بسم الله الرحمن الرحيم

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Bacteria and Risk factors associated with Bovine Mastitis in Eastern Nile Locality Khartoum-Sudan

البكتيريا وعوامل الخطر المرتبطة بإلتهاب الضرع في الأبقار بمحلية شرق النيل الخرطوم – السودان

A thesis Submitted in Fulfillment for Requirement of the Degree of Master of Preventive Veterinary Medicine (MPVM)

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الآية

بسم الله الرحمن الرحيم

قال تعالي:

وَإِنّ لَكُم فِي الأَنعامِ لَعِبرَةً نُسقيكُم مِمّا في بُطونِهِ مِن بَينِ فَرثٍ وَدَمٍ لَبَنًا خالِصًا سائِغًا لِلشّارِبينَ ﴿٦٦﴾

صدق الله العظيم

سورة النحل الآية 66

DEDICATION

This thesis is dedicated to my parents, my brothers, my sisters, my teachers and my friends with best wishes.

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Abstract

This study was conducted to isolate and identify the bacteria that associated and causing mastitis among the herds and to determine the risk factors which contributed for spread of the disease in East Nile locality. A total of 99 milk samples were collected from cows with clinical and subclinical mastitis from different four units (East soba, Alsheglla, Alseleet,and Kuku) for the period from February to April 2017. California mastitis test, pHmeasurement for milk and used to identify different types of mastitis pathogens. Culture characteristics and biochemical testsincludeprimary tests such as: gram stain, catalase test, oxidase and sugars tests. And secondary tests such as: (urease, nitrate,mannitol test, also analytical profile index APIE20) .From the 99 milk samples were used to identify isolated bacteria the result revealed that 18 (18%) werepositiveto California mastitis test and their pH was above normal. The total number of isolates was 123. Outof the 123 bacterial isolates 98 were gram positive, theisolated gram positive bacteriawere as follows: ***Staphylococcus***71 (72.4%), ***Micrococcus*** 27(27.6%).Secondary tests were performed and two species of Staphylococci were identified as:**S.** ***epidermidis*** 5 (18.5%) in Kuku farms, 6 (22.2%) in East soba farms, 16(59.3%) in Al Shegllafarms. and ***S. sciuri***9(21.4%) in Al Sheglla,15 (35.7%) in Alsellet farms,18(42.9%) in Kuku . other isolated bacteriainclude ***Micrococcus*** **species** from Kuku farms17 (63.0%) , in AlSheglla 4 (14.8%) , Alsellet 6 (22.2%) . one isolate from gram negative bacteriawas identified as: ***Psuedomonasoryzihabitans***while thetwo bacteria were not identified .

Analysis of risk factors according to the studydetermined that some of the risk factors which were contributed for the occur of thedisease include: body condition (p value=0.10%), sanitarypractice (p value=0.10%) and quarter type (p value=0.05) out of twenty one risk factors.

مستخلص البحث

هدفت هذه الدراسة الي عزل وتصنيف البكتيريا المسببة المصاحبةلإلتهاب الضرع في الابقار والي تحديد عوامل الخطورة التي ساعدت علي إنتشار المرض في محلية شرق النيل . جمعت 99 عينة من اللبن من أبقار مصابة بالتهاب الضرع السريري وتحت السريري من أربعة وحدات مختلفة في الفترة من شهر فبراير وحتي شهر أبريل 2017. بإستخدام إختبار الكاليفورنيا وإختبار الأس الهيدروجيني للبن وإستخدام صبغة الجرام تمت معرفة أنواع البكتيريا المسببة لإلتهاب الضرع ثم كذلك إجراء الإختبارات الأولية لصبغة الجرام,الكاتليز,الأوكسديز,اختبارات السكر.والاختبارات الثانوية:كإختبار اليوريز,النتريت, المانيتول,إختبارAnalytical Profile Index APIE20.ثم أخذ عينات اللبن من 99بقرة حلوب,أشارت نتائج الدراسةأن 18(18%)كانت موجبة لفحص الكاليفورنيا وعدد18بقرة كان الأس الهيدروجيني أعلي من الطبيعي .تم عزل123عزلة بكتيرية, 98 عينة كانتموجبة لصبغة الجرام.كانت نسبة البكتريات كالأتي:*Staphylococcus*71(72.4%)27*Micrococcus*(27.6 %).بصورة عامة بعد اجراء الإختبارات الثانوية تم التعرف علي نوعين من البكتيريا الموجبة لصبغة الجرام هي:***S.epidermidis***في منطقةحلةكوكو بنسبة5(18.5%)وبنسبة 6(22.2%)في مزارع سوبا شرق والشقلة بنسبة16 (59.3%). ***S.sciuri*** بنسبة 9(21.4%) في الشقلة ونسبة 15 (35.7%)في السليتو18 (42.9%)في كوكو.

وكذلك تم عزل بكتيريا ***Micrococcus***من منطقة حلة كوكو بنسبة 17(63%).وفي الشقلة بنسبة4(14.8%) وفي السليتبنسبة6(22.2%). كما تم التعرف علي نوع واحد من البكتيريا السالبة لصبغة الجرام من مجموع 3 بكتيريات وهي كانت***Pseudomonas oryzihabitans*** ولم يتم التعرف علي النوعين الاخرين.

وعند التحديد لعوامل الخطورة عند التحليل الاحصائي لكل عامل خطورة حددت الدراسة بعض عوامل الخطورة التي ساهمت بشكل وثيق في حدوث المرض منها حالة الجسم الطبيعية للحيوان (P-value = %0.10) والاجراءات الصحية(P-value = %0.10) وتدلي الضرع(P-value = %0.05) من مجموع 21 عامل خطورة.

Introduction:

Livestock production systems in Africa are classified into intensive and semi – intensive systems according to practice and distribution of pasture that varies with the rainfall, season or cultivated crop. In Sudan, 92 % of livestock population is possessed by nomads that follow extensive system of husbandry in eastern, western, and southern part of the Sudan. (Nuol ,. *et al* 2009).

Milk which is a very nutritional food i.e. rich in carbohydrate, proteins, fats, vitamins, and minerals, provides an important dietary source for the majority of rural as well as considerable number of the urban and peri – urban population (Haftom ,. *et al* 2015).

Bovine mastitis is the inflammation of the mammary gland often due to microorganisms that invade the udder, multiply and produce toxins that are harmful to the mammary tissue (David,. *et al* 2013).

Mastitis has been known to cause a great deal of loss or reduction of productivity to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age (Mekibib,. *et al* 2010).

Mastitis is one of the most important diseases that causes economic loss in dairy industry worldwide (Chaiwat,.*et al* 2012).

Unfortunately, most producers regard young heifers as uninfected, and the presence of mastitis is not observed until calving and showing the first signs of clinical mastitis in early lactation. Thus,an animal may carry an intra mammary infection for a year or more before it's diagnosed with mastitis (Ayhan,. *et al* 2015), also Mastitis can cause devastating effects to farmers because of the serious economic losses and the danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption(Zeryehun,. *et al* 2013).

Dairy cows are most efficient converters of forage to food for humans Mastitis is the most common infectious disease encountered in dairy cattle , that decreases milk yield and quality in lactating cows .(Biniam,. *et al* 2015).

Mastitis is a disease that can be met all over the world, in all animals, but particularly at the dairy cows. The disease may be caused by the interaction of the environmental factors, of theinfectious agents, with the host`s resistance sub clinically. Mastitis interferes not only with the milk quantity, but also with the qualitative features such as the composition or otherphysicochemical factors. In addition to inflammatory cells, mediators of the inflammation, and bacterial toxins that can be found in milk, modifications of nutritional components are encountered. Owing to these, the level of fats, of lactose, casein and of the calcium are decreasing, whereas the level ofAlbumin, sodium, and chloride, increase. The increasedlevels of lipases, proteases, oxidases, plasmin, and plasminogen caninfluence the stability of milk, its flavor, and the quality of the processed products. The lesions that can occur in mastitis can cause the atrophy of the glandular akin or of the affected quarter. *(* Căşaru,.*et al* 2016*)*.

Efficient production of high quality milk is challenged when udder health problems occur. Despite huge efforts, and although progress has been made, udder health still is an important issue on the dairy farms throughout the world. In General, the rate of intra-mammary infection is established by a combination of exposure of the teat-end to pathogens and the effectiveness of the defense mechanisms of the cow. Therefore, the teat mastitis, so the changes in teat tissue around the teat canal may favor penetration of bacteria into the udder (Nakov and Trajcev, 2013*)*.

Objectives of this study were:

1- To investigate the risk factors associated with bovine mastitis

2- To isolate and identify the bacteria associate with bovine mastitis.

Chapter one

Literature Review

1.1 Definition:-

- Bovine mastitis is the major problem for milk producers throughout the world and responsible for substantial losses of revenue annually (Suzan,. *et al* 2016).

- Mastitis, the common problem of dairies, that is known by an inflammation of the mammary gland is the leading one, that can contribute to reduce milk production (Birhanu ,. *et al* 2013).

- Mastitis is a multifactorial disease, results from injury, chemical irritation and infection caused by different bacterial species. Bovine mastitis is the inflammation of the parenchyma cells of the mammary glands of cattle, buffalo and other animals associated with microbial infections and physiological changes.**(**Afaf**,.***et al* 2016).

- Mastitis is caused by a group of infective and potentially pathogenic bacteria, viruses, fungi, and parasite (Hala, 2016*)*.

