بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Frequency of Aerobic Bacteria Associated with Subclinical and Clinical Mastitis among Lactating Goats in Khartoum State

تردد البكتيريا الهوائية المرتبط بالتهاب الثدي غير الظاهر والظاهر عند الماعز الحلوب في ولاية الخرطوم

A Dissertation submitted in partial fulfillment for the requirement of

M. Sc in Medical Laboratory Sciences (Microbiology)

By

Mustafa Mohammed Madani Eshag

B. Sc (Honors) Medical Laboratory Sciences

Shendi University (2007)

Supervisor

Dr. Ali M. S. El-Eragis

B.V. Sc, M.V. Sc, PhD

Department of Pathology and Diagnosis

Veterinary Research Institute (VRI)

December 2011

بسم الله الرحمن الرحيم

قال تعالى:-

الرَّحْمَـنُ ﴿١﴾ عَلَّمَ الْقُرْآنَ ﴿٢﴾ خَلَقَ الْإِنسَانَ ﴿٣﴾ عَلَّمَهُ الْبَيَانَ ﴿٤﴾

صدق الله العظيم

سورة الرحمن الآيات 1-4

Dedication

I dedicate this work

To my parents

To my brothers and sisters

To my teachers and friends

With love and respect

ACKNOWLEDGEMENT

First of all my thanks and eulogize were due to ALMIGHTY ALLAH, the beneficent and merciful for giving me health and strength to accomplish this work.

I express my deepest gratitude to my supervisor Dr. Ali M. S. EL-Eragi for his guidance, insight, encouragement and valuable supervision throughout this study.

My appreciation and gratitude were also to Dr: Mogahid Mohamed Elhassan for his support and encouragement.

Last but not least my thanks and appreciations were due to anyone who helped me directly or indirectly in the preparation and revision of this study during the research work, my best regard to all without any exception.

My thanks and gratitude also goes to Dr. Asia M. Saeed, Ustaza Sohair Ustaz Ramadan, Wesal Jaafr, Sami Ahmed, Emad Elden Mohamed, Kihdir Elhadi, Abd Elmukarum Ahmed, Eltayeb Mahmood, Taha Mohamed and Shams Elden Abdalrasool.

ABSTRACT

The aim of this study was to isolate and identify the bacterial agents that have association with subclinical and clinical mastitis among lactating goats in Khartoum State and to assess their antibiotic susceptibility patterns.

One hundred and thirty two milk samples were collected from Khartoum North and Omdurman, 100 samples from apparently healthy goats, and 32 from mastitic one under aseptic condition; 15ml of milk were collected from each udder and transported to the laboratory in an ice box.

Bacteria were identified to the species level using standard bacteriological procedures such as colonial morphology, Gram stain and their biochemical utilization behavior.

Thirty two out of hundred (30%) apparently healthy goats showed bacterial growth, where as19/32(59.37%) mastatic goats milk showed bacterial growth from their milk. Isolated bacteria from both subclinical and clinical mastitis were as follows: subclinical bacteria were 22/100 (22%) gram positive cocci, of which 17/22(77.27%) were coagulase negative *Staphylococci* (CNS) and 5/22(22.72) were *Staphylococcus aureus.* Gram negative bacilli were 8/100(8%), of which 6/8(75%) were *E. colli* and 2/8(25%) were *Pseudomonas aerogenosa.*

Bacteria isolated from clinical mastitis were 16/19(84.21) as Gram positive cocci of which, 5 *Staphylococcus aureus*, 5 CNS and 6 *Streptococcus spp*, while 3/19(15.78%) were Gram negative bacilli (2 *E.colli* and 1 *Pseudomonas aerogenosa*).

Thirty out of sixty (50%) of Saanene revealed bacterial growth where as nineteen out of seventy two (26.38) of local breed showed bacterial growth.

The recovered *Staphylococci* and *streptococci* were found to be resistant to methicillin 38/38(100%), penicillin G 18/38(47.37%), on the other hand they were sensitive to vancomycin 38/38 (100%), gentamycin 35/38 (92.1 %), while Gram negative were sensitive to ciprofloxacin.

In conclusion, CNS was significantly higher than other bacteria, so it was considered as the main cause of mastitis; the use of CMT is useful in detection of subclinical cases because thirty three out of hundred (33%) milk samples revealed CMT positive. Gentamycin, vancomycin and ciprofloxacin, were active against the recovered bacterial isolates.

الخلاصة

الهدف من هذه الدراسة دراسة تردد البكتيريا المرتبطة بالتهاب الثدي غير الظاهر والظاهر عند الماعز الحلوب بولاية الخرطوم وتحديد المضادات الحيوية الأكثر فاعلية ضد الأنواع المعزولة.

تم جمع١٣٢ عينة في كل من الخرطوم شمال وأم درمان من ماعز سليمة ظاهريا وأخرى مصابة في ظروف معقمة أخذت ١٥مل من كل ضرع وأرسلت إلى المعمل في صندوق ثلج.sتم التعرف على البكتيريا حتى مستوى النوع باستخدام الطرق المعيارية مثل شكل المستعمرة, صبغة الجرام و الاختبارات الكيماوية.

ثلاثون من مائة عينه (٣٠٪) من الماعز السليمة أظهرت نموا بكتيريا بالإضافة إلى ٣٢/١٩ (٥٩,٣٧٪) من الماعز المصابة أظهرت نموا بكتيريا, البكتيريا المعزولة من ألبان الماعز السليمة والمصابة كانت كما يلي:

١٠٠/٢٢من الماعز السليمة (٢٢٪) كانت موجبة الجرام منها ١٧ /٢٢ (٧٧,٢٧%) المكورات العنقودية الذهبية سالبة إنزيم تختر البلازما بينما ٥ /٢٢ (٢٢,٧٢ ٪) العنقودية الذهبية إما العصوية سالبة الجرام ١٠٠/٨(٨٪) منها ٨/٦ (٧٥٪) المتوشقة الخيطية و٢ /٨ (٢٥٪) الزائفة الزنجبارية, أما الماعز المصابة ١٦ /١٩ (٨٤,٢١٪) منها كانت موجبة الجرام, ٥ عنقودية ذهبية, ٥ عنقودية ذهبية سالبة انزيم تختر البلازما و٦ كروية سبحية بينما ٣ /١٩ (١٥,٧٨) هى عصوية سالبة الجرام منها ٢ المتوشقة الخيطية و١الزائفة الزنجبارية.

ثلاثون من ستون٥٠٪ من السعانين أظهرت نموا بكتيريا بينما تسعة عشر من اثنان وسبعون ٢٦,٣٨ ٪ فقط من الماعز المحلية أظهرت نموا بكتيريا.

