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BACTERIAL COUNTS OF CAMEL´S RAW MILK FROM SOME LOCATIONS AT KHARTOUM STATE

العد الكلي للبكتيريا في لبن الأبل من بعض محليات ولاية الخرطوم

A graduation research in fulfillment of the requirements for the degree of Bachelors in Veterinary Medicine

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DEDICATION

We dedicate our success to everyone who taught us something new and nourished intellectually by knowledge ,to all who stood beside us and helped us to overcome all difficulties ,to our professors and doctors in our college ,to each of their pains to give us a moment of happiness ,to our fathers, mothers, brothers, sisters and friend**.**

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LIST OF CONTENTS

LIST OF TABLES

ABSTRACT

The objective of this research is to make a study on bacterial count of camels milk and identification of associated bacteria in Khartoum State. A total of 31 arow milk samples were collected from four locations at Khartoum State (al Samrab ,Gandhar ,Shambat and Althora) as follows : 19 directly from udder,7 from milking equipments ,and 5from aselling centers .Addittionally 17 samples were collected randomly from the same farms for bacterial identification.The laboratory procedure included standerd total plate count for bacterial count, for identification of bacteria: gram stain, oxidase test, catalase test, and oxidation fermentation test. The total viable Bacterial counts of the udder samples ranged between (9.5×10^6 to 11.3×10^9 cfu/ml) with 4 uncountable sample, and the count of samples taken from equipment were between (1.4×10^9 to 2.4×10^9 cfu/ml) with 4 sample reveal uncountable bacterial colonies ,and for the samples taken from selling centers counts ranged between (2.6×10^9 to 6×10^9 cfu/ml) with one uncountable sample Bacterial samples identification of the camel's milk were revealed *Enterobacterial* spp. , *Staphylococcus* spp. and *Bacillus* spp.

مستخلص الاطروحه

الهدف من هذا البحث هو دراسة العد الكلي للبكتيريا من حليب الإبل و عزل و تحديد البكتيريا في حليب الإبل في ولاية الخرطوم . ٣١ عينه من لبن الابل تم جمعها من بحري و أم درمان في ولاية الخرطوم مقسمة على النحو التالي: ١٩ عينه تم جمعها مباشرة من الضرع ، ٧ عينات من معدات تجميع اللبن و ٥عينات من مراكز البيع في الخرطوم .ايضا ١٧ عينه تم جمعها عشوائيا من نفس المزارع ثم تزريعها والبكتريا والتعرف عليها. الاختبارات المعمليه الاوليه التي اجريت مجموع العد الكلي البكتيري، صبغبه الجرام، اختبار الاكسيديز، واختبار الكتاليز واختبار الاكسده والتخمير للتعرف علي نوع البكتيريا. كل العينات تم تزريعها في اجر الدم.عدد البكتيريا من العينات التي أخذت من الضرع بين (٢٠ -١١٣٠) بكتيريا مع ٤ عينات لا يمكن عدها، وعدد البكتيريا من العينات المأخوذة من معدات تجميع اللبن بين (١٤٠-٢٤٠)بكتيريا مع ٤ عينات لا يمكن عدها. و عدد البكتيريا من العينات التي أخذت من مراكز البيع بين (٢٦٠ - ٦٢٠)بكتيريا ، مع عينه واحده لا يمكن عدها. كانت العينات التي تم جمعها من أجل العزل والتحديد هي : العنقودية، المعوية والعصوية.

INTRODUCTION

In the past man used animal's milk for food, the increase in the demand of camel's milk has raised, this concern over the hygienic management and preservation of its milk, Quran and Sauna mentioned the high benefits of camel's milk. According to the international classification Sudan is the first country that have the largest number of camel (4.5 million head) (FOW 2009). The Ministry of Animal Resources and Fisheries (1996) gave an estimate of annual milk production in Sudan of about 7.58 million tons, of which 0.033 million tons is camel's milk Camel's milk is extremely popular and widely consumed by nomadic tribes in Sudan (fresh, soured milk) especially in East and West region (Shahani and Chandan,1979). Approximately 50% of the examined raw camel's milk samples were produced and handled under poor hygienic conditions with high health risk to the consumer (EL-Ziney and AL-Turk, 2007). Boiling of the milk is not common as it is known to remove its goodness, and if there is no good cooling and refrigeration with high temperature it facilitates hygienic problems (Radwen *et al*., 1992. Semereat &Molla,2001). Milk is an ideal habitat for the growth and multiplication of microorganisms due to its nutritional constitution which contain protein, carbohydrate, minerals and vitamins. All these components support the growth of many types of bacteria (Omer and Eltinay, 2008). Microbial contamination of milk can generally occur from three main sources within the

