بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Veterinary Medicine

Bacteriological Study on Mastitis in Dairy Cattle -Eldamazine Locality – Blue Nile State, Sudan

دراسة الإلتهاب البكتيري لضرع الأبقار الحلوبة بمحلية الدمازين – ولاية النيل الأزرق،السودان

BY

Mysoon Ali Babiker Idrees

B.V.M. (2003)

University of Khartoum

A Thesis Submitted in Fulfillment of the Requirement of the Graduate College for Degree of Master in Veterinary Medicine (Microbiology)

Supervisor

Prof. Siham Elias Suliman Mohammed

August 2021

DEDICATION

I dedicate this work to my dear parents,

brothers, sisters and to my husband,

To all who helped and supported me.

ACKNOWLEDGEMENTS

Firstly, thanks Allah for giving me the strength to finish this work, then I would like to express my thanks to my supervisor professor, Siham Elias Suleiman, College of Veterinary Medicine, Sudan University of Science and Technology for advice and supervision throughout the process of research during practical work and thesis, Professor Mohammed Abd Elsalam AbdAlla, College of Veterinary Medicine, Sudan University of Science and Technology for encourage and continuous support. I would like to thank Eldamazine Veterinary Research Laboratory staff for facilities, Special thanks to Dr. Ahmed Haroun, who facilitated all difficulties during the laboratory. Thanks to senior technician Najwa Abd Allah Mohammed in Microbiology Laboratory- Sudan University of Science and Technology for her help. My appreciation is extended to all who helped me in this study.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Abstract

This study was conducted to examine the clinical mastitis, to isolate and identify the bacteria causing the disease and to determine the sensitivity pattern of isolated bacteria to commonly used antibiotic in Eldamazine locality - Blue Nile State, Sudan from June to November 2020. A total of 45 milk samples were collected from dairy cows clinically infected with mastitis and transported to Microbiology Laboratory in Eldamazine town for bacteriological examination. All samples were cultured in blood agar, MacConkey agar and purified in Nutrient agar. Gram stain was used for identification of morphological characteristics of bacteria. Antibiotics sensitivity test to gentamycin, ampicillin, vancomycin, ciprofloxacin, oflaxacin, tetracycline and erythromycin. Biochemical tests were done to all isolates. Three types of inflammation were detected: acute mastitis with high prevalence (62.2%) followed by chronic mastitis (35.6%) and gangrenous mastitis (2.2%). The isolates were: 73.4 % *Staphylococcus* spp, 4.4% *Streptococcus* spp, 8.9% *Bacillus* spp , 4.4% *Pseudomonas* spp and 8.9% *Escherichia* spp. The results revealed that the most sensitive antibiotics on isolated bacteria were gentamycin, vancomycin, ciprofloxacin, oflaxacin and tetracycline, while resistance was ampicillin and erythromycin. *Pseudomonas* spp was resistant to all antibiotics used. In conclusion mastitis is associated with huge economic loss in the study area.

المستخلص

أجريت هذه الدراسة لإختبار إلتهاب الضرع السريرى، لعزل ومعرفة البكتيريا المسببة للمرض وإختبار حساسية البكتيريا التي تم عزلها للمضادات الحيوية الشائعة التى تستخدم في محلية الدمازين _ ولاية النيل الأزرق، السودان في الفترة من يونيو حتي نوفمبر ٢٠٢٠. العدد الكلى للعينات ٤٥ عينة حليب جمعت من أبقار مصابة سريريا بإلتهاب الضرع وتم نقلها إلي معمل الأحياء الدقيقة بمدينة الدمازين لإجراء الفحص البكتيري. كل العينات تمت زراعتها في أغار الدم ، أغار المكونكي وتمت تنقيتها بزراعتها في الأغار المغذي . إستخدمت صبغة جرام لمعرفة أشكال البكتيريا . إختبار الحساسية للمضادات الحيوية علي جنتامايسين ، أمبسلين ، فانكومايسين ، سبروفلوكساسين ، أوفلاكساسين ، تتراسايكلين و إريثرومايسين. أجريت الإختبارات الكيميائية الحيوية لجميع المعزولات. رصدت ثلاثة أنواع من الإلتهاب: إلتهاب الضرع الحاد بنسبة حدوث عالية (٦٢.٢%) يليه إلتهاب الضرع المزمن (٣٥.٦%) و إلتهاب الضرع الغرغريني (٢.٢%) . المعزولات كانت: ٧٣.٤% أنواع العنقودية ، ٤.٤% أنواع العقدية، ٨.٩ % أنواع العصيات ٤.٤، % أنواع الزائفة و٨.٩% أنواع الإشريكية. أظهرت النتائج أن المضادات الحيوية الأكثر حساسية علي الباكتيريا التي تم عزلها هي جنتامايسين، فانكومايسين ، سبروفلوكساسين ، أوفلاكساسين وتتراسايكلين، بينما كانت مقاومة للأمبسلين والإريثرومايسين .أنواع الزائفة كانت مقاومة لكل المضادات الحيوية التي إستخدمت. ختاما إلتهاب الضرع مرتبط بخسائر إقتصادية كبيرة فى منطقة الدراسة.

Introduction

Mastitis is a global problem as it adversely affects animal health, quality of milk and the economics of milk production, affecting every country, including developed ones and causes huge financial losses (Biffa *et al*., 2005; Suzan *et al*., 2016). Mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labor, milk withheld following treatment, death, and premature culling (Kaneene and Hurd, 1990; Miller *et al*., 1993). Due to the heavy financial implications involved and inevitable existence of latent infection, it is obvious that mastitis is an important factor limiting dairy production. Additional economic incentives to control mastitis include consumers' acceptance and product shelf- life factors. Although all of these factors result in considerable economic losses, decreased milk production is the single most important economic consideration and this requires the development of methodologies of control program under prevailing husbandry system (Gera and Guha, 2011).

Mastitis is often the end result of the interaction of several factors such as man , cow , environment , microorganisms and management (Blood *et al*., 1989; Berhanu, 1997; Awale *et al*., 2012). Mastitis is a difficult problem to comprehend because, it is a disease caused by many factors, both in large and in small herds. Micro-organisms are responsible for the infection, but for them to enter the mammary gland and establish themselves to the point that they cause an infection, a multitude of factors may be involved. Mastitis maybe infectious caused by microbial organisms or non infectious resulting from physical injury to the gland (Campus, 2007). The infectious etiology is the most important and caused by one or more types of pathogens ,

such as bacteria , virus , Mycoplasma , yeast and algae (Welleberg *et al*., 2002; Malinosuki *et al*., 2006; Chanetonl *et al*., 2008 ; Osumi *et al*., 2008).

Mastitis is the most common disease of dairy cows and the most common reason that cows are treated with antibiotics (Saini *et al*., 2012). The most important changes in the milk are discoloration and presence of clot (Blood *et al*., 1983). So for the continuous complain of the owners in the study area about bovine mastitis, more efforts will be prevent .

Objectives

1- To identify the types of clinical mastitis among dairy cows in study area.
2- To isolate and identify the species of bacteria that causes clinical mastitis.
3- To determine the sensitivity pattern of isolated bacteria to commonly used antibiotic in study area.

CHAPTER ONE

LITERATURE REVIEW

1.1. Mastitis in general

Mastitis means breast inflammation. Mastitis is defined as inflammation of the mammary gland, infectious or non-infectious etiology (Bradley, 2002). It is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues of the udder that affects the quality and quantity of milk (Radostits *et al*., 2000; Sharma *et al*., 2012). It is also defined as inflammation of mammary gland parenchyma which is caused by microorganisms, usually bacteria, that invade the udder, multiply and produce toxins, which are harmful to the mammary gland (Sharma *et al*., 2006; Osman *et al*., 2009).

Inflammation of the affected mammary tissue is characterized by gross abnormalities in the udder (swelling, heat, redness, pain). Persisting inflammation leads to tissue damage and replacement of the secretory tissues with non-productive connective tissues. There are changes in composition and appearance of milk. Abnormalities in milk may include flakes, clots or a watery appearance (Hillerton, 1999).

Mastitis must have been one of the first observed diseases of farm animals when cattle were domesticated over 5000 years ago. It is one of the costly diseases in dairy animals and causing severe losses to the dairy industry. The losses due to mastitis are not only economic but issues like animal health and welfare, quality of milk, antibiotic usage and the image of the dairy sector are also important reasons to focus on mastitis control

programme. Mastitis causes a great deal of loss or reduction of productivity to influence the quality and quantity of milk and to culling of animals at annual acceptable age (Singh and Singh, 1994).

1.2. Etiology

Mastitis may be caused by wide variety of microorganisms including bacteria, fungi, yeast and mycoplasma. However, bacteria are the most frequent pathogens of these diseases (Lim *et al*., 2007). The causative bacteria can be classed as major or minor pathogens (Harmon, 1994).

Herd mastitis can be caused by both environmental and contagious pathogens (Bodman and Rice, 2003). In addition to origin-based classification of mastitis -causing agents, they can be divided into major and minor pathogens according to their prevalence and the severity of symptoms (Heikkilä *et al*., 2018 and Saidani *et al*., 2018). Yeasts are also responsible for causing mastitis. Overuse of antibiotics and poor sanitation contribute to yeast mastitis (Ganguly, 2018). Mastitis can be caused by physical injury such as cuts or bruises or by chemical agents or infectious agent but in most cases it is caused by several bacterial pathogens (Shawgi, 2003).

1.2.1. Causative agents

Various infectious agents numbering more than twenty different groups including bacteria, viruses, yeast, fungi and rickettsia, being the major cause. One hundred and thirty seven infectious causes of bovine mastitis are known to date; 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 *Actinomyce spyogenes*, *Pseudomonas aeruginosa, Nocardia*

asteroi , *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), Streptococcus agalactiae (St. agalactiae), Corynebacterium bovis, Mycoplasma* species, which are wide spread from the infected quarters, primarily during milking (man hands, milking machines), and those causing environmental mastitis such as *Streptococcus uberis, Streptococcus dysgalactiae, Streptococcus bovis*, *Klebsiella oxytoca*, *Klebsiella pneumonia, Enterobacter aerogenes*, *Serratia species, Escherichia coli* (*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).

B. abortus was 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 supra mammary 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).

1.2.2. The important agents of bovine mastitis

According to Sharif *et al*. (2009) the most contagious pathogens causing intramammary inflammation are *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus uberis.*

1.2.2.1. *Staphylococcus* spp

Staphylococci are gram positive cocci, catalase positive and ferment glucose. They are classified according to the coagulation of human or rabbit plasma coagulase positive *Staphylococci* (CPS), represented by *Staphylococcus aureus*, and coagulase negative *Staphylococci* (CNS) *such* as *Staphylococcus epidermidis* (Baird- Parker, 1962). *Staphylococci* were found to be the most frequent causative agents of mastitis among cattle (Cargil and Bootas, 1970; Kapur and Singh, 1978). Coagulase negative *Staphylococci* were identified as primary caustive agent of cattle mastitis during first lactation (Derieze and Keyser, 1980; Timms and Schultz, 1987). 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).

1.2.2.1.1. *Staphylococcus aureus*

It is known to cause per acute, sub-acute and chronic mastitis in addition to gangrenous mastitis (Radostitis *et al*., 1994). Khan and Khan (2006) stated that infections caused by *S. aureus* remained the largest mastitis problem in dairy cattle because the cure rate using antibiotics is very low during lactation, and, in many cases, the infection become chronic, making culling of the affected animal frequently necessary. Mastitis caused by this pathogen is only successfully controlled through preventing new infections and the

culling of affected animals. Similar to other contagious pathogens, it spreads via milking machine components, the hands of milking personnel, and through wash cloths (Petersson *et al*., 2010).