- Mastitis is identified by an increase in the number of somatic cells in the milk as well as pathological changes in the mammary tissue (Pourtaghi,.*et al* 2016).

1.2 Causative agents:

Various infectious agents numbering more than twenty different groups including bacteria, viruses, yeast, fungi and rickettsia, being the major cause. 137 infectious causes of bovine mastitis are known todate and in large animals, the commonest pathogens are ***Staphylococcus aureus, Streptococcus agalactiae***, other ***Streptococcus*** species and Coliforms. It may be also associated with many other organisms including ***Actinomycespyogenes,Pseudomonas aeruginosa ,Nocardiaasteroids , Clostridium perfringens*** and others like ***Mycobacterium ,Mycoplasma , Pastuerella and Prototheca*** species and yeasts (Lidet,. *et al* 2013). Mastitis can be caused by a series of pathogens,differentiated into two broad categories: those causing contagious mastitis such as ***Staphylococcus aureus (S. aureus), Streptococcusagalactiae (St. agalactiae)***, ***Corynebacteriumbovis ,Mycoplasma*** species. which are widespread from the infected quarters, primarily during milking (man hands, milking machines), and those causing environmental mastitis such as ***Streptococcus uberis, Streptococcus dysgalactiae,Streptococcus bovis ,Klebsiellaoxytoca ,Klebsiella pneumonia, Enterobacteraerogenes, Serratia species, Escherichiacoli (E.coli)***which are present in the environment (bedding, flooring, droppings) and generally transmitted in any time of cow's life: during milking, between milking and during the dry period, especially at first calving in heifers. ***S. aureus***(25.8%) followed by ***E.coli***(18.7 %) and ***Streptococcus agalactiae***(11.8 %) **(**Sayed,. *et al* 2015).

Mastitis can be caused by physical injury (cuts or bruises) or by chemical agents or infectious agent but in most cases it is caused by several bacterial pathogens (Shawgi, 2003).

B. abortuswas isolated more frequently from milk samples than from mammary tissues. Organisms were often demonstrated immunohistochemically and by culture in tissues showing moderate to severe histological changes (Xavier,.*et al*

2009) .bacteria replicate to high numbers in the gravid uterus and also infect the udder and lymph nodes. The udder and supramammary lymph node are the most common sites for localization. Infected mammae intermittently or continuously excrete brucellae into the milk throughout lactation. Clinical findings are typically limited to decrease milk production and increased numbers of leukocytes in the milk. (Meador,. *et al* 1989).

1.2.1 *Staphylococcus*:-

Mastitis is a problem; more than 50% of cows may have chronic, subclinical infections. ***Staphylococcal*** mastitis leads to duct obstruction with cells and cellular debris (Rofaida, 2006). Thegenus***Staphylococci*** is classified into two major groups: coagulase-negative staphylococci (CNS) and coagulase-positive *Staphylococci* (CPS). CNS, comprising the majority of species, is considered to be saprophytic or, rarely, pathogenic, but the importance of CNS due to it increasing number in the environment and the veterinary medicine. Recently, eleven staphylococcal species have been sequenced: ***S. aureus, S. epidermidis, S. saprophyticus, S. haemolyticus, S. hominis, S. cohnii, S. auricularis, S. capitis, S. simulans, S. warneri and S. lugdunensis***(Uranchimeg, 2006).

1.2.1. 1 *Staphylococcus aureus* :-

An opportunistic pathogen, a gram positive, spherical or ovoid bacterium, non capsulated, non-motile, catalase positive non-sporulated organism, 0.8-1.0 μm in diameter, its occurs as normal flora of the skin, nose and mucous membranes, that causes serious diseases in men and animals (Parmar,.*et al* 2014).

1.2.2 *Streptococcus agalactiae*:-

Streptococci are gram positive cocci form chains of different lengths. They are fastidious bacteria and require the addition of blood or serum to culture media, *Streptococcus* species are non motile, facultative anaerobic which are catalase negative. *Streptococcus agalactiae*produces beta-haemolysiswhich is complete haemolysis indicated by clear zones around colonies (Quinn and Marky, 2003). Mastitis due to these organisms is largely subclinical .The main reservoir of bovine strains is the udder of cows generally, milk becomes contaminated after multiplication of the organism in the udder and the pathogen will be spread to other cows by milking (Quinn *et al*,. 1994). The organisms are susceptible to penicillin and once eliminated, usually does not return to the herd (Merk, 1998).

1.2.3 *Streptococcus dysagalactiae :-*

Streptococcus dysgalactiae(*S. dysgalactiae*) has the unique characteristic of being considered both a contagious and an environmental pathogen. These organisms can spread from cow to cow at milking time and are also commonly found in the cow's environment .Infections most likely occur in early lactation are at increased riskfor new infections due to the increased stress and immune suppression associated with the postpartum period. Also, following milk cessation, cows do not experience the daily flushing of the gland and are at an increased risk for mastitis in the early dry period. Cows with high milk production are not at greater risk than cows with low milk production **(**Christina,.*et al* 2012).Gram-positive cocci or oval cells in short- to medium-length chains. Growth in glucose nutrient broth is poor and has a final pH of **4.7** to **4.9.** On blood agar plates colonies are surrounded by a wide zone of alpha hemolysis (greenish).The optimum temperature for growth is **37°C (**ELLEN,.*et al* 1983).

1.2.4 Pseudomonas Species:

Pseudomonas spp. including (*aeruginosa* and *oryzihabitans*) are environmental mastitis-causing pathogens that are Gram-negative and similar in structure to other coliform mastitis pathogens. *Pseudomonas* spp. has been isolated from milking parlor drop hoses and are known to cause mastitis through the use of water during milking. When grown on blood agar, *Pseudomonas* spp. have been found to smell like grapes. *Pseudomonas* spp. can also be found in wet bedding, cooling ponds, pools of standing water, muddy lots or corrals, marshy areas, and manure and urine. New infections can occur at any time during lactation. Cows in early lactation are at greater risk for new infections due to the increased stress and immune suppression associated with the postpartum period (Turner**,.***et al* 2016**).**

1.2.5 Coliforms(*E. coli* and *Klebsiella* spp):

Belong to the colonic flora and infect the udder via fecal contamination of the cow´s surroundings. They do not survive long inside the udder. Coliform mastitis is common during the puerperal period and symptoms are often acute to peracute as a consequence of endotoxin production. (Sandra, 2013).

1.3 Pathogenesis:

Mastitis in dairy animals occurs when the udder becomes inflamed and bacteria invade the teat canal and mammary glands. These bacteria multiply and produce toxins that cause injury to the milk secreting tissue, besides, physical trauma and chemical irritants. These cause increase in the number of leukocytes, or somatic cells in the milk, reducing its quantity and adversely affecting the quality of milk and milk by products. The teat end serves as the first line of defense against infection. From outside, a sphincter of smooth muscles surrounds the teat canal which functions to keep the teat canal closed. It also prevents milk from escaping, and bacteria from entering into the teat. From inside, the teat canal is lined with keratin derived from stratified squamous epithelium. Damage to keratin has been reported to cause increased susceptibility of teat canal to bacterial invasion and colonization. The keratin is a waxy material composed of fatty acids and fibrous proteins in the teat. The fatty acids are both esterified and non-esterified, representing myristic acid, palmitoleic acid and linolinic acid which are bacteriostatic. The fibrous proteins of keratin in the teat canal bind electrostatically to mastitis pathogens, which alter the bacterial cell wall, rendering it more susceptible to osmotic pressure. Inability to maintain osmotic pressure causes lyses and death of invading pathogens. The keratin structure thus enables trapping of invading bacteria and prevents their migration into the gland cistern. During milking, bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus. The canal of a teat may remain partially open for 1-2 hour after milking and during this period the pathogens may freely enter into the teat canal. Bacterial pathogens which are able to traverse the opening of teat end by escaping antibacterial activities establish the disease process in the mammary gland which is the second line of defense of the host. In dairy animals, the mammary gland has a simple system consisting of teats and udder, where the bacteria multiply and produce toxins, enzymes and cell-wall components which stimulate the production of inflammatory mediators attracting phagocytes. The severity of inflammatory response, however, is dependent upon both the host and pathogen factors. The pathogen factors include the species, virulence, strain and the size of inoculums of bacteria, whereas the host factors include parity, the stage of lactation, age and immune status of the animal, as well as the somatic cell count. Neutrophils are the predominant cells found in the mammary tissue and mammary secretions during early stage of mastitis and constitute > 90% of the total leukocytes. The phagocytes move from the bone marrow toward the invading bacteria in large numbers attracted by chemical messengers or chemotactic agents such as cytokines, complement and prostaglandins released by damaged tissues. The Neutrophils exert their bactericidal effect through a respiratory burst and produce hydroxyl and oxygen radicals that kill the bacteria. During phagocytosis, bacteria are also exposed to several oxygen-independent reactants such as peroxides, lysozymes, hydrolytic enzymes and lactoferrin. In addition to their phagocytic activities, neutrophils are a source of antibacterial peptides called defenses, killing a variety of pathogens that cause mastitis. Masses of neutrophils pass between the milk producing cells into the lumen of the alveoli, thus increasing the somatic cell counts and also damaging the secretary cells. Increased number of leukocytes in milk causes increase in the number of somatic cells. Clots are formed by aggregation of leukocytes and blood clotting factors which may block the ducts and prevent complete milk removal, resulting in scar formation with proliferation of connective tissue elements. This results in a permanent loss of function of that portion of the gland. The milk ducts remain clogged, secretary cells revert to non-producing state, and alveoli begin to shrink and are replaced by scar tissue. This helps in formation of small pockets making difficult for antibiotics to reach there and also prevents complete removal of milk. Macrophages are the predominant cells found in milk and tissue of healthy involutes and lactating mammary glands. Macrophages ingest bacteria, cellular debris and accumulated milk components. The phagocytes activity of macrophages can be increased in the presence of opsonic antibody for specific pathogens. Because of indiscriminate ingestion of fat, caseinand milk components, the mammary gland macrophages are less effective at phagocytosis than are blood leukocytes. Macrophages also play a role in antigen processing and presentation. Conditions which contribute to trauma of mammary gland include: incorrect use of udder washes, wet teats and failure to use teat dips, failure to prepare milking animals or pre-milking stimulation for milk ejection, over milking, insertion of mastitis tubes or teat canulae, injury caused by infectious agents and their toxins and physical trauma (Khan,. *et al* 2006).