udder, the exterior of the udder and surface of milk handling and storage equipments. All these sources of contamination influence the total bacteria count (TBC) or Standard Plate Count (SPC) regulatory standard when bacterial count in raw milk are met, pasteurization is highly effective in destroying pathogenic microorganisms that can represent a threat to human health (Boor and Murphy,2002).

OBJECTIVES:

- To determine the bacterial counts in camel's milk (from udder, milk equipment and market level).

- To isolate and identify the bacteria encountered in camel's milk.

CHAPTER ONE

LITERATURE REVIEW

1.1. Chemical composition of camel milk:

Camel's milk is white and, although it has a pH of 6.5, has a slightly salty taste. The changes in taste are influenced by the type of fodder consumed and availability of water to the animal. The density of camel milk is between 1.025-1.032 g/ml with an average of 1.029g/ml. Both the pH and density are lower than those of the cows milk the total solids in camel milk range between 11 and 14%. The fat content is between 3-5%, Protein ranges between 2.7-5.4% while lactose is 3.4-5.6% as compared to 4.6% of the cow. (Yagil and Etzion , 1984). The mineral content of camel milk is not well known but calcium is said to be lower than that of the cow's milk. Casein is lower in camel's milk than in cow's milk but camel's milk has a higher content of whey proteins The total free fatty acids (FFA) concentration in camel milk is 1.36 µmol/ml. Saturated fatty acids content is 62.5% of FFA and is the same as that of the cow milk,while that of the goat milk is 74.5%. Camel milk lacks short chain (C4- C8) fatty acids (FA) while the middle chain (C9 – C14) FA are lower than those of goat and cow milk. The long chain (C16 –C20) FA content of the camel milk is higher than that of both goat and cow milk (Cardak *etal*., 2003).

The natural antimicrobial proteins like lysozyme in camel milk, is higher (648 µg/100ml) than the cow's milk, which is 120 µg/100ml (Farah, 1996).

1.2. Health Benefits of Camel Milk:

1.2.1. Diabetes Treatment:

Camel milk has a wealth of nutrients, including insulin, which is an essential component of human health. Insulin and glucose balance is very important for the prevention of diabetes, making it a potential natural solution for diabetes, eliminating the need for insulin injections if a steady stream of camel milk is included in the diet. If used as a preventive measure, it can also prevent developing the disease in the first place. (Wernery; 2003).

1.2.2. Immune System:

There is a surprisingly high level of proteins and other organic compounds in camel milk, some of which have powerful antimicrobial abilities. This means that it can help to boost the immune system and keep us healthy from the inside out (W.N-Sawaya *etal;*1984).

1.2.3 Growth and development:

The high level of animal proteins found in camel milk, many of which are not found in goat and cow milk, can help to stimulate proper growth and development

of bones and organ systems. Protein is one of the most basic building blocks of life, and camel milk provides a lot of it. In fact, in many cultures, camel milk is given to malnourished infants and children, as it can improve health and wellness so dramatically. Camel milk was first used as a way to prolong journeys through the desert, when food and other chances for liquid were limited (Katie- Wellness Mama; 2017).

1.2.4 Stimulate Circulation:

The high iron content found in camel milk makes it ideal for preventing anemia. Iron is a crucial component of red blood cells, which means that camel milk can increase circulation of the blood and oxygenation of the body's organ systems and extremities. Following childbirth, injury, or period of malnutrition, camel milk can significantly help maintain health (Sawaya, *et al .,*1984).

1.2.5 Autism Treatment:

The high concentration of unique organic compounds has been known to have particularly powerful effects on the neurological system, and can even prevent certain autoimmune disorders. Numerous case studies have shown that autistic symptoms have been lessened or erased completely when camel milk was taken regularly. The exact pathways are unknown, but this is a potentially invaluable new remedy for a tragic affliction (katie- Wellness Mama; 2017).