وجد إن العنقودية الذهبية والكروية السبحية مقاومة للمثيسلين و البنسلين بينما الفانكوميسين والجنتاميسين فعالة جدا ضد الأنواع المعزولة.وجد إن العنقودية الذهبية سالبة إنزيم التختر أكثر من البكتريا الأخرى فهي تعتبر الأكثر تسببا في التهاب الثدي عند الماعز, كما وجد إن استخدام ال CMT ذو جدوى في كشف الحالات السليمة ظاهريا بحيث إن ثلاثة وثلاثون من مائة عينة كانت موجبة اختبار ال CMT.

TABLE OF CONTENTS

LIST OF TABLE

CHAPTER ONE

1. INTRODUCTION

1.1: Goats and mastitis

The goat's population in Sudan is about 6 million; two thirds of which are distributed north of latitude 10˚N. Nubian and Sudanese desert breeds are the main breeds in the Sudan. This wealth is exposed to many endemic and epidemic viral, bacterial and parasitic diseases. Among these mastitis though not often fatal, but remains one of the most serious factors that negatively affect the dairy production in goats (Omiema 2003).

Mastitis means the infection of the mammary glands. It is characterized by physical, chemical and bacteriological changes in the milk and by pathological changes in the mammary tissue. Because of the large number of sub-clinical cases, mastitis is defined as disease which is characterized by the presence of significant increase in the leucocytes count in milk (Grant and Steve, 2001).

Mastitis is common in lactating goats. Both acute and chronic forms may be encountered. The disease is caused by a number of different types of pathogenic bacteria such as *Mycoplasma spp, Staphylococcus spp, Streptococcus spp,* coagulase negative *Staphylococci* (CNS), *Yersina,* and *Nocardia* are of the common causes of mastitis in cattle but only limited reports are available in goats (Omiema 2003).

Professional dairy goat farming is a growing industry all over the world because of an increasing public interest in alternatives for cow milk. Also goat milk is destined for a vulnerable group of people, for example children with an allergy against cow milk, it is very important to ensure the quality of goat's milk. An important hazard to milk quality can be the infection of the udder, which can result in mastitis. These infections of the udder bring along potential zoonotic risks due to the presence of bacteria in milk for human consumption. Mastitis is also causing economic losses mainly due to reduced milk production and discarding of milk because it may contain antibiotics due to treatment of the animal. After (CNS), *Staphylococcus aureus* (*S. aureus)* is the most important pathogen causing (sub) clinical mastitis in dairy goats *S. aureus* is responsible for 35.4% of all the mastitis cases in dairy goats. In the United Kingdom it was found that *S. aureus* caused 13% of all subclinical mastitis cases in dairy goats. Diagnosis of subclinical mastitis in dairy goats is unlike the diagnosis of subclinical mastitis in cattle, furthermore there is some evidence that somatic cell count (SCC) of uninfected udder halves in the goats were not always lower than SCC of infected udder halves. Because of these difficulties with using SCC as a diagnostic tool for mastitis, research has to concentrate on finding practical and useful diagnostic tools. The only definitive diagnosis of subclinical mastitis in goats so far requires bacteriological culture of milk samples (Poutrel *et al.*, 1997).

1.2 Rationale

Mastitis has been the focus of much attention for almost many decades. It's importance lies in it's frequency as a cause of decreasing milk yield and economic impact, despite all the advances made by medical science and research.

Many studies have been done in many countries in addition to Sudan reflecting the emergence of different microorganism as etiological agents of mastitis.

During the last ten years their is tremendous increase in goat farming in Khartoum State. This situation renders the diagnosis of mastitis, detection of the causative agents and their antimicrobial susceptibility so important. In addition, the use of California Mastitis Test (CMT) in the detection of subclinical cases of mastitis should also be addressed.

1.3 Objectives

1.3.1 General objective

To study the frequency of the aerobic bacteria associated with subclinical and clinical mastitis among lactating goats in Khartoum State.

1.3.2 Specific objectives

- To use the California mastitis test for the detection of subclinical mastitis in apparently healthy goat udders.
- To isolate and identify the common causes of mastitis from lactating goats in Khartoum State farms.
- To determine the antimicrobial susceptibility of the isolates.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Types of goats in Sudan

There are two historical main types of goats in Sudan

2.1.1 Nubian goats

Nubian goats are distinctive breed, and were originated in the Sudan .It is widely distributed in Ethiopia and in the Sudan the colour is variable but red, brown and black are common. It is one of the African breeds that have been selective for milk production. Their average daily milk yield is about 1-2kg per day (Omiema, 2003).

2.1.2 Desert goats

The Sudanese desert breed is an important breed and has the following common characteristics: long legs, fine coat and special adaptation to the dry region, for this reason they are known as Sahel or desert goats' .The coat colour is variable and range from grey, red and black colours. They were reared primarily for meat and skin production (Devendra, and mcleray 1983).

2.2 Diseases and parasites

Diseases and parasites form one of the main constraints to goat production in many parts of the tropic including the Sudan. In addition to disease and parasites, low plan of nutrition, poor managements, sanitation and hygiene all these factors affect the health and performance of the goats .In general goats in tropics are hardy animal and show good resistance to disease .They exhibit remarkable ability to adapt to variety of situations and fluctuation

feed supplies, where often cattle and sheep are not so able to survive .Never the less, there are number of diseases (Clostridial disease, Brucellosis, Gaseous and parasites (helminthes, fashiola, etc) that affect goats (Omiema, 2003).

2.3 Pathogenesis

During the course of mastitis, bacteria and secretory cells within the mammary gland produce various chemical messengers that enter the blood circulation within the udder. These chemical messengers attract a specialized type of somatic cell called neutrophils to the mammary glands.

Therefore, as a result of the intramammary bacterial infection, a tremendous amount of neutrophils is mobilized into the udder in order to combat the infection. The increase of this cell type within milk is the primary cause of increased milk somatic cell counts associated with mastitis (Grant and Steve, 2001).

2.4 Mastitis in goats

Mastitis is defined as an inflammation of the mammary gland. The inflammation is the result of a localized immune response to an irritant within the gland. The irritant can be in the form of pathogens, toxins, or physical trauma. The goat mounts an immune response in an attempt to destroy or neutralize the irritant and returns the mammary gland to normal function (Grant and Steve, 2001).

Mastitis implies that an infectious agent is present in the mammary gland and is nearly always caused by bacteria. Bacteria invade the udder by entering the teat orifice, multiply and die within the gland and in the process, produce and release toxins that cause injury to secretory tissue and stimulate

an immune response. Besides bacteria other pathogens such as yeast, *Mycoplasma*, and algae can infect the mammary gland. Bacteria which infect the mammary gland are classified into two major categories, contagious or environmental pathogens. Mastitis caused by these pathogens is generally referred to as contagious mastitis or environmental mastitis.