1.2.2.2. *Streptococus* spp

Streptococci are the second most common pathogens isolated from cows' milk (Sharma and Pasker, 1970; Ahmed *et al*., 1991). They are classified according to precipitation reaction of specific carbohydrate antigens into 12 groups (Merchant and Packer, 1967).

1.2.2.2.1. *Streptococcus agalactiae*

Streptococcus agalactiae represent the major *Streptococcus* species that cause mastitis in cattle (Costa *et al*., 1998). This organism causes mainly contagious subclinical mastitis, which is usually spread by milking leading to considerable losses of milk quality and yield. The prevalence of *Streptococcus agalactiae* demonstrates that this bacterium is a significant cause of mastitis, especially in herds that are not well managed and have poor hygiene (Tolla, 1996; Sharif *et al*., 2009; Kassa *et al*., 2014).

1.2.2.2.2 .*Streptococcus dysgalactiae*

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 risk for 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).

Streptococcus dysagalactiae infection with injured teat and improper milking hygiene promote the spread of the organisms within the herd. The presence of this pathogen in dairy herds is serious because the inflammation caused by this agent is usually acute (Rantamäki and Müller, 1995) .They can be controlled with proper sanitation and are moderately susceptible to antibiotics (Watts, 1988).

1.2.2.3. Coliforms

Bovine mortality survey carried out in 1992, identified Coliform mastitis as the single most important cause of death in dairy cows (Menzies *et al*., 1992).

One of the most important pathogens that cause environmental mastitis is *Escherichia coli*. It was defined as the most common Gram- negative bacilli associated with clinical and sub clinical mastitis (Elliot *et al*., 1976; Jha *et al*; 1994) and causes sudden sharp drop in production of milk (Mustafa *et al*., 1977). *Escherichia coli* were isolated from udders of cattle at calving and during dry period (Timms and Schultz, 1987), it usually attacks the mammary gland during early lactation, occasionally resulting in lethal consequences if left untreated (Burvenich *et al*., 2003). *Escherichia coli* may cause acute and per acute form of clinical mastitis (Radostitis *et al*., 1994). Hyper acute *E. coli* mastitis is considered the most common cause of fatal cases(Menzies *et al*.,1992) The clinical outcome of *E. coli* mastitis depends upon the severity of infection (Lehtolainen, 2004), energy balance

(Suriyasathaporn *et al*., 2000), stage of lactation and vaccination status (Burvenich *et al*., 2003).

When *E. coli* occurs in the mild form, cows show only local signs in the udder and milk, and the duration of symptoms is short. In other more acute cases, it can have very severe or even lethal consequences (Lehtolainen, 2004).

Klebsiella is the second most common Gram- negative *Bacillus* isolated from cattle milk infected with mastitis (Howell, 1972; McDonald *et al*., 1977). *Enterobacter spp* were found to cause bovine mastitis (Park, 1979; Haghour and Ibrahim, 1980). Coliform mastitis is common during the puerperal period and symptoms are often acute to per acute as a consequence of endotoxin production (Sandra, 2013).

1.2.2.4. *Pseudomonas* spp

1.2.2.4 .1. *Pseudomonas aeruginosa*

Environmental mastitis-causing pathogens that is Gram-negative and similar in structure to other coliform mastitis pathogens. *Pseudomonas spp*. has been isolated from milking parlor drop hoses and is known to cause mastitis through the use of water during milking. When grown on blood agar, *Pseudomonas aeruginosa* has been found to smell like grapes. *Pseudomonas aeruginosa*. 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).

1.3. Classification of mastitis

1.3.1. According to mode of transmission of pathogen

1.3.1.1. Contagious mastitis

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 cow udder, milkers hands and clothes, or milking machines. Use of milking gloves and individual towels will help to prevent this.

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 one to three days. Among the contagious organisms, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* have been identified as the major causes of bovine mastitis. Contagious mastitis can be further classified into three major groups based on the symptoms associated with infection; clinical, sub-clinical and chronic mastitis (Basdew and Laing, 2011).

1.3.1.2. Environmental mastitis

Environmental bacteria, as the name implies, come from the 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*, *Klebsiella spp K. Pneumonia* , and *Enterobacter* , whose main origin is

manure and soil, and the environmental *Streptococcus uberis* and *Streptococcus agalactiae* 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).

1.3.2. According to the clinical symptoms

Radostitis *et al*. (1994) classified mastitis into two forms, clinical and subclinical mastitis. Clinical mastitis is characterized by apparent changes of both milk and mammary gland and sub clinical mastitis in which there are no apparent changes.

1.3.2.1. Clinical mastitis

Clinical mastitis refers to inflammation of mammary gland with grossly visible changes on the udder and milk. It is characterized by abnormalities such as discoloration of milk, redness, increased temperature, pain, and disturbance of function of the udder (Bishi, 1998). The detection of clinical mastitis depends upon the examination of the mammary gland and its secretion. The affected gland may show swelling, heat, pain and hardness. The secretion may be clotted, serous or occasionally blood stained (Andrews *et al*., 2004)

1.3.2.1.1. per acute mastitis

It is characterized by gross inflammation, reduction milk yield and changes in milk composition .This form of mastitis is fairy uncommon and includes depression, raised pulse and respiratory rates, loss of muscle coordination, cold extremities, reduced papillary reflex, dehydration and diarrhea (Philpot and Nickerson, 2000.

1.3.2.1.2. Acute mastitis

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

1.3.2.1.3. Sub-acute mastitis

It is characterized by only minor alteration in the milk and affected quarter such as clots, flakes or discolored secretions. The quarter may also be slightly swollen and tender (Philpot and Nickerson, 2000).

1.3.2.1.4. Chronic mastitis

The chronic form may begin as any clinical form or as sub- clinical mastitis and may be evidenced by intermittent signs of clinical mastitis. There is usually a progressive development of scar tissue and a change in size and shape of the affected gland, accompanied by reduced milk yield (Philpot and Nickerson, 2000).

1.3.2.2. Sub clinical mastitis

Refers to inflammation of mammary gland in the absence of visible changes in the udder but presence of pathogenic organisms in the milk and can only be diagnosed with indirect screening tests or laboratory culturing (Suttie, 2003).

Sub clinical mastitis is the multimedia logical complex disease which consists of infectious and noninfectious agent as potential risk factors. It cannot be detected by visual observation though it can be identified by conducting tests to detect the presence of infecting microorganism or the product of inflammation such as somatic cell count (Philpot and Nickerson, 2000).

1.4. Signs of mastitis

Michel (2000) described the signs of mastitis according to the mastitis form. In clinical mastitis, the infected quarter often becomes swollen, sometimes painful to touch and the milk is visibly altered by the presence of clots, flakes or discolored serum and sometimes blood. In severe cases (acute mastitis), the cow shows signs of generalized reaction, fever, rapid pulse, loss of appetite and sharp decline in milk production. In contrast, subclinical mastitis is subtle and more difficult to detect. The cow appears healthy, the udder does not show any signs of inflammation and the milk seems normal. However, microorganisms and white blood cells (somatic cells) that fight infections are found in elevated numbers in the milk. Schroeder (1997) characterized subclinical mastitis by lack of consistent, visible and elevation of somatic cells count of the milk. Bacteriological culturing of milk will detect bacteria in milk and this form causes the greatest loss in dairy farms through lowered milk production.

1.5. 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 (Khan and Khan, 2006). 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 one to two 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 (Khan and Khan, 2006). 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, casein and 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 and Khan, 2006).

1.6. Diagnosis of mastitis

Early diagnosis of mastitis is essential because changes in the udder tissue take place much earlier than they become apparent the California mastitis test can easily be detected by inspection of udder and or systemic sign of inflammation whereas diagnosis of subclinical mastitis is more problematic since the milk appears normal but usually has an elevated somatic cell count the California mastitis test applied for the detection of mastitis based on alteration of pH of milk (Kelly, 1984). Other type of test is white side test which is simple and rapid test to evaluation of nonspecific bacterial genital infection of repeat breeding cattle. But the diagnosis of clinical mastitis based on the appearance of abnormally appearing milk /milk may be off color /watery /bloody or have the appearance of serum. Abnormal milk may also contain varying amount of pus and clots, the amount of swelling

severity of pain and the overall appearance of the cow will indicate the severity of infection and serve as a guide for the course of treatment (Muhammed *et al*., 2011). Diagnosis of clinical mastitis can be achieved by visual examination of both milk and mammary gland where the abnormality could be detected easily (Blood *et al*., 1989) .Confirmation of diagnosis is usually done by the isolation of causative agent.

1.6.1. Physical examination

1.6.1.1. Visual examination

According to Kelly (1984), clinical mastitis may be detected by examination of the udder for warm, swollen quarters, which are indicative of acute mastitis. Misshapen, hard atrophied and fibrotic quarters indicating chronic mastitis. In gangrenous mastitis the gland reveals initially the presence of swelling and blue color of the udder.

1.6.1.2. Palpation of the udder

According to Kelly (1984), in acute mastitis palpation reveals increased local temperature, pain, abnormal texture and increased size of supra mammary lymph nodes. In chronic mastitis palpation reveals abnormal texture, no pain, normal local temperature and increase in size of supra mammary lymph nodes. In gangrenous mastitis palpation on reveals decrease of local temperature abnormal texture and increased size of supra mammary lymph nodes, in late stage of gangrenous mastitis then is desquamation of the udder from the body with smelling of offensive odour.

1.6.2. Chemical examination

1.6.2.1. California Mastitis test

The California Mastitis test (CMT) which is called Rapid Mastitis Test is commonly used for detection of mastitis and has proved to be highly efficient (Blood *et al*., 1983). Two ml of fore milk were squeezed from each quarter into the cup of the paddle where equal volume of California mastitis test reagent (Alvetera rapid mastitis test kit - Alvetera Gmbh-Germany) was added. The milk and reagent were mixed together and the reaction between them was interpreted (Schalm and Noorlander, 1957). It is direct test that grossly measures the amount of DNA, primarily function of the number of nucleated white blood cells in the milk (Quinn *et al*., 1994).

1.6.2.2. Modified white side test

The test is performed by adding one to two drops of Sodium hydroxide solution (0.4%) to five drops of cold milk on glass on black back ground and then stirring the mixture vigorously for 20 second. In positive reaction the milk was separate to water and shreds or flakes but in negative the mixture remains uniformly opaque (Kelly, 1984).

1.6.2.3. PH indication papers

The test strips detect the more alkaline pH in quarters with mastitis. Normal milk has a pH of approximately 6.5 to 6.7 where as mastitis milk often approaches plasma pH of 7.4 (William, 1995).

1.6.2.4. Somatic cells count

Somatic cells are defined as epithelial cells or neutrophils derived from the blood (Schalm and lasmanis, 1968). 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).

1.6.2.5. Culturing

Bacteriological culture of milk samples is required to determine the etiological agents involved (Anon, 1987).

1.7. Treatment of mastitis

Therapeutic success of mastitis depends mainly on accurate diagnosis, severity of udder pathology, drug selection, and relevance of route of administration, supportive treatment, and elimination of predisposing factors (Du Preez., 2000). A program for mastitis treatment starts with clinical cases

and teats in earliest stage. In sub clinical mastitis quarters are identified using survey or representative sampling during a routine check.