1.4 Epidemiology of mastitis:

Udder health depends on a balanced interaction between host and its microbiota, which may contain microorganisms ranging from probiotic to potentially infectious. Obviously, there are relevant differences among mammals regarding the number, size, position and structure of the mammary glands. In addition, mammals (even within a same species) differ widely in their ecosystems management and use (e.g., milk producing versus meat-producing domestic species). Therefore, there are many microbial, host and/or environmental factors that may play important roles in the development of mastitis (Andres and Juan 2011).*streptococcus (S. uberis*) is considering a reservoir, not a mastitis pathogen that is obligatorily adapted to the udder. In fact, it is a ubiquitous microorganism which colonizes animals as well as their environment, Detection of *S. uberis* in bovine teat canals, remains unclear -unlike cow-associated mastitis pathogens like *S. aureus* and *St. agalactiae* are abscent. the environmental-associated pathogen. *S. uberis* is able to colonize the teat canal epithelium (Volker ,. *et al* 2014) .

1.5 Transmission:

If we know how a disease is spread, we also know how to stop the spread. There are two main modes of transmission for mastitis: contagious and environmental. In the case of contagious transmission, also called cow-to-cow transmission, cows with mastitis are the main source of infection. Spread of the bacteria that cause the infection primarily happens during milking, e.g. via the milkers' hands, udder cloths, or the milking machine. use of milking gloves and individual towels will help to prevent this. Environmental mastitis originates in the environment, e.g. in bedding, manure or water. Occasionally, bedding contains high numbers of bacteria even before it is used. Routinely, bedding is contaminated with manure, which contains everything bacteria need: moisture, warmth and nutrients. Water can be contaminated with bacteria from manure, or it may accumulate in a milking machine, particularly if hoses don't have the correct slope and if the temperature of the cleaning water is too low. In addition to those common modes of transmission, there is also the possibility of transmission by people. This may happen via animal health products, such as teat wipes or teat dip. Alternatively, people may be a direct source of infection for animals because many mastitis-causing bacteria occur in people as well as in cattle. The human-to-animal route of transmission has various names, none of which are particularly attractive: humanosis, reverse zoonosis, or anthroponosis. Without knowledge of the most important sources or transmission routes of bacteria on a farm or during a multi-farm outbreak of mastitis, we cannot take adequate control measures. Strain typing can help us to identify sources and transmission routes so that we can target our control efforts**. (**Ruth and Zadoks, 2014)**.**

1.6 Types of mastitis:

1.6.1 Accordingto mode of transmission of pathogen:

1.6.1.1 Contagious mastitis:

With contagious diseases, the mammary glands and teat skin serve as the primary reservoirs of infections with colonies establishing at the teat end and slowly growing through the teat canal over 1-3 days. Among the contagious organisms, *Staphylococcus aureus*, *Streptococcus agalactiae* and*Streptococcus dysgalactiae*have been identified as the major causes of bovine mastitis. Contagious mastitis canbe further classified into three major groups based on the symptoms associated with infection; clinical, sub-clinical and chronic mastitis (Basdew and Laing, 2011).

1.6.1.2 Environmental mastitis:

Environmental bacteria, as the name implies, come fromthe cow's environment (bedding, soil, manure, etc.) and thus are highly influenced by management practices. It is therefore impossible to completely eliminate them, as they are endemic to where the animals live, and can only be controlled by improving cleanliness of both the cows and their surroundings. The most common environmental bacteria are the coliforms (*E. coli,Klebsiellaspp, and Enterobacter*), whose main origin is manure and soil, and the environmental *streptococcus* (*St. uberis*and *St. dysgalactiae*) that come from the environment but also from infected udders. The fact that this last group is also present in the udder increases the likelihood of them being also contagious. Environmental bacteria thrive under wet conditions in the presence of the adequate substrate (manure). When the cow lies on soiled bedding, wades through mud, or even when contaminated water is splashed on the udder (water pools, footbaths, etc.), these bacteria can colonize the udder skin and eventually enter through the teat canal at milking time (Alvaro, 2004).

1.6.2 According to the clinical symptoms:

1.6.2.1 Clinical mastitis:

The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supramammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder is also inspected for the presence of any grossly visible injury and ticks. Injuries caused by ticks and vigorous calf suckling were described based on location, size, and nature. Injuries caused by ticks were identified as indurate necrotic lesions following detachment of the parasites; these could be with or without abscess formation. Injuries caused by vigorous calf suckling were identified as circumscribed lesions around the teats (Demelash,.*et al* 2005).

1.6.2.1.1 Peracute mastitis:

Is characterized by a sudden onset, severe inflammation of the udder, serous milk and systemic illness. The systemic illness is due to septicemia or toxemia, results in fever, anorexia, depression, decreased rumen motility, dehydration, and sometimes death of the cow. Systemic illness often precedes the symptoms manifested in the milk and mammary gland (Amare, 2016).

1.6.2.1.2 Acute mastitis:

Similar to per acute mastitis, but with lesser systemic signs like fever and mild depression (Awale*et al*., 2012).

1.6.2.1.3 Sub acute Mastitis:

When symptoms include only minor alteration in the milk and the affected quarter such as clots, flakes or discolored secretion. The quarter may also be slightly swollen and tender (Philpot and Nickerson, 2000).

1.6.2.1.4 Chronic Mastitis:

Chronic mastitis: It's a headache for every dairy. However, there are four tools to manage chronic mastitis infections:

• Individual cow Somatic Cell Counts (SCC) (diagnose).

• Milk culture results

• Culling

• Pharmaceuticals (manage).

We need individual-cow SCC to identify chronically infected animals. If a cow's SCC is greater than 200,000, or Linear Score (LS) greater than 3.9, for two months in a row or two out of the last three months, this is chronically infected. It's easy to select chronically infected animals, just look at their reproductiveStatus, production records and the presence of other health problems (Jack and Linda 2008).

1.6.2.2 Subclinical mastitis:

The non observable form of mastitis, such as no visible abnormalities of either the milk or the udder, is known as subclinical mastitis.In excess of 50% of animals in a herd can have subclinical mastitis at any given time.A sudden rise in milk somatic cell count observed in normal milk from normal udders may indicate the presence of subclinical mastitis. Animals which have subclinical mastitis are usually not producing milk to their full potential and can serve as a potential source of infection to healthy udders. The subclinical form of mastitis in dairy cows is important because this form is (a) 15 to 40 times more prevalent than the clinical form, (b) it usually precedes the clinical form, (c) it is of long duration, (d) it is difficult to detect, (e) It reduces milk production, (f) it adversely affects milk quality and (g) constitutes a reservoir of microorganisms that can affect other animals within the herd due to its contagious nature.Besides causing huge losses to milk production, the sub clinically affected animals remain a continuous source of infection to other herd mates. If the infection persists for longer periods, then it may form a fibrous tissue barrier between the organisms and the antibiotic preparations, thus, limiting their efficacy (Amare ,2016).

1.7 Diagnosis of Mastitis:

1.7.1White Side Test (WST):

The WST was performed as per procedure described by Kahir (2006). In brief, 50 µl (five drops) of milk were placed on a glass slide with a dark background by micropipette. And then 20 µl of WST reagent (4% NaOH) were added to the milk sample and the mixture was stirred rapidly with a toothpick for 20-25 seconds. A breaking up of milk in flakes, shreds and viscid mass was indicative of positive reaction. On the other hand, milky and opaque and entirely free of precipitant was indicative of negative reaction. (Islam,.*et al* 2010).

1.7.2Surf Field Mastitis Test (SFMT):

The samples were subjected to surf test. for thisPurpose, 3% Surf solution was prepared by addition of three grams of commonly used detergent powder (Surf Excel) in 100 ml of water. Milk samples and surf solution were then mixed in equal quantities in Petri dishes. The formation of gel indicated the positive samples and the reaction developed almost immediately with milk containing a high concentration of somatic cells (Muhammad,.*et al* 2011).

1.7.3Somatic cell count:

The somatic cell counts have become the most widely used index of the level of the infection within individual cows and herds (Bartelett*et al*,. 1992). Somatic cell count consists primarily of leukocytes that are present in the udder in response to infection and to repair damaged tissue, somatic cell also include epithelial cells which make up the internal lining of the mammary gland tissue and are normally replaced during the early stage of lactation(Harmon and Langlois , 1986). When the udder or teat is severely injured there are large increase in somatic cell counts (De Graaf and Dwinger , 1996). The direct microscopic somatic cell count (DMSCC) is the procedure of evenly spreading a measured volume of milk over a calibrated area of a microscope slide, staining the film and counting somatic cell within specified area of the film (Packard *et al*,. 1992). The count is then converted to cells per milliliter (ml) by a factor which is determined by magnification and area counted. Low somatic cell count herds are considered to have higher levels of environmental mastitis (Peeler *et al*,. 2000).