1.2.6. Allergic Reactions:

Camel milk has been connected to reducing allergic reactions in those who regularly consume it. Furthermore, camel milk does not cause the same sorts of lactose intolerance reactions of cow milk, as it has a significantly different chemical makeup (katie- Wellness Mama; 2017).

1.2.7. Heart Health:

With such a healthy and comprehensive set of fatty acids, camel milk can greatly improve the balance of cholesterol in the body. By reducing "bad" cholesterol in the body, camel milk helps to reduce atherosclerosis, heart attacks, and strokes, and even lowers blood pressure in regular users(katie- Wellness Mama; 2017).

1.3. Health hazards of milk with respect to poor quality:

Milk is an excellent culture medium for the growth of microorganisms. Their rate of multiplication depends mainly on storage temperature and handling conditions. The handling of Milk during informal marketing has been reported to affect the quality of the milk (Bachmann, 1992). It influences bacteriological quality by adding to the milk some externally acquired Microbial contaminants. The external sources of such microbes include the equipment, the persons and water. The time taken and temperature at which milk is kept influences generation time of

microbes, hence the rate of multiplication of bacteria in the milk and the ability of microorganisms to cause disease depends upon the type of microorganisms present, the initial load of contamination of the milk, handling conditions and the time elapse from production before consumption. The potential health hazards associated with raw camel milk are well documented. The genera *Salmonellae, Shigellae, Brucellae ,Mycobacterium, Campylobacter* and *Staphylococci,* have been reported to be transmitted through milk The growth of contaminating bacteria in raw camel milk poses a threat to consumer health when milk of unknown microbial quality is sold (James *etal;* 1985).

1.4 . Source of Contamination of Raw Milk:

Different sources of bacterial contamination include: air, milking equipments, feed, soil, feces and grass. The number and type of microorganism in milk affected by animal and equipments cleaning , season ,feed and animal health.

Microbial contamination can occur within the udder, from the exterior of the udder and from the surface of milk handling and storage equipment (Varnan *et al*.; 1994).

1.4.1. She Camel

Microorganisms can enter the milk from the skin of teat which often contaminated by dung, soil, dust, flacks of skin, hair, and dirt from feed and flacks. (Walsta, *et al 1999,*and Bachanan *et al.,* 1997).

1.4.2. Soil , Dung and Dust

All of this contaminant can reach the milk and thereby increase bacterial count. More over spore of Bacteria, yeast, and mold also occur in air. (Varnan *et al*.,1994).

1.4.3. The Feed

Feed often contain large number of microorganisms. Feed can some time fall directly into the milk but more significantly certain microorganisms in the feed survive passage through the digestive tract and subsequently enter milk through dug. Spore forming bacteria occur in silage of inferior quality. (Walstra, *et al* ., 1999).

1.4.4. The Milker

The milker may affect the Microbiological quality of milk direct contamination Ex: from his hand If the milker suffers from a microbial infection, might directly contaminate with pathogen. (Walstra, *et al* .,1999).

1.4.5. Water

Tap water usually have good quality, any private water supply must be examined at interval. Surface water can contain many microorganisms including human pathogen, and it must therefore on no account be used for rinsing. (Walstra,, *etal* ;1999).

1.4.6. Milking unit

Infection by contact represent the most important threat contamination to almost all of food including milk. Poorly cleaned and disinfected milking equipment can contain large number of Microorganisms; since these organisms generally originate from milk they will grow rapidly and can decrease quality. (Walstra,, *et al* .,and Bachanan *et al*., 1999)**.**

1.5. Microorganism Associated With Diseases oregenated from Milk:

1.5.1.Brucella spp:

Brucella species are found in many animal species including cattle, sheep, and goats. *Brucella spp*. are destroyed by pasteurization. *Brucella spp.* causes illness with symptoms that are flu-like which include fever, sweats, headaches, back pain and physical weakness. In some cases long-lasting symptoms of fever, joint pain and fatigue may occur. (Steel, *etal*; 1997).

1.5.2. Escherichia coli

Escherichia coli O157:H7 is one strain in a large family of bacteria. Strains of *Escherichia coli (E. coli*) are considered as fecal coliforms. Most strains of *Escherichia coli* do not cause illness and live in the intestinal tracts of healthy

humans and animals. *E. coli* O157:H7 is found in the intestinal tract and feces of cattle. It is destroyed by pasteurization (peng robin 1998).