Treatment of mastitis should be based on bacteriological diagnosis and take national and international guidelines on prudent use of antimicrobials into account. In acute mastitis, where bacteriological diagnosis is not available, treatment should be initiated based on herd data and personal experience. Rapid bacteriological diagnosis would facilitate the proper selection of the antimicrobial. Treating subclinical mastitis with antimicrobials during lactation is seldom economical, because of high treatment costs and generally poor efficacy. The treatment of mastitis is mostly based on hit and trial, it makes condition beyond repairable. Major use of antibiotics in dairy cattle is towards the treatment and prevention of mastitis. Involvement of multiple etiological agents makes it necessary to perform antibiotic drug sensitivity prior to select the final line of treatment (Kumar *et al*., 2010).

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). Effective and appropriate treatment of mastitis used all over the season called lactation therapy or dry cow therapy at the end of the season is vital (Du Preez,1989) . The use of intramammary antibiotics at dry off is common in US dairy herds. Dry cow therapy (**DCT**) is typically administered as a treatment for existing subclinical mastitis infections and for prevention during the no lactating period (McEwen and Fedorka-Cray, 2002; Aarestrup, 2004).

1.7.1. Intramammary infusion

Most farmers treat clinical mastitis based on symptoms and without microbiological analysis, thus treatments are often given regardless of etiology (Hoe and Ruegg, 2006).

In the Sudan study conducted by Abdel Basit (2003) found that using of Neomastipra intramammary infusion in treatment of clinical mastitis was effective against Streptococcal, Staphylococcal and coliform mastitis. It was found to be ineffective against nocardial infection. Mastitis caused by *Nocardia spp is* prevalent (14.3%) and *Nocardia was* proven experimentally to be highly virulent and difficult to treat.

Study conducted by Khadiga (2008) revealed that treatment of mastitis with antibiotics penicillin and oxytetracycline intramammary for five consecutive days resulted in inhibition of the bacterial growth ten days post-treatment. This indicating the good response to these products. Some researchers have reported no difference in bacteriological cure rates for untreated cows compared with cows treated for mastitis caused by gram-negative pathogens, and the majority of antimicrobials labeled to treat mastitis have limited activity against these organisms (Pyörälä *et al*., 1994; Suojala *et al*., 2013).

1.7.2. Intramuscular treatment

Intramuscular treatment of dairy cows with systemic drugs, including Oxytetracycline and penicillin maintained minimal inhibitory concentrations during lactation and dry period. Thus systemic treatment of mastitis has been evaluated, intramuscular treatment is as effective as intramammary treatment in eliminated mastitis in dry cows.

A combination of intramuscular and intramammary treatment increased cure rates and maintained greater concentration of antibiotics in the mammary tissue (Lents *et al*., 2002).

1.8. Mastitis in the Sudan

In the Sudan mastitis 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. Mastitis was first reported in the Sudan in 1953(Annual Report of the Sudan veterinary service, 1953).Since that it was described as being common (Annual Report of the Sudan veterinary service, 1953-1955 and Annual Report of the Department of Animal production, 1956-1957).Later, prevalence of mastitis in dairy herds in the Sudan was investigated by Wakeem and ElTayeb (1962). The investigation was carried out to determine the incidence, prevalence rate of infection, the causative agents and the response to control efforts, which include treatment.

Bagadi, (1970) investigated both clinically and bacteriologically the etiology of bovine mastitis in seven herds of cattle in three areas in Sudan. He found that *Staphylococcus aureus* was the most common causative agent representing 92.2% of the isolated bacteria from clinical cases and44.2% from subclinical cases. Adlan *et al*. (1980) isolated *Streptococcus agalactiae*, *Bacillus cereus* and *Staphylococcus epidermidis* from bovine mastitic milk. *Staphylococcus aureus* was isolated from bovine clinical mastitis by Mamoun and Bakheit (1992). *Corynebacterium spp* was isolated by Jha *et al*. (1994) from clinical mastitis. Costa *et al*. (1998) isolated *Corynebacterium bovis* from clinical and subclinical cases of bovine mastitis. Ibrahim *et al*. (1997) isolated *Actinomyces pyogenes* (9.8%) from 173 mastitic milk. Elsayed (2000) isolated *Staphylococcus aureus* from mastitic milk of some domestic animal. AbdAlbasit (2003) found that mastitis caused by *Nocardia spp* prevalent (14.3%). *Nocardia* was proven experimentally to be highly Virulent and difficult to treat. 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. About 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. 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 *Staphylococcus aureus* and *Staphylococcus hyicus* amounted to 24% and the percentage of chronic mastitis caused by *Staphylococcus aureus* was 44% and that caused by *Staphylococcus hyicus* was 8%. Afaf (2012) isolated 48% *Staphylococcus aureus* ,8% *Staphylococcus hyicus* , 28% *Streptococcus agalactiae*, 4% *Streptococcus dysgalactiae* and 12% *proteus spp* from 50 mastitic milk sample in Hilat Kuku.

1.9. Economic impact of mastitis

1.9.1. Milk production losses and change in milk quality

1.9.2. 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).

1.9.3. 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. 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).

1.9.4. Labour

Costs of labour are difficult to interpret. Opportunity costs 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).

1.9.5. 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 and 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. Prevention and Control of mastitis

Prevention of mastitis primarily depend 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 disinfectants, pre-milking strip cup, post milking teat dipping and cow therapy (Radositis *et al*., 1996).

1.10.1 Nutrition

Deficiencies of selenium and vitamin E in the diet have been associated with an increased rate of new mammary infection. Proper nutrition will reduce the risk of environmental mastitis, adequate levels of vitamin E and selenium reduce the incidence of environmental mastitis (Awale *et al*., 2012).

1.10.2. 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 weeks before their anticipated calving date. This timing was

based on the Periods of greatest risk for 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 third dose at calving, whereas cows vaccinated with JVac® did not. Immunization by either of these vaccines did not affect the severity of clinical coliform mastitis (Rebecca, 2014)**.**

1.10.3 Control of contagious mastitis

Contagious mastitis can be effectively controlled through air grouse program of teat dipping and dry cow antibiotic treatment. Teat must be dipped in germicide after each milking (this decrease incidence of the disease).Each quarter must be treated with dry cow antibiotics at end of lactation (this decrease prevalence of the disease). Cows with contagious mastitis should be milked last or a separate milking claw used for the infected cows. Milking cows should be flushed with hot water or germicide after milking infected cows (Called back flushing). Individual cloth /paper towels should be used to wash /dry teats. Milked should have clean hands and wear latex gloves. New addition to the herd should be cultured and persistently infected cows should be culled. Teat lesions should be minimized (from chapping, bite, stepped on teats, lacerations, or machine damage). Heifers can be given dry cow antibiotic treatment during gestation if *Staphylococcus aureus* is a problem in the heifers (Awale *et al*., 2012).

1.10.4. Control of environmental mastitis

Environmental pathogens are more difficult to control than the contagious pathogens, many of these organisms are resistant to germicides in teat dip and antibiotics in dry cow therapy. Identification of the source and removal

(bedding, ponds, and mud) is the key to control. Udder can be clipped to minimize the amount of manure clinging to the glands, only clean dry teats should be milked. Teat should be pre-dipped with germicide before milking Cow should be kept standing after milking (offer them feed). Sterile single dose infusion products should be used and sterile infusion techniques (alcohol swab) should be used. The milking parlor should be kept clean. The teat dipper should be kept clean; organisms survive in many germicide. Pipelines /water heater may need to be replaced in cases of *Pseudomonas* contamination (Awale *et al*., 2012).

The national mastitis council developed a five point mastitis control program in 1969 to control the incidence rate of mastitis. This five point mastitis control program includes: Dipping teats in an antiseptic solution before and after milking, Proper cleaning and maintenance of milking equipment, Early detection and treatment of infected animals, Dry cow therapy with long acting antibiotics to reduce duration of existing infection and to prevent new intramammary infection and Culling chronically infected animals (Neave *et al*., 1969; Blowey, 2010).

CHAPTER TWO

MATERIAL and METHODS

2.1. Study area

This study was conducted in Eldamazine locality, Blue Nile State, Sudan.

2.2. Collection of milk samples

A total of 45 mastitic cross breed cows (two to four years) were examinated clinically. This was done during the period extending from June to November 2020. Forty five milk samples were taken (five ml) under aseptic condition as possible for bacteriological examination in sterile disposable bottles after cleaning the outer surface of the udders and teats with cotton wool soaked in 70% Alcohol, after stripped off the fore milk. All samples collected were immediately placed on ice in a thermo flask after collection and transported to the Microbiology Laboratory in Eldamazine town.

2.3. Sterilization

All steps of sterilization were done according to Barrow and Feltham (2003).

2.3.1. Autoclaving

Culture media, Solutions, Bijou and universal bottles were sterilized in the autoclave at 15 pound pressure for 15 minutes at 121°C.

2.3.2. Hot air oven Sterilization

Petri dishes, graduated pipettes, swabs, glass rods, flasks and test tubes were sterilized in hot air oven at 160°C for one hour and half.

2.3.3. Flame Sterilization

Sterilization by flame was used for sterilization of the metal wire and loops, which were used in the laboratory to transfer of bacterial colonies or spreading them on glass slides. Forceps were sterilized by flaming after dipping in spirit.

2.3.4. Disinfection

Laboratory benches were cleaned and disinfected by ethyl alcohol solution (96%). This step was done by cotton before, during and after each work in the laboratory. Ultraviolet irradiation (UV) was also used for 15 min to sterilize media pouring room and safety cabinet before and after use. Hands were washed with soap and alcohol.

2.4. Preparation of culture media

Preparation of media was used according to Oxoid (2006) as instructed by the manufacturer.

2.4.1. Nutrient agar

It is prepared by Suspending 28.0 grams from HIMEDIA (M001-500G) in 1000 ml purified/ distilled water. Heating of the mixture to dissolving the medium completely and sterilized by autoclaving at 121 °C for 15 minutes then cooled to 45 -50 °C in water bath before dispended into sterile Petri plates.

2.4.2. Blood agar

This medium was prepared using 40.0 grams from HIMEDIA (M073-500G) in 1000 ml purified/ distilled water. Dissolving of the medium completely by

heat and sterilized by autoclaving at 121 °C for 15 minutes then cooled to 45- 50 °C in water bath and aseptically added 5% de -fibrinated sheep blood, then mixed well and poured into sterile Petri plates

2.4.3. MacConkey's agar

The medium was prepared by suspending 51.53 grams from HIMEDIA (M001-500G) in 1000 ml purified/ distilled water. Boiling of the medium and sterilized by autoclaving at 121 °C for 15 minutes then cooled to 45 -50 °C in water bath before dispended into sterile Petri plates.

2.4.4. Peptone water

Fifty grams of peptone water powder (Oxoid, CM9- CM10) were added to one liter of distilled water, mixed well, distributed in three ml amount into clean test tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.4.5. Sugar test media

4.5grams of sugar dissolved with 45 ml of peptone water and phenol red o.5 ml in 100 ml distilled water and transferred to conical flasks, and dispensed into test tubes. Insertion of Durham tubes into all tubes. Steaming in 65 °C for 30 minutes for sterilization.

2.4.6. Motility media (semi-solid medium)

It was prepared by Suspending 20.0 grams from HIMEDIA (M 260- 500G) in 1000 ml purified/ distilled water. Heating and boiling to dissolving the medium completely. Dispense in tubes and sterilized by autoclaving at 15 Ibs pressure 121 °C for 15 minutes, allowed the tubes to cool in an upright position.