1.8 Treatment of Mastitis:

A program for mastitis treatment starts with clinical cases and treats in earliest stage. Treatment of mastitis should be targeted towards the causative bacteria wheneverpossible, but in acute situations, treatment is initiated based on a herd data and personal experience (Pyorala, 2009). In subclinical mastitis quarters are identified using survey or representative sampling during a routine check. Another treatment during dry period to cure the infection and to protect from a new infection which occurs during dry period. A broad spectrum antibiotic is therefore the first essential of dry period intramammary infusion (Blood,. *et al* 1994). The lack of appropriate mastitis therapy results in the development of resistant organisms to antibiotics (Linhart and Weiskopf, 1989). Especially in improper treated cows (Rabinson,. *et al*, 1988). Moreover use, misuse and often abuse of antimicrobial agents have encouraged the evolution of bacteria towards resistance resulting into therapeutic failure (Straut ,. *et al* ,1995). Furthermore resistance can be transferred between species of different genera (Maff, 1998).

1.9 Prevention and control:

The five points plan for mastitis control has been the corner stone of control strategies for many years worldwide. The main aim of the control program was to Eradicate ***S. aureus***and ***Strept. Agalactiae***from dairy herds. The elements were post-milking teat disinfection, dry cow therapy, and treatment of clinical cases during lactation, proper maintenance of the milking machinery and culling of chronically infected cows. The five points plan, or some of its components, has considerably reduced ***Strept. Agalactiae*** mastitis, but for ***S. aureus***mastitis the effect has been less satisfactory. Separation of infected cows alone has not shown sufficient cure rates for dry-cow therapy have been low and ranged from 40 to 70% and there is no scientific evidence to suggest that culling alone is of economic importance. Epidemiological studies ofS***. aureus***in the environment of dairy cows have increased knowledge on the dynamics of***S. aureus***intramammary infections. Current strategies for control and prevention of ***S. aureus***mastitishave been expanded to include isolation or elimination of the reservoir by segregation, therapy, and/or culling, isolation or removal of the fomites by applying improved milkingHygiene, evaluation of teat skin condition, teat disinfection and back flushes. In some countries, host resistance has been enhanced by improving management of the cows and vaccinating against mastitis. In spite of the introduction of large-scale mastitis control programs, ***S. aureus***remains a major mastitis pathogen. It causes mastitis Epidemics even in well-managed dairy herds and can persist for long periods in the mammary glands. The current control practices may fail to prevent the spread of particularly virulent strains (Ayman, 2011). Prevention of mastitis depends primarily on good hygiene (before, during and after milking) practices and effective animal management which include treatment of clinical cases as they occur, use of udder disinfection and pre-milking strip cup, post milking teat dipping and dry cow therapy. (Sohiela , 2002**)**.

1.9.1 Vaccines:

Mastitis vaccine research dates back at least three decades. Throughout this time, several vaccines have become commercially available. In the United States, there are 40 vaccines that guard against ***S. aureus***and ***E. coli***, but none are currently available that afford protection against any ***Streptococcus***species .The purpose of a vaccine is to enhance the immune response. However, an improved immune response correlates to an increased somatic cell count (SCC), so this can be a difficult situation for dairy producers. Whenever vaccines are used as part of a mastitis control program, it is imperative that they are handled properly, used before the expiration date, Tomita and coworkers looked at the efficacy of two different vaccines against ***E. coli-*** JVac® and J5 bacterin®. All cows were vaccinated at drying off and at two weeksbefore their anticipated calving date. This timing was based on the periods of greatest riskfor acquiring coliform mastitis, which has been shown to be during the early dry period,late dry period, and at calving. Cows vaccinated with J5 bacterin® received a thirddose atcalving, whereas cows vaccinated with JVac® did not. Immunization by either of thesevaccines did not affect the severity of clinical coliform mastitis. (Rebecca,2014)**.**

1.10 Economic impact of mastitis:

The economic consequences of mastitis (clinical or Subclinical) are due to treatment, production losses, culling, changes in product quality and the risk of other diseases. The associated costs can be divided among the following factors:

-Milk production losses

-Drugs

-Discarded milk

-Veterinary services

-Labour

-Product quality

-Materials and investments

-Culling

1.10.1 Drugs:

Drugs necessary to treat infected animals are a direct cause of economic damage, owing to their costs. The costs of drugs vary between countries, Depending on the legislation and the infrastructure of the country. (Halasa,.*et al* 2007).

1.10.2 Culling:

Culling is a difficult factor to estimate since it is a result of other effects (except in the case of death from causes other than culling). Culling is a decision of the dairy farmer. A cow is culled when replacement is the optimal decision. Cows with mastitis have a higher risk of being culled the cost of premature replacement of animals due to mastitis is probably one of the largest areas of economic loss. However, it is very difficult to calculate precisely. When a cow is culled, there are direct costs that are the costs of rearing or buying a replacement animal (mostly heifers). Indirect costs are a decreased efficiency of milk production by the replacement animal, since the milk yield of multiparous cows is higher than that of primiparous cows. (Halasa,.*et al* 2007).

1.10.3 Labour:

Costs of labour are difficult to interpret. Opportunitycosts of labour may differ from farm to farm. If the labour is external, then the cost of labour for the time that has been used to prevent mastitis is quite easy to calculate (hours x hourly wage). If the labour comes from the farmer's free time, the Opportunity costs are zero. However, if because of mastitis the farmer spends less time on other management tasks, the opportunity costs are the decrease in income due to skipping these tasks. (Halasa,.*et al* 2007).

1.10.4 Diagnostics:

Diagnostics costs that are relevant to mastitis must be included in the calculations, for instance costs of technicians and bacterial cultures. (Halasa,.*et al* 2007).

1.10.5 Veterinary services:

Besides delivering drugs (in many countries), the veterinarian might have to spend time on diagnosis of a (clinical) mastitis case. Veterinary services may be mandatory for each (clinical) mastitis case, if required by national legislation, or is only provided upon request by the farmer (Halasa,.*et al* 2007).

1.11 Situation of Bovine Mastitis in Sudan:

In the Sudan the disease has become one of the major problems in recent years, given the fact that many herd owners shifted to increase milk productivity by selecting local or foreign breeds.The most common major pathogens include *Staphylococcus aureus, Streptococcusagalactiae, Coliforms, Streptococci* and *Enterococci*, while *Corynebacteriumbovis*were considered to be minor pathogens (Sohiela, 2002**)**.

From the annual report of veterinary services of the Northern State, Sudan, mastitis was the second disease after pneumonia(13,15%).causing loss in milk production in the dairy cows and this ratio may be considered only for clinical mastitis, the other types (subclinical and chronic) were not investigated before and the economic impact of the disease was not estimated. (Albagir ,. *et al*.2015).

The first survey on bovine mastitis ever made in Sudan was by Wakeem and Eltayeb (1962) who found that 96% of the examined cows had chronic mastitis, the predominant organisms being *Staphylococci* followed by *Streptococci* and they attributed this to lack of periodic examination .Bagadi(1970) investigated the etiology of mastitis in seven herds of cattle in three provinces in Sudan both clinically and bacteriologically . He found that *Staphylococcus aureus* was the most common causative agent representing 92.2% of the isolates from clinical cases and 44.2% of the isolates from subclinical cases .Adlan*et al* (1980) isolated *Streptococcus agalactiae* , *Bacillus cereus* and *Staphylococcus epidermidis* from bovine mastitic milk. Costa *et al* (1998) isolated

*Corynbacterium*bovis from clinical and sub clinical cases of bovine mastitis. Elsayed (2000) isolated *Staphylococcus aureus* from normal and mastitic milk.

Sohiela (2002) isolated from CMT positive samples and clinical mastitis samples in Kafory and Azaheer dairy farms. Gram-positive bacteria represented (72.5%) of the isolates while Gram negative-bacteria accounted for 27.5%of the isolates. From the isolated Gram-positive bacteria 32% were *streptococci* and 2.7% *enterococci.* 87.5% of the isolated streptococci were from cases of sub clinical mastitis while 12.5% were from clinical cases. The incidence of *Streptococcus spp*. in sub clinical mastitis was high compared with clinical mastitis.

In the river Nile State, the isolated microorganisms from infected quarters were *staphylococcus*, *Streptococcus*, Coliform, *Micrococcus* and fungi, Cows were investigated to confirm the effect of mastitis on milk yield. The average of total and daily milk yield had been adopted. The result showed irregularity in average of total and daily milk yield for the mastitic cows compared to healthy ones (Isam, 2007).

Nuol*et al* (2009) in Khartoum north (Hillat Kuku dairy farms), which is considered to be the largest milk producing and marketing area in Khartoum State and regarded as semi-intensive system (small holder) of milk production, isolated*Corynebacterium striatum* 9 (33.3%), *Arcanobacteriumpyogene*4 (14.8%)*Corynebacteriumpseudotuberculosis*2(7.4%), *Corynebacteriumulcerans*5 (18.5%), *Corynebacteriumbovis*7 (25.9%). The result showed that age, stage of lactation, teat lesion could be risk factors for presence of bovine mastitis.