E. coli O157:H7 produces toxins that cause illness in humans. Symptoms of illness include bloody diarrhea and abdominal cramps. In some time, particularly in young children*, E. coli* O157:H7 infection causes hemolytic uremic syndrome, which destroys red blood cells and causes kidney damage or failure, and in some cases death.(Jayarao, *et al., 2001).*

1.5.3. Listeria monocytogenes

Listeria monocytogenes is found in soil and water and has been isolated from a large number of environmental sources. *Listeria monocytogenes* is destroyed by pasteurization, but if food products are contaminated after pasteurization, *Listeria monocytogenes* can grow at refrigerator temperatures. Illness can occur as sporadic events or large outbreaks. *Listeria monocytogenes* typically causes illness in pregnant, adults, newborns, the elderly, and patients with compromised immune systems, but healthy adults and children may also become infected (DAFM 2014). Symptoms of Listeriosis are flu-like symptoms including , fever, muscle aches, stiff neck, headache, septicemia, meningitis, miscarriage, stillbirth, premature delivery, abortion, or death.*(Steel et al., 1997 and padhye 1991).*

1.5.4. Salmonella spp

Salmonella species contain several strains that cause illness in humans; the most common are the serotypes *Enteriditis* and *Typhimurium*. *Salmonella* has been found in the intestinal tracts of all warm-blooded animals including humans. *Salmonella* is destroyed by pasteurization. *Salmonella spp.* causes illness that can develop 12 to 72 hours after exposure, and can last 4 to 7 days. *(Van Kessel ,et al.,2004).* Symptoms of *Salmonellosis* include diarrhea, abdominal cramps, and fever. Most people recover without treatment other than fluid and electrolyte replacement. Some cases may be severe and require hospitalization. A small number of people may develop Reiter syndrome, which is a reactive arthritis that may affect multiple joints, particularly the knee joint (*Jayarao, et al.,* 2001).

1.5.5 . Coliforms

Coliforms are a large group of bacteria that are found in the intestines of warm-blooded animals. Most coliforms are not pathogenic, but their presence indicates contamination, usually from fecal sources. Coliforms are destroyed by pasteurization (*Van Kessel, et al., 2004*).

1.5.6. Enterotoxigenic Staphylococcus aurous:

Staphylococcal food poisoning (or entero intoxication) is the result of the ingestion of thermo tolerant toxins produced by these staphylococci during growth in foods. The staphylococcal enterotoxins (SE) form a heterogenic group, differing

at the nucleotide and aminoacid levels, and there are 11 recognized enter toxins (denoted A, B, C1-3 and D–I) that represent the pyrogenic group. Further, toxin production may be affected by a number of factors including pH, and temperature. Staphylococcal entero intoxication usually occurs 2–4 h after ingestion of contaminated food and lasts for a period of 24 h. Symptoms include nausea, vomiting and on occasionlly diarrhea. Enterotoxin poisoning is usually self-limiting with rapid recovery *(DAFM 2013)*. A number of studies have reported the isolation of *S. aureus* and staphylococcal enter toxins directly from milk cheese produced from cow's raw milk and from the bulk milk tank (*Jayarao etal;2001*).

1.5.7. Streptococcus agalactiae

Streptococcus agalactiae or Group B Streptococcus (GBS) are spherical cell shaped, non-motile, chain-forming and non spore-forming, Gram-positive bacteria. In Gram-positive bacteria the cell wall is composed predominantly of peptidoglycan on which various carbohydrates, bacterial polysaccharides (teichoic Acid) and surface antigens are attached. The cell wall polysaccharides of streptococcal species are critically important in determining the Lancefield serological grouping of strains on the basis of surface protein antigen Capsular polysaccharide antigen and surface protein antigen determined ten serotypes Ia, Ib and II to IX in Group B Streptococcus. Majority of the neonatal infections in

humans are caused by types I, II, III, and V (*Whiley and Hardie, 2009; Imperi et al., 2010).*