2.4.7. Urea Agar Base (Christensen)

The medium was prepared by suspending 24.51 grams from HIMEDIA (M112S-500G) in 950 ml distilled water. Boiling of the medium and sterilized by autoclaving at 15 Ibs pressure in 121 °C for 15 minutes then cooled to 50 °C and aseptically added 50 ml of sterile 40% Urea Solution(FD048) and mixed well . Dispended into sterile tubes and allowed to set in the slanting position.

2.4.8. Simmons Citrate Agar

The medium was prepared by suspending 24.28 grams from HIMEDIA (M099-500G) in 1000 ml distilled water. Boiling of the medium and dispended in tubes sterilized by autoclaving at 15 Ibs pressure in 121 °C for 15 minutes.

2.4.9 Hugh and Lifson's O-F Medium

Preparing by adding two grams of peptone, five grams of sodium chloride,1.5 grams potassium phosphate and three grams of agar to 1000 ml distilled water. Dissolving by heating and added 15 ml of bromthymol blue 0.2 % sterilized by autoclaving at 115 °C for 20 minutes. Added glucose solution to give final concentration of 1%, and dispensed in ten ml in test tubes (Cowan and Steel, 1974).

2.4.10 Mueller Hinton sensitivity testing agar

This medium was prepared using 38.0 grams from HIMEDIA (M173-500G) in 1000 ml distilled water. Heating and boiling to dissolve the medium completely. Sterilization by autoclaving at 15 Ibs pressure 121 °C for 15 minutes. Then mixed well and poured into sterile Petri dishes.

2.4.10.1 Preparation of turbidity standard

Preparing of 1% v/v solution of sulphuric acid by adding one ml of concentrated sulphuric acid to 99 ml of water. The ingredients were mixed well, the preparation of (1% w/v) solution of barium chloride by dissolving 0.5 g of dihydrate barium chloride in 50 ml of distilled water, added 0.6ml of the barium chloride solution to 99.4 ml of the sulphuric acid solution, and mixed and transferred small volume of the turbid solution to a capped tube of the same type as used for preparing the test Cheesbrough (2005).

2.5. Culturing and purification of culture

The bacteriological culture was performed following the standard microbiological technique (Quinn *et al*., 1994). A swab of each milk samples was streaked on the two media: blood agar and MacConkey's agar. After culturing of the samples then the plates were incubated for 24 to 48 hours at 37 °C, the plates were examined for growth, morphologic features of the colonies and hemolytic characteristic. Purification was achieved by culturing on nutrient agar and incubated at 37 °C for 24 hours.

Presumptive identification of the isolated bacteria was done on the basis of colony morphology, heamolytic characteristics.

2.6. Gram's stain technique

Films were made from purified cultures with a sterile loop, emulsified in a drop of normal saline on clean microscopic slides then dried and fixed by flame. The stages of the method were crystal violet (1 min), lugol's iodine (1 min), decolorized by alcohol 95% (20seconds), and then stained with dilute carbol fuchsin (15 sec). All slides were washed after each step by water. Examination of the slides under the microscope in oil immersion lens.

Positive organisms identified by blue coloration, negative organisms showed red coloration and also the shape of the bacteria was seen (Barrow and Feltham, 2003).

2.7. Biochemical tests

Biochemical reactions were done according to standard (Barrow and Feltham, 2003).

2.7.1. Motility test

Motility medium (semi-solid medium) inoculated with a straight wire, made single stab down the center of the tube to about half the depth of the medium. Then incubated at 37 °C for 24 hour. Motile organism grew migrating beyond the stab line (Turbid) while the growth of non-motile organisms was confined to the stab line.

2.7.2. Oxidase test

The fresh culture of the tested organism was smeared by oxidase strips and those were done in a filter paper impregnated with 1% Tetra methyl – p-phenylene diamine dihydrochloride (oxidase reagent), positive result was shown by the development of dark purple color within five to ten seconds.

2.7. 3. Oxidation Fermentation test (O.F)

Two tubes of O.F. medium (Hugh and lifson's media) were stabbed by the organism under test with straight loop. To one of the tubes, a layer of paraffin oil was added. The two tubes were incubated at 37°C for48 hrs, with inoculated tubes as a control. Development of yellowish color in the two inoculated tubes indicated fermentation, where as oxidation reaction was indicated by the development of yellow color in the open tube

Changes of the color of the medium in both tubes = fermentative= (F)

Changes of the color of the medium in the open tube = oxidative = (O)

No changes of the color of the medium in both tubes = negative = (-)

2.7.4. Catalase test

A drop of 3% hydrogen peroxide (H2O2) was placed on a clean microscopic slide. Sterile Pasteur pipette was used to transfer a portion of a bacterial colony to be tested from solid media to the drop of hydrogen peroxide and mixed together. Immediate production of gas bubbles was considered a positive test.

2.7. 5. Coagulase test

All colonies of the sample s were inoculated in peptone water. A drop of the bacteria from fresh culture was placed on a Tube Coagulase Test approximately one ml of coagulase reagent (human plasma) in a labeled test tube and incubated at 37 °C for 24 hours. The development of clumping indicates a positive test (clot formation).

2.7.6. Fermentation of sugars test

Carbohydrate media were inoculated with the tested organism. Changing of the color to pink was regarded as appositive result. The cultures were monitored for seven days before they were discarded.

2.7.7. Urease test

The slope of Christensen's medium agar was streaked with the isolate under test, incubated at 37 °C and examined after 24 hours and daily for seven

days. Urea hydrolysis was indicated by the changes of the medium from yellow to pink color.

2.7.8. Citrate utilization test

A single well isolated colony of the tested organism was streaked over the surface of the slope of Simmon's citrate medium, incubated at 30 °C and examined daily for seven days. The change of the medium color from green to blue indicates utilization of citrate (Positive test).

2.7.9. Indole test

Peptone water was inoculated with tested bacteria and inoculated at 35 °C for 24 hours. After incubation 0.2 - 0.3 ml Kovac´s reagent was added and the tube was well shaken and examined after one minute. Appositive reaction was indicated by the red color in the reagent layer and yellow color means negative.

2.7.10. Anti microbial sensitivity test

Using a sterile wire loop, three to five well isolated colonies of the same type from the culture plate were taken and emulsify in three to four ml of sterile physiological saline, the turbidity was checked by suspension to turbidity of the chemical standard. Using a sterile swab the inoculated plate of Mueller Hinton agar, removing of excess fluid by pressing and rotating the swab against the side of the tube, swabbing the surface of the sensitivity testing agar and ensure even distribution, using sterile forceps placing antimicrobial discs on the inoculated plate, pressed down to ensure its contact with the agar, within 30 minutes of applying the disk and incubated aerobically at 35°C over night and measuring the zone of inhibition in mm

Cheesbrough (2005). The sensitivity of isolates was examined by using the following antibiotics:

Ampicillin (AS) 20mcg, Oflaxacin (OF)5mcg, Gentamycin (GN)10 mcg, Vancomycin (VA) 30mcg , Tetracycline (TE) 30mcg, Ciprofloxacin (CIP) 5mcg and Erythromycin (E)15 mcg.

2.8. Statistical analysis

The data of sensitivity to antibiotics was analyzed using SPSS (Version 17.0) computer soft ware program. The method used was one way ANOVA and the statistical significance was set at P- value of ≤ 0.05.

CHAPTER THREE

RESULTS

3.1. Clinical status of mastitis

The result showed high incidence of acute mastitis (62.2%) followed by chronic mastitis (35.6%) and gangrenous mastitis (2.2%) as shown in Fig (1).

3.2. Identification of bacteria isolates

3.2.1. Reaction to Gram Stain

Microorganisms isolated in this study were 86.7% Gram positive bacteria, while Gram negative bacteria were 13.3 % as shown in Fig (2).

3.2.2. Identification of bacteria genera

The bacteria isolated from milk samples included Gram positive cocci and rods and Gram negative rods. The bacteria isolated were *Staphylococcus* spp (73.4%), *Bacillus* spp (8.9%), *Streptococcus* spp (4.4%), *Escherichia* spp (8.9%) and *Pseudomonas* spp (4.4 %) as shown in Fig (3).

3.3. Biochemical tests

Table one showed the biochemical tests done for the identification of *Staphylococcus* spp, table two showed biochemical tests were done for identification of *Streptococcus* spp and table three showed biochemical tests were done for identification of *Bacillus* spp. whereas table four showed biochemical tests were done for identification of *Pseudomonas spp* and table five showed biochemical tests were done for identification of *Escherichia* spp.

3.4. Isolated bacteria and type of inflammation and number of affected quarters

The different types of inflammation that affected different number of quarters, *Staphylococcus* spp was found to infected one or two quarter causing chronic mastitis, and acute mastitis, but sometimes gangrenous mastitis. *Streptococcus* spp was infected one or two quarter causing acute mastitis. *Pseudomonas spp* infected one quarter causing acute mastitis, but *Bacillus* spp was infected two or three quarters causing acute mastitis. While *Escherichia* spp involved the four quarters causing mainly acute mastitis.

3.5 Antibiotic sensitivity test

All of isolates were subjected to the antibiotic sensitivity test. The results showed high susceptibility to antibiotics ciprofloxacin, gentamycin, tetracycline then vancomycin, oflaxacin, the resistances were to ampicillin and erythromycin (Table 6).

Fig (1): Types of mastitis in mastitic cows (n=45) in Eldamazine locality-Blue Nile State

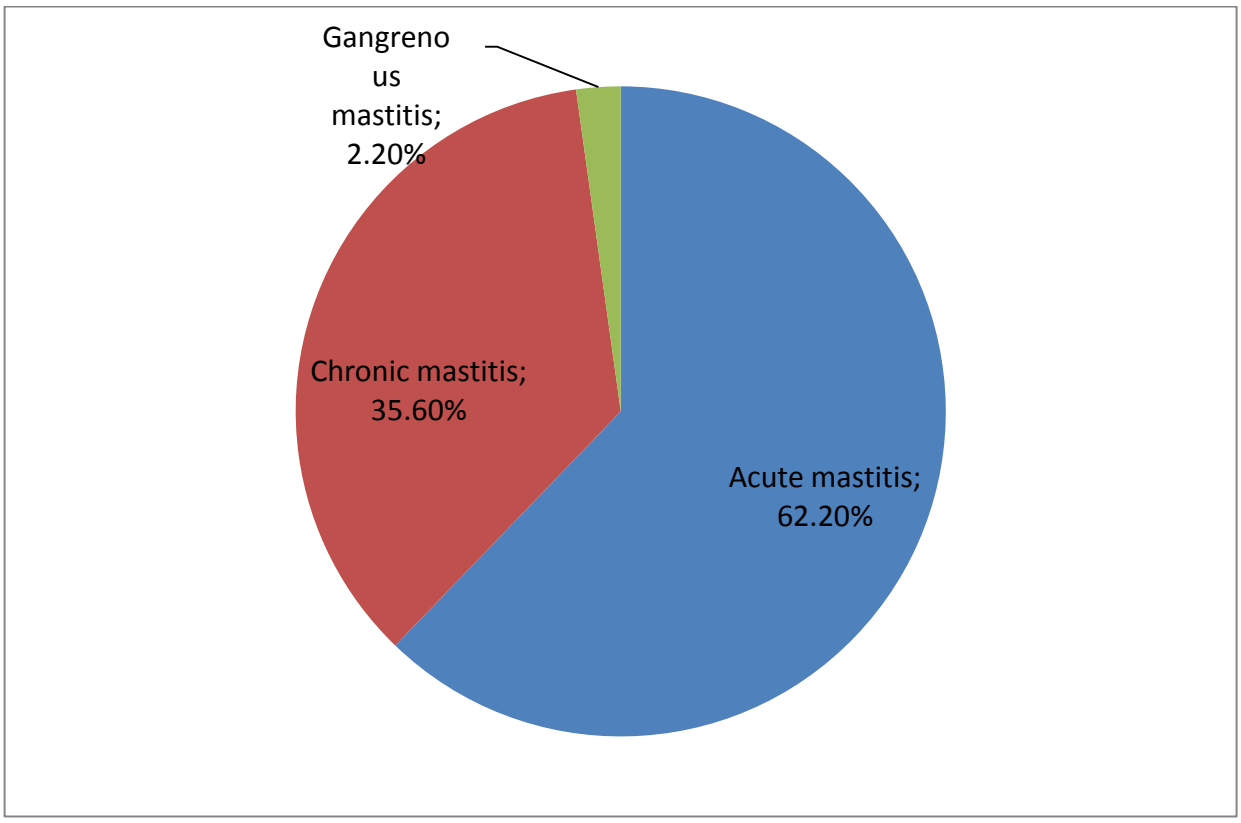

Fig (2): Number of the Gram positive and Gram negative bacteria isolated from milk samples of mastitic cows (n=45) in Eldamazine locality- Blue Nile State

