بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Studies on Clinical, Aetiological and Antibiotic Susceptibility of Mastitis in She-camel (*Camelus dromedarius*) in Butana area, Sudan

دراسات سريرية، المسببات واستجابتها للمضادات الحيوية لإلتهاب الضرع في إناث الإبل في البطانة-السودان

A Thesis submitted in fulfillment of the requirements of the Graduate College for the degree of master of Veterinary Medicine (Medicine and Therapeutics)

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الآيـة

قال تعالى:

(وَمِنَ الْأِبِلِ اثْنَيْنِ وَمِنَ الْبَقَرِ اثْنَيْنِ)

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(الإبل عز لأهلها) .

وقال أيضاً:

(الفخر والخيلاء لأصحاب الإبل).

Dedication

To the soul of my father

To my mother who surrounded me with love and care

To my grandmother, brother and sisters for their encouragement and support

To my wife to whom I live and breathe

To best friend Fisal Aseel

To all whom I love, I dedicate this work.

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TABLE OF CONTENTS

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER THREE: RESULTS

List of Tables

List of Figures

Abstract

This study was performed to investigate the types of clinical mastitis among camel herds reared in Butana region. The effects of season, age, stage of lactation, number of calving on occurrence of mastitis causative agents and antibiotic sensitivity of the bacterial isolates from mastitic she-camel were included. The study also involved treatment trial in Tamboul area for selected cases of acute and chronic mastitis. The occurrence of clinical mastitis among 319 milking camels during both summer and winter were found at the rate of 9.09% (29 case). Among camels with anti-suckling devices 69% (20 case) were found to be mastitic. Tick infestation which causes teat lesions was also found to increase risk to mastitis.

Three forms of clinical mastitis were diagnosed according to the obvious clinical signs and the visible alteration of milk. Chronic form was of the highest occurrence (72.41%) followed by acute form (24.14%) and the least was the gangrenous form (3.45%).

The highest occurrence of clinical mastitis was found at the age between 11-15 years (65.52%). Few incidence of mastitis were detected in first stage of lactation (17%) and increased at the middle stage (28%) and the highest was found at late stage of lactation (55%).

This study revealed that there was a direct relationship between number of calving and the occurrence of clinical mastitis. The highest incidence of clinical mastitis was found at the first, second and third calving (65.52%).

The percentage of isolated Gram positive bacteria from clinical mastitis was 81.08% and Gram negative bacteria were 18.92%. The predominant isolated organism was *Staphylococcus spp.* (37.8%) followed by *E.coli* (18.9%), *Streptococcus spp.* (13.5%), *Bacillus spp*. (10.8%), *Micrococcus spp.* (8.1%), *Corynebacterium spp*. (5.4%) and *Salmonella spp.* (5.4%).

The antimicrobial susceptibility test of the isolated bacteria generally showed high susceptibility to the most of the examined antimicrobial agents. There was high sensitivity to Gentamicin, Ciprofloxacin, Cloxacillin and Amikacin, moderate sensitively to Ampicillin/Sulbactam and Trimoxazole and the greatest resistance was found with Tetracycline and Chloramphenicol.

The treatment trial for mastatic cases showed that the most effective antimicrobial drugs for camel mastitis was Pen&Strep®(Procaine Penicillin, Dihydrostreptomycin Sulphare).

الخلاصة

تم إجراء هذه الدراسة لتحقيق مفصل عن انواع التهاب الضرع السريري في قطعان الإبل التي تربى في منطقة البطانة. وقد تم التحقيق من تأثيرعوامل الموسم والعمر ومرحلة الأدرار، وعدد الولادات على حدوث التهاب الضرع و أيضا العوامل المسببة والحساسية للمضادات الحيوية للعزلات البكتيرية من ضرع النوق المصابة. شملت الدراسة أيضا تجربة للعلاج في منطقة تمبول لحالات مختارة من إلتهاب الضرع الحاد والمزمن. نسبة الإصابة بالتهاب الضرع السريري بين 319 من الإبل الحلوب خلال فصلى الشتاء و الصيف و جدت بمعدل 9.09%(29 حالة). بينما وجدت 69% (20 حالة) من الإبل المصابة بالتهاب الضرع مستخدمة لإدوات منع الرضاع الرضاعة. كما وجد أيضا ان الأفات الموجودة في الحلمات و الناتحة من تفشي القراد تذيد من خطر الإصابة بالتهاب الضرع.

تم تشخيص ثلاثة أنواع لإلتهاب الضرع السريري عن طريق العلامات السريرية الواضحة و التغييرالمرئي في الحليب. كان الشكل المزمن هو الأعلى معدل للإنتشار (72.41%)، يليه الشكل الحاد (24.14 %) و أخيراً كان الشكل الغرغريني(3.45%).

وجدت أعلى نسبة حدوث لإلتهاب الضرع السريري في الأعمار بين 15-11سنة كانت (65.52%). و جدت حالات قليلة من إصابات إلتهاب الضرع في المرحلة الأولى من الإدرار كانت(17%) وارتفعت في المرحلة المتوسطة (28%) وعثر على أعلى المستويات في المرحلة المتأخرة من الإدرار (55%).

وجدت هذه الدراسة أن هناك علاقة مباشرة بين عدد الولادات وحدوث إتهاب الضرع السريري .حيث وجدت أعلى نسبة حدوث بالتهاب الضرع السريري في الولادات الأولى والثانية والثالثة كانت (65.52%).

نسبة البكتيريا الموجبة لصيغة جرام المعزولة من التهاب الضرع السريري كانت 81.08% والبكتيريا السالبة لصيغة جرام كانت 18.92%. الجراثيم المعزولة الغالبة هي المكورات العنقودية (37.8%)، يليه إسكيريتشيا القولونية (18.9%)، العقدية (13.5%)، العصوية (10.8%)، ميكروكوكس(8.1%)، الوتدية(5.4%) والسالمونيلا(5.4%).

أظهرت العزلات الجرثوميه عموما حساسية لمعظم الأدوية المضادة للميكروبات المستخدمة. وكان هناك حساسية عالية لجنتاميسين، سيبروفلوكساسين، كلوكساسيلين والأميكاسين .وحساسية معتدلة إلى الأمبيسلين وموكسازول وأكثر مقاومة وجد مع التتراسيكلين والكلورامفينيكول.

التجربة العلاجية لعدد من حالات التهاب الضرع أظهرت ان اكثر الأدوية المضادة للميكروبات فاعلية كانت(بروكايين البنسلين، ثنائي هيدروستريبتوميسين) Pen& Strep ® .

Introduction

According to animal population Sudan ranks the first in the Arab world and second in African. Concerning camels population Sudan is the second largest country worldwide with in camels population estimated at more than four millions (Ministry of animal Resource, 2013). Camels in the Sudan are spread in a belt configuration; it extends between latitudes 12˚ -16˚ N. (Eisa and Mustafa, 2011).

Camels play a central role as milk suppliers. Under the difficult environmental conditions camels produce more milk and at a longer period of time than any other dairy animals (Farah, 1996). Also they re-hydrate very quickly (Abdelmagied and Taha, 2003).

Camel milk is one of the main components of diet of the nomads in semiarid and arid zones and is an essential food for livelihood of people and it may be the only milk available in places where other milking animals cannot be maintained (Abdurahman, 2006). Little work has been done on mastitis in camels comparing to studies on sheep and cows. During a decade ago mastitis in the camel has been reported from a number of camel-rearing countries of the world (Khedid *et al*., 2003; Al-Ani, 2004 and Mohammed *et al.*, 2005),but little attention of mastitis as a problem was paid at herd level.

Mastitis is a complex disease occurring world-wide among dairy animals, with heavy economic losses (AL-Ani, 2004). It can be defined as an inflammation of parenchyma tissue of the mammary gland, regardless of cause. It is therefore characterized by a range of physical and chemical changes in milk and pathological changes in the glandular tissue, there are swelling, heat, pain, and edema in mammary gland. In clinical cases the most important changes in milk include discoloration, presence of clots and presence of large number of

Leukocytes (Radostits *et al*., 2007) . It takes two forms: clinical mastitis and subclinical mastitis (Matofari *et al.*, 2005).

Mastitis has both an extreme zoonotic and economic importance. It is the cause of multiple hazardous effects on human health and animal production (Hegazy *et al.*, 2004 and Al-Majali *et al.*, 2008).

Many different bacteria have been isolated from mastitic mammary glands in camels either in the form of pure or mixed infection (Younan et *al*., 2001; Hegazy *et al*., 2004; Sanaa, 2005; Abdurahman, 2006; Abdel Gadir *et al.*, 2006; Kalla *et al.*, 2008*;* AL-Tofaily and Al rodhan, 2011; Alamin *et al.*,2013) There are various studies which have been conducted worldwide on the isolation and identification of bacterial organisms in mastitic camel milk and their effect on quantity and quality of milk.

