obtained during this study was 0.61±0.103%. Also higher ash content were found for the milk samples collected during summer than those collected during winter season (0.63%±0.11 and 0.593±0.0896%, respectively) as shown in Table 18. These results differ from those reported by Hussein (1985) who obtained higher ash values and Hamid (1994) who obtained lower ash content. The differences might be attributed to breed factors (Hamid, 1994) and stage of lactation (Hussein, 1985). However, the findings were in accordance with Bakhiet (1995) who obtained ash of 0.59±0.09%.

Results of this study revealed that lactose content were found to be 4.01±0.59% for both seasons, while those during summer and winter were 3.95±0.561% and 4.06±0.618%, respectively (Table 18). These results were in disagreement with those reported by Ibrahim (1989), Harding (1999) and Ballou *et al.* (1995) who obtained higher results. The reduction might be due to breed differences (Logacheva, 1975 and Bergmann, 1980), feed differences (Ibrahim, 1989) and health status of the udder (Mohamed *et al.,* 1997 and Mohamed *et al.,* 1998) and bacteria contamination fermentation (Edwin *et al.,* 1958). The findings were similar to those reported by Buchberger *et al.* (1976) who obtained lactose content of 4.18 to 5.03%.

Higher total solids were obtained during winter season than those during summer season (12.77±0.919% and 12.47±1.39%, respectively) as shown in Table 18. These were conflicting with those obtained by Casper *et al.* (1992) who obtained lower total solids and Hussein (1985), Abdalla (1987) and Hamid (1994) who obtained higher total solids. They reported 12.91% to 14.78%, 13.3%, 13.6% and 14.3% and 13±0.99% to 14.02±1.43%, respectively. This might be due to the fact that milk constituents were affected by diversity of factors. Among the important ones are the breed of the animal, the feeding regimes, the length of dry period and the level of supplementation (Elhaj, 1994).

From Table 16 it was observed that means of fat %, protein %, ash %, and lactose % of milk samples showed non significant differences ($P > 0.05$) for milk samples collected from different cities and during different seasons. However, total solids showed significant differences ($P< 0.01$) due to the different cities from which the milk samples were collected. Also Table 20 indicated that total solids of milk samples collected from Omdurman were significantly differences ($P< 0.05$) from those which collected from Khartoum and Khartoum North. This might be due to effect of breed, feeding and management as stated by Nickerson (1999). He also reported that synthetic secretary tissue of the mammary gland, the initiation and establishment of lactation, the milk ejection reflex, the breeds and genetics factors, the nutrition, the environment and the milking management practice, all have important effects on milk composition and quality. So higher total solids of milk samples collected from Omdurman might be due to one or other factors included above. Also Elfaki (1988) reported that University of Khartoum dairy farm showed 12.9% total solids of milk for high grade dairy cows and 13.1% for low grade cows. Also as

shown in Table 1 Omdurman dairy farms have local breed and most crossed with low percentage of foreign blood.

Table 16 clearly presents the effect of season on milk properties, which showed highly significant different ($P< 0.001$) for temperature degree. Similarly higher significant variations ($P< 0.001$) were obtained for freezing point, pH, acidity, standard plate count, laboratory pasteurization count, staphylococcus count, Enterobacteriaceae count and coliforms counts. However non significant differences ($P> 0.05$) were observed for fat, protein, ash, lactose and total solids. This indicated that season had highly significant effect on quality of raw milk produced by different farms, especially during summer season where the temperature was high combined with the absence of cooling facilities (Table 18). As multiplication of bacterial was higher, it leads to increase acidity and therefore decrease pH (Edwin *et al.,* 1958). Also Harding (1999) stated that total bacteria count of cooled milk, produced under good hygienic conditions should be lower than 10000 bacteria/ml. So to achieve a high bacterial quality at farm level it is important for farmers to be aware of the sources of contamination and to understand how they can be controlled (Murphy and Boor, 2000). Also mastitic cows produce milk with very high bacteria, therefore controlling mastitis is important in order to produce high quality milk (Mohamed *et al*., 1999). It was also observed that the milk samples collected from Khartoum had high significant differences ($P< 0.05$) than those collected from Khartoum North and Omdurman for acidity, standard plate count, laboratory pasteurization count, staphylococcus count, Enterobacteriaceae count and coliforms count as shown in Table 19. It was also observed from Table 20 that means of temperature were 34.85± 6.56º C for the milk samples collected from Khartoum, 35.15± 3.64º C for the milk samples collected form Khartoum