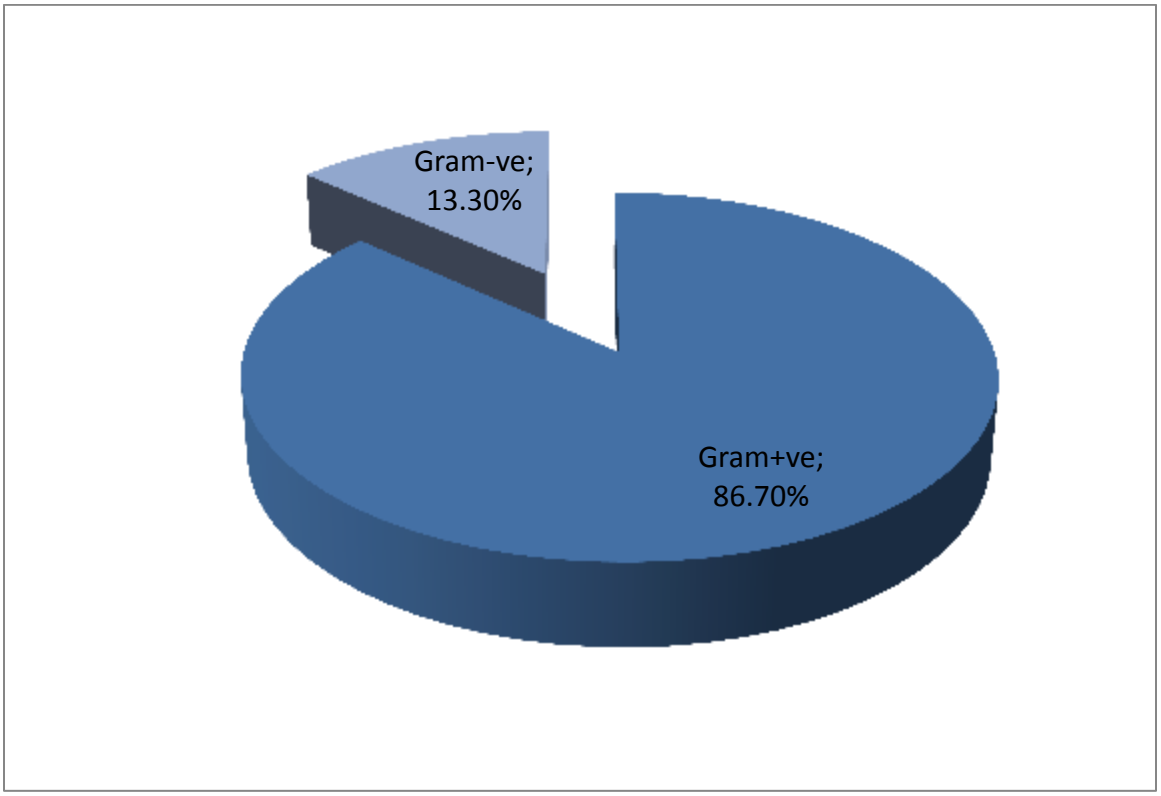

Fig (3): Types of Bacterial Genera isolated from milk samples of mastitic cows (n=45) in Eldamazine locality- Blue Nile State

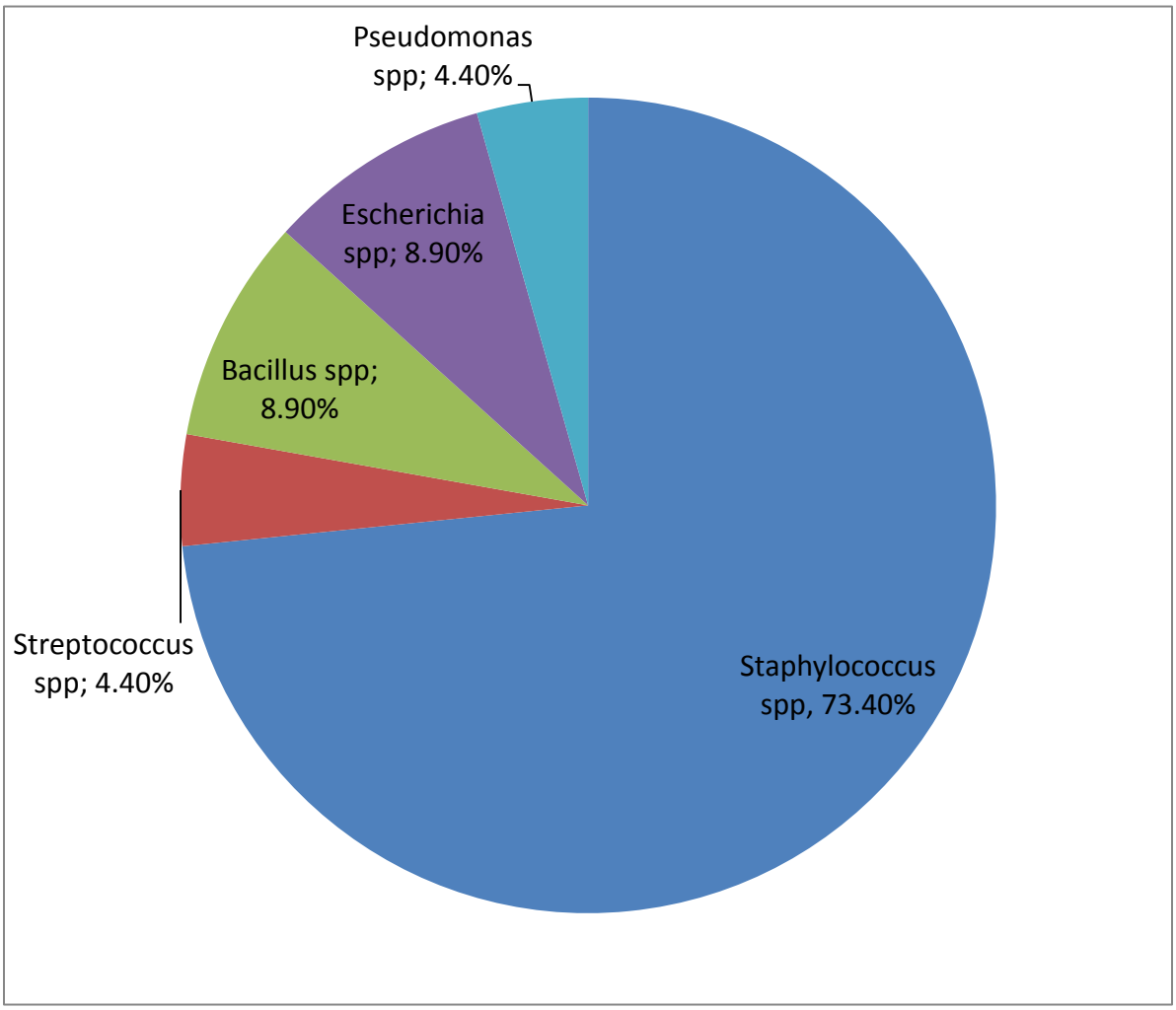

Table (1): Biochemical tests for identification of *Staphylococcus* spp from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Biochemical test	*Staphylococcus* spp		
	1	2	3
Gram stain	+	+	+
Cocci/ Bacilli	Cocci	Cocci	Cocci
Haemolysis in blood agar	+	-	-
Growth motility	-	-	-
Catalase	+	+	+
Co agulase	+	-	-
Oxidase	-	-	-
O.F	+ F	+ F	+ F
Glucose	+	+	+
Lactose	+	+	+
Sucrose	+	+	+
Maltose	+	+	-
Fructose	+	+	+
Trehalose	+	-	+
Xylose	-	-	-
Urease	+	+	+
Indole	-	-	-
Mannitol	+	-	-

Key:

F : Fermentative

1, 2, 3 : *Staphylococcus* spp (three species).

Table (2): Biochemical tests for identification of *Streptococcus* spp from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Biochemical test	*Streptococcus* spp
Gram stain	+
Cocci/ Bacilli	Cocci
Haemolysis in blood agar	+
Growth motility	-
Catalase	-
Co agulase	-
Oxidase	-
O.F	Fermentative
Glucose	+
Lactose	+
Sucrose	+
Maltose	+
Fructose	+
Trehalose	+
Xylose	-
Urease	-
Indole	-
Mannitol	-

Table (3): Biochemical tests for identification of *Bacillus* spp from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Biochemical test	*Bacillus* spp
Gram stain	+
Cocci/ Bacilli	Bacilli
Haemolysis in blood agar	+
Growth motility	+
Catalase	+
Co agulase	-
Oxidase	-
O.F	Fermentative
Glucose	+
Lactose	-
Sucrose	+
Maltose	+
Fructose	+
Trehalose	+
Xylose	-
Urease	+
Indole	-
Mannitol	-

Table (4): Biochemical tests for identification of *Pseudomonas* spp from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Biochemical test	*Pseudomonas* spp
Gram stain	-
Cocci / Bacilli	Bacilli
Growth in MacConkey agar	+
Motility	+
Oxidase	+
Catalase	+
Co agulase	-
O.F	+Oxidative
Glucose	+
Lactose	-
Sucrose	-
Fructose	+
Mannose	-
Maltose	-
Trehalose	+
Xylose	+
Manitol	+
Simmon’s citrate	+
Urease	-
Indole	-

Table (5): Biochemical tests for identification of *Escherichia* spp from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Biochemical test	*Escherichia* spp
Gram stain	-
Cocci / Bacilli	Bacilli
Growth in MacConkey agar	+
Motility	+
Oxidase	-
Catalase	+
Co agulase	-
O.F	+Fermentative
Glucose	-
Lactose	-
Sucrose	+
Fructose	-
Mannose	+
Maltose	+
Trehalose	+
Xylose	+
Manitol	+
Simmon's citrate	-
Urease	-
Indole	+

Table (6): Degree of antibiotics susceptibility on isolated bacteria from milk samples of mastitic cows (n=45) in Eldamazine locality-Blue Nile State

Isolated bacteria	AS	OF	GN	VA	TE	CIP	E
Staph spp (1)	5.73±3.77	16.62±2.21	18.62±1.50	15.04±2.63	13.81±2.17	18.85±1.35	7.00±2.67
Staph spp (2)	4.00±3.37	16.25±2.22	19.50±1.29	15.00±0.82	16.25±2.06	18.00±1.63	2.50±3.32
Staph spp (3)	4.00±3.61	15.33±1.53	19.33±1.15	13.67±1.53	16.33±2.08	18.67±2.08	8.67±2.52
Streptococcus spp	3.50±4.95	20.00±1.41	19.50±0.71	16.50±2.12	15.00±1.41	19.50±2.12	11.00±2.83
Pseudmonas spp	0.00±0.00	1.50±2.12	6.00±1.41	0.00±0.00	2.00±2.83	6.00±1.41	6.50±2.12
Escherichia Spp	7.00±2.16	7.25±2.63	17.75±2.22	3.25±3.95	14.00±3.16	21.00±1.83	11.75±2.50
Bacillus Spp	0.50±1.00	16.00±1.83	19.25±0.96	11.25±3.30	17.50±1.91	15.50±1.91	13.00±1.63
Sig	*	**	**	**	**	**	**

Sig = Significance * = p≤ 0.05 ** = p ≥ 0.01 ns = not significant

Key: AS : Ampicillin OF : Oflaxacin

GN : Gentamycin VA : Vancomycin

TE : Tetracycline CIP : Ciprofloxacin

E : Erythromycin

Staph spp (1), (2), (3): *Staphylococcus* spp (three species)

CHAPTER FOUR

Discussion

Mastitis among bovine is a common disease, several surveys conducted in Sudan showed that the prevalence of bovine mastitis is high (Mustafa *et al*., 1977; Ibrahim and Habib alla, 1978).