Acute or chronic mastitis is one of the important diseases of she-camel in Eastern Sudan. The isolates *Staphylococcus aureus, Escherichia coli, Corynebacterium spp.* were the main causes of mastitis in camels (Amel, 2003; Suheir, 2004 and Sanaa, 2005).

Objectives of the study:-

General objective:

To study the clinical status of mastitis problem among she-camels in Butana region.

Specific objectives:

1-Clinical identification of the different types of mastitis in she-camel in Butana region.

2-Isolatation and identification of the bacterial causative agents.

3- Determination of the antibiotic sensitivity of the isolates.

4- Trials of different regimes of therapeutic management of mastitis.

Chapter one

Literature Review

1.1Camels in Sudan:

The history of the dromedary camel in Sudan is obscure. It is believed camels have entered the Sudan from Egypt, dating about 2980-2474 B.C., the oldest evidence is a bronze figure of camel with saddle found at Merwi dated between 25-15 B.C. (Adison, 1934 and Robinson, 1936). Probably the camels entered the Sudan through three routes: the first route through North West Africa during the 4^{th} to 6^{th} century, the second was the Egyptian route and the third route was via the Red Sea (Salman, 2002).

In many semiarid and arid regions of the Horn of Africa (e.g. Sudan), camel keeping is the most sustainable livestock enterprise. Due to climatic changes and desertification, cattle numbers are decreasing in such regions while camel numbers are increasing (Eisa and Mustafa, 2011). The camel is of significant socio-economic importance in many arid and semi-arid parts of the world and its milk constitutes an important component of human diets in these regions (Elhatmi. *et al.*, 2007). According to breeds camels are used for racing, ridding or as back-animals (Gillispie, 1962 and Elamin, 1979). Camel is used for other purposes including meat, milk and wool. Camel's Milk is used for treatment of some diseases.

Camels in the Sudan are spread in a belt configuration; it extends between latitudes 12˚ -16˚ N.(Wardeh, 1989).This belt is characterized by erratic rainfall, less than 350mm. Camels are found in Eastern Region (Butana plain , Red Sea hill) and the Western Regions(Darfour and Kordofan) (Eisa and Mustafa, 2011). In Butana area of Sudan camels are commonly raised under nomadic conditions in a geographical zone, which lies approximately between latitude 14-16 N and longitude 33-36 E. Atbara River to the East, River Nile to

the West and Blue Nile bind the area to the South and Southwest (Elamin, 1979). 25.7% of the country camel stock is found in the Eastern region of Sudan (Dorosa, 2005).

The following breeds exist in Sudan based on conformation and tribal ownership. First the Arab breed of camel is a sandy-grey, large, heavily built animal used as a pack animal for draught work. This breed is widely distributed in northern Darfur and northern Korodofan provinces, and is bred by the Kababish, Hamar, Kawahla, Zaghawa, Maidob and Zayadya. A mature animal weight about 400-500kg and can carry about 275kg over 25-30 km per day. Second the Rashaida camel is aurburn in color, has a heavy body weight, and is used as pack animal. The Rashaida nomeds of eastern Sudan herd this breed. Third the Sudanese riding camel is light weight and runs fast. This breed includes two types: the Anafi camel and Bishari camel. Both types are very fast and used for racing (AL-Ani, 2004). The main camel keeping tribes in Butana region are the *Lahawiyin*, *Kawahla*, *Shukriya*, *Rashaida*, *Bija* and *Bawadra* (Dorosa, 2005).

Herd family members of camels in Butana region are found in a percentage of male: female ratio 60% versus 40%, respectively (Sakr and Majid, 1998) and another study reported the percentage of male: female ratio 57.2% versus 42.8%, respectively (Darosa, 2005).

1.2 Anatomy of udder in she-camels:

In the prepuberal and nulliparous females, only the small teats are visible as the mammary tissue does not develop until the end of the first pregnancy. At the peak of lactation, udder increases in size and shows well-developed venous drainage. The udder of the camel consists of four glandular quarters, each with its own teat (Nosier, 1974). The left and right halves of the udder are separated from each other by fibro elastic tissue extending from the linea alba and

glandular units of the lobule, the alveoli or acini, are separated from each other by the interlobular connective tissue (Smuts and Benzuidenhout, 1987). The duct system begins with small interlobular ducts that enlarge progressively and each duct is lined by an epithelium resting on a distinct basement membrane. The duct epithelium is low, simple and secretary in the smallest interlobular duct but becomes columnar in the larger ducts (Nosier, 1974). The color of mammary gland is brown to black tinge. The anterior and posterior quarters are independent, but no visible separation between them is observed. The teats are directed cranio-ventrally, but the conformation of teats changed markedly with change in physiological state, turned noticeably round at the tips in lactating females. The circumference and diameter of teat increased from tip to base. The most striking feature observed was the presence of two-streak canals in all four teats of female camels which are longer in lactating periods (Kausar , 2001)**.** There is a great variety in different udder and teat shapes and sizes of she-camels according to age and stage of lactation (Albrecht, 2003 and Wernery *et al.,* 2004). In lactating animals, mammary gland is characterized by major changes including increase in number of alveoli, alveolar lumen and decrease in connective tissue (Holland and Holland, 2005 and Patel *et al.,* 2007).

Ultrasonographic appearance of mammary gland and teat demonstrated that teat wall could be divided into 3 layers and the base of the teat, the annular folds, appear as a hyperechoic linear structure extending into the lumen. The glands of sinuses appear as an anechoic area continuous with the teat sinus. The lining of the wall of the glands sinus appear as mixed hyper to hypoechoic areas within the hypoechoic material of the glands(Abshenas *et al.,* 2007).

1.3 Camel milk:

Milk is an important nutrient in human nourishment. In some communities, camels represent the most important source of this nutrient. Some projects, for example, the one sponsored by SNV (Netherlands Development

Organization) through the Resource Mobilization Center in Kenya, have demonstrated that the rational use of this animal is highly valuable for feeding poor populations (Musinga *et al.,* 2008).

The milk is either consumed in the raw state (fresh), soured or used to produce yogurt or cheese. There is no need of its being boiled as much as cow or goat milk. It has a strong flavor and salty taste because camels are fond of grazing on sodium-rich herbs and shrubs. It must be drunk slowly to allow the stomach to digest it. Consequently it has an apparent effect, especially on the foreigner (person who drank it in first time); but after a short time usually gets accustomed to it, likes it very much and suffers no ill effects (AL-Ani, 2004). Also camel milk has properties that it can be kept for long periods than cow’s milk when refrigerated and even with the desert heat it does not spoil shortly (Thiagarajan, 2001). Moreover, the milk composition of dromedary camel is excellent from a nutritional view point (Gran *et al.*, 1991). Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and higher concentration of vitamin C in comparison with cow milk (Barłowska *et al.*, 2011). It can be considered as a good source of minerals, vitamins and characterized by higher ratio of lactoferrin Moreover, camel milk could meet a big part of the daily needs of humans from these nutrients because camel milk has most the essential nutrients (Al-Otaibi and El-Demerdash, 2013).

The milk of camel has several beneficial characteristics, such as the absence of diabetes in populations that consume it (Agrawal *et al.,* 1984**)** and tolerance by patients who show intolerance to lactose. Even though camel milk does contain lactose (Cardoso *et al.,* 2010), it is a nutrient for individuals who are allergic to cow milk (Ehlayel *et al.,* 2011 and Shabo *et al.,* 2005). Also is much more nutritious than that of cow milk because it is low in fat and lactose contents, and higher in potassium, iron and vitamin C (Anonymous, 1996).

Camel milk has medicinal properties and contains protective proteins, which may have a possible role for enhancing the immune defense mechanism (Yagil, 1982). Its specific properties, particularly its anti-infectious action, should be used to replace other milks (Roberto *et al.*, 2013). The triglycerides, which contain a great variety of fatty acids, are accompanied with small amounts of di- and mono-acylglycerols, cholesterols, free fatty acids and phospholipids (Christie and Clapperton, 1983). The ability of camel milk to inhibit growth of pathogenic bacteria and its relation to whey lysozyme has been demonstrated by Barbour *et al.* (1984).

The milk let-down of camels is usually stimulated by a suckling calf and is of short duration. Therefore, the calf is quickly removed and the camel is milked by milkers on both sides of the animal simultaneously. There are a number of scientific reports concerning the milk yield of camels in nomadic areas of the world (Knoess , 1976). Machine milking of camels has been carried out in Russia, Saudi Arabia, United Arab Emirates and India. The calf is still to initiate letdown, but exogenous oxytocin has also been used. Unlike cows, camels do not milk in the udder, and any distraction at milking can stop the milk flow entirely (Al-Ani 2004).