North and 33.2± 2.57º C for the milk samples collected from Omdurman. Also from Table 16 it was observed that temperature showed significant differences between seasons (P< 0.01) and non-significant differences (P> 0.05) between cities. These might be due to higher temperature of the milk samples collected from Khartoum dairy farms (34.85± 6.56º C) except Arab dairy farm (4 and 15º C during summer and winter, respectively). So standard deviation was clearly higher than those of Khartoum North and Omdurman. So the absence of cooling combined with higher temperature might activate and enhance the growth and multiplication of bacteria and therefore increase the acidity of milk. Also the poor management practices found in Khartoum dairy farms as shown in Table 1 to Table 12 might be anther reasons. Furthermore Table 1 indicates the educational level of the farm's owners was low. Higher illiteracy was found among the farms found in Khartoum than those found in Khartoum North and Omdurman. Other practices, which raised those bacterial count are milking soiled cows, maintaining an unclean milkers, milking and housing environment, improper condemnation of abnormal milk and failure of cooling facilities for the milk. Also mastitic cows can cause significant failure in milk quality (Mohamed *et al*., 1999). This is in accordance with those reported by Murphy and Boor (2000). This is also supported Zingeser *et al.* (1991) findings that mis management such as poor milking techniques, poor or absent of equipment maintainance, improper use of antibiotic and failure to cull defective cows lead to production of unhygienic milk. Unhygienic milk, shed environment, high ambient temperature, and unclean clothes, which is used to clean the udder lead to high incidence and persistence of mastitis (Ibrahim and Habiballa, 1978; Hinckley *et al.,* 1988 and EL amin and EL Zubeir, 2002).

CONCLUSIONS AND RECOMMENDATIONS

Conclution

The majority of farms were poorly constructed and they are with limited management skills. The current type of management seems to be derived from peasant farmers husbandry practices where management standard were not usually very high. Moreover most of those farms could not be considered as specialized or optimum dairy farms.

With regard to breed type in dairy herds in Khartoum State cross bred cows representing 92% of the total breeds. But of unknown foreign blood percentages because farmers do not keep records. Highest number of calves were raised in Arab dairy farm. It was also observed that there was a high mortality rate among calves especially those with high percentages of foreign blood. Farm's owners tend to sale calves after parturition in the majority of farms studied and no planning for herd replacement was applied. Calf rearing system in the majority of farms was traditional and husbandry methods such as artificial nursing, dehorning, identification and bedding were absent.

Ideal building material such as corrugated iron and concrete were very rare. The same was true for using machine milking and mastitis prevention practices. Similarly it was found that vaccination against diseases were not administrated in regular way and preventive measures practices, veterinary supervision, general hygiene, cleaning programs and sanitation practices were not optimum. A serious problem arises due to the absence of records keeping in the majority of the farms studied, especially health records. So cows treated with drugs were milked with the rest of cows. This might create risk of antibiotics in milk. Also treatment in most of the dairy farms which studied were practiced without veterinarian

inspections that might cause health hazards, another problem was that abnormal milk was milked directly on the floor of the pens.

Most of the dairy farms in Khartoum and Khartoum North sell their milk in Omdurman. This might be due to the fact that Omdurman has small number of herds and most of them were local breed coupled with high population, since most of the milk which was produced was sold in the market, irrespective of its quality. Hence from the present study, it is concluded that hygienic control measures applied in the farms studied were either poor or completely lacking and improvement of hygiene necessitates health supervision of milking animals and education and health supervision of the milkers. Also the farms need well planned construction, operation and management. However, during the present survey many problems faced with the complete evaluation of the farms studied especially about incidences of diseases. Some farms owners are not aware about diseased cows and conditions of their animal health.

Suggestion and recommendation for further improvement

1- Provision of essential services such as clean potable water, health care and education to the owners of dairy animals.
2- Extension services among dairy farmers, labours and milkers were urgently needed on dairy faming practices such as housing, milking techniques and hygiene, proper sanitary practices and cleaning programs, diseases prevention measures and culling strategy.
3- Establishment of milk collection centers with supported services such as cooling and trasport.
4- Enforcement of legislations and laws for milk production and dairy products, adoption of standard methods for production and

establishment of procedures to control milk grading and marketing.

5- Setting milk pricing structure in order to stimulate the awareness of the quality for milk production.

6- Application of HACCP-based quality assurance programs on the farm.

7- Further studies are urgently needed for detection of residual contaminants such as antibiotics, preservatives and neutrlizers and enumeration of some pathogenic microorganisms and their toxins.

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