1.5.8. Haemophilus species

Are Gram negative spherical, oval or rod-shaped cells less than 1μm in width, variable in length, with marked pleomorphism, and sometimes forming filaments. The optimum growth temperature is 35–37°C. They are facultative anaerobic and non-motile. Members of the *Haemophilus* genus are typically cultured on blood agar plates as all species require at least one of the following blood factors for growth: haemin (factor X) and/or nicotinamide adenine dinucleotide (factor V). Chocolate agar is an excellent *Haemophilus* growth medium as it allows for increased accessibility to these factors. Alternatively, *Haemophilus* is sometimes cultured using the "Staph streak" technique: both Staphylococcus and *Haemophilus* organisms are cultured together on a single blood agar plate. In this case, *Haemophilus* colonies will frequently grow in small "satellite" colonies around the larger Staphylococcus colonies because the metabolism of Staphylococcus produces the necessary blood factor by-products required for *Haemophilus* growth. All *Haemophilus* species grow more readily in an atmosphere enriched with CO_2; *H. ducreyi* and some non typable *H. influenza* strains will not form visible colonies on culture plates unless grown in CO_2- enriched atmosphere. *Aggregatibacter aphrophilus* and *Haemophilus paraphrohaemolyticus* require CO_2 for primary

isolation. On chocolate blood agar, colonies are small and grey, round, convex, which may be iridescent, and these develop in 24 hours. Iridescence is seen with capsulated strains. Carbohydrates are catabolised with the production of acid. A few species produce gas. Nitrates are reduced to nitrites (*Ledeboer, 2011*).

1.6. Microbiological test of raw milk:

1.6.1. Standard Plate Count

Classically SPC procedure are used to determine the total plate count TPC or aerobic plat count APC or total viable count TVC. SPC is stander method comparing other screening test like chemical and microbiological analysis product (*Ramakant Sharma, 2014*).

1.6.2. Preliminary Incubation Count

The Preliminary Incubation Count (PIC) reflects milk production practices. This procedure involves holding the milk at 55°F for 18 hours prior to plating. This step encourages the growth of groups of bacteria that grow well at cool temperatures (psychotrophs). Bacteria in the incubated sample are counted with the standard plate count (SPC) procedure and compared to the SPC from the same sample to determine if a significant increase has occurred. PIC counts are generally higher than the SPC. Counts with a 3-4-fold increase are considered significant. Some consider counts greater than 50,000 cfu/ml to be of concern regardless of the SPC,

though in some cases the counts may be equal and in rare cases the PI may be lower. (Ramakant Sharma, 2014).

1.6.3. Coliform Count

The Coliform Count (Coli Count) procedure selects for bacteria that are most commonly associated with manure or environmental contamination. Milk samples are plated on a selective nutrient media that encourages the growth of coliform bacteria, while preventing the growth of others. Although coliforms are often used as indicators of fecal contamination, there are strains that commonly exist in the environment. (Ramakant Sharma, 2014).

1.7. Previous Studies in total Bacterial count and their results :

1.7.1. In Sudan:

Total count result 5×10^9 cfu/ml (Intisar; 2013).

El tahir (2005) had found that total bacterial count of Camels milk was ranged 1.1×10^6 - 3.9×10^{10} cfu/ml.

1.7.2. United Arab Emirates:

The microbial quality of camels raw milk was investigated, 50 samples were analyzed for: Aerobic plates count, total *coliform*, total *Staphylococcus aureus,* total yeast, and mold. Sixty eight samples were examined for *Bacillus cereus, Salmonella spp. Clostridium perfringens, and Listeria monocytogenus*. The results indicated that the mean value of aerobic plate count was 1.8×10^5 cfu/ml, mean

value of total coli form 6.8×10^{-1}, mean value of *staphylococcus aureus* 1.2×10^{-3}, yeast mean value 4.1×10^{-1} cfu/ml. (Omer and. Eltinay ;2008).

1.7.3. In Kenya

Total Bacterial count range from10^3-10^5 colony forming units per ml (cfu/ml). Over 90% of the samples from the processing and market levels ranged from 10^6-10^8 cfu/ml. Gram negative rods (GNR) were the majority and included the genera *Escherichia*, *Enterobacter* and *Pseudomonas*. *S.enterica* are isolated. (Matofari; 2007).

1.7.4.In Egypt:

The microbiological results revealed that camel raw milk contaminated 13×10^6, 12×10^4, 13×10^2 , 1×10^4 and 3×10^3 cfu/ ml for total bacterial count (T.B.C), total coliform (T.C.), faecal coliform (F.C.), total fungi (T.F.) and lactic acid bacteria (L.A.B) respectively. (Neamat,*etal* ;2014).