Camel dairy farming has not been properly developed. However, in certain countries such as Saudi Arabia, United Arab Emirates, Mauritania, and Kazakhstan large-scale camel dairy farms have been established. Camel's milk is one of the most valuable food resources in arid and semi-arid zones. Camel milk products such as ice cream, butter, cheese, yogurt and fermented camel milk have been produced (AL-Ani, 2004).

1.4 Camel breeds for milk production:

Dairy breed camels are characterized by high milk production which is not less than 2,500kg of milk per year produced under natural grazing

conditions. The general appearance is characterized by well-developed udder, prominent milk vein, small hump and less beef body conformation with relatively big abdomen (Kohler-Rollesfson *et al.*, 1990 and Kohler-Rollesfson and Rathore, 1995).

The main breeds for milk production in camel (*Camelus dromedarius*) recorded by (AL-Ani, 2004) are: Rashaida breed is found in the Kasala area of Eastern Sudan and are raised by the well-known Rashaida tribe. The milk production ranged between 2000-3000kg/year. The average milk production per years is around 3500 liters. They withstand harsh desert condition well. Pakistani breed is originated in Pakistan has real production potential for milk. A good Pakistani milking camel can produce more than 5000 liter in 365days. Shallageea breed or Coast breed is found at coastal strip of the Red Sea northeast of Sudan. Upon milking three times a day each animal gives a daily yield of 15-18kg and gives about 18-21kg of milk daily. Average yearly milk production is around 3000kg. Sirtawi breed is found mainly in the Sirt area in the middle coastal zone in Libya. Annual milk production is between 3000 to 4000kg. Ould Sidi Al-Sheikh breed is found in the Ain Safra area among the north eastern borders of Mauritania, the south eastern borders of Morocco and south western borders of Algeria. Under desert conditions, milk production is about 2000kg annually. However, milk production might reach 3500kg/350 days under good feeding conditions. Fakhreya breed is found in southern and western areas of Benghazi in Libya. Milk production under natural feeding is around 3500kg annually. Hoor breed is the most common type of camel distributed in different parts of Somalia. A small body size, short legs and white color characterize general features of camels in this breed. Average milk production in these animals is 7-8 kg/day. Average yearly milk production is around 2000kg.

Data on the actual amount of milk produced by Sudan camels are not very accurate for judging the milk yielding capability. Mainly because camels exist in desert areas with difficult accessibility, calves are still suckling and therefore, the actual volumes of milk secreted are higher than the figures presented among the different herds and breeds studied. On the other hand milking frequency varies among the different camel nomads. Camel may be milked twice a day among the Rashaida tribe and from two to five times or more among the other nomads in Sudan (Eisa, 2006).

1.5 Mastitis in Camel:

Mastitis is a complex disease occurring world-wide among dairy animals, with heavy economic losses. Mammary infections results in milk compositional changes such as increase in leukocyte counts, leakage of plasma proteins into the milk and cell damage, resulting in leakage of intracellular constituents into milk, change in ion composition and decrease in milk production (Bhikane and Kawitkar, 2000 and AL-Ani, 2004). This result in reduced milk yield, degradation of milk quality and additional cost in the care and treatment of mastitis (Eyssu and Bekele, 2010). Incidence of mastitis may increase in dairy camel due to hand milking and teat malformation (Almaw and Molla, 2000). Cases of mastitis in camels have been reported from a number of camel keeping countries including Egypt (Mostafa *et al.,* 1987 and Younan and Abdurahman, 2004), Saudi Arabia (Barbour *et al.*,1984; Saleh and Faye, 2011and Aljumaah *et al.,* 2011), United Arab Emirates (Al-Juboori *et al.,* 2013), Iraq (AL-Tofaily and Alrodhan, 2011), Jordan (Hawari and Hassawi, 2008), Morocco (Khedid *et al.,* 2003),Ethiopia (Abdel Gadir *et al.,* 2006 and Abera *et al.,* 2010), Kenya (Younan, 2002; Matofari *et al.,* 2005 and Wanjohi *et al.,* 2013), Pakistan (Ahmad *et al.,* 2012) , Nigeria (Shittu *et al.,* 2012), India (Mody *et al.,* 1998), Israel (Guliye *et al.,* 2002) and from different parts of Sudan (Obied *et al.,* 1996; Amel, 2003; Suheir, 2004; Sanaa, 2005 and Alamin *et al.,* 2013).

The causative agents of bovine mastitis are well defined. There is an extensive literature on bovine mastitis and to a lesser extent on ovine and caprine mastitis. In contrast, there is paucity of information about the etiological agents associated with camel mastitis. Few available studies indicate that some bacterial infections have been implicated as causes of mastitis in camels. Some of these are *Staphylococcus aureus, Streptococcus spp.* (Younan *et al.,* 2001; Amel, 2003; Suheir, 2004; ;Sibtain *et al.*, 2012; and Alamin *et al.,* 2013), *Micrococcus spp.* (Al-Ani and Al-Shareefi, 1997 ; Hawari and Hassawi, 2008 and Al-Juboori *et al.*,2013), *Streptococcus agalactiae* (Younan *et al.,* 2001 ; Abera *et al.,* 2010 and Husein *et al.,* 2013), coagulase negative staphylococci (Abdurahman *et al.,* 1995), *Staphylococcus epidermides, Pasteurella haemolytica* (Al-Ani and Al-Shareefi, 1997 and Hawari and Hassawi, 2008), *Escherichia coli* (Al-Ani and Al-Shareefi, 1997; Kalla *et al.*, 2008 and Eyassu and Bekele, 2010). and *Corynebacterium spp* (Barbour *et al.*, 1984; Abdel Gedir, 2001; Suheir, 2004 and AL-Tofaily and Alrodhan, 2011).

Camel mastitis has been estimated to affect more than 25% of lactating she-camel (Saleh and Faye, 2011 and Alamin, *et al.*, 2013). It is also known to cause approximately 70% losses in milk production (Fazhani *et al.*, 2011). Mastitis can be divided into subclinical mastitis and clinical mastitis.

1.5.1 Subclinical mastitis:

Subclinical mastitis is very common but cannot be detected by physical examination of either the camel or udder or milk. However, there can be large numbers of somatic cells produced by the inflammation in the affected gland. In such cases the diagnosis of mastitis depends largely on the leukocyte count of milk by indirect tests such as California Mastitis Test(CMT) and Somatic Cell Count (SCC) as well as bacteriological examination (Abdurahaman *et al.*,1996; Obeid and Bagadi, 1996 and Almaw and Molla, 2000). The results in milk with

a high somatic cell count (SCC) which is expressed as cells/ml, with subclinical mastitis can contribute a significant proportion of bulk tank (SCC). If found above 250 000 cells/ml detected that quarter of she-camel was affected with subclinical mastitis (Radostits *et al.*, 2000).

1.5.2 Clinical mastitis:

Clinical mastitis causes abnormalities in udder or milk and these can be detected during physical examination and systemic signs. The clinical mastitis in camel is diagnosed by palpation and examination of udder or milk, acute mastitis has been reported to occur during the first few days following parturition by alarming including anorexia, fever, general depression, swelling, severe inflammation and pain of the udder (Quandil and Oudar, 1984; Obeid and Bagadi, 1996 and Tibary and Anuassi, 2000). Chronic mastitis can be observed by presence of pus or high bacterial cell count using California Mastitis Test (CMT), atrophy of one or more quarters and presence of pustules on the surface (Barbour *et al.*, 1984,Saad and Thabet, 1993).

1.6 Camel mastitis in Sudan:

In Sudan the first investigation of mastitis in camel has been done by filed survey in Eastern Sudan (Obied, 1983). A total of 763 milk samples were tested, many bacterial organisms were isolated. The most common isolated organisms were *Streptococcus aglalactiae* (26.5%), *Staphylococcus aureus* (16.9%), *Staphylococcus albus*(6%), *Streptococcus spp*(1.7%), *Micrococcus spp*(1.6%) *and Coliforms*(0.7%).

Salwa, (1995) collected and tested a total of 180 milk samples form Eastern Sudan. The bacterial isolates were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus aglalactiae, Micrococcus spp, Aerococcus spp, Corynebacterium spp,Bacillus spp and Fungi.*

Nuha, (2001) studied Staphylococci in normal and mastitic milk of cow, goat and she-camel in Khatoum, a high prevalence of Staphylococci in normal camels were *Staphylococcus epidermidis, Staphylococcus chromogenes, Staphylococcus lugdunenes, Staphylococcus caesolyticus, Staphylococcus hyicus* and *Staphylococcus hominis.* And from mastitic she-camel was *Staphylococcus aureus.*

Amel, (2003) collected 269 milk samples from she-camels at Red Sea Area. The clinical mastitis was diagnosed in 16 cases (6.15%) and the bacterial isolates were *Staphylococcus aureus* (27.27%*), Staphylococcus chromogenes* (13.6%*), Staphylococcus caesolyticus* (4.54%*), Staphylococcus epidermidis* (4.54%*), Staphylococcus simulans* (4.54%*), Streptococcus aglalactiae* (18.18%*), Escherichia coli*(18.18%*), Micrococcus spp* (4.54%*)* and *Proteus panneri* (4.54%*),*while the sub-clinical mastitis was diagnosed in 101 cases (38.85%) by CMT and Leukocyte count.