2.5 Types of mastitis:

Mastitis is an inflammatory condition of the mammary gland, characterized by changes in the physical characteristics of the udder or milk. Mastitis can be classified into three major types: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Anonymous, 2003).

2.5.1 Subclinical mastitis:

In SCM, there are no clinical signs of disease other than an increased somatic cell count (SCC) in the milk, the presence of pathogenic organisms in the milk, and an inflammatory response that can only be detected by screening or laboratory tests. Obviously SCM is one of the most important infectious diseases in small ruminants. Furthermore, SCM represents a constant risk of infection for the whole stock. As there is a need for higher milk yields and more stringent requirements on milk quality in dairy goat herds, udder infections must be prevented or detected at an early stage not only to protect the farmer but rather the consumer (Mohammad *et al.,* 2011)

2.5.2 Clinical mastitis:

In CM, there are changes in milk color, clots are present in the milk and there are large numbers of leukocytes in the milk. Swelling, heat, pain, and indurations may be observed in the mammary gland in clinical cases; these symptoms can be detected by visual observation of the udder. (Mohammad *et al.,* 2011).

2.5.3 Chronic mastitis

Is an inflammatory process that has lasted for months and may continue from onset of lactation to another (Mohammad *et al.*, 2011).

2.5.4 Contagious mastitis

Contagious pathogens are spread from an infected udder to a non infected udder during the milking process. The source of bacteria is an infected udder. The most prevalent contagious pathogens associated with mastitis are *Streptococcus agalactiae* and *Staphylococcus aurous*. (Catherine and Poutrels, 1984; Grant and Steve, 2001).

2.5.5 Enviromental mastitis

As the name implies, environmental pathogens that infect the mammary gland are present in the goat's surroundings. The reservoirs for these pathogens include feces, soil, and bedding. Transmission of pathogens from the environment to the udder mainly occurs between and during milking. Environmental pathogens commonly isolated from infected udders are coliform bacteria, *Streptococcus* species other than *Streptococcus agalactiae* and *Staphylococcus* species other than *Staphylococcus aureus,* Mastitis also is common in lactating goats where ever they are kept. Both acute and chronic forms may be encountered .The pre disposing causes of the disease are bad hygiene and poor management at milking time .The disease is caused by different type of pathogenic bacteria .The reported causal agent of goats mastitis in Sudan are *Staphylococci*, *Corynebacteria*, *Coliform Streptococci*. *Nocardia asteroid* was associated with goat mastitis (Dafaala and gharib, 1958; grant and Steve, 2001).

2.6 Staphylococcal mastitis

Mastitis is one of the most costly diseases in the dairy industry, and although much information is available concerning mastitis in cows, few studies deal with mastitis in goats. Researchers studying subclinical mastitis in goats agree that intramammary infection (IMI) caused by coagulase-negative *Staphylococci* (CNS) are the most prevalent. Despite the high prevalence of IMI caused by CNS, CNS are considered to be minor pathogens. However, IMI caused by CNS are associated with clinical mastitis, changes in milk composition, and reduced milk yield. IMI caused by CNS are also capable of persisting throughout lactation and the dry period. The use of a single milk sample for bacteriological analyses to detect IMI in dairy cows might contribute to miss diagnosis (Contreras *et al.*, 1997).

Several pathogens can cause mastitis but *Staphylococcus spp*. is the most frequently diagnosed causal microorganisms of intra mammary infection (IMI) in goats. Other pathogens such as *streptococcus. , Enterobacteriacea, Pseudomonas aerogenosa, Mannheimia haemolytica Corynebacteria* and fungi can produce IMI in small ruminants. The number of species of the genus *Staphylococcus* is steadily increasing (Contreras *et al.*,2004). About 36 species are listed in the 2006 review some species of this genus cause a variety of diseases by production of a series of enzymes and toxins, invasion of host cells and tissues. Staphylococcal alpha-hemolysin or alpha-toxin is the most studied and characterized cytotoxin, and is considered a main pathogenicity factor because of its hemolytic, dermonecrotic and neurotoxic effects. Additionally, beta-hemolysin is a sphingomyelinase that is highly active against sheep and bovine erythrocytes while delta -hemolysin as well as alpha-hemolysin induce pore formation, perturbing the cell membrane permeability In reports from different parts of the world the oxacillin

resistance of coagulase-negative *Staphylococci* (CNS) lies between 70% and 80% studies showed that 22.5% of CNS isolates from 750 human subjects were resistant to methicillin. The public health significance of *Staphylococci* isolated from milk and dairy products is important because these products can be a source of, toxins and antibiotic-resistant strains for humans.In goat the role of *Staphylococcus* aureus and (CNS) in udder infection was the subject of some recent studies, however, there are few studies on the antimicrobial susceptibilities and exotoxin production by *Staphylococci* isolated from goat mastitis in Iran (Azizollah *et al.,* 2010).

2.7 Gangrenous goat mastitis

Mastitis is considered one of the most important diseases of domestic animals, caused by several etiological agents. Transmission of the microorganisms primarily occurs in the teat canal, usually involving agents from animals and environmental origin and from the milking process. *Staphylococcus aureus* is recognized as the most common causal agent of goat mastitis, followed by minor occurrence of *Escherichia coli, Clostridium perfringens*, *Streptococcus*, *Pseudomonas* and *Nocardia* genera. Gangrenous mastitis in goats is a severe clinical inflammatory process in the mammary glands. Clinical signs commonly occur in the first weeks of lactation, committing one or two sides of the glands and are characterized by fever, anorexia, dyspnea and systemic signs of toxemia. Initially, the udder is hot, painful as well as swelling of the affected side, with watery milk, containing flocculent pus and/or blood secretion. Evolution of the process is characterized by udder becoming discolored (blue–blackish or blue–greenish), cold, with demarcation line of the affected tissue, development of abscess and draining pus. Fatal clinical course is characterized by worse corporal condition, pneumonia, septicemia and/or toxemia. Usually,

gangrenous goat mastitis is associated with *S. aureus* or *M.haemolytica* infections. Treatment involves antimicrobial and anti-inflammatory drugs associated with fluid therapy, surgical drainage, debridation and demotion of necrosis tissue. Some reports described an unusual case of combined infection by *S.aureus*, *E. coli* and *C. perfringens* isolated from gangrenous mastitis in goat (Ribeiro *et al.*, 2007).