In this study *Staphylococcus* spp was high percentage (73.4%) might be attributed to the survival of the bacteria in the environment and high infectivity to the udder, this finding supports the previous finding of Radostitis *et al*. (1994) who mentioned that *Staphylococcus aureus* is the first microorganism increminted in bovine mastitis. Predominance of *Staphylococcus aureus* in mastitis in cows has been reported by Watts (1988). This result agreement with Bagadi (1970) and Adlan *et al*. (1980) who reported that *Staphylococcus aureus* was the most frequent cause of mastitis in British dairy cows. The same results obtained by Mamoun and Bakheit (1992) and Mwahib (2010) who reported that *Staphylococcus aureus* was the most frequently isolate udder pathogen, Reem (2008) revealed that the high incidence might be attributed to the increases in number of animals per farm and spread of strain resistance and agrees with Elsayed (2000) who isolated *Staphylococcus hyicus* as 8.85%. Also this result agrees with Mashaer (2017) who isolated *Staphylococcus epidermidis* (8%) from mastitic cows in Khartoum North.

Isolation of *Escherichia* spp (8.9%) in this study agreement with Radostitis (2000) who reported that in contrast to contagious mastitis, environmental mastitis caused by coliform bacteria *E.coli* is primarily associated with clinical mastitis.

Isolation of *Bacillus* spp (8.9%) from mastitic milk samples could be attributed to failure of sanitary programmes which help in the elimination of the causative agents, this supported by Quinn *et al.* (1994) who mentioned that *Bacillus* spp was isolated from mastitic milk of bovine and this is agreed with Nail *et al*. (2003) and Reem (2008) who isolated *Bacillus* spp from mastitic cows in Khartoum State. The species *Streptococcus* (4.4%) was isolated in this study is similar to Afaf (2012) who isolated 28% *Streptococcus agalactiae* and 4% *Streptococcus dysgalactiae,* Ibtisam and Elowni (2006) isolated *Streptococcus* spp as 1.08% from the air of studied farms suffering from bovine mastitis. Also, the isolation of *Pseudomonas* spp (4.4%) is similar to Madut *et al.* (2009) who isolated *Pseudomonas* spp from mastitic milk of bovine.

Most of isolates tested revealed high percentage of susceptibility to antibiotics, this finding is agreed with Ibtisam *etal*. (2006) who stated that in Sudan most of mastitis caused by bacteria, were highly susceptible to antimicrobial agents. In this study gentamycin, ciprofloxacin and tetracycline showed the best antimicrobial effects against the tested isolates. Tetracycline possesses antimicrobial activity by binding to the ribosomal subunit (30 S) of the susceptible organism that interfering with bacterial protein synthesis in growing or multiplying organisms (Gale and Folkes, 1953; Suzuka *et al.,* 1966). Because of this tetracycline group is inhibiting the growth of a wide variety of bacteria. Followed by vancomycin and oflaxacin, the tricyclic glycopeptide vancomycin is active against gram positive cocci, enterococci and aerobic gram negative bacteria, but N-alkyl vancomycin is five times more active than vancomycin (Nagarajan *et al.*, 1989). However, aminopenicillins generally active against some

Enterobacteriaceae and gram positive (Adams, 2001). Also, ofloxacin is bactericidal that inhibiting bacterial DNA replication and transcription (Ferrero *et al.*, 1995; Drlica and Zhao, 1997). This drug activity against most of gram-negative and gram-positive bacteria. All isolates resistant to erythromycin except *Bacillus* spp and all isolates resistant to ampicillin. *Escherichia* spp resistant to vancomycin and oflaxacin and *Pseudomonas spp* resistant to all antibiotics used including gentamicin in contrast to finding of Adams (2001) who stated that this organism is sensitive to the therapy.

CONCLUSION

Mastitis in Eldamazine locality is common among herds, this indicates that mastitis is serious problem across herds in this area. Continuous monitoring of mastitis, and its management, is essential for the well-being of a dairy herd, which can be achieved through the detection of mastitis in its early stages and treatment of the mastitis infection. The major findings of the present study could be concluded in: acute bovine mastitis is common with (62.2 %), chronic bovine mastitis (36.5 %) and gangrenous bovine mastitis (2.2 %) in Eldamazine locality, the isolated bacteria caused mastitis from mastitic cows were *Staphylococcus* spp (73.4 %), *Streptococcus* spp (4.4 %), *Bacillus* spp (8.9 %), *Pseudomonas* spp(4.4 %) *and Escherichia* spp (8.9%). *Staphylococcus* spp were the most frequent isolated bacteria. The effective antibiotics used were gentamycin, vancomycin, ciprofloxacin, oflaxacin and tetracycline and ineffective antibiotics used were erythromycin and ampicillin with high resistant.

RECOMMENDATIONS

1- Hygienic measures and practices procedure in dairy farm must be sustainable to prevent from mastitis.

2- Examination of the udder and milk sampling from dairy cows should be taken for routine examination.

3- The usage of antibiotics in dairy farms should be under supervision of

veterinarian to avoid misuses which leading to the development of antibiotic resistant bacteria.

4- Antimicrobial sensitivity testing should be practiced before treatment of mastitis with antibiotics.

5- More epidemiological studies on the occurrence of mastitis and its association with environmental factors are needed to adopt the suitable control measures.

References

Aarestrup, F.M. (2004). Aarestrup Monitoring of antimicrobial resistance among food animals: Principles and limitations *J. Vet. Med. B Infect. Dis. Vet. Public Health*, 51 (2004), pp. 380-388.

AbdelBasit, O. (2003). *Bovine mastitis etiology, clinical aspects and treatment with special reference to actinomycetes infection*. (Ph.D) Thesis, University of Khartoum., Khartoum: Sudan.

Adams, H. (2001). *Veterinary Pharmacology and therapeutics*, 8th,(ed). A Blackwell Publishing company, Lowa State. : USA.

Adlan, A. M., Shommein, A. M and Elmin, E. D. M. (1980). A survey of Bovine mastitis in four dairy farms in the Sudan. *Sudan, J. Vet. Res*., 2: pp.37-40.

Afaf (2012). *Bovine mastitis: the causative agents and the antimicrobial susceptibility* (M. V. Sc). Thesis, Sudan University of Science and Technology. :Khartoum

Ahmed, R.; Javiad, S. and Lateef, M. (1991). Studies on prevalence, aetiology and diagnosis of subclinical mastitis in dairy animals, *Pakistan, Vet. J.*, 11: 138-140.

Alvaro Garcia. (2004). Extension dairy specialist Contagious vs. environmental mastitis. College of Agriculture& Biological Science/ South Dakota State University / USDAEx 4028. *Dairy Science.*

Andrews, A. H., Blowey, R.W., Boyd, H and Eddy, R.G. (2004). *Bovine Medicine Disease and Husbandry of Cattle*, second edition, black well science.

Anon (1953). Annual Report of the Sudan veterinary service. Ministry of Animal Resources, Sudan.

Annual Report of the Sudan veterinary service, (1953-1955). Ministry of Animal Resources, Sudan.

Annual Report of the Department of Animal production, (1956-1957). Ministry of Animal Resources, Sudan.

Anon (1987). Bovine mastitis, *Definition and guidelines diagnosis Buletin of the international diary Federation* No.211.

Awale, Dudhatra, Kumar, A., Chauhan, Kamani, M . ; Patel ,M. (2012). Bovine mastitis: A Threat to economy. Open access scientific reports. http:// dx.doi.org /10.4172/ scientifisreports.295.

Bagadi, H.O. (1970). The etiology of bovine mastitis in three areas in the Sudan. *Trop. Anim. Hlth. Prod* 2:pp.28-34.

Baird-Parker, A.C. (1962). The occurrence and enumeration according to a new classification of Micrococci and Staphylococci in bacon and human, pig skin. *J. of Appl . Bact*., 25: 352-361.

Bartlett, P.C., Miller, GY, Lance, SE and heider , L. E (1992). Environmental and managerial determinants of somatic counts and clinical incidence in Ohio dairy herds.*Prev.Vet. Med* . 14(3-4): pp. 195- 207.

Barrow GI and Feltham RKA. (2003). *Cowan and Steel's Manual for the Identification of Medical Bacteria*. 3rd,(ed), Cambridge press. : British.

Basdew I. H. and M.D. (2011). Laing Mini-Review: Biological control of bovine mastitis using bacteriophage therapy.*Sci.Microbpathog.Commun.Curr.Res.*

Technol. Adv.World Sci.Singapore, 386-393.

Berhanu, S. (1997). *Bovine mastitis in dairy farms in Dire Dawa administration Council and eastern Hararge Zone.* Prevalence, isolation and "*in vitro*" antimicrobial susceptibility studies. DVM thesis, FVM, AAU, Debra Zeit, Ethiopia.

Biffa D, Debela E, Beyene F (2005). Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia. *International Journal of Application Research in Veterinary Medicine.* 3: 189-198.

Bishi AS (1998). *Cross sectional and longitudinal prospective study of bovine mastitis in urban and peri-urban dairy production system in the Addis Ababa region, Ethiopia.* MSc Thesis, FVM, School of graduate studies, Freie University, Berlin, DWLFO, 2014: Debretsige Woreda Livestock and Fishery Office.

Bodman, R. C and Rice, N.G (2003). Mastitis is a disease control and every day task. NebGuide, published by cooperative Extension, Institute of Agriculture and natural resources, University of Nebraska, Linocoln.

Blood, D.C; Radostits, O.M and Henderson, JB. (1983). *Veterinary Medicine.* 6th,(ed), Bailliere Tindal, London, Pp. 451.

Blood, D.C; Radostitis, O.M and Henderson, JA. (1989). *Veterinary Medicine*. 7th, (ed), Balliere Tindal, London Pp. 501- 550.

Blowey RW. (2010). *Mastitis Control in Dairy Herds.* 2nd, (ed), *Cambridge, MA: CABI, Cambridge, Mass.*

Bradley, A.J. (2002). Bovine mastitis: An evolving disease. *Vet. J.*, 164: 116-128.