Suheir, (2004) investigated sub-clinical mastitis by the California mastitis test(CMT) for a total of 160 milk samples; 85 samples from Kordofan, 60 from Khartoum and 15 from Port Sudan. The bacterial isolated were *Staphylococcus aureus, Staphylococcus spp, Streptococcus aglalactiae, Streptococcus spp, Enterobacteria spp, Micrococcus spp, Corynebacterium spp, Bacillus spp, Mycoplasma arginini and Fungi.*

Sanna, (2005) conducted a field survey of bacterial diseases of the reproductive system of camel in Eastern Sudan. This author reported that acute and chronic mastitis among the most important diseases and the isolated bacterial were *Staphylococcus aureus* (38.7%), *Staphylococcus spp* (4.3%), *Escherichia coli* (36.3%) and *Corynebacterium spp* (30.7%) .

Alamin *et al.,* (2013) in North Kordofan State showed that the most predominant causes of mastitis in camel were *Staphylococcus spp*. (80.30%)

followed by *Bacillus spp.* (9.09%), *Pasteurella spp.* (6.06%), *Corynebacterium spp.* (3.03%) and *Streptococcus spp*. (1.52%).

1.7 Predisposal factors of camel mastitis:

Traditional husbandry systems and bad milking habits include tying the teats with soft bark to prevent the calf from suckling and cauterization of the udder skin by the piece of wood and cloth, which aggravates the existing lesion like wounds on teats and leaves behind scar tissue. Through these wounds the *Staphylococcus spp.* which is usually found in wounds, may invade the mammary gland tissues and contribute to the development of mastitis in camels (Younan and Abdurahman, 2004 and Alamin *et al.*, 2013).

Tick infestation causes skin lesion which may facilitate bacterial entry and leaves behind permanent tissue damage especially by *Staphylococcus spp.* and *Streptococcus spp.* In a limited study in Kenya, 22% of tick bite lesions were shown to harbour *Streptococcus agalactiae* (Younan and Abdurahman, 2004). In one study in Ethiopia, 72% of udders were infested by ticks. The incidence of mastitis was higher (30%) in heavily infested udders than in non-infested udders (9%) (Almaw and Molla, 2000). Mastitis prevalence was significantly affected by tick infestations according to study reported by Abera *et al*., (2010).

Camel-pox was an important predisposing factor; causes skin lesions on teats or canal orifices. It is a contributing factor in spreading the intramammary gland infection caused by *Streptococcus agalactiae* (Younan *et al.,* 2001*)*. Teat canal blockage with dilatation of the gland is a common predisposal factor for camel mastitis (Younan *et al.,* 2001).

1.8 The important causative agents of she-camel mastitis:-

1.8.1 *Staphylococcus aureus:*

Staphylococci are Gram-positive cocci, catalase positive and oxidase negative. *Staph aureus* is the most important cause of mastitis and in many cases of mastitis begins as a consequence of the penetration of pathogenic bacteria through the teat duct in to the interior of the mammary gland (Quinn *et al.*, 1994).

The capacity to coagulate plasma, the principal characteristic of the *Staphylococcus aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host (Murray *et al.*, 2006), and can contaminate milk when there is an infection of the mammary gland by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking.

1.8. 2 Coagulase negative *Staphylococci:*

They are non motile, non spore forming, Gram-positive, facultatively anaerobic, clustering cocci that produce catalase and glucose fermentation (Barrow and Feltham, 2003).

Coagulase negative *Staphylocci* are found in skin of the external orifice of teat canal, are normal floras of the skin and considered to be opportunistic pathogens (Irlinger, 2008). The proportions of isolated from camel of these were by (Abdurahman *et al.*, 1995 and Hawari and Hassawi, 2008) especially high in subclinical mastitis and also more commonly isolated in clinical mastitis.

1.8. 3 *Streptococcus agalactiae:*

These microorganisms are Gram-positive, cocci, 0.6-1.2 μm diameter, not motile, do not form spores, are catalase-negative and grow in pairs or chains,

based on the presence of a polysaccharide in the cell wall.This polysaccharide is composed of galactose, N-acetylglucosamine, rhamnose and glucitol phosphate (Schuchat, 1998).

Streptococcus agalactiae which inhabits ducts and cisterns of the gland. It causes an inflammation which blocks the ducts, leading to decreased milk production, increased somatic cell count, and eventually to involution (Harmon, 1994 and Myllys and Rautala, 1995). Among the various pathogens causing mastitis, *Streptococcus agalactiae* is of particular importance (Meiri-Bendek *et al.,* 2002) as a causative agent in she-camel. Clinical mastitis exists in different countries; Kenya (Younan *et al.,* 2001and Abera *et al.,* 2010) Sudan (Sanna, 2005 and Alamin *et al.,* 2013),Jordon (Hawari and Hassawi, 2008) and United Arab Emirates (Al-Juboori *et al.,* 2013) .

1.8.4 *Corynebacterium:*

They are Gram-positive, catalase positive, non spore-forming, non motile, rod-shaped bacteria that are straight or slightly curved form small grayish colonies with a granular appearance, mostly translucent, but with opaque centers, convex, with continuous borders (Yassin *et al.*,2003), with a length of 1 to 8 µm and width of 0.3 to 0.8 µm, which form ramified aggregations in culture.

Corynebacterium bovis is a pathogenic veterinary bacterium that causes mastitis and pyelonephritis in cattle, and spread from cow to cow most commonly through improper milking technique. (Hirsbrunne *et al.,* 1996).

In some studied on she-camel mastitis isolated *Corynebacterium bovis* as mean causative agent of mastitis (Suheir, 2004 and Alamin *et al.,* 2013) Sudan, (AL-Tofaily and Alrodhan, 2011) Iraq and (Abdel Gedir, 2001) Ethiopia.

1.8.5 *Escherichia*:

Escherichia is Gram negative rod, non-sporing rod, often motile, catalase positive, oxidase negative, attack sugars fermentatively and aerobic and facultatively anaerobic grows (Barrow and Feltham, 2003).

The proportion of *Escherichia coli* as a causative agent in she-camel clinical mastitis varies between countries, (Amel, 2003 and Sanna, 2005) Sudan, (Kalla *et al.,* 2008) Nigeria, (Eyassu and Bekele, 2010) Ethiopia and (AL-Tofaily and Alrodhan, 2011) Iraq.

1.8.6 Micrococcus:

Micrococcus is Gram-positive cocci in small or large clusters, aerobic, not motile, non-sporing, catalase positive, usually oxidase-positive and attack sugars oxidatively or not at all (Barrow and Feltham, 2003).

Members of this genus have been associated with camel mastitis and it was isolated from same mastatic milk by Suheir, (2004) in Sudan Hawari and Hassawi, (2008) in Jordan and Al-Juboori *et al.,* (2013) in United Arab Emirates as important causative agent of camel mastitis.

1.8.7 *Bacillus cereus*:

Bacillus cereus is a Gram-positive, facultatively anaerobic, spore-producing, motile, rod shaped bacterium. Its spores are ellipsoidal, sub terminal and do not swell the sporangium. *Bacillus cereus* cells tend to occur in chains and the stability of these chains determines the form of the colony, which may vary from strain to strain (Logan and De Vos, 2009).

The *Bacillus cereus* is a main causative agent of all types of she-camels mastitis (Hafez *et al.,* 1987 and Ramadan *et al.,* 1987), and were isolated from

various countries; Sudan (Salwa, 1995 and Alamin *et al.,* 2013), Ethiopia (Eyassu and Bekele, 2010) and Kenya (Wanjohi *et al*., 2013).

1.8.8 *Salmonella* :

Gram-negative rods, motile, aerobic facultatively anaerobic, catalase positive, oxidase negative and attack sugars by fermentation with production of gas (Barrow and Feltham, 2003).

In mastitic milk in one study in Iraq was found 9.52% of bacterial isolated in clinical mastitis (AL-Tofaily and Alrodhan, 2011).

1.8.9 Fungal infection:

Mycotic mastitis in camels is relatively uncommon. But some yeast was isolated from camel mastitic milk samples (Salwa, 1995; Amel, 2003 and Suheir, 2005).