2.8 Effect of subclinical mastitis in milk composition

The proportion of udder halves with subclinical IMI in goats in different countries ranges from 35 to 70%, the main pathogen group in infected udder halves comprises various species of coagulase negative staphylococci (CNS), mainly *Staphylococcus caprae* and *Staphylococcus Epidermidis* the main influence on SCC was IMI, and milk yield was significantly higher in uninfected than in infected halves. However, a direct comparison between infected and uninfected glands was not possible because the measurements were based on the whole udder level and not on a single gland level. Therefore, a glandular level model was developed in which each animal had one udder half infected with an identified CNS species, and the contra lateral gland was free of bacteria, to focus on how subclinical mastitis (SM) affected milk yield and compositional changes in relation to curd yield (CY) Applying this experimental design in sheep showed that subclinical IMI was associated with increased plasminogen activator (PA) and plasmin (PL) activities as a result of accelerated conversion of plasminogen (PLG) to PL in the infected glands. These changes were associated with accelerated apparent casein (CN) degradation, reduced Yc, and increased milk-clotting time (CT). These modifications indicate that the changes in milk composition negatively affect the yield and quality of cheese made from milk that originates from infected glands. Plasmin is the main proteolytic

enzyme in cow and sheep milk (Leitner *et al.,* 2004) in which it occurs mostly as the inactive zymogen PLG, which is activated by PA. However, only residual PLG activity was found in goat milk, which was consistent with the unusually high PA activity compared with values for ovine and bovine milk. Nevertheless, PA and PL activities in late-lactating goats are negatively correlated with the coagulating properties of milk, which suggests that this system is important in goats as well. Some study applied the glandular level model to dairy goats to test the effect of IMI on milk yield and on milk quality as reflected in CY and CT To achieve this goal, animals were chosen that had one udder half infected with an identified CNS species and the contralateral gland free of bacteria. In each gland, inflammation indices were analyzed along with total milk protein, CN, whey proteins, the PA-PL system activity, and measures of proteolysis (Leitner *et al.,* 2004).

2.9 Infection and Milk Yield

Various CNS bacteria are the most abundantly occurring isolates associated with SM in goat herds in a number of countries (Contreras *et al.,* 1997). The CNS are not considered as major pathogenic bacteria, and their occurrence is usually ignored by farmers and veterinarians (Leitner *et al.,* 2004). However, CNS infection induced the inflammatory response, reflected in the high SCC, which is consistent with previous findings in goats and sheep. The inflammatory response was associated with a marked reduction in milk yield in the infected gland compared with that of the uninfected one, which is consistent with earlier results in sheep (Leitner *et al.,* 2004). In sheep with both glands infected, the reduction in milk yield was significant, whereas when only one gland was infected, the contra lateral gland compensated for about 80% of the reduction. Thus, although the point has not yet been tested, this possibility is not rule in goats too; compensation in the uninfected gland

mitigates the effect on milk yield as measured on a whole-animal basis when only one gland is infected. Nevertheless, the extensive survey of milk records of goat farms in France by leaves no doubt that increased SCC associated with IMI reduced milk yield in comparison with farms with low SCC (Leitner *et al.,* 2004).

2.10 Interrelationships between milk yield and composition in sub clinically mastitic goats

It was found that an up regulation in the activity of the PL system in glands infected with SM is consistent with previous findings in dairy cows and dairy sheep (Leitner *et al.,* 2004).

Higher protein and fat concentrations were found in infected glands than in uninfected ones at the same time, milk volume decreased, suggesting that this response is related to a mild increase in PL activity (30 to 50%) over the basal level. Under such conditions, plasmin-induced hydrolysis of CN liberates a peptide from β-CN (β-CN 1-28), which in turn down regulates milk secretion in cows and goats; its activity was correlated with its ability to block potassium channels in the apical membranes of mammary epithelia However, in some individual cases, the protein and fat concentrations were found to be lower in infected glands than in uninfected ones. Such a response was observed when the increase in PL activity was large (2-fold or more), as is the case during milk stasis it has been shown that CN hydrolysis under high PL activity induces rapid drying-off of mammary secretions in goats and cows despite the doubled PL activity, the reduction in milk yield more or less matched the reductions in protein and fat secretion, so that overall there was no net change in protein and fat concentrations. However, as discussed previously, there is compelling evidence that the CN was degraded and modified in the infected gland. The marked reduction in

lactose concentration resembled the response in sheep and cows under high PL activity. Thus, the degree of PL activation determines not only the reduction in milk volume, but also the changes in the secretion of organic components and, consequently, milk composition and CY, probably because certain structural modifications in the molecule affect its ability to aggregate, owing to the proteolytic action of enzymes on the CN micelle. (Batanani *et al.*, 2007; and Silanikove *et al.*, 2000).

2.11 Epidemiological aspects of small ruminant mastitis

The annual incidence of clinical mastitis in small ruminants is generally lower than 5%, but this incidence can increase sporadically. The prevalence of subclinical mastitis has been estimated at 5–30% or even higher. But there are only limited data about incidence of intramammary infection (IMI) of goat and sheep in the literature but occurrence rates are lower. In addition, severe cases of mastitis related to incorrect preventative strategies have been attributed to the pathogens *Aspergillus fumigatus*, *Serratia marcescens*, *P. aerogenosa* and Lentiviruses are also known to infect goats and sheep, but because they rarely produce clinical symptoms or elevated MSCC, they are not usually considered as classic small ruminant intramammary pathogens. Nevertheless, caprine lentiviruses should still be included in the general plan for controlling mastitis. Because contagious agalactiae syndrome produces symptoms other than mastitis, some authors fail to consider *Mycoplasma* spp. as the etiology of sheep or goat IMI. However, the intense effects of this pathogen in reducing milk production and increasing the MSCC, means that contagious agalactiae should be considered as one of the most important causes of mastitis in endemic areas, where subclinical cases are frequent. In herds clinically infected by *Mycoplasma* spp., besides significant losses due to mortality or the need to cull animals, producers cannot comply with the

milk quality standards demanded by consumers, industry and public health organizations. Rather than risk a human health hazard that could be caused by some mastitis-causing bacteria, milk is generally heat treated to minimize this effect, however in regions where cheese is made from raw milk (Contreras *et al.,* 2004; Tanga and Karen, 2010).

2.12 Diagnosis

2.12.1California mastitis test (CMT)

The California Mastitis Test (CMT) and Somatic Cell Counts (SCC) of milk are useful monitoring tools to detect the presence of mastitis in the mammary glands of dairy goats. The CMT is a simple rapid means for detecting mammary gland infection and irritation. It has had wide acceptance and use by veterinarians and dairymen in routine mastitis prevention and control programs. There is widespread belief that a higher CMT is normal for goats than for cows. Until that argument is definitely settled, a CMT of 1 or higher should be cause for concern in goats. Somatic Cell Counts are a more accurate measure of udder health. Healthy dairy goat herds can be expected to produce milk with a somatic cell count under 500,000. The presence of mastitis infection in dairy goat herds is reflected in bulk tank milk samples with a CMT of 1 or higher and a somatic cell count exceeding 1,000,000 cells per milliliter. Regular use of the CMT or SCC can give both the owner and the milk consumer confidence (Ibrahim *et al.*, 2009).