1.7.5. In Ethiopia:

Semreab and Molla (2001) had mentioned that total bacterial count of camels milk was 4×10^5 to 1×10^5 cfu/ml.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Description of the study area:

The study was conducted at Khartoum state. Camel milk samples were collected from four different farms, Shambat, Gandhar, Thawrat, and Samrab.

2.2. Collection of sample:

Sterile plastic containers were used for collection of samples. The camels used for the milk collection were healthy and uninfected. The udder was cleaned and disinfected with Alcohol (70%).

Samples taken were classified into three groups as follows:

- Samples taken directly from udder (group A).

- Samples taken from milking utensils (group B).

- Samples taken from milk selling center (group C).

Samples were preserved in container with ice until reaching the laboratory .

2.3. Equipment:

The equipments used for sterilization, media preparation, culturing of sample and biochemical tests were :

glass slides and cover slips, pipette 0.01 ml, one ml, five ml and ten ml, bottles, syringes, test tubes, Petri dishes ,loops, , droppers , clean 250 ml beakers, balance, incubator, Water baths, autoclave and oven .

2.4. Sterilization:

Sterilization was done according to Barrow and Feltham (2003). The glass wares such as Petri dishes, test tubes, flasks and pipettes were sterilized using dry heat oven regulated at 160°C for 1 hour. The media, automatic pipette tips and distilled water were sterilized using steam autoclave at 121°C for 15 minutes (15

Ib/ inch2).

2.5. Preparation of blood agar for culturing:

The Blood was collected from sheep, from the farm of Sudan University of science and technology kuku by sterile syringes in to sterile bottle containing anticoagulant agent.

Procedure:

Weigh 3.7gram of blood agar base by sensitive balance, added to 100ml of distilled water heated in water bath and mix well, sterilized in autoclave at 121° C and 15 pressure for 15 minute, add sterile sheep whole blood as10% value (v/v) and cast it in Petri dish and left to dry.

2.6. Total viable bacterial count

A number of 31 milk samples were processed for total viable count as follows :

2.6.1. Preparation of serial dilution:

Samples was diluted in sterile test tubes using (in 9 ml aliquots) normal saline by taking 1 ml from the sample9 by pipettes and mixing in the first tube contain 9 ml of normal saline and repeated serially for at least 7 tubes , After that 1drop from each dilution was streaked onto a quarter of a blood agar plate and spread it by sterile loop and incubated in 37˚c for 24 hour and finally the number of colonies were counted for each dilution .

Counting of colonies:

The counting done manually by using colony counter and recorded as colony forming unit per milliliter (cfu\ml).the total number of the colonies in the selected dilution was multiplied by the reciprocal of the dilution (Marshall,1992).

2.6.2. Standerd plate count :

The total viable count was carried out using the pour plate method described by (Harrigan and Mac Cance 1976). 7 fold serial dilutions were made by using sterile buffer saline, and prepared for each specimen1×10^{-6}, 1×10^{-7} are taken. 5 micro

liters from each milk sample dilution was spreaded by sterile loop on plate count

agar, and were incubate at37°C over night.

Table 1: farm location and mean milk production collections.

Farm	Location	Samples	Nutrition	Daily milk production
1-	1-Bahri shambat .	A1, A2 and, B1).	-alfa Alfa and cafe ration.	6,5litter/animal.
2-	Omdurman gandhar market). .	A3,A4,A5 ,A6,A7, A8,A9, A10,A11, A12,A13, A14,A15, A16, B2,B3,B4, and B5).	Café ration and grassing.(in these farm animals are uses for fattening)	Daily milk production:- 8liter/animal
3-	Omdurman al thwara alhara 103).	(A3, A4, B6).	animals feed by grassing.	
4-	Al samrab – dardoge).	(A17, A8, A19, B7).	animals feed on cafe ration and also grassing in external pasture.	Daily milk production:-4 liter /animal.

A: samples taken directly from udder.
B: samples taken from milker,s utensils .

2.7. Bacterial Isolation and Identification:

Seventeen samples were collected and examined for identification of bacterial content. Identification of bacteria in raw milk was carried out according to (Cowan and Steel 2003). Each sample was culture on blood agar plate and incubated at 37°C for over night .Individual colonies were picked up and further sub cultured for purification then were examined for cell morphology, gram staining ,oxidase,catalase and oxidation fermentation test.