1.9 Diagnosis of mastitis:-

1.9.1 Physical examination:-

1.9.1.1 Visual examination:

Visual check may detect the three types of clinical mastitis by examining the udder for edematous swelling, redness and visible alteration of the color and consistency of milk, watery and with clots are signs of acute mastitis. Hard atrophied, misshapen and fibrotic quarters, massive dilatation of quarter and accumulation of dried pus, exudates and hypertrophy of the teat all these are signs of chronic mastitis. Gland reveals initial, enlargement, redness and darkness end of teats or blue color of the udder are signs of gangrenous mastitis (Kelly, 1984 and AL-Tofaily and Alrodhan, 2011).

1.9.1.2 Palpation:

In acute mastitis palpation of mammary gland will reveal the presence of heat, swelling and pain in the effected quarters and increase or moderate enlargement of supramammary lymph nodes. The inflammatory reaction related to severity of mastitis and is indicated by elevated temperature, increased respiratory and pulse rates.

Chronic mastitis is characterized by hypertrophy and with palpation the fibrotic regions are painless and hard with an uneven surface. The udder temperature is normal; there is an increase in size of the supramammary lymph nodes with hard content.

Gangrenous mastitis is characterized by abnormal texture and there may be desquamation of the udder from the body with swelling of offensive odour. Restlessness, poor appetite, and fever are found (Kelly, 1984 and AL-Tofaily and Alrodhan, 2011).

1.9.2 Chemical examination:-

1.9.2.1 California Mastitis Test (CMT):

Also called Rapid Mastitis Test (RMT). It is a direct test that grossly measures the amount of DNA, primarily a function of the number of nucleated white blood cells in milk. California Mastitis Test (CMT) is based on the amount of gelling that occurs as equal amounts of milk and reagent interact; the test subjectively read after about 20 seconds. The reaction is scored visually as negative (N) no infection, trace (T) possible infections, slightly or weak positive (1), moderate or distinct positive (2) heavy or strong positive (3) (Schalm and Noorlander, 1957). It is economical, easy and rapid and can be used to detect sub-clinical camel mastitis (Sena *et al.*, 2000; Hawari and Hassawi, 2008 and Eyassu and Bekele, 2010).

1.9.2.2 Modified White Side Test:

The white side test is performed on glass slide onto black ground, by adding 4% sodium hydroxide solution to be mixed with the milk of each quarter in a ratio of 1:5.The she-camel is considered mastitic when it's milk become viscid and thick (separate to water and shred or flakes) (Saad and Thabet, 1993).

1.9.2.3 Somatic Cell Count (S.C.C):

The somatic cell count (S.C.C.) is done according to standardized cell count methods (Packard *et al*., 1992). An amount of 0.01 ml milk sample is spread over an area of 1 cm2 on a glass slide. The smear is dried and heated slowly to prevent cracking and peeling. The smears are stained with Newman's stain for two minutes, then washed gently in water and counted. Reading above 250,000 cell/ml is considered positive (Radostits *et al.,* 2000).

The leukocytes were counted according to the following equation:

Leukocyte count= $\frac{\text{Number of leucocytes counted} \times \text{MF}}{\text{Number of field counted}}$

magnification factor (MF) $\frac{40000}{3.1416 \times d2}$ (d=diameter of microscopic lens)

1.10 Treatment of mastitis:

The program for treatment should be started in earliest stage with clinical cases of mastitis (Blood *et al.*, 1994). Anti microbial therapy was considered in all cases of mastitis, knowledge of the causative agent is helpful when making decisions about therapy of clinical mastitis episodes during lactation and the systemic reaction can usually be brought under control by standard doses of anti microbial agents (Radostits *et al.,* 2007). Supportive therapy such as fluids and electrolytes is also crucial to the survival of the animal and minimization of the severity of the mastitis and extent of permanent injury to the udder. The lack of

appropriate mastitis therapy results in the development of resistant organisms to antibiotics.

The anatomical structure of much more long limbs compared with these of cattle that lead to raise the udder from the ground until when sitting the animal, and the dry nature of feces of camel and environment .However, camel milk contains substances that inhibit the growth of pathogenic bacteria, these inhibitors are proteins in nature and have been described as lysozyme, immunoglobulins, lactoferrin and lactoperoxidase, these proteins have been shown with higher potential and concentrations in camel milk than in bovine milk (Farah, 1996 and Kappeler,1998).

The most common method for controlling mastitis is antibiotic treatment, however the improve serious problem is antibiotic-resistant for some bacterial strains which may transfer this resistant between human and animal strains (Nobakht and Shahriyar, 2010).

The teat of the camel udder contains two sometimes three separate teat canals, that open independently into the teat sphincter. Intramammary treatment of mastitis is complex because each quarter should be treated separately and also each gland in quarter as well (Younan and Abdurahman, 2004).

In Iraqi one study in mastitis of she-camels by (AL-Tofaily and Alrodhan, 2011) the Ciprofloxacin, Doxycycline, Gentamicin and Chloramphenicol were the most effective antimicrobial for mastitis causative agents and the greatest resistance for Ampicilline, Erthromycine and Trimethiprime was recorded.

Chapter two

Materials and methods

2.1 Study area:

The study was conducted in Butana area which occupies the middle of the North-eastern Sudan in an area extending over 120,000 km² representing one third of the area of Eastern Sudan. It is in a geographical zone which lies approximately between latitude 14-16 N and longitude 33-36 E (Elamin, 1979). (Figure, 1).

2.2 Animals:

One hundred and sixty she-camels in winter (160) from twelve herds and one hundred fifty nine she-camels in summer (159) from fifteen herds were examined for mastitis by clinical examination and California Mastitis Test (CMT) She-camels that showed clinical mastitis had been subjected to further examinations including the body temperature, plus rate, respiratory rate and general health condition.

2.3 Temperature:

The body temperature was measured of she-camels by using digital thermometer. The measurement was conducted by lubricating the bulb end of the digital thermometer then inserting gently with a circled action through the anal sphincter into the rectum contacted with mucous membrane of the rectum for one to two minutes (Kelly, 1984).

2.4 Pulse rate:

The pulse rate was measured from the middle coccygeal artery on the under aspect of the tail about 10cm below the level of the anus. The number of thrills per minute taking by ball part of one or more fingers is pulse rate (Kelly, 1984).

2.5 Respiratory rate:

The respiratory rate was determined and measured by observing nostril movements or/and flank movements for one minute (Kelly, 1984).

2.6 Diagnosis of mastitis:

Clinical examinations of each milking she-camel was carried out by observation of the macroscopic changes in milk and palpable signs of udder according to Kelly,(1984); Younan, (2002) and AL-Tofaily and Alrodhan, (2011). Milk samples were examined for visible abnormalities and they were screened by California Mastitis Test (CMT) according to Quinn *et al.*, (1999) from each quarter of the udder, a squirt of milk sample was placed in each of the cups on (CMT) paddle and an equal amount of 3% (CMT) reagent was added to each cup and mixed well. The interpretation was in such a way that (CMT) score: 0 was taken as negative, while CMT scores trace, 1+, 2+ and 3+, were considered positive, thus forming five categorical classes. The reactions were interpreted as follows: score 1 = no reaction; score 2 = slight slime which tends to disappear with continued swirling; score 3= distinct slime but without gel formation; score 4 = immediate formation of gel which moves as a mass during swirling; score 5 = gel develops a convex surface and adheres to the bottom of the paddle.

2.7 Questionnaire:

This included information about camel's traditional husbandry system used by camel owners, stage of lactation, udder hygiene and examinations including the body temperature, plus rate, respiratory rate.

2.8 Collection of milk samples:

The first stream of milk was allowed to flow out and a volume about 5-10ml was collected into labeled sterile universal bottles and kept in cool box with ice pack or kept in 4-5°C and transported to laboratory immediately. Isolation and

identification of microorganisms was done in Microbiology Laboratory _College of Veterinary Medicine_Sudan University of Science and Technology.

2.9 Bacteriological examinations:

Positive mastitis milk samples were collected from infected quarters for bacteriological analysis. Bacterial isolation and identification was done according to standard procedures. A loop full of milk sample was streaked onto a plate of blood agar (5% defibrinated sheep blood) and MacConkey agar (Oxoid, Hampshire, England). Plates were then aerobically incubated at 37°C for 18- 24 hours. The preparation of media and the pure colonies were sub-jected to the primary and secondary biochemical tests for identification used according to Barrow and Feltham, (2003).

2.10 Sterilization:

Metal wire and loops were sterilized by flame and also forceps after treated by spirit. Petri dishes, graduated pipettes, flasks and test tubes were sterilized in hot air oven at 160 °C for one hour. Universal bottles, Peptone water, MacConkey's agar, blood agar and nutrient agar were sterilized in autoclave at pressure for 15 minutes at 121°C.