2.12.2 Somatic cell count

The somatic cell count (SCC) is an indicator of the intensity of the cellular immune defense and it represents a marker of the sanitary state of the udder. During the course of intramammary infection, leucocytes migrate from the blood towards the mammary gland leading to increase somatic cells in the milk. SCC represents a valuable tool for prevalence assessment and screening mastitis, a common accepted SCC values have not been established for milk produced by healthy animals (Ghaleb *et al.*, 2005).

2.13 Control and prevention strategies

Vaccines against clinical gangrenous mastitis, that are available on the market for small ruminants, are widely used when there is a high incidence of clinical gangrenous mastitis. However, owing to the reported different effectiveness of these vaccines for dairy cows and sheep, and their inability to prevent new infections, it has been suggested that vaccines should be used in dairy herds with a high prevalence of *S. aureus* IMI to reduce clinical symptoms. The effectiveness of vaccination programs against mastitis caused by *S. aureus* has been reported for sheep but not for goats (Contreras *et al.*, 2007).

SCC is reliable enough to be useful for predicting IMI in goats as previously proposed. Antibiotic treatment of goats at drying-off is an efficient method to control mastitis and systematic treatment should be recommended when SCC in bulk milk is high (>1,000 103 cells/ml), even when CNS are involved in IMI (Poutrel *et al.*, 1997).

2.14 Prevention and Treatment

Tender loving care may be the most important basic requirement for mastitis prevention and treatment. Dairy goats are very sensitive, intelligent animals. When the person milking the goat likes the animals and handles them gently, quietly and patiently, goats willingly and eagerly participate in the milking procedure. With ideal milking management, goats show abundant evidence of affection for the person doing the milking job, letting their milk down for maximum ease and speed of milking.

Both hand and machine milking require good milking preparation -clean dry teats and clean dry hands and/or teat cup inflations. Rough handling, irregulars milking times, over milking or inadequate preparation for milking, all take their role in providing stress and injury. These directly affect mastitis resistance and susceptibility. Mastitis in dairy goats, like mastitis in dairy cows, is rarely an important disease in herds where animals are thoroughly prepared for milking by massaging and washing udders. The use of a bactericidal solution to cleanse the udder and teats also stimulates good milk let-down. Dry the udder and teats with an individual paper towel before milking begins. With hand milking, it is very important that milkers' hands be thoroughly washed and dried before milking.

Milking machine teat cups should not be attached to the goat until udder and teats are thoroughly washed and massaged, cleaned and dried. Hand or machine milking which is hurtful or excessive beyond normal let-down contributes to teat end injury and the spread of mastitis from goat to goat in the milking procedure. Systemic Treatment in severe acute attacks of mastitis, systemic administration of antibiotics by intravenous or other parenteral means is indicated. Frequent udder massage with gentle hand

milking may be helpful to relieve pressure in the affected gland to aid recovery. Strict attention should be paid to milk with holding instructions on the label of the product used. When mastitis cases are treated by a veterinarian, be sure that you follow milk withholding instructions given.

Mastitis in dairy goats is usually the result of defective milking management which gives the organisms the opportunity to spread and produce disease. Adequate sanitary preparation for milking which results in clean dry udders, clean dry milkers' hands or milking equipment are fundamental requirements for mastitis prevention. Regular use of the California Mastitis Test and/or Somatic Cell Counts can successfully monitor the progress of mastitis control and the health status of udders in the herd (Sharif and Mohamed., 2009).

Twenty eight Antibiotics udder treatments available are excellent for treatment of infected mammary glands, but success with their use is determined by the level of milking management and sanitation used in milking the herd. Of course, milk from treated does must not be withheld from human consumption according to label instructions; nor can meat of treated goats go to butcher before usually 30 days (Joanne, 2005).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

Descriptive prospective study.

3.2 Study area

This study was carried out in Khartoum State, specimen were collected from different farms from Khartoum North and Omdurman.

3.3 Study duration

The study was conducted during the period from May to October 2011.

3.4 Sample size

One hundred milk samples were collected from apparently healthy goats and thirty two from mastitic goats.

3.5 Sampling protocol

Milk samples were collected after the teat area was disinfected with a piece of cotton soaked in 70% alcohol, the first steam of milk was discarded, then 15 ml were collected from each halves in sterile capped bottles and immediately transported in an ice box to the laboratory for analysis or stored at 4˚C.

3.6 Laboratory investigations

3.6.1 California mastitis test (CMT)

The CMT is based on a reagent destroying the membranes of the somatic cells in milk and binding to the cellular DNA. This process results in an increase of the milk viscosity depending on the amount of cells, 100 milk samples were subjected to CMT by adding 4-5 drops from the CMT reagent (Alkyl Aril Sulphonate) to 3-5ml of each sample.

3.6.2 Preparation of the samples

From each sample, 10 ml were centrifuged at (x12000 rpm) and the deposit was inoculated in culture media.

3.6.3 Primary culture

Deposits of milk samples were streaked with a sterile loop onto Tryptic soya agar (TSA) or Brain heart infusion agar (BHA) media plates, incubated at 37 ˚C 18-24hrs, then checked for bacterial growth.

3.6.4 Purification of the isolates

Typical colonies from the primary culture plates were picked with a sterile loop and streaked on TSA, BHA, blood agar and MacConckey agar. Pure cultures were obtained by replanting the subculture two to three times.

3.7 Identification of isolates

3.7.1 Colonial morphology

Colonies identification was based in comparison of isolates with that described in Barrow and Felltham (1993) and Cheesbrough (2006). Where they were white, yellow to creamy, grey in colour, slightly raised and easily emulsified on slide and some are mucoid.

3.7.2 Gram's stain

This step was done to differentiate between Gram positive and Gram negative, cocci or bacilli. By taking small part from the colony smeared on the slide, fixed by flame then add crystal violet washed add iodine washed add safranein then dried examined under microscope by the oil immersion lens (x100).

3.8 Biochemical Identification

Selected biochemical tests were performed for identification of the isolates, tests were done according to standard protocol described by (Cheesbrough, 2006)

3.8.1 Catalase test

A drop of 3% aqueous solution of hydrogen peroxide (H_2O_2) was placed on clean sterile test tub, a small amount of the bacterium under test was mixed with the H_2O_2, and production of gas bubbles indicated the release of O_2 by catalase enzyme from the bacteria was considered as positive.

3.8.2 Coagulase test

A drop of sterile human plasma was placed in clean sterile microscopical slide. Loop full of the bacterium under test was mixed with the human plasma. production of clots indicated the release of coagulase enzyme from bacteria which was taken as positive result.

3.8.3 DNA-ase test

This test was used to help in the identification of *S.aureus* which produces the enzyme deoxyribonuclease (DNA-ase). Small parts from the colony of Gram positive coagulase positive was cultured in DNAase media, after 24hrs hydrochloric acid was flowed the precipitation of DNA indicated by clear zone around the colonies means positive result.