2.7.1. Gram stain:

Techniques

1. Flood air-dried, heat-fixed smear of cells for 1 minute with **crystal violet** staining reagent. Please note that the quality of the smear (too heavy or too light cell concentration) will affect the Gram Stain results.

2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

3. Flood slide with the mordant: **Gram's iodine.** Wait 1 minute.

4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

5. Flood slide with **decolorizing agent (Acetone-alcohol decolorizer)**. Wait 10-15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear .

6. Flood slide with counterstain, **safranin**. Wait 30 seconds to 1 minute.

7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.

8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

Results:

- Gram-negative bacteria will stain pink/red and

- Gram-positive bacteria will stain blue/purple.

2.7.2. Oxidation\Fermentation test:

OF medium(hugh and leif son) in two tubes were inoculated with the organism by straight wire ,then on one of the tubes a layer of sterile oil was added 3cm above the medium level ,then both tubes were incubated at 37° C and examine daily for up to one week (Hugh and Leif son),the result was read as follow :

1_Oxidative if the tube without oil was changed to yellow color.

2_Fermentative if both tubes were change to yellow color.

3_Negative result was indicated by no color changes in both tubes.

2.7.3 .Catalase test

Few of colonies of the culture were picked using a sterile loop and put on a clean Petri dish, A drop of 3%hydrogen peroxide (H_2O_2) was added to the tested

organisms using a sterile pipette at room temperature .Bubbles indicated catalase positive reaction.

2.7.4 . Oxidase test

This test was done to separate the oxidative and fermentative gram negative organisms. Pure colonies of the isolates were smeared on the test Oxidase strip. Color change to deep blue was positive for the test.

2.8. Statistical analyses

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SSPS Inca nd Chicago, IL, USA). All bacterial counts were analyzed and **ANOVA** was performed.

CHAPTER THREE

RESULTS

3.1 The Total Bacterial Count:

A Number of 31 samples were collected for total bacterial count: 19 samples from the udder (A) the average count was between 9.5×10^6 to 11.3×10^9 cfu/ml. Mean 2.8×10^9 cfu /ml and 4 samples out of the 19 samples were uncountable. 7 samples from the milk utensils (B) the average of count was between 1.4×10^9 to 2.4×10^9 cfu/ml, Mean 2.8×10^9 cfu /ml and4 samples out of these seven samples were uncountable. 5 samples from the market (C) the average of count was between 2.6×10^9 to 6×10^9 cfu/ml. Mean 4.6×10^9 cfu /ml one sample out of these five samples was uncountable .See Table (2).

Table (2):Number of Bacterial count (cfu/ml) for each group: A, B, C

Sample	Total bacterial count(cfu/ml)
A1	1.5×10^9
A2	9.5×10^8
A3	11.3×10^9
A4	3.7×10^9
A5	2.6×10^9
A6	2.8×10^9
A7	1.9×10^9
A8	1.8×10^9
A9	2×10^8
A10	7×10^8
A11	Un countable
A12	Un countable
A13	Un countable
A14	4×10^8
A15	Un countable
A16	1×10^9
A17	4×10^9
A18	6.2×10^9
A19	4×10^9
B1	1.4×10^9
B2	Un countable
B3	Un countable
B4	Un countable
B5	Un countable
B6	1.7×10^9
B7	2.4×10^9
C1	Un countable
C2	3.7×10^9
C3	2.6×10^9
C4	6.2×10^9
C5	6×10^9

3.2. Isolation and identification

17 samples were isolated for identification by primary biochemical test including gram stain, oxidase test, catalase test and oxidation/fermentation test. There were different Shape in gram stain, 5 (29.4%) cocci, 6 (35.3%) bacilli, and 6 (35.3%) mixed (cocci + bacilli). All the smears were gram positive except 2 smears were gram negative. Table (3).

3.3 .Biochemical test:

All samples were Catalase positive, Oxidase negative, and fermentative. According to the primary test the organisms were identified as : *Staphloccous spp, Bacillus spp* and *Enterobactia.* Table (3).

Table (3) Result of primary test for identification of bacteria.