2.11 Disinfection:

The teat tips were disinfected using disposable paper towel immersed in 70% ethyl alcohol. Also Laboratory benches were cleaned and disinfected by using cotton dipped in 70% ethyl alcohol solution. Hands were washed with soap and disinfectant.

2.12 Culture media:

2.12.1 Blood agar:

The contents of proteose peptone 15g, agar 12g, yeast extract 5.0g, sodium chloride 5.0g, liver digest 2.5 and pH 7.4. 40g were dissolved of in one

liter of distilled water, sterilized in autoclave at pressure for 15 minutes at 121°C, cooled to 50°C and 10% of defibrinated sheep blood was added aseptically. The mixture was then dispensed into sterile Petri dishes.

2.12.2 Nutrient agar:

This is composed of peptone 5.0g, yeast extract 2.0g, lab-lemco powder 1.0g, sodium chloride 5.0g and agar 15g. 28g of mixture was suspended in one liter of distilled water, then sterilized by autoclaving at pressure for 15 minutes at 121°C and dispended into sterile Petri dishes.

2.12.3 MacConckey's agar:

This media contents: Peptone 20 g, sodium chloride 5.0g, Sodium taurocholate 5.0 g, Agar 20g, Lactose 10 g Neutral red 1% 10 ml and Water 1000 ml were dissolved the peptone, sodium chloride and bile salt in the water by heating. Adjusted pH 8.0, boiled for 20 min, cooled and filtered. Added and dissolved the agar by boiled and adjusted pH 7.4. Added the lactose and indicator solution then sterilized by autoclaving at pressure for 15 minutes at 121°C and dispended into sterile Petri dishes (Barrow and Feltham, 2003).

2.12.4 Peptone water:

Fifteen grams of peptone water were added to 1000ml of distilled water, mixed well distributed into tube after adjusting the pH to 7.4 and sterilized by autoclaving at 121°C for 15 minutes.

2.13 Gram's stain:

Ammonium oxalate-crystal violet stain was applied to smear for 90 seconds, then Lugol's iodine solution was added for one minute, this was washed with distilled water and decolorized with alcohol for 2-3 seconds and

again washed thoroughly with water, counter staining was done with diluted carbol fuchsin for 15 seconds, then washed and drained or blotted to dry.

Gram-positive organisms were blue or purple and Gram-negative organisms were red color (Barrow and Feltham, 2003).

2.14 Motility:

This was done by the hanging drop technique according to Barrow and Feltham, (2003). The Motility medium was inoculated with the organism under test by stabbing to depth of 5mm. The medium then was incubated at 37°C for 24hours. Motile organisms grew, migrating beyond the stab line while the growth of non motile organisms was confined to the stab line.

2.15 Biochemical methods for identification of bacteria:

All biochemical tests were performed according to Barrow and Feltham, (2003).

2.15.1 Catalase test:

Organism was grown on a nutrient agar slope and one ml of 3%hydrogen peroxide was allowed to run over slope in microscopic slide. Gas bubbles seen immediately or after 5 seconds indicated catalase positive.

2.15.2 Oxidase test:

A piece of filter paper 7 cm diameter was placed in a Petri dish and 2 to 3 drops of tetramethyl-p-phenylenediamine dihydrochloride were added. The test organism was taken by a sterile loop and smeared across the surface of the impregnated paper. A positive reaction was shown by development of dark purple color within 5-10 seconds**.**

2.15.3 Urease test:

Aslope of urea agar medium was inoculated with the test organism, incubated and examined after 24 hours and daily for 5 days. Change in color to pink or red indicated positive reaction**.**

2.15.4 Fermentation of sugars:

Carbohydrate media were inoculated with a 24 hours peptone water culture by a loop and monitored for seven days before discarded. A positive reaction was indicated by change of the color to pink. Sugars used of identification were Lactose, Fructose, Sucrose, Maltose, Raffinose and Mannose.

2.15.5 Oxidation Fermentation (OF) test:

Duplicate tubes of Hugh and Lifson's medium were inoculated by stabbing with a straight wire. The medium in one of the two tubes was covered with a layer of paraffin to depth of about one cm and the tubes were incubated at 37°C for up to 14 days and examined daily. If color changed to yellow in both opened and sealed tubes, this indicated fermentative organism, but a change in the uncovered tube only indicated that the organism was oxidative.

2.15.6 Nitrate reduction:

Nitrate broth was inoculated with the test organism and examined after 48 hours. One ml of nitrate reagent A followed by one ml nitrate reagent B were added. Red color indicated positive reaction. To tubes showing no red color within 5 minutes powder zinc up to 5 mg /ml of culture was added. Yellow color indicated negative reaction.

2.15.7 Indole test:

Peptone water medium was incubated with test culture and inoculated at 37°C for 48 hours. One ml of Kovac's reagent was run down the side of the tube where a pink ring appeared on the reagent layer within a minute is positive result.

2.15.8 Novobiocin sensitivity test:

A volume of two ml of diluted culture were spread on the surface of nutrient agar. The excess fluid was discarded and the plate was allowed to dry then oxoid discs of novobiocin 5 mg was placed on the surface of the medium by sterile forceps and incubated at 37°C for 24 hours. Zone of inhibition was determined to record whether the organism was sensitive to novobiocin or not.

2.16 Antibiotic sensitivity test:

The isolates were streaked on nutrient agar and by sterile forceps; multidisc of different antibiotic was put on culture then incubated for 24-48 hours at 37°C aerobically. Diameter of Inhibition zone in millimeters (ADIZ) was measured and compound with the following standard table.

Millimeters	Sensitization
20<	Very high effectiveness
18-20	High effectiveness
14-18	Moderate effectiveness
10-14	Low effectiveness
0-10	Resistant

Multidisc for antimicrobial susceptibility testing for Gram positive isolates Axion® Code no: MD001 were Ampicillin/Sulbactam (AS) 20mcg, Co-Trimoxazole (BA) 25mcg, Cephalexin (PR) 30mcg, Tetracycline(TE)30mcg, Cefotaxime (CF) 30mcg, Ciprofloxacin (CP) 5mcg, Levofloxacin(LE) 5mcg,

Linezolid(LZ) 30mcg, Cloxacillin(CX) 5mcg, Roxithromycin(RF) 15mcg, Lincomycin(LM) 2mcg and Gentamicin(GM)10mcg. While multidisc for antimicrobial susceptibility testing for Gram negative isolates Axion® Code no:MD002 were Ampicillin/Sulbactam (AS) 20mcg, Co-Trimoxazole (BA) 25mcg, Cefotaxime(CF)30mcg, Tazobactam/Piperacillin (TZP)100/10mcg, Chloramphenicol (CH)30mcg, Ciprofloxacin (CP) 5mcg, Ceftizoxime(CI) 30mcg, Tetracycline(TE)30mcg, Ofloxacin (OF)5mcg, Gentamicin(GM) 10mcg, Amikacin(AK) 30mcg and Levofloxacin(LE) 5mcg.

2.17 Treatment trials:

Five cases of clinical mastitis were observed and treated by the famous antibiotic for camel owners (Pen and strep ®, Oxyteteracycline ® and Almoxla®) and Udroit®, in Tamboul town (Figure, 2). The treatment was done before undertaking of the antibiotic sensitivity to the isolated bacteria.

Antibiotic	Active ingredient
Pen and strep ®	Procaine Penicillin, Dihydrostreptomycin Sulphare
Oxyteteracycline ®	Oxyteteracycline HCL 5%
Almoxla®	Amoxicillin 15%
Udroit®	Camphor 2g, Phenol 2.5, Eucakyptus oil 2g and Iodine 600mg..

2.18 Statistical analysis:

Data collected from this study were compiled using an appropriate statistical package SPSS version 16. The result was summarized as means ± standard error and Levels of Significance was taken at ($P \leq 0$ .05).