3.8.4 Urease test

Urea medium slope was inoculated with each of Gram negative bacilli strains incubated aerobically for 24 hrs at 37 ^{o}C and examined, a pink red colour indicated positive other wise negative.

3.8.5 Indole test

Testing for indole production is important in identification of enterobacteria. A Gram negative bacilli were inoculated in alkaline peptone water for indole production, incubated for 24 hrs after that kovac's reagent was added. Appearance of red surface layer indicated positive result.

3.8.6 Oxidase test

Apiece of filter paper was soaked with fresh few drops of oxidase reagent(Cytochrom Oxidase), a colony from Gram negative bacilli were smeared on the filter paper, blue –purple colour indicated positive result.

3.8.7 Citrate utilization test

Strains from Gram negative bacilli were inoculated in citrate broth checked after 24hrs, blue colour indicated positive result.

3.8.8 Manitol salt agar

A useful selective media for the detection of *S.aureus*, Gram positive cocci with coagulase positive were inoculated examined after 24h, change of the colour of the media to yellow colour indicated positive result.

3.8.9 KIA media

KIA slopes were inoculated with Gram negative bacilli incubated for 24hrs. Yellow colour of the slopes indicated lactose and glucose fermentation .Red colour in all slope indicated no sugar fermentation while cracking in slope indicated oxygen production.

3.8.10 Antibiotic susceptibility pattern of the isolates

All isolates was tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method, Muller and Hinton agar used for susceptibility test. Barium chloride standard (McFarland standard tube 0.5) used as turbidity standard, suspension prepared by taken one to four colony from tested organism in 1-2 ml sterile normal saline compare with McFarland standard, then cotton swab soaked in suspension and covered the media by it. Then

antibiotic discs of penicillin (p), gentamycin (Gn), amoxicillin (Amx) vancomycin (V), tetracycline, ciprofloxacin, novobiocin, methicillin, placed on the culture medium containing bacterial samples.

3.8.11 Preparation of suspension

The suspension was prepared by direct method by taking 4-5 colonies from each isolates transferred to 3-5ml normal saline in test tub. Mixed well and match against Mac Farland standard turbidity to adjust the density of the suspension. Then Muller Hinton is inoculated using sterile cotton swab. Disc of antibiotics were applied to the surface of media. Then incubated over night at 37˚C.

CHAPTER FOUR

4. RESULTS

4.1 California mastitis test (CMT)

Thirty three out of hundred milk samples were found positive for CMT due to gelling of the milk sample indicating subclinical mastitis.

4.2 Bacteriological results

A total of 132 samples, 100 samples of them apparently normal and (32) milk samples of mastatic goats were collected and subjected to bacteriological examinations. Thirty out of hundred (30%) from apparently healthy goats revealed bacterial growth indicating the presence of subclinical mastitis condition, whereas nineteen out of thirty two (59.37%) revealed bacterial growth from clinically mastitic goats (Table 1).

Table 1: Bacterial growth according to the type of mastitis

Type of mastitis	**culture**		
	Positive	**Negative**	**Total**
Subclinical mastitis	30 30%	70 70%	100 100%
Clinical mastitis	19 59.37%	13 40.63%	32 100%
Total	49 37.12%	83 62.87%	132 100%

4.3 Biochemical identification

4.3.1 Biochemical tests of Gram positive cocci

S.aureus was positive to Catalase, Coagulase, DNAase, Manitol salt agar and negative to Oxidase, CNS were positive to catalase only, but *Streptococcus* spp were negative to catalase and coagulase (Table 2.1).

Table2.1: Biochemical tests of gram positive isolates

Biochemical test / **Isolated species**	**Catalase**	**Coagulase**	**DNAase**	**MSA**	**Ox**
S. aureus	+	+	+	+	-
Coagulase negative *staphylococci*	+	-	-	-	-
***Streptococcus* spp**	-	-	-	-	-

- MSA : manitol salt agar
- Ox: Oxidase

4.3.2 Biochemical tests of Gram negative bacilli.

E.colli was positive to indole, kligler iron agar without H_2S gas production. Negative to Urea, Oxidase and citrate. *Pseudomonas aerogenosa* was positive to Oxidase and negative to all the above mentioned tests (Table 2.2).

Table 2.2: Biochemical test of gram negative isolates

Biochemical test / **Isolated species**	**Urea**	**Ox**	**Cit**	**Ind**	**KIA Medium**			
					Slop	**But**	**H2S**	**Gas**
Escherichia colli	-	-	-	+	Y	Y	-	+
Pseudomonas aerogenosa	-	+	-	-	R	R	-	-

Ox: Oxidase; **Cit:** Citrate; **Ind:** Indole; **KIA:** kligler iron agar; **Y:** Yellow; **R:** Red.

4.4 Bacteria from subclinical mastitis

Twenty two out hundred (22%) were Gram positive cocci, of which 5/22 (22.72%) were identified as *Staphylococcus aureus*, while 17/22(77.27%) were identified as Coagulase negative *staphylococci* (CNS). 8/100 (8%) were gram negative bacilli, where 6/8(75%) were *Escherichia colli*, and 2/8 (25%) were *Pseudomonas aerogenosa* (Table 3).

Table 3: Bacterial isolated from sub clinicallys mastitic goats

Isolated bacteria	**Positive samples (%)**	**Negative samples (%)**
Gram positive cocci		
Staphylococcus aureus	5/22 (22.72%)	17/22 (77.27%)
Coagulase negative *Staphylococci*	17/22(77.27%)	5/22(22.72%)
Gram negative bacilli		
Escherichia colli	6/8(75%)	2/8(25%)
Pseudomonas aerogenosa	2/8(25%)	6/8(75%)

4.5 Bacteria from clinical mastitis

Sixteen out of nineteen samples (84.21%) were Gram positive cocci, of which 5/16 (31.25%) were *Staphylococcus aureus*, 5/16 (31.25%) were CNS and 6/16 (37.5%) were *Streptococcus spp*. 3/19 (15.78%) were Gram negative bacilli of which 2/3(66.66%) were *E.colli* and 1/3(33.33%) was *Pseudomonas aerogenosa* (Table 4).

Table 4: Bacterial isolated from milk samples of clinically mastitic goats

Isolated bacteria	Positive samples (%)	Negative samples (%)
Gram positive cocci		
Staphylococcus aureus	5/16 (31.25%)	11/16 (68.75%)
Coagulase negative *staphylococci*	5/16 (31.25%)	11/16 (68.75%)
Streptococcus spp	6/16(37.5%)	10/16 (62.5%)
Gram negative bacilli		
Escherichia colli	2/3 (66.66%)	1/3 (33.33%)
Pseudomonas aerogenosa	1/3 (33.33%)	2/3 (66.66%)

4.6 Bacteriological result according to the location of collected sample

Out of 82 samples collected from Khartoum North 30(36.58%) revealed bacterial growth, while 19/50 (38%) from Omdurman revealed bacterial growth (Table 5).