Sample	Gram stain	Catalase	Oxidase	Shape	O/f test
1	+ve	+ve	-ve	Cocci	F
2	+ve	+ve	-ve	Cocci	F
3	+ve	+ve	-ve	Cocci	F
4	+ve	+ve	-ve	Cocci	F
5	+ve	+ve	-ve	Bacilli	F
6	+ve	+ve	-ve	Bacilli	F
7	+ve	+ve	-ve	Bacilli	F
8	+ve	+ve	-ve	Bacilli	F
9	-ve	+ve	-ve	Mixed	F
10	-ve	+ve	-ve	Mixed	F
11	+ve	+ve	-ve	Cocci	F
12	+ve	+ve	-ve	Bacilli	F
13	+ve	+ve	-ve	Mixed	F
14	+ve	+ve	-ve	Mixed	F
15	+ve	+ve	-ve	Mixed	F
16	+ve	+ve	-ve	Mixed	F
17	+ve	+ve	-ve	Mixed	F

F: fermentative

Table (4):Percentage of Bacterial Isolates From 17 Samples:

Bacterial spp.	Percentage	Number
Staphylococcus spp.	29.4%	٥
Bacillus spp.	23.5%	٤
Enterobacterial spp.	11.8%	٢
Un identified	35.3%	6
Total	100	١٧

There are no significant differences of total bacterial count between the sources

of milk: udder, utensils and markets

Table (5): the result of ANOVA test

Source of sample	TBC (log CFU/ml)
Udder (n=19)	9.65±0.73
Container(n=7)	9.92±0.62
Market(n=5)	9.64±0.16
Significant	NS

Values are means ± standard deviation.

 NS = not significant differences

CHAPTER FOUR

DISCUSION

The raw Camel milk can be contaminated with pathogenic and spoiling microorganisms if proper handling practices are not followed. This study monitored the source of camel milk microbial infections in the udder, milk container and markets, using total bacterial counts and bacterial isolation and identification. The bacterial counts in camel milk from market reflect higher count than that present in containers and udder due to improper handling and storage. These problems were minimized by having proper milking. In this study total bacterial count (TBC) ranged from 9.5×10^6-1.3×10^9 cfu/ml (mean 5.3×10^7cfu/ml) Generally there is no large difference in (TBC) when comparing with other studies as follow: Joseph (2007).10^6-10^8cfu/ml (mean 10^7cfu/ml) and Neamat *et al.,* (2014). Found TBC 10^7 cfu/ml results were relatively similar to our results although there is a different in environment. Eltahir (2005) found the TBC as 1.1×10^6-3.9×10^{10}cfu/ml (mean 2.5×10^8) and Intisar (2013), found TBC 10^9cfu/ml. these results were higher than our results, this may be due to differences in environment and milk processing. Semreab and Molla (2001) found TBC as 10^5cfu/ml. which are lesser than our results possibly attributed to better hygiene and milk storage.

Conclusion

The present study revealed that the raw camel´s milk being produced and marketed in Khartoum State has a high load of micro flora ranging from (9.65±0.73) cfu/ml from the udder to (9.92±0.62) cfu/ml from the container and (9.64±0.16) at market level. The milk was mainly contaminated with the pathogen of the genus *Staphelococcus* , genus *Bacillus* and *Enterobacteria* which indicate the existence of health problems in both camels and humans. The risk factors in the Camel milk is the environment that were associated with camel´s milk microbial infections in this study included equipments, animals and humans. The approach to improving camel milk hygiene should be based on the principles of food sanitary practice like avoiding contamination and using appropriate equipment for processing.

RECOMMENDATIONS

The following recommendations have been suggested in this study based on the results obtained to improve the hygiene of camel´s milk production and marketing chain and hence its safety to the consumers:

1. Currently there is a practice of mixing evening milk and morning milk at collection centers, to reduce the chances of contamination evening milk should not be mixed with morning milk, each batch should be treated separately and be sold separately.

2. Before taking any sample from the udder it must be examined for subclinical mastitis.

3. Training and guidance programs should be started in order to develop awareness among farmers emphasizing the need for hygienic practice at farm level.

4. Good management practices should be directed, such as cleaning, applying personal and equipment hygiene during milking process.

5. The udder and teats should be washed and cleaned before milking.

6. Transportation and storage of raw milk should be at low temperature in clean cold steal or plastic tanks to avoid bacterial growth in raw milk.

7. Containers should be properly washed prior to transferring milk to them.

8. The raw camel's milk must be pasteurized or heated before direct drinking to avoid growth of contaminating bacteria.

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