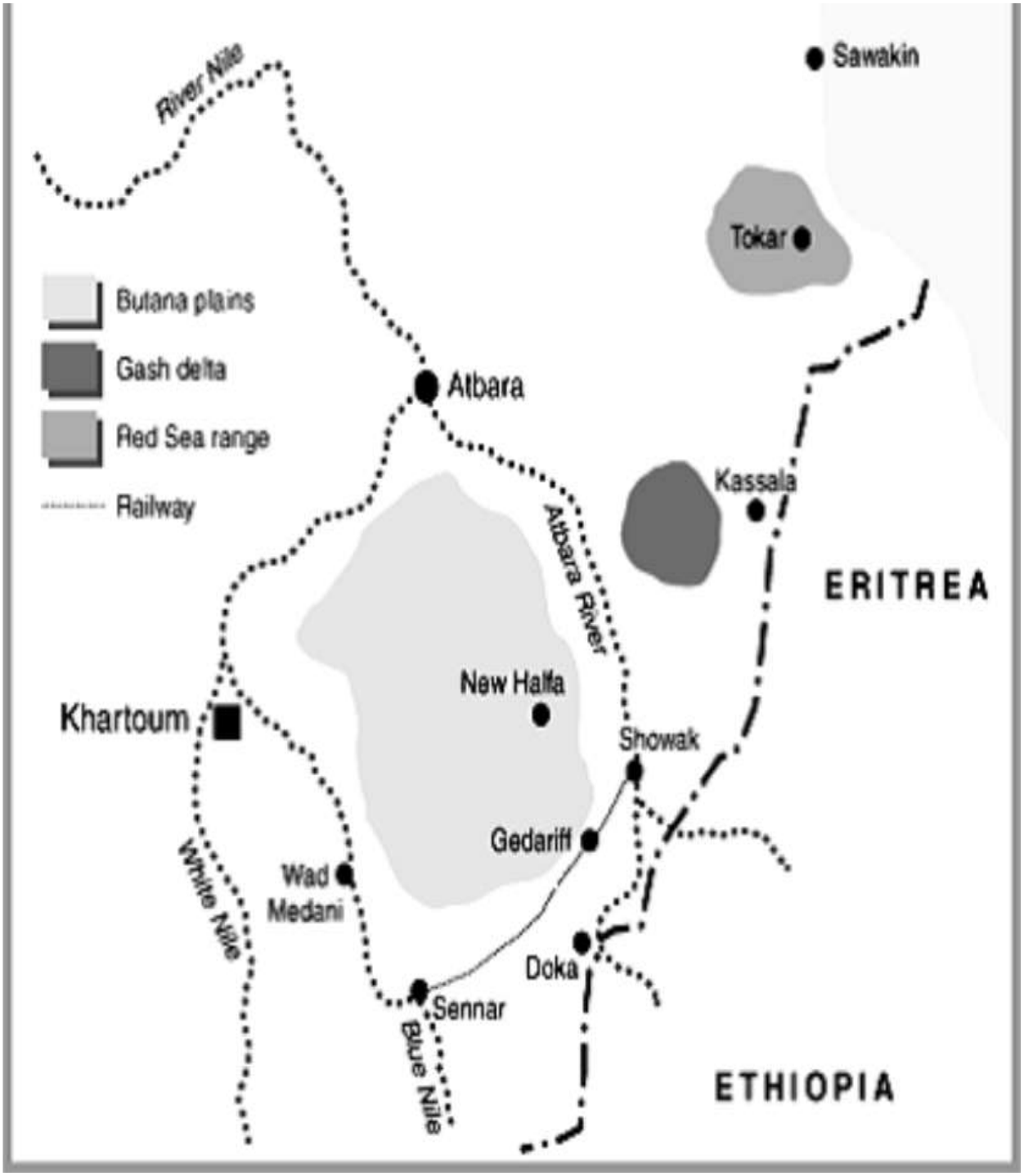
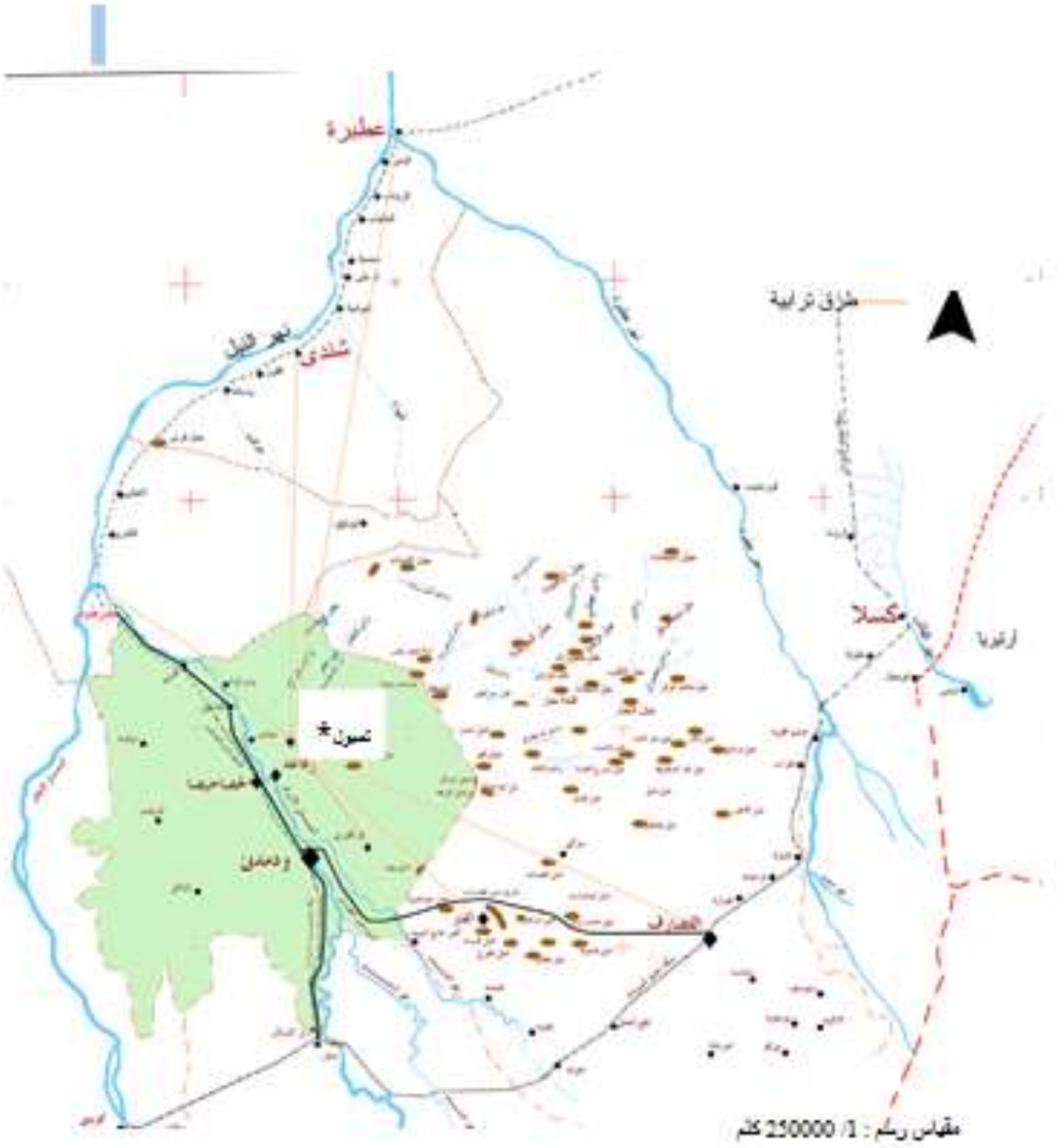


Figure (1) Butana Region in the middle of the North-eastern Sudan



Source: Government of Gezira State

Figure (2) Tamboul town on Butana Region

Chapter three

Result

3.1 Survey:

The survey was conducted in Butana region to investigated camel diseases with special reference to mastitis in camel herds during different seasons of the year (winter and summer). The analysis indicated that the main breeds were *Arabi* and *Anafi* types. The most common diseases problems of herds were trypansomiasis, mange, tick infestation, ringworm and contagious skin necrosis.

The survey also showed that mastitis disease is one of the most worrying diseases to camel owners and is known by the local name (Hadaia). The clinical form mastitis was characterized by swelling of the udder and change of milk contents and color.

Temperature, pulse rate and respiratory rate were found leading to increased temperature and pulse rate from milking camels and decreased respiratory rate on summer than winter season, this difference was significant at ($P. < 0.05$). (Table, 1).

In winter season among 656 camels in 12 herds, 160 were found as milking camels; among these milking she-camels 13 cases clinical mastitis were diagnosed. (Table, 2).

In summer season among 758 camels in 15 herds, 159 were found as milking camels; among these milking she-camels 16 cases of clinical mastitis were diagnosed. (Table, 3).

The owners of these camels used two kinds of anti-suckling devices (Surar and Gourab). Surar is pieces of wood and cloths tying one or two pair of teat together which definitely causes injuries or traumas to the udder (Figure, 3). Gourab is dirty plastic sac or cloth covering the udder and flank which are considered as sours of contamination to the udder (Figure, 4). These devices are usually used to prevent suckling milk from the udder. Fifteen (52%) from

mastitic she-camels were found to use (Surar). Five (17%) from mastitic she-camels were found to use (Gourab) and nine (31%) from mastitic she-camels were found to be free from the two devices (Figure, 5). Those two devices did not show any significant effect on the occurrence of mastitis at ($p. < 0.05$).