Burvenich, C.; van Merris, V.; Mehrzad, J.; Diez-Fraile, A.; Duchateau, L. (2003). Severity of *E. coli* mastitis is mainly determined by cow factors. *Vet. Res.*, *34*, 521–564.

Campus, M. (2007). Mastitis in dairy cows. *Dairy Cattle Production* , Pp 342-345.

Cargil, C.F. and Boots, R.W. (1970). Field observation on the control of Staphylococcal bovine mastitis. *Austr. Vet. J.,* 46: 249-252.

Chanetonl ,Tirantel .; Matio, J.; Chaves,J.; Bussmann, LE. (2008). Relationship between milk lacto ferrin and etiological agent in the mastitis bovinmammary gland. *J.Dairy Sci* 91: 1865 -1873.

Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries.* Part 2 Cambridge University Press.British

Christina S. Petersson-Wolfe, Assistant Professor, Dairy Science, VirginiaTech John Currin, Clinical Instructor, Virginia-Maryland Regional College of Veterinary Medicine(2012). *Streptococcus dysgalactiae*: A Practical Summary for Controlling Mastitis. Produced by Communications and Marketing, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, VT/0712/WEB/DASC-5P.

Costa EO, Ribeiro AR, Watanabe ET, Melville PA (1998). Infections of bovine mastitis caused by environmental organisms, *ZL Bl .Vet. B.* (B) 45: 65 -71.

Cowan, S.T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria,* 2nd, ed. Cambridge, Cambridge University press. British.

De Graaf. T and Dwinger , R.H (1996). Estimation of milk production losses due to sub clinical mastitis in dairy cattle in Costa Rica.Prev . *Vet. Med*. 26(3- 4): pp-215- 22.

Deries, L.A. and Keyser, H.DE. (1980). Prevalence of different species of coagulase negative Staphylococci on teats and in milk samples from dairy cows. *J. of Dairy Res*., 155-158.

Drlica, K. and Zhao, X.(1997). DNA grase ,TopoismeraseIVand the 4-quinolones.*Microbiology and molecular Biology Reviews*,16: 377-392.

Du Preez, JH.(1989). Reasons why mastitis therapy is unsuccessful. S. *Afr J Dairy Sci*.; 21: 21–29.

Du Preez,JH.(2000). Bovine mastitis therapy and why it fails. *Tydskr.S.Afr.vet.Ver.;* 71: 201–208.

Elliot REW, Jeain GT, Brookbanks EO (1976). New Zealand National mastitis. The microflora of bovine composite milk samples. *New Zeal. J. Vet*. 24: 80-84.

Elsayed, NI. (2000). *Staphylococcal species in normal and mastitic milk of some domestic animals.* (M.V.Sc). Thesis, University of Khartoum.

Ferrero, L.; Cameron, b. and Crouzet, J. (1995). Analysis of gyr A and grl A mutations in stepwise – selected ciprofloxacin resistant mutants of *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy,* 39:1554-1558.

Gale, E. F. and Folkes, J. P. (1953). The assimilation of amino acids by bacteria: actions of antibiotics on nucleic acid and protein synthesis in *Staphylococcus aureus*. Biochemic. J., 53:493-498.

Ganguly, S. (2018). *Mastitis, an Economically Important Disease in Milching Ruminants.* Agri-BioVet Press (a unit of Prashant Book Agency), Daryaganj, New Delhi, India. ISBN 978-93-84502-65-2.

Gera, S. and Guha, A. (2011). Assessment of acute phase proteins and nitric oxide as indicator of subclinical mastitis in Holstein Haryana cattle. *Indian Journal of Animal Sciences* 81(10): 1029-1031.

Haghour, R. and Ibrahim, A.E. (1980). Incidence of mastitis and brucellosis in some dairy farm in Syria. *Sudan. J.Vet. Sci. and Anim. Husb.,* 21: 10-12.

Halasa.T. K. Huijps, O. Osterds and H. HogeveenL.(2007). Economic effects of bovine mastitis and mastitis management: *Veterinary Quarterly* ;29(1): 18-31ISSN: 0165-2176 (Print) 1875-5941 (Online) Journal homepage

Harmon, R. J., and B. E. Langlois. (1986). Prevalence of minor pathogens and associated somatic cell counts. Page 11 in *Proc. 25th, Annu. Mtg. Natl. Mastitis Counc.,* Columbus, OH. Natl. Mastitis Counc., Inc., Arlington, VA.

Harmon, R.J. (1994). Physiology of mastitis and factors affecting pp.somatic cell counts. *J. Dairy Sci*. 77, 2103–2112.

Heikkilä, A.M.; Liski, E.; Pyörälä, S.; Taponen, S. (2018). Pathogen-specific production losses in bovine mastitis. *J. Dairy Sci.* 101, 9493–9504.

Hillerton, J. E. (1999). Balancing mastitis and quality. In: *Proceedings of British Mastitis Conference*, pp 31-36, Stonelergh, United Kingdom.

Hoe, F.G., P.L. Ruegg. (2006). Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being *J. Dairy Sci.*, 89 , pp. 2297-2308.

Howell, D. (1972). Survey on mastitis caused by environmental bacteria. *Vet. Rec*., 90: 654-657.

Ibrahim ,A.E.and Habib alla, W.(1978). Persistant mastitis in dairy herd of breeding center.*Sudan J. Vet .Sci.Anim Husb*.19(2):96-101.

Ibrahim,E.M,Elowni,O.A.O.and Mohamed,G.E.(1997). Effect of bacteria causing mastitis on milk constituents.*Sud.J.Sci.Anim.Husb*.36:125-136.

Ibtisam ,E.M. E and O.A.O Elowni (2006). Seasonal variation of incidences and Etiological Agents of Bovine Mastitis in Friesian Cattle in Sudan. *Research Journal Animal and Veterinary Sciences*1(1): 25-29.

Ibtisam ,E.M.E,P.Kutzer and O.A.O Elowni (2006). Frequencies of antibiotic susceptibility pattern of bacteria causing mastitis among cows and their environment in Khartoum Stats. *Research Journal of microbiology* 1(2):101-109.

Jha VC, Hakur RP, Yadar JN (1994). Bacterial species isolated from bovine mastitis and their antibiotic sensitivity patterns. *Sud. Vet. J. Anim. Sci*. 9: 21-23.CAB.

Kaneene, J. B. and H. S. Hurd, (1990). The National Animal Health Monitoring System in Michigan. III. Cost estimates of selected dairy cattle diseases. *Preventive Veterinary Medicine*, 8, 127–140.

Kapur, M. and Singh, R.P. (1978). Studies on clinical cases of mastitisin cows, buffaloes and goats in Haryana State. *Indian. Vet. J.,* 55: 803-806.

Kassa, F.; Ayano, A.A.; Abera, M.; Kiros, A. (2014). Longitudinal study of bovine mastitis in Hawassa and Wendo Genet Small Holder Dairy farms. *Glob. J. Sci. Frontier Res. 14*, 33–41.

Kelly ,W .R.(1984). *Veterinary Clinical Diagnosis*.3rd .ed. Bailliere Tindal.Pp196-298.

Khadiga, M.O. (2008). *Comparative efficacy and economic impact of some commonly used antibiotic and anthelmintics in the veterinary field in Sudan*.Ph.D thesis University of Khartoum.

Khan, M.Z.; Khan, A. (2006). Basic facts of mastitis in dairy animals: Review. *Pak. Vet. J. 26*, 204–208.

Kumar GSN, Apannavar M M, Surnagi MD, Kotresh AM (2010). Study on incidence and economics of clinical mastitis. *Karnataka Journal of Agricultural Sciences* 23(2): 407-408.

Lehtolainen, T. (2004). *Escherichia Coli Mastitis: Bacterial Factors and Host Response*. Ph.D. Thesis, Faculty of Veterinary Medicine, University of Helsinky, Helsinki, Finland.

Lents CA, Wettemann RP, Paape MJ, *et al* (2002). Efficacy of intramuscular treatment of beef cows with oxytetracycline to reduce mastitis and to increase calf growth. *J Anim Sci*. 80: 1405-1412.

Lidet G/Michael, BentiDeressa, FeyissaBegna and AbebeMekuria (2013). Study on Prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka town, Southern of Ethiopia .*African Journal of Microbiology Research* October.VOL.7 (43), pp.5051-5056, 25.ISSN 1996-0808.Academic Journal.

Lim, G.H., Leslie, K.E., Kelton, D. F., Duffield, T. F.,Timms, L. L. and Ding well, R. T. (2007). Adherence and efficiency of an external sealant to prevent new intramammary infection in the dry period. *Dairy Sci*.90: pp: 289- 1300.

Linhart , A. and S. Weiskopf. (1989). Developmentof drug resistance in bovine mastitis pathogens .*Dairy Sci.Abstract*, 51:6524.

Madut, N.A. Atif Elamin Abdel Gadir and Isam Mohamed El Jalii (2009). Host determinants of bovine mastitis in semi-intensive production system of Khartoum state, Sudan. *Journal of Cell and Animal Biology* Vol. 3 (5), pp. 071-077.

Malinosuki, E.;Lassa,H.; Kllossowska, A.; Smulski,S. ;Markiewicz ,H .; Kaczmarowski, M. (2006) . Etiological agent of dairy cows mastitis in western part of Poland. *Pol . J. Vet ⁄ Sci* .9:191- 194.

Mamoun,I.and Bakheit,M.R.(1992). Bovine staphylococcal mastitis in the Sudan.*Sud.J.Vet.Sci Anim.Husb.*31:62-64.

Mashaer, M. (2017) . *Isolation and characterization of Bacteria Causing Mastitis in Dairy cattle and evaluation of Efficacy of Antibiotics Used Routinely for Treatment in Khartoum North, Khartoum State.* (M .V. Sc) Thesis University of Khartoum.

Meador .V. P, B.L. Deyoe , and N. F. Cheville (1989). Pathogenesis of *Brucella abortus* Infection of the Mammary Gland and Supramammary Lymph Node of the Goat. *Vet, Pathol*. 26:357-368.

Merchant, I.A. and Packer, R.A. (1967). *Veterinary Bacteriology and Virology*, 7th ed. University Press. Ames, Iowa, USA.

McDonald, J.S. (1977). Streptococcal and Staphylococcal mastitis. *J.Am. Vet. Med. Assoc.*, 170: 1157-1159.

McEwen S.A. and P.J. Fedorka-Cray (2002). Antimicrobial use and resistance in animals. *Clin. Infect. Dis*., 34 pp. S93-S106.

Menzies, FD; Bryson, DG;McCallion ,T. and Matthews, DA. (1992). Astudy of mortality among suckler and dairy cows in Northern Ireland in 1992. *Vet. Rec*, 137: 531-536.

Michel, A. Wattiaux (2000). Mastitis: The disease and its transmission.1-5. From, http:// www. geocities. com/ Heartland/ 8815/mastitis. Html.

Miller, G. Y., P. C. Bartlett, S. E. Lance, J. Anderson and L. E. Heider, (**1993).** Costs of clinical mastitis and mastitis prevention in dairy herds. *Journal of the American Veterinary Medical Association*, 202, 1230–1236.

Muhammad Irshad. Muhammad Rizwan and Sajjad Ahmed. (2011). Diagnosis of subclinical Mastitis in Bovine Using Conventional Methods and Electronic Detector ARPN *Journal of Agricultural and Biological Science*VOL.6 No 11.ISSN 1990-6145.