A Study conducted at Khartoum State (Eltebna, Falasteen, Shambat, Hilat Kuku, Elhalfaia, Elsamrab and University of Khartoum farms) by Reem and Basit(2012) showed that mastitic cows were found in all investigated farms. The percentages of acute mastitis caused by *Staphaureus*and *Staph hyicus*amounted to 24% and the percentage of chronic mastitis caused by *Staph aureus*was 44% and that caused by *Staph hyicus*was 8%.

Chapter two
2. Materials and Methods

2.1 The study area:

Khartoum State, the capital of the Sudan, is located in the semi-desert zone between latitudes 15.08 and 16.39 North, and longitudes 31.36 and 34.25 East. It's divided into three major localities (Khartoum, Khartoum North and Omdurman).

The topography is flat, except for some scattered mountains. It is hot and dry with rains in summer, and cool and dry in winter, the annual rainfall range from 75 to 160mm, falling mainly in July and august. Generally the dry period extends for 8-10 months. The daily average minimum temperature in winter is 5ºC. The evaporation is 7.7mm/day but during April it reaches 9.3mm/day .The daily average relative humidity is 38% at (8AM) and 21%at (12 noon).The wind speed is generally about 14.48km/hour. The population of Khartoum State is grown rapidly in recent years and is estimated as more than 7million people, including 2 million refugees from neighboring countries such as Chad and Ethiopia. The resident live stock in Khartoum is about 249083 head of cattle, (Ministry of Animal Resources and Fishers –information center2015).The system of animal breeding or animal production in this area is generally semi-intensive depending on natural range in the vicinity of the village and the town outskirts as well as individual houses. However ,different intensive system have recently been introduced in animal nutrition, improvement of breeds and its productivity, Moreover ,there are various breeds of these animals such as Kennana ,Butana , cross breeds and Frisian for cattle ,Nubian , desert, cross Saaneen and other foreign breeds for goats .Similarly there are different types of sheep, camels and poultry. Furthermore, milk production is the essentialactivity in Khartoum and the usefulness of these animal products and requirements for the public in this state are increasing continuously parallel with the unique progressive increase of human population due to the importance as fundamental food or in food security. Cattle believed to be the most essential source of this product producing about 94%of the total milk produced in Khartoum State followed by goats, which produce 6% of total milk for marketing.

2.2 Target populations:

One locality (sharagAlneel) in Khartoum State, farms were individually selected by simple random sampling.

2.3 Study type:

A cross sectional study design in which all the study animals were seen visually for clinical mastitis by physical examination of the udder and then tested for subclinical mastitis by California Mastitis Test (CMT). Information regarding the potential risk factors was collected by questionnaire survey and by the observation of the investigators. In this study, multistage random sampling was carried out in East Nile locality.

2.4 Sampling method:

Probability sampling method was used to select animals, The study involved a multistage random sampling technique to select study farms associations, farms households and animals, randomly 4 Administration units included (Kuku farms, AlSheglla farms, Alsellet farms, East soba farms). Associations of farms were selected randomly and finally appropriate herds were selected from each farm followed by sampling lactating cows from each randomly selected herd.

2.5 Sample size determination:

A total of 100 samples were collected from 99 lactating Cows healthy or mastitic from small holder dairy herds from four unites found in East Nile locality from February to April 2017.

2.6 Questionnaire execution:

A semi structured questionnaire was prepared and filled to evaluate the effect of potential risk factors on the occurrence of mastitis. All the dairy cows in the farms which were selected were examined and the questionnaire was filled out by asking the owner.

- Risk factors considered were divided into:

1- Individual risk factors including:

locality, age, breed, body condition, stage of lactation, parity, previous exposure of mastitis, teat injury, present of tick in udder, quarter type, milk yielding, herd size.

2- Housing and management risk factors including:

Sanitary practice, drainage system, washing hand before milking, source of water, dung removing, cow restrain for milking, milking technique, type of fencing , educational level.

2.7 Diagnostic techniques:

2.7.1 Physical examination of the udder:

The udder was first examined visually and then through palpation to detect pain reaction upon palpation, possible fibrosis, cardinal signs of inflammation, visible injury, tick infestation, atrophy of the tissue and swelling of the supramammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous healthhistory of the mammary quarters and cause of blindness was obtained from case record sheets when available. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions (Elbably,.*et al* 2013**)**.

2.7.2 Milk sample collection:

Before the collection of quarter milk samples from the tested cow, the udder was thoroughly cleaned with soap and water, rubbed dried and the teats disinfected with cotton wool moistened with 70% ethyl alcohol, which was been allowed to be air dried, then 5-10 ml of milk was collected in a sterile universal bottle .All milk samples were cooled and transferred to the laboratory in an ice box. either for identification of the clinical mastitis bacteria pathogen or to determine the pH parameter of milk.(IDRISS,. *et al* 2014).

2.7.3 California mastitis test (CMT):

CMT was used to detect subclinical mastitis. Two (ml) of milk from milk of each udder quarter was milked in a plate that had four separated cup (buddle). Three (ml) CMT liquid was added to each cup and mixed gently by rotating the plate. The reaction was then visually scored depending upon the amount of gel formation. Theresults were classified into four scores: 0 = negativeor traces (no change in consistency), 1 = slightlypositive (+), 2 = positive (++) and 3 = highly positive (+++). Scores 1, 2 and 3 depend on the degreeofgelatin that were indicated by gelatinous mass. **(**Endale ,. *et al* 2016**).**

2.8 Bacteriological Examination of Milk Samples:

2.8.1 Culturing methods:

One loopfull of milkwas streaked on 5% sheep blood agar to detectbacteria that could grow on this medium. The plates were incubatedaerobically at 37°C for 24 - 48 h. The plates were examinedfor growth, morphologic features of the colonies and hemolytic characteristic.Presumptive identification of bacteria on pure culturewas done on the basis of colony morphology, heamolytic characteristics,and Gram-stain and biochemical tests such as: (Nuol ,. *et al* 2009).

2.8.2 Catalase Test:

A drop of 3% aqueous solutionofhydrogen peroxide (H2O2) was

Placedon a clean microscope glass slide. A small amount of the organism undertest was mixed with H_2O_2. Production of gas bubbles indicated the releaseof$O_2$by catalase enzyme from the organism under test which was taken as positive result(Sohiela. 2002).

2.8.3 Oxidase Test:

Two to three drops of 1% tetramethyl-p-phenylenediamine dihydrochloride were placed on a filter paper. The test organisms which were grown on nutrient agar plates, were removed with a sterile glass rod and smeared across the impregnated paper. The development of dark purple color within ten seconds indicated a positive reaction(Sohiela.2002).

2.8.4 Sugars Test:

(A)Glucose Test

Glucose medium was inoculated with 24 hours growth in peptone water medium then incubated at 37ºC. The change of color to pink indicated a positive reaction; gas was accumulated in the Durham's tubes when produced. Cultures were examined for 7 days.

(B)Oxidation Fermentation Test (OF):

Duplicate tubes of Hugh and Leifson's medium were inoculated by stabbing with a sterile straight wire. A layer of melted soft paraffin oil of 1Cm depth was added to one of the tube. The tubes were incubated at 37 ºC and examined daily for up to 14 days. A change of color in the open tube only indicated oxidation while change of color on both open and sealed tubes indicated fermentation.

2.9 Secondary Tests:

2.9.1 Analytical Profile Index Test (API 20E):

1- Enterobacteriacea

2-Non fastidious

3- Gram negative rod

Principle:

20 strips consist of 20 micro tubes containing dehydrated substrates.

Procedure:

These tests are inoculated with a bacterial suspension and incubated for (24h) metabolism produces color change that is either spontaneous or revealed by the addition of reagents. The reactions read according to the reading table and the identification is obtained by referring to the analytical profile index.

-Reagents:

Indol, Voges-Proskauer(VP1, VP2).

2.9.2 Urease Test:

A slope of Christensen's urea medium was heavily inoculates and examined after incubation for 4 hours and daily for 5 days .Red colour indicated positive reaction.

2.9.3 Nitrate Test:

Lightlyinoculated nitrate broth was incubated for up to 5 days. Gas formation in the inverted inner tube was noted. Add 1 ml of nitrate reagent A followed by 1 ml of reagent B. A deep red colour which shows the presence of nitrate and thus shows that nitrate has been reduced indicates a positive reaction. If tubes not showing a red colour within 5 minutes and powdered zinc up to 5mg/ml of culture and allow to sand. Red colour= nitrate present in the medium, absence of red colour = nitrate absent in the medium.

2.9.4 Mannitol, Lactose, Sucrose, and Fructose Test (Acid from Carbohydrates):

Each sugar medium was inoculated and examined daily for 7 days for acid or acid and gas production, reversion to alkalinity was noted. Negative test were examined at regular intervals for up to 30 days. Suspected results were obtained the tests should be repeated at lower temperature.

2.10 pH Test:

As described by the manufacturer, pH reading was recorded by pH meter for checking sample. The pH meter level more than 6.8 indicated the incidence of sub clinical mastitis. The pH of normal cow's milk lies in the range from 6.6 to 6.9 which indicates that it is slightly acidic, the acidity of cow's milk is due to the presence of phosphates, proteins and to some extent CO_2 and citrates.

-**Advantages**: cost effective and rapid.