Table 5: Bacteriological result according to the location of collected samples

Location	**No. positive**	**No. negative**	**Total**
Khartoum north	30 36.58%	52 63.41%	82 62.12%
Omdurman	19 38%	31 62%	50 37.87%
Total	49 37.12%	83 62.87%	132 100%

4.7 Relationships between location and type of mastitis

In Khartoum North 17/30 (56.66%) were subclinical cases, while 13/19 (68.42%) were clinical cases, whereas in Omdurman subclinical cases were 13/30 (43.33%) and clinical cases were 6/19 (13.57%) (Table 6).

Table 6: Relationship between location and type of mastitis

locations / **Type of mastitis**	**Khartoum North**	**Omdurman**
Subclinical mastitis	17/30(56.66%)	13/30 (43.33%)
Clinical mastitis	13/19(68.42%)	6/19 (31.57%)

4.8 Association between type of breed and infection

Thirty out of sixty (50%) Saanene were revealed bacterial growth while 19/72(26.38%) of the local breeds showed bacterial growth (Table7).

Table 7: Association between type of breeds and infection

Type of breed	Number of samples	Infection (%)
Saanene	60	30/60 (50%)
Local breeds	72	19/72(26.38%)
Total	132	49/132(37.12%)

4.9 Antibiotic susceptibility results

All identified isolates from both subclinical and clinical samples were tested to study their antibiotic susceptibility patterns. It was performed against the following drugs; vancomycin, gentamycin, erythromycin, novobiocin, amoxicillin, penicillin G, ciprofloxacin, tetracycline, and methicillin.

Gram positive cocci were found to be resistant to Methicillin 38/38 (100%) and penicillin G 18/38 (47.37%) and so was for the amoxicillin, on the other hand they showed higher sensitivity to Vancomycin 38/38(100%) and Gentamycin 35/38(92.1%).

Gram negative bacilli were found to be resistant to Tetracycline, while they were mostly sensitive to Ciprofloxacin (Table 8 and table 9)

Table 8: Antibiotic susceptibility of Gram positive isolates (S.aureus, CNS, and *Streptococcus spp*)

Antibiotic	*S.aureus* (no=10)				CNS (no=22)				*Streptococcus* spp(no=6)			
	susceptible		resistant		susceptible		resistant		susceptible		resistant	
	no	(%)	no	(%)	no	(%)	no	(%)	No	(%)	no	(%)
Vancomycin	10	100	0	0	*22*	*100*	*0*	*0*	6	100	0	0
Gentamycin	10	100	0	0	22	100	0	0	3	50	3	50
Erythromycin	8	80	2	20	17	77.3	5	22.7	4	66.7	2	43.2
Novobiocin	5	50	5	50	10	45.4	12	54.5	4	66.7	2	43.2
Amoxicillin	8	80	2	20	11	50	11	50	6	100	0	0
Penicillin G	7	70	3	30	13	59	9	41	5	83.3	1	16.7
Ciprofloxacin	10	100	0	0	*22*	*100*	*0*	*0*	6	100	0	0
Tetracycline	9	90	1	10	16	72.7	6	27.3	4	66.7	2	43.2
Methicillin	0	0	10	100	0	0	22	100	0	0	6	100

Table 9: Antibiotic susceptibility of Gram negative isolates (*E.colli* and *Pseudomonas aerogenosa*)

Antibiotics	*E.colli*(no=8)				p. *aerogenosa* (no=3)			
	susceptible		resistant		susceptible		resistant	
	no	(%)	no	(%)	no	(%)	no	(%)
Vancomycin	7	87.5	1	12.5	2	66.6	1	33.3
Gentamycin	6	75	2	25	2	66.6	1	33.3
Erythromycin	4	50	4	50	2	66.6	1	33.3
Novobiocin	3	37.5	5	62.5	1	33.3	2	66.6
Amoxicillin	5	62.5	3	37.5	1	33.3	2	66.6
Penicillin G	3	37.5	5	62.5	1	33.3	2	66.6
Ciprofloxacin	8	100	0	0	3	100	0	0
Tetracycline	6	75	2	25	2	66.6	1	33.3
Methicillin	2	25	6	75	1	33.3	2	66.6

CAPTER FIVE

5. DISCUSSION

Mastitis is one of the major diseases affecting dairy goats, causing major economic losses; Bacterial contamination of milk from affected quarters may pass for human consumption and provide a mechanism for spread of diseases to human such as tuberculosis and brucellosis (Omiema 2003; and Ibrahim *et al.,* 2009).

The main objective of this study was to isolate and identify the bacteria involved in subclinical and clinical mastitis among lactating goats in Khartoum State and to determine their antibiotic susceptibility patterns.

In the present study, 10/100(10%) the Nubian goat's milk showed no bacterial growth despite the bad hygiene, management practiced in the farms and type of milking so the frequency of bacterial species isolated from clinically normal goat's milk is affected by several factors factor such as breed difference which is clear in this study, a finding that that was consistent with that of (Ebrahimi, *et al.,* 2007).

In the current study, coagulase negative *staphylococci* (CNS) are the major group of the isolated bacteria in both clinical and subclinical mastitis in goats a finding that was in agreement with many studies around the world (Azizollah *et al.,* 2010, Ibrahim *et al.,* 2009 and Joanne, 2005).

However; *Staphylococcus aureus* in the present investigation constitutes about10/49(20.40 %) of the total recovered bacteria in both subclinical and clinical mastitis. This finding was in agreement with most of studies around the world (Azizollah *et al.,* 2010, Grant and Steve, 2001, Ribero *et al.,* 2007and Contreras *et al.,* 2007).

According to this study Gram negative bacilli especially *E.colli* were reported to play an important role in inducing subclinical mastitis.

In current study, CMT was used to predict the subclinical cases, where 33% of the collected specimens were found to be CMT positive. On culturing, only 3(3%) showed no bacterial growth, it may be due to antibiotic administration to these goats according to owners behavior.

In this study, 30/60 (50%) Saanene and Shame goats showed bacterial growth while only 19/72(26.38%) of the local breeds showed bacterial growth. This finding may suggest that foreign breeds (Saanene and shame) were more susceptible to bacterial infections than the native breeds this finding was in agreement with (Donkin and Boyazoglu 2004).

The isolated bacteria showed variable susceptibility patterns when they were subjected to antibiotic sensitivity tests. All tested Gram positive bacteria were found to be resistant for Methicillin 38/38 (100%), while all of them were sensitive for Vancomycin 38/38(100%). Sensitivity for Gentamycin, erythromycin, novobiocin, amoxicillin, penicillin, ciprofloxacin, tetracycline and methicillin among both Gram negative and Gram positive bacteria were variable. In conclusion the present study showed that gentamycin, vancomycin, and ciprofloxacin were very active against all isolated organism especially CNS.