Mustafa, A.A., Abbas, B. and Ghalib, H.W. (1977). Incidence, control and economic significance of mastitis in a dairy herd. *Sudan. J.Vet. Sci. and Anim. Husb.*, 18. 70-76.

Mwahib,E. (2010). *Prevalence of bovine mastitis and causative agents in Sennar State.* M.V.SC. Thesis University of Khartoum.

Nagrajan, R.; Schabel, A. A.; Occolwitz, J. L.; Counter, F. T.; Ott, J. T. and Felty-Duckworth, A. M. (1989). Synthesis and evaluation of N-alkyl vancomycins. *J. Antibiotics*, 42(1): (63-72).

Nail JR, George CC, Gemmell G and Lain SH (2003). Production of Diarrhoeal Enterotoxins and other Potential Virulence factors by Veterinary of Bacillus species Associated with Non Gastrointestinal infections. Appl. Environ. Microbiol 69 (4): 2372-2376.

Neave, F; Dodd, F; Kingwill, R;Westgarth, D (1969). Control of mastitis in the dairy herd by hygiene and management. *Journal of dairy Science*.:696-707.

Osman, K. M., El–Enbaawy, M. I., Ezzeldeen, N. A. and Hussein H. M.G. (2009). Mastitis in dairy buffalo and cattle in Egypt due to Clostridium Perfringens: prevalence, incidence, risk factors and costs. *Rev. Sci. Tech*. off. Int. E PIZ. vol.28, No. (3), pp: 975-986.

Osumi, T .;Kishimoto ,Y. ; Kano, R.; Maruyama, H. ; Onozaki, M. Makimura , K.; I to ,T.; Matsubara, K. ; Hasegaw, A. (2008) . Prototheca zopfii genotypes isolated from cow barns and bovine mastitis in Japan *Vet.Microbial* 131:419 -423.

Oxoid,LTD.(2006). *The Oxoid manual* 9th, (ed) ,Oxoid limited,Wade Road,Basingstoke, Hampshire.

Packard, V.S, Tatini, S., Fugua , R., Heady, J. and Gilman, C.(1992). Direct microscopic methods for bacteria or somatic cell in: *Marshall, R.T.Standard*

method for the examination of dairy products, 16th edition, American public Health Association, USA, Washington. D.C. pp. 309-325.

Park, C.K. (1979). Gram negative baccilli isolated from bovine udder infection. Korean .J. Vet. Res., 19: 131-134.

Petersson-Wolfe, C.S.; Mullarky, I.K.; Jones, G.M. (2010). Staphylococcus aureus Mastitis: Cause, Detection, and Control. *VA Coop. Ext. 404*, 1–7.

Philpot, W. N. and Nickerson, S. N. (2000). Winning the fight against mastitis.info @ham by dairy supply.com.

Pyörälä, S.L. Kaartinen, H. Kack, V. Rainio.(1994). Efficacy of two therapy regimens for treatment of experimentally induced *Escherichia coli* mastitis in cows *J. Dairy Sci*., 77 pp. 453-461.

Quinn,P.J.;Carter,M.E.and Markey,B.K.andCarter,G.R.(1994). Mastitis; In Quinn,P.J(ed) clinical vetrenary Microbiology.Wolfe,BaltimorePp.327-344.

Rabinson , T. C., E . R Jackson and I. Mair .(1988). Mastitis incidence in quarters with different infection status at during off and calving in two treatment groups. *British Vet. J.* 144: 166- 173.

Radostitis, O. M.; Blood, D.C. and Gay, J. (1994). *Veterinary Medicine*. 12 ed. Tindall, London,p. 510-560.

Radostitis, O. M.;Leslie, K.E. and Fetrow ,J.(1996). Mastitis control in dairy herd .In Radostitis, O.M; Leslie,K.E.and Fetrow,J.(eds).Herd Health: *Food Animal production Medicine*.2ed Philadelphia:W.B.Saunders company.Pp229-276.

Radostits, O.M.; C.C. Gay; D.C. Blood and K.W. Hinchkliff, (2000). *A Text Book of Veterinary Medicine.* 9th Edn., W.B. Saunders, New York, pp: 563-618.

Rantamäki, L.K.; Müller, H.P. (1995). Phenotypic characterization of Streptococcus dysgalactiae isolates from bovine mastitis by their binding to host derived proteins. *Vet. Microbiol.* 46, 415–426.

Rebecca Gooder (2014). *A Review of Mastitis Control Practices*. The Faculty of the Dairy Science Department California Polytechnic State University, San Luis Obispo.

Reem,R. (2008). *Mastitis Etiological, Clinical and Treatment Aspects in Khartoum State- Sudan* M.V.Sc.Thesis university of Khartoum.

ReemRabie Mohammed Salih and A/ Basit Osman Ahmed (2012). Staphylococcal Species Responsible For Bovine Mastitis in Khartoum State, SUDAN.U of K. *J. Vet.Med. &Anim Prod.* Vol.3, No 1. (149- 156)-- 159; 25, Volume 5, Issue 6: 153-- 159, 2 V015.

Saidani, M.; Messadi, L.; Soudani, A.; Daaloul-Jedidi, M.; Châtre, P.; Ben Chehida, F.; Mamlouk, A.; Mahjoub, W.; Madec, J.Y.; Haenni, M. (2018). Epidemiology, antimicrobial resistance, and extended-spectrum Beta-lactamase-producing enterobacteriaceae in clinical bovine mastitis in Tunisia. *Microb. Drug Resist.*, *24*, 1242–1248.

Saini , V ;McClure, J.T ;Leger , D;Dufour ,S;Sheldon,A .G ;Scholl, D .T.and Barkema,H .W.(2012). Antimicrobial use on Canadian dairy farms.*J. Dairy Sci*.95:1209-1221.

Sandra Björk. (2013). Clinical and subclinical mastitis in dairy cattle in Kampala, Uganda, *Examensarbete*.ISSN 1652-8697 .*Examensarbete*:65.

Sayed, M.L., Shell, W.S, Hanan, A. Ahmed, Hanan, M. Ibrahim, Nasr, E.A. and Ali, A.M.(2015). Efficacy of a locally prepared bovine mastitis vaccine. *Benha Veterinary Medical Journal*,Vol. 29,NO.2:309-318.

Schalm, O.W. and Noorlander, D.O. (1957). Experiments and observations leading to development of C.M.T., *J. Am. Vet. Med. Assoc.*, 130: 199-204.

Schalm, O.W. and Lasmanis, J. (1968). The leucocyte origin and function in mastitis. *J. Am. Vet. Med. Assoc.* 153: 1688-1690.

Schroeder, J.W. (1997). Mastitis Control Programs: Bovine mastitis and milking management. NDSU. Extension service. North Dakota Statae University. From http:// w.w.w. ext. nodak.edu./ extpups/ ansci /dairy /as1129 w.htm. page 1-16.

Sharif, A.; Umer, M.; Muhammad, G (2009). Mastitis control in dairy production. *J. Agric. Soc. Sci. 5*, 102–105.

Sharma, R.M. and Pasker, R.A. (1970). Occurrence and ecological features of *Streptococcus ubris* in the dairy cow. *Am. J . Vet.Res.,* 31:1197-1203.

Sharma, N., A. Gautam, S.R. Upadhyay, K. Hussain, J.S. Soodan and S.K. Gupta, (2006). Role of antioxidants in udder health: A review. *Indian J. Field Veterinarian*, 2: 73-76.

Sharma, N., A.K. Srivastava, G. Bacic, D.K. Jeong and R.K. Sharma, (2012). Epidemiology. In: *Bovine Mastitis.* 1st Edn., Satish Serial Publishing House, Delhi, India, pp: 231-312.

ShawgiDerhimGasim El gadasi.(2003). *Identification of bacteria isolated from mastitic milk of cattle, sheep and goats in Khartoum State and study of antimicrobial sensitivity.* B.V. Sc. (1996) Faculty of Veterinary Science.University of Khartoum.

Singh, PJ; Singh ,KB. (1994). A study on economic losses due to mastitis in India. *J Dairy Sci* 47: 265-271.

Sohiela Mohammed El Hassan Abd El Rahman El Hassan.(2002). *Prevalence of Streptococcal Mastitis in Dairy Cattle in Khartoum State.*B.V. Med., University of Khartoum.

Straut , M ., M . Surdeanu, G. Oprisan, D. Otelea and M. Damian.(1995). Antibiotics and antibacterial resistance.Afew elements of genetic basis for this relationship. *Roun . Arch. Microbial .Immunol* ., 54: 241-254.

Suojala, L. Kaartinen, S . Pyörälä (2013). Treatment for bovine *Escherichia coli* mastitis—An evidence-based approach *J. Vet. Pharmacol. Ther*., 36 (2013), pp. 521-531.

Suriyasathaporn, W.; Heuer, C.; Noordhuizen-Stassen, E.N.; Schukken, Y.H.(2000). Hyperketonaemia and the impairment of udder defence: A review. *Vet. Res. 31*, 397–412.

Suttie JM, Reynolds SG (2003). FAO-STAT. FAO Statistics Database on the World Wide Web. In Suttie JM, Reynolds ST (Eds.), pp. 1-36.

Suzan, K. Murad; Hatil H. EL Kamali and Manal ,A. Ibrahim(2016). Isolation, Identification and Antibiotics Treatment of Some Bacterial Strains Caused Bovine Mastitis in LIBYA.*European Journal of Basic and Applied Sciences*. Vol. 3 No. 3, ISSN 2059-3058.

Suzuka, I.; Kaji, H. and Kaji, A. (1966). Binding of specific sRNA to 30S ribosomal subunits: effect of 50S ribosomal subunits. *Proc. Natl. Acad. Sci*.; 55: 1483-1486.

Timms, L.L. and Schultz, L.H. (1987). Dynamics and significance of coagulase negative Staphylococcus intramammary infection. *J. Dairy. Sci.,* 70: 43-47.

Tolla, T.(1996). *Bovine Mastitis in Indigenous Zebu and Borona Holstein Crosses in Southern Wollo.* Ph.D. Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Turner Swartz, Student, Dairy Science, Virginia TechChristina S. Petersson-Wolfe, Dairy Specialist, Virginia Cooperative Extension. (2016). Pseudomonas spp: A Practical Summary for Controlling Mastitis.Produced by Communications and Marketing, College of Agriculture and Life Sciences, Virginia Tech,VT/0316/DASC-70P.

UranchimegTsegmed.(2006). Staphylococci Isolated from Raw Milk of Yak and Cattle in Mongolia. *Swedish University of Agricultural Sciences* (SLU).ISSN 1403-2201.

Wakeem A.A and Eltyeb. E.(1962). A survey of causal organisms of chronic mastitis in a herd and its treatment with oxytetracycline .*Sudan J. vet. Sci .Anim . Husb*.3 (1):28-32.

Watts JL (1988). Etiologic agents of bovine mastitis.*Vet. Microbiol*, 16: 41-66.

Wellengberg,G . J.; Vander poel, W.H.; Vanoirschot, J. T. (2002). Viral infections and bovine mastitis .*Vet Microbial* .88 :27-45 .

William,G.R. (1995). *Disease of Dairy Cattle 1st* ed .philadelphia:Alea and Febiger book.Williams and Willins pp.287.

Xavier, M.N; T.A.Paixao;F. P. Poester A. P. and Lage, R. L.(2009). Pathological ,Immunohistochemical and Bacteriological Study of Tissues and Milk of Cows and Fetuses Experimently Infected with *Brucella abortus. Journal of Comparative Pathology*. Volume 140, Issues 2-3, Page 149-150.