- **Disadvantages**: not as sensitive as other tests.

2.11 Data management and statistical analysis:

The Risk factors data collected during the study periods were entered into MS excel and analyzed using SSPS version (16) the statistical analysis used included comparison of proportions and chi square tests which appliedstatistically significant association existed between predisposing risk factors with mastitis positivity.For all the analysis performed $p<0.05$ was taken as statistically significant**.**

Chapter Three

3- Results

California Mastitis Test (CMT) performed on milk samples of 99 cross breed dairy cows results were as following: 6(100%) in EastSoba farms were negative, 7(24.1%) in AlSheglla farms were positive, 6(28.5%) in Alsellet farms were positive, 5(11.6%) in Kuku farms were positive and total of Negative California mastitis test in all these farms were 81 (81.8%) Table (1)

Table (1): distribution of sub clinical mastitis in 99 cattle are the positive examined by California mastitis test (CMT) in East Nile locality:

Locality	No. of Samples	Negative	Sub clinical (positive to CMT)	Frequency
East Soba	6	6	-	100%
Alsheglla	29	22	7	24.1%
Alseleet	21	15	6	28.5%
Kuku	43	38	5	11.6%
Total	99	81	18	18%

There was association between the somerisk factors and mastitis which were sanitary practice (χ^2 2.6, PV= 0, 10),body condition (χ^2 2.4, PV = 0.10),quarter type (χ^2 3.8, PV= 0.05) which were less than or equal to 0.10 under Confidence Interval (CI 90%). under CI 90% quarter type (Pendulous significant 0.05 more than non pendulous). Table (2):

Table (2): Frequency distribution of 99 dairy cows and univariate chi-square analysis of Mastitis Risk factors examined in East NileLocality:

Variable	Number Tested	Positive (%)	χ^2	Df	P-value
Locality			4	3	0.26
Soba	6	6(100)			
Alsheglla	29	29(100)			
Alseleet	21	21(100)			
Kuku	43	40(93)			
Age(month)			2.5	2	0.29
13-18	55	52(94.5)			
7-12	42	42(100)			
1-6	2	2 (100)			
Stage of lactation			0.2	1	0.69
Late	55	53(96.4)			
Early	44	43(97.7)			
Parity			0.8	2	0.66
Many	30	29(96.7)			
Moderate	49	47(95.9)			
Few	20	20(100)			
Washing hand before milking			–	–	–
Yes	0				
No	99	96(97)			
Sanitary practice			2.6	1	0.10
Bad	54	51(94.4)			
good	45	45(100)			
Breed			–	–	–
Cross	99	96(97)			
Body condition			2.4	1	0.10
Fair	43	43(100)			
Good	56	53(94.6)			
Previous exposure to mastitis			0.7	1	0.39
+ve	19	19(100)			
-ve	80	77(96.2)			
Teat injury			0.1		0.76
Present	3	3 (100)		1	
Absent	96	93(96.9)			
Presence of tick in udder			0.01	1	0.91
Yes	36	61(96.8)			

No	63	35(97.2)			
Quarter type Pendulous Non pendulous	 21 78	 19(90.5) 77(98.7)	3.8	1	0.05
Milk yielding High Moderate Low	 39 54 6	 39(100) 51(94.4) 6 (100)	2.6	2	0.28
Herd size Small Large	 71 28	 69(97.2) 27(96.4)	0.04	1	0.84
Draining system Present Absent	 50 49	 98 95	0.4	1	0.55
Source of water Pipeline Wells	 50 49	 98 95	0.4	1	0.55
Dung removing Yes No	 99 0	 97 0	–	–	–
Cow restraining for milking Yes No	 0 99	 0 97	–	–	–
Milking technique **Finger** **Striping**	 99 0	 97 0	–	–	–
Type of fencing Bricks Iron	 34 65	 94.1 98.5	1.4	1	0.23
Locality Soba Alsheglla Alseleet Kuku	 6 29 21 43	 6(100) 29(100) 21(100) 40(93)	4	3	0.26
Age(month) 13-18 7-12 1-6	 55 42 2	 52(94.5) 42(100) 2 (100)	2.5	2	0.29
Stage of lactation Late Early	 55 44	 53(96.4) 43(97.7)	0.2	1	0.69
Parity Many Moderate Few	 30 49 20	 29(96.7) 47(95.9) 20(100)	0.8	2	0.66
Washing hand before milking Yes No	 0 99	 96(97)	–	–	–
Sanitary practice Bad good	 54 45	 51(94.4) 45(100)	2.6	1	0.10
Breed **Cross**	 99	 96(97)	–	–	–

Body condition			2.4	1	0.10
Fair	43	43(100)			
Good	56	53(94.6)			
Previous exposure to mastitis			0.7	1	0.39
+ve	19	19(100)			
-ve	80	77(96.2)			
Teat injury			0.1		0.76
Present	3	3 (100)		1	
Absent	96	93(96.9)			
Presence of tick in udder			0.01		0.91
Yes	36	61(96.8)		1	
No	63	35(97.2)			
Quarter type			3.8	1	0.05
Pendulous	21	19(90.5)			
Non pendulous	78	77(98.7)			
Milk yielding			2.6	2	0.28
High	39	39(100)			
Moderate	54	51(94.4)			
Low	6	6 (100)			
Herd size			0.04	1	0.84
Small	71	69(97.2)			
Large	28	27(96.4)			
Draining system			0.4	1	0.55
Present	50	98			
Absent	49	95			
Source of water			0.4	1	0.55
Pipeline	50	98			
Wells	49	95			
Dung removing			–	–	–
Yes	99	97			
No	0	0			
Cow restraining for milking			–	–	–
Yes	0	0			
No	99	97			
Milking technique			–	–	–
Finger	99	97			
Striping	0	0			
Type of fencing			1.4	1	0.23
Bricks	34	94.1			
Iron	65	98.5			

In this study monitoring the pH of milk samples of 99 cows revealed that there was association between pH of milk and subclinical mastitis, all samples which were positive to CMT showed alkaline pH of more than 6.8 .Samples with alkaline pH were as follows: AlSheglla farms 7(24%), Alseleet farms 6(28%) and Kuku farms 5(11%). Table (3):

Table (3): pH of milk samples collected from 99 cows in East Nile Locality:

Locality	No. of Samples examined	Normal PH	Alkaline PH
East Soba	6	6	-
Alsheglla	29	22	7 (24%)
Alseleet	21	15	6 (28%)
Kuku	43	38	5 (11%)
Total	99	81	18 (18%)

The pathogens isolated from 99 milk samples collected from the four units were 123 isolates out of which 98 isolates were gram positive identified by using primary tests (gram stain,catalase, oxidase, sugar fermentation tests) and secondary tests (urease, nitrate, manitol, lactose, sucrose, fructose), they were related to two genera ***Staphylococcus***and ***Micrococcus*** species were: ***S.epidermidis*** 6(22.2%) in East Soba farms, 16 (59.3%) in Al Sheglla, 5(18.5%) in Kuku. ***S.sciuri*** 9(21.4%) in Al Sheglla, 15(35.7%) in Alseleet, 18 (42.9%) in Kuku farms.***Micrococcus spp*** 4 (14.8%) in Al Sheglla, 6(22.2%) in Alseleet, 17 (63.0%) in Kuku farms. The three bacterial isolates were negative to gram stain, one ofthem identified by using analytical profile index test (API 20E) as ***Pseudomonas oryzihabitans*** in Kuku area while the other two were not identified. Table (4):

Table (4): Bacteria Species isolated from 99 milk samples:

		Locality				Total
		East Soba	Alsheglla	Alseleet	Kuku	
S.epidermidis	Count	6	16	0	5	27
	% within Bacteria_Spp	22.2%	59.3%	0.0%	18.5%	100.0%
S.sciuri	Count	0	9	15	18	42
	% within Bactria_Spp	0.0%	21.4%	35.7%	42.9%	100.0%
micrococcus.spp	Count	0	4	6	17	27
	% within Bacteria_Spp	0.0%	14.8%	22.2%	63.0%	100.0%
Total	Count	6	29	21	40	96
	% within Bacteria_Spp	6.2%	30.2%	21.9%	41.7%	100.0%

Chi-square

	Value	Df	P-value
Pearson Chi-Square	42.077[a]	6	.000

Locality	Frequency	Percent	Cumulative Percent
East Soba	8	6.7	6.7
Alsheglla	38	31.7	38.3
Alseleet	26	21.7	60.0
Kuku	48	40.0	100.0
Total	120	100.0	

Staphylococcal species isolated within localities were: 8(100%) in East Soba farms, 25(86.2%) in Al Sheglla farms, 15(71.4%) in Alseleet , 23(57.5%) in Kuku . ***Micrococcus*** species were as follows: 4(13.8%) in Al Sheglla, 6(28.6%) in Alseleet , 17(42.5) in Kuku farms . this result revealed that the higher percentage of ***Staphylococcus*** species were reported from Al Sheglla than others localities. while the higher percentage of ***Micrococcus*** species were reported from Kuku farms. Table (4) and (5) showing a significant association between bacteria type and localities (P-value=.o16).