Conclusions

In conclusion different pathogens (*Staphylococci, Streptococci, E.colli,* and *Pseudomonas*) were isolated and identified from subclinical or clinical mastitis.

The predominant causative agent of subclinical mastitis is CNS species, the use of CMT in the detection of subclinical mastitis cases helped in the protection of clinical cases occurrence, Currently gentamicin, vancomicin, and ciprofloxacin were very active against all isolated organism especially CNS.

Recommendations

1. It highly recommended using CMT in all milking farms for early detection of subclinical mastitis.
2. Bacterial culture method must be use for the detection of the causative agents and their antimicrobial susceptibility pattern.
3. Further research is required to determine the most effective antimicrobial agents.

REFERENCES

Anonymous,M (2003). Mastitis in dairy cow. Department of animal science faculty of agriculture and environmental science, McGill University, **Anne de Bellevue**, Canada; Pp 12.

Azizollah, E., Naser, S., Somayeh, S., Pezhman, M. (2010). *Staphylococci* isolated from mastatic goats milk in Iranian dairy herd. J. vet. World. **3(5)**: 205-208.

Batanani, R. A., Asri, **S**., and Naebzadeh, H. (2007). The effect of subclinical on milk composition in dairy cow. Iranian. J. vet. Research. **8(3):** 205-210.

Catherine, L.and poutrel, B. (1984). Characteristic of non clinical mammary infection of goats. Ann. Rech. Vet. **15(1):** 105-112.

Cheesbrough, M. 2006. District laboratory practices in tropical countries.2nd Ed. New York: Cambridge University Press; (Pt).

Contreras, A., Corales, J.C., Sanchez, A. and Sierra, D. (1997).Persistent of intramammary pathogen in goats through lactation. J. Dairy. Sci. **80**: 2815-2819.

Contreras, A., Sierra, D., Sanchez, a., Corrales, J. C., Marco, J. C., Paape, M. J., Gonzalo, C. (2007). Mastitis in small ruminants. Small. Ruminant. Research. **68**:145-153.

Dafaalla, E. N. and Gharib, H. M. (1958). A study of mastitis on goats caused by Nocardia asteroid. Brit. Vet. J. 114:143-145.

Devendra, C. and Mcleray, B. G. eds (1983) Goat & sheep production in tropic. Pp74-83.

Donkin, E.and Boyazoglu, P. (2004). Disease and mortality of adult goats in a South African milk goat herd .south. afr. J.animal.sci.**34:**254-257.

Ebrahimi, A., Lotfalian, sh. And Karimi,S.(2007)Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in sharekord district. Iranian. J. vet. Research. **8(1):**76-79.

Ghaleb, A., Dauod, A., Rateb, A., Jamal Abo, O. (2005). Prevalence of microorganism associated with intramammary infection in cow and small ruminant in north of Palestine. The Islamic University of Gaza.**13 (1):** 165-173.

Grant, M., T And Steve, P. (2001).the mastitis problem. Goat. Field. Day. **16**: 6-9.

Ibrahim, A., Kursat, K., Hasi, A. (2009).Identification and Antimicrobial susceptibility subclinical mastitis pathogen isolated from hair goat's milk. J.animal.Vet.Advances.**8 (6):**186-1090.

Joanne, K. (2005).Intramammary antibiotic in dairy goats: with drawal period and tissue tolerance (MSc) University of Pretoria.

Leitner, G., Merin, U.and Silanikove, V., (2004). Change in milk composition as affected by sub clinical mastitis in goats'. Dairy.Sci.**87:**1719-1726.

Mohammad, R. I., Mohamed, S. A., Mohamed, M. R., Tazminaz, S. (2011). Identification and antibiotic sensitivity of the causative organism of subclinical mastitis in goats & sheep. Pakistan. Vet. J. **31:** 1-5.

Omiema, A.G. (2003). Caprine mastitis caused by Nocardia and related actinomycetes (the MSc) University of Khartoum.

Poutrel, B., Decremouex, R., Ducelliez,M. and Verneau,D. (1997). Control of intramammary infection in goats: Impact on somatic cell count. J. Anim. Sci. **75:**566-570.

Ribeiro, M. G., Lara, G. H. B., Bicudo, S. D., Souza, A. V. G.(2007). An unusual gangerenous goat mastitis caused by staphylococcus aureus, clostridium perfringens and Escherichia colli co infection. Med. Vet. Zootec. **59(3):** 810-812.

Sharif, A. and Mohamed, G. (2009). Mastitis control in dairy animals. Pakistan. Vet. J. **29(3):** 145-148.

Silanikove, N. A., Shamay, D. and Moran, A. (2000). Stress down regulates milk yield in cow by plasmin induced ß casein product that blocks k^+ channels on the apical membranes. Life. sci. **67:** 2201-2212.

Tanga, S. and Karen, A. (2010) Intra mammary infection in dairy goats: recent knowledge and indicator for detection of sub clinical mastitis. Vti.agriculture and forestry research4. **60:**267-280.

APPENDIX

Preparation of culture media and biochemical test

1.1Culture media

1.2 blood agar

A non selective media for the isc of many pathogenic and non pathogenic microorgani n used to observate the forms of haemolysis from pa ns.

This culture medium can be used without blood for setting up blood culture and as base for preparing special culture media.

Composition:

Ingredient s	Grams/Liter
Meat extract …… ……..	10.0
Peptone……	10.0
Sodium chloride …….	5.0
Agar ……	15.0

Directions:

Suspend 40 gm in 1litre of distilled water. Bring into boil for completely dissolve. Sterile by autoclaving at 121˚C for 15 minutes. For blood agar, cool at 45-50˚C and add a aseptically 6% (5-10%) sterile defibrinated blood.

Tryptic soya agar (difco 0369 -01-4)

Tryptic soya agar 40gm, distilled water 1 liter, pH 7.2, autoclave at 121C for 15 minute.

Brain heart agar (Merck, G) 1.13825.

Brain heart agar 52g, Distill water 1 liter, sterilized by autoclaving at 121 C for 15 minute then distributed in sterile Petri dish

1.4 Mueller Hinton agar NO 2M1084

Mueller Hinton Agar No 2 is used for testing susceptibility of common and rapidly growing bacteria using anti microbial discs by the Bauer-Kirby method. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

Composition

Ingredient	GMS/liter
Casein acid hydrolysate……	17.500
Beef heart infusion…….	2.000
Starch, soluble ……...	1.500
Agar……	17.000
Final pH (at25 C)……	7.3±0.2

Directions:

Suspend 38 grams in 1000 ml distilled water. Heat boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121C) for 15 minute. Mixed well and dispense as desired.