Table (5): Type Bacteria isolated from 99 milk samples:

			Locality				Total
			East Soba	**Alsheglla**	**Alseleet**	**Kuku**	
bacteria	Staphylocous	Count	8	25	15	23	71
		% within bacteria	11.3%	35.2%	21.1%	32.4%	100.0 %
		% within Locality	100.0%	86.2%	71.4%	57.5%	72.4%
	Micrococcus	Count	0	4	6	17	27
		% within bacteria	0.0%	14.8%	22.2%	63.0%	100.0 %
		% within Locality	0.0%	13.8%	28.6%	42.5%	27.6%
Total		Count	8	29	21	40	98
		% within bacteria	8.2%	29.6%	21.4%	40.8%	100.0 %
		% within Locality	100.0%	100.0%	100.0%	100.0 %	100.0 %

Chi-Square

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.282[a]	3	.016

	Frequency	Percent	Cumulative Percent
Soba	8	6.7	6.7
Alsheglla	38	31.7	38.3
Alseleet	26	21.7	60.0
Kuku	48	40.0	100.0
Total	120	100.0	

Chapter Four

Discussion

Mastitis is the inflammation of the mammary gland and udder tissue, a major endemic disease of dairy cattle; it usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical or thermal injury to the cow's udder. Mastitis, as one of the most costly disease in the dairy industry, is the result of the interactions between combinations of microbiological factors, host responses in the udder, and management practices (Eglė ,. *et al* 2016).

This study has given a due attention to isolate and identify the bacteria associated with bovine mastitis and investigate the risk factors associated with bovine mastitis.

Twenty one risk factors were entered into SPSS using cross tabulation and chi-square to estimate significant statistical association between risk factors and mastitis. In this study investigations were applied through combination of California mastitis test (CMT) and udder inspection.

This study showed that subclinical mastitis in the all four selected units were higher than that of clinical mastitis; this could be due to the reason that in Khartoum State sub clinical mastitis receives little attention and efforts have been concentrated only on the treatment of clinical cases (Kundu, 2013).

In the current study body condition score showed a significant statistical association with mastitis (p-value =0.10) this result is in agreement with the findings of previous works conducted in Tanzania by Kivaria ,. *et al* (2006) and by Uddin ,.*et al* (2009) in Mymunsingh, Bangaladesh . However it is well suggested that poor body condition is usually associated with debilitating disease which may produce high somatic cell count which is detected as intra mammary infection and have negative effect on milk quality and milk production (Kivaria ,.*et al* 2004).

In this study association of quarters type was studied, pendulous one had the highest rate of clinical and sub clinical mastitis (p- value =0.05) compared with non pendulous, this result is in agreement with the findings of previous work conducted in Khartoum State by Kundu ,.*et al* (2013) due to the fact that quarters are more exposed to dirt when the cow lies down on floor as well as they are more contaminated with fecal material.

In this study sanitary practice showed a significant statistical association (p-value =0.10) with mastitis, this result is in agreement with survey conducted on milk hygiene in Kuku area at the farm level by Nuol*et al* (2009), the survey proved that Kuku is the most bad area in this concern, this due to poor management of farms because owners didn't know the basics of farm production management and also they have not consulted professionals to help them on managing their farms.

CMT and pH have been used for early diagnosis of disease. In the current study, quarters wise of sub clinical mastitis among different farms of the locality showed that 18(18%) of animals showed a pH more than 6.8(alkalinity) this result is in agreement with the findings of the study conducted in Pakistan by Muhammad, .*et al* (2011), The alkaline pH was due to increase in somatic cell count in milk and activity of sodium and chloride ions, thus represent mastitis.

Bacteriological examinations of the milk samples were made to isolate and identify the main etiological agents involved in the disease process. The organisms were identified on the bases of their culture, staining characteristics and biochemical reactions. The results revealed that ***Staphylococcus*** species 71(72.4%) were the predominant gram positive pathogens in the four units than ***Micrococcus***species 27(27.6%) this result is supported by previous study conducted in Ethiopia by Tesfaye (2016).this may be due to the fact that bacterial species may originate from udder infection or poor hygienic husbandry practice like poor personal hygiene because bacteria transmit from infected to uninfected quarter by the contaminated hands of the milkers (Biruke and Shimeles ,2015).

The current study showed that there's a significant association between type of bacteria and locality (p-value =.016) this is in agreement with previous study conducted in Southern Ethiopia by Biffa,.*et al* (2005) this might be due to different management practices that were applied in farms in different localities. In addition, during this study most of surveyed farms were small with poor drainage system.

The most common organisms isolated in this study were coagulase negative Staphylococcus species in the study herds, regardless of inflammatory reaction in the udder quarter and they were *S. epidemidis* and *S .sciuri.* The result showed that *S. sciuri* 42(100.0%) is a common species followed by ***S.****epidermidis* 27(100.0%), this result is in agreement with a Canadian study by Davidson,.*et al* (1992).

The only gram negative bacteria isolated in this study was *Pseudomonas oryzihabitans,*it was isolated from some quarters but in a lower proportion compared with those mentioned above .This findings is generally in disagreement with astudy reported by.Hussein (2008).

The natural habitat of Pseudomonas spp is water and cooling ponds, wet bedding. New infection can occur at any time during lactation thus they are classified as environmental mammary gland pathogen (Turner,.et al 2016).

Conclusion

Mastitis in East Nile locality is common among herds; this indicates that mastitis is serious problem across herds in this area. The main risk factors associated with the mastitis were sanitary practices and body condition. Mastitis infections were largely caused by environmental ***Staphylococcus***and ***Micrococcus*** species , these micro organisms are associated with poor hygiene and contamination of udder and milking equipment and sanitary practices.

Recommendations

- To reduce the prevalence of the disease by using different epidemiological methods.
- Factors that interplay in mastitis occurrence should be well studied.
- A practical mastitis control strategy in the herd and national approach is needed.
- Using California Mastitis Test (CMT) in all farms for early monitoring ofthe disease.
- Adequate housing with proper sanitation and regular screening for early detection and treatment, follow up of chronic cases, culling of older cows with repeated attacks are recommended to control the disease.
- Divide herd into groups according to udder status in order to establish a milking order.
- Apply correct treatment of mastitis based on bacteriological culturing by consulting a veterinarian.
- Do not keep high parity cows with a poor udder health.

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Appendix

Questionnaire

1- Locality:

		Frequency	Percent	Cumulative Percent
Valid				

2- Age:

		Frequency	Percent	Cumulative Percent
Valid	1-6 years			
	7-12 years			
	13-18 years			
	Total			

3- Breed:

		Frequency	Percent	CumulativePercent
Valid	kenana			
	botana			
	cross			
	Total			

4- Body Condition:

		Frequency	Percent	Cumulative Percent
Valid	Good			
	poor			
	fair			
	Total			

5- Stage of lactation:

		Frequency	Percent	Cumulative Percent
Valid	Late			
	early			
	Total			

6- Parity:

		Frequency	Percent	Cumulative Percent
Valid	Few 1-2			
	Moderate 3-5			
	Many >5			
	total			

7- Previous exposure to mastitis:

		Frequency	Percent	Cumulative Percent
Valid	Yes			
	No			
	total			

8- Teat injury:

		Frequency	Percent	Cumulative Percent
Valid	present			
	absent			
	total			

9- Present of tick in udder:

		Frequency	Percent	Cumulative Percent
Valid	yes			
	No			
	total			

10- Quarter type:

		Frequency	Percent	Cumulative Percent
Valid	Non pendulous			
	Pendulous			
	total			

11- Milk yielding:

		Frequency	Percent	Cumulative Percent
Valid	High			
	Low			
	total			

12- Herd size:

		Frequency	Percent	Cumulative Percent
Valid	Small			
	Large			
	total			

13- Sanitary Practice:

		Frequency	Percent	Cumulative Percent
Valid	good			
	bad			
	total			

14- Drainage System:

		Frequency	Percent	Cumulative Percent
Valid	present			
	Not present			
	total			

15- Washing hand before milking:

		Frequency	Percent	Cumulative Percent
Valid	Yes			
	No			
	total			

16- Source of water:

		Frequency	Percent	Cumulative Percent
Valid	Pipeline			
	Wells			
	total			

17- Dung removing:

		Frequency	Percent	Cumulative Percent
Valid	yes			
	No			
	total			

18- Cow restrain for milking:

		Frequency	Percent	Cumulative Percent
Valid	yes			
	No			
	total			

19- Milking technique:

		Frequency	Percent	Cumulative Percent
Valid	striping			
	5 fingers			
	total			

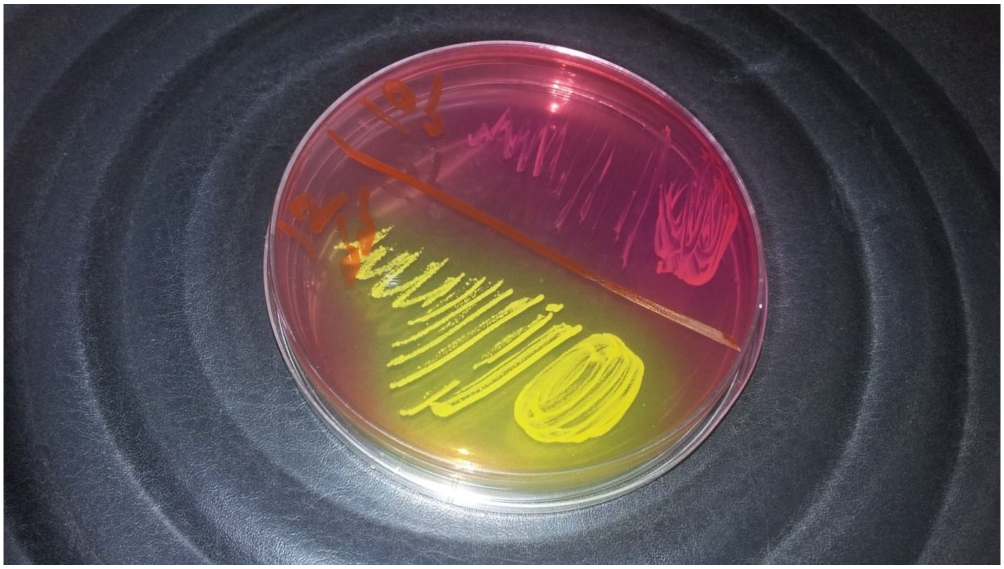
20- Type of Fencing:

		Frequency	Percent	Cumulative Percent
Valid	walls			
	bricks			
	iron			
	total			

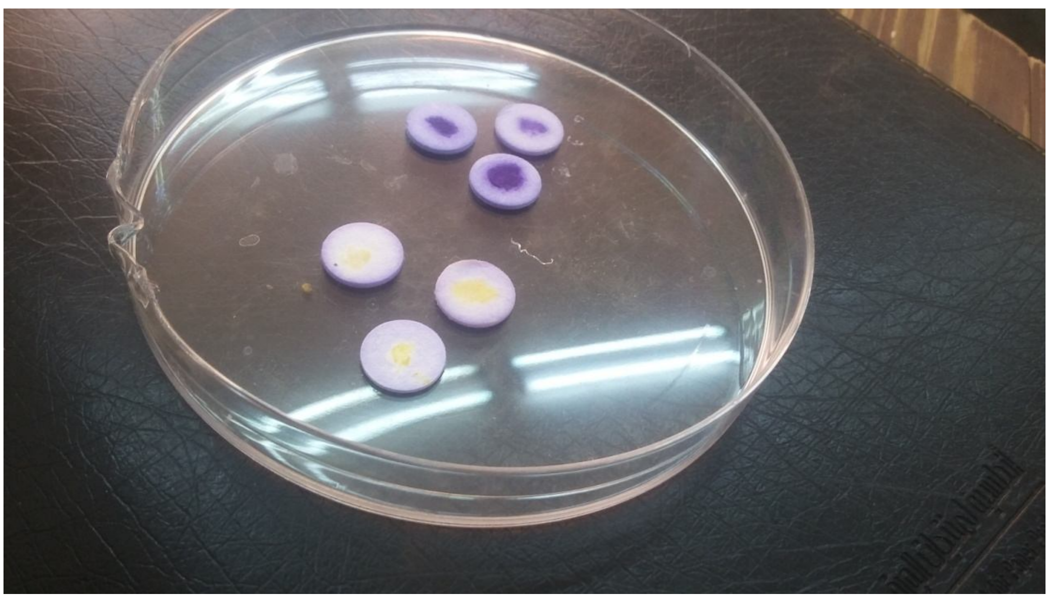
21- Educational Level:

		Frequency	Percent	Cumulative Percent
Valid	educated			
	illiterate			
	total			

Appendix (2)

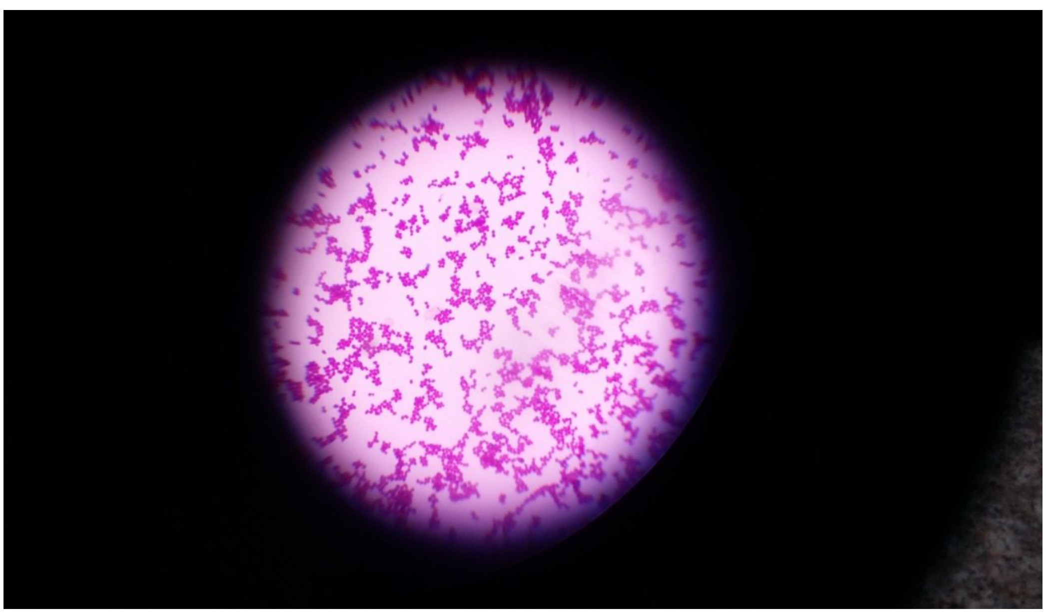


Staphylococcus spp **in manitol salt agar**

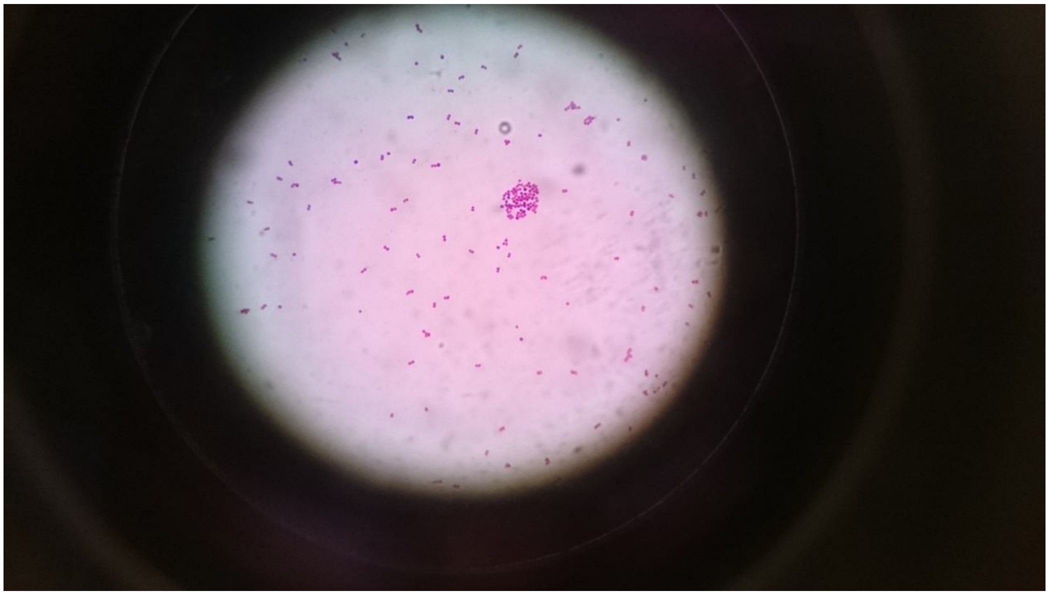


Oxidase test

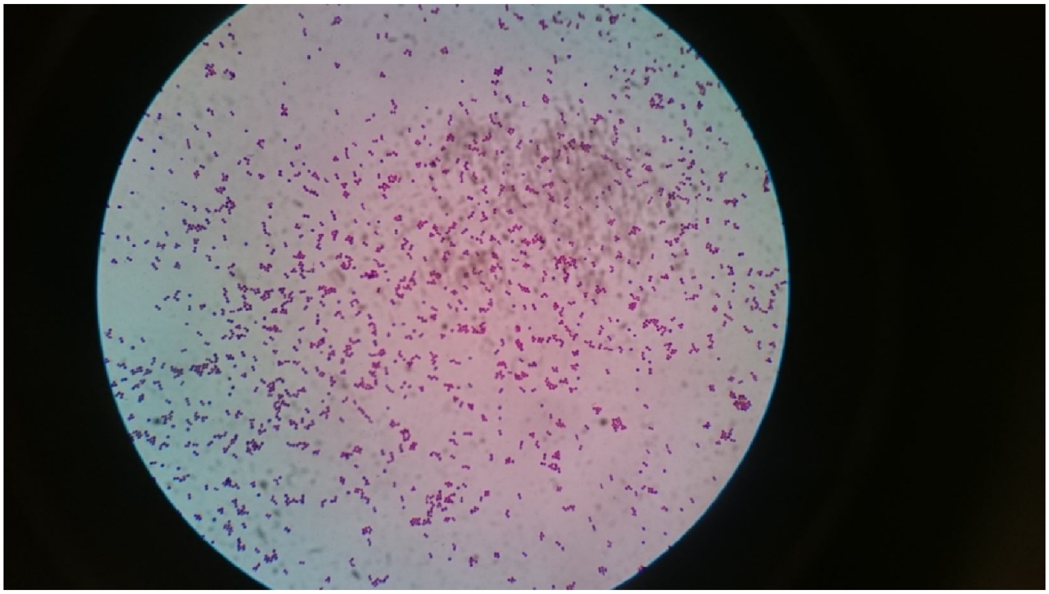
Sugars Fermentation test



Micrococcus species



Staphylococcal species



Staphylococcal species