The high incidence of clinical mastitis was found in mastitic she-camels infested by tick in 21cases (72%) (Figure, 6), but they did not show any significant effect on the occurrence of mastitis at ($p. < 0.05$).

The occurrence cases of clinical mastitis in she-camels among 319 milking camels during summer and winter were found in 29 cases (9.09%) (Figure, 7).

The she-camels have a clinical mastitis showed slightly systemic reaction, there were increased of body temperature (37.9±1.44 C°), respiratory rate (16.03±2.57/min per minute) and pulse rate (53.97±6.64/min per minute) than negative she-camel from mastitis (36.97±1.24 C°),(14.63±2.42/min) and (48.22±4.95/min) respectively, this difference was significant at ($p. < 0.05$). (Table, 4).

3.2 Type of clinical mastitis:

Table (5) Shows that were 29 clinical mastitis cases among the (319) examine camels. According to type of mastitis the acute form was diagnosed in 7 cases (24.14%), chronic 21cases (72.41%) and gangrenous form was only one case (3.45%). Clinical signs for acute mastitis were severe inflammation of supramammery lymph node and enlargement with redness of the udder and swelling. Also pain in the affected quarter, the mammary secretions were watery, yellowish or containing blood and clots or flakes (Figure, 8, 9, and 10). Clear signs of udder chronic mastitis were hypertrophy of the mammary gland and watery secretions with pus (Figure, 11). Some of chronic mastitis showed obstruction of the teat canal and hypertrophy of the teat (Figure, 12). Certain chronic mastitis showed firm, hypertrophy and painless in affected quarter

(Figure, 13). The gangrenous mastitis showed bluish discoloration of udder, this was found to associate with injury by anti-suckling devices (Surar) (Figure, 14).

3.3 Effect of Season on clinical mastitis:

Three cases of acute, nine cases of chronic and one case of gangrenous mastitis were diagnosed during winter season. Four cases of acute and twelve cases of chronic mastitis were diagnosed during summer season. The effect of season on the incidence of mastitis was not significant (P. <0.05) (Figure, 15).

3.4 The effect of age on clinical mastitis:

The age of she-camels affected with clinical mastitis varied between 6 to 23years. The incidence of clinical mastitis was 2 cases (6.89%), one case (3.45%) at the age ranging from 6-10 years in winter and summer respectively. Nine cases (31.03%) in summer and 10 cases (34.48%) in winter were at the age ranging from 11-15 years. Wherever 2 cases (6.89%) in winter and 5 cases (17.24%) in summer at the age from 16≤ years. The highest incidence of clinical mastitis was found at the age between 11-15 years was 19 cases (65. 52%) in the year (Figure, 16).

3.5 Effect of stage of lactation on clinical mastitis:

Few observed mastitis cases in the first stage of lactation were 5 cases (17%). increased cases were observed at the middle stage were 8 cases (28%) and the highest incidence of clinical mastitis was diagnosed at in late stage of lactation were 16 cases (55%) (Figure, 17).

3.6 Effect of number of calving on clinical mastitis:

There was a direct relationship between number of calving and clinical mastitis. The incidence of clinical mastitis during the first, second and third calving was 19 cases (65.52%), during the fourth and fifth calves was 7cases (24.14%)and during the sixth calving the incidence decreased to 3 cases (10.34%) (Figure, 18).

. **3.7 Biochemical tests:**

Different biochemical tests were used for the presumptive identification for all isolates. The differences between *Staphylococcus aurues*, *Staphylococcus hyicus* and *Staphylococcus lentus* were that *Staphylococcus aurues* was coagulase +ve, mannose –ve and urease –ve. While the differences between *Staphylococcus hyicus* and *Staphylococcus lentus* were *Staphylococcus hyicus* was arginine +ve, novobiocin sensitive, mannitol –ve, maltose–ve and raffinose–ve (Table, 6 and 7).

3.8 Bacteriological examinations:

The results showed that 27 (93.1%) of the 29 culture of samples gave positive bacterial growth. 37 microorganisms were isolated, 30(81.08%) were Gram + ve and 5(18. 92%) were Gram – ve bacteria (Figure, 19).

Table (8) showed the main bacterial isolates were *Staphylococcus spp*. 14 (37.8%), *E.coli* 7 (18.9%), *Streptococcus spp*. 5 (13.5%), *Bacillus spp*. 4(10.8%) *Micrococcus spp*.3 (8.1%), *Corynebacterium spp*. 2 (5.4%) and *Salmonella spp.* 2 (5.4%). (Figure, 20) showed the effect of season on bacteria isolated.

3.9 Antibiotic sensitivity test:

All the bacterial isolates were subjected to antibiotic sensitivity test. The antibiotics used for Gram positive bacteria were Ampicillin/Sulbactam (AS) 20mcg, Co-Trimoxazole (BA) 25mcg, Cephalexin (PR) 30mcg, Tetracycline (TE)30mcg, Cefotaxime (CF) 30mcg, Ciprofloxacin (CP) 5mcg, Levofloxacin (LE) 5mcg, Linezolid(LZ) 30mcg, Cloxacillin(CX) 5mcg, Roxithromycin(RF) 15mcg, Lincomycin(LM) 2mcg and Gentamicin(GM)10mcg. While antibiotics used for Gram negative isolates were Ampicillin/Sulbactam (AS) 20mcg, Co-Trimoxazole (BA) 25mcg, Cefotaxime (CF)30mcg, Tazobactam/Piperacillin (TZP)100/10mcg, Chloramphenicol (CH)30mcg, Ciprofloxacin (CP) 5mcg, Ceftizoxime(CI) 30mcg, Tetracycline(TE) 30mcg, Ofloxacin (OF)5 mcg, Gentamicin (GM) 10mcg, Amikacin(AK) 30mcg and Levofloxacin(LE) 5mcg.

The effectiveness of each antibiotic was read by average of diameter of growth inhibition zone in millimeters (ADIZ). Table (9) Shows effectiveness percentage of antibiotic used against the different isolates. All isolates were sensitive to Ciprofloxacin, Gentamicin and Levofloxacin 100%, 100% and 97.3% respectively. The moderate sensitive antibiotic were Ampicillin/Sulbactam (81.08%) and Co-Trimoxazole (75.68%) and resistant were Tetracycline (40.54%) and , Cefotaxime(40.5%). The results of antibiotic sensitivity test against Gram +ve bacteria showed that the high sensitive on Roxithromycin (97.3%) and Cloxacillin (94.6%). The moderate sensitive antibiotic was Linezolid (78.38%) and resistant were , Lincomycin (54.05%) and Cephalexin (35.14%). While that the Gram –ve bacteria showed that the high sensitive antibiotics were Amikacin and Ofloxacin (100%and 91.89%) respectively, the resistants were Tazobactam/Piperacillin (43.24%), Chloramphenico (54.06%) and Ceftizoxime (31.8%).

3.10 Treatment trials:

Five cases of clinical mastitis were observed and treatment by the famous antibiotic for camel owners (Pen and strep ®, Oxytetercyclline ® and Almoxla®) and Udroit®, this treatment was done before undertaking of the antibiotic sensitivity to the isolated bacteria. The most effective antibiotic for mastitis for common drug used was Pen and strep ® (Table, 10) (Figure, 21).

Table (1) Effect of season on temperature, pulse rate and respiratory rate of she-camels in Butana region

Clinical parameter / Season	**Temperature C°**	**Pulse rate thrill/minute**	**Respiratory rate Murmur/minute**
Winter(160)	36.05±0.75	46.72±2.7	15.26±2.38
Summer (159)	38.06±0.83	50.78±5.26	14.26±2.45

A.

B.

Figure (3) Anti-suckling device (Surar)

B. Made of plastic sac

C. Made of cloth

Figure (4) Anti-suckling device (Gourab)

Table (2) The diagnosed clinical mastitis among twelve herds during winter season in Butana region

Number of herds	**Number of she-camels**	**Milking she-camel**	**Clinical mastitis**	**Type of mastitis**
1	57	12	1	chronic
2	37	8	1	Gangrenous
3	60	20	3	1Acute +2chronic
4	75	18	2	2chronic
5	51	15	1	Acute
6	70	14	-	-
7	33	4	-	-
8	45	19	1	chronic
9	53	21	1	chronic
10	62	9	1	Acute
11	69	13	1	chronic
12	44	7	1	chronic
Total	656	160(24.4%)	13(8.125%)	3A+1G+9C

A: Acute mastitis.

C: Chronic mastitis.

G: Gangrenous mastitis.

Table (3) The diagnosed clinical mastitis among fifteen herds during summer season in Butana region

Herd No.	**Number of she-camels**	**Milking she-camel**	**Clinical mastitis**	**Type of mastitis**
1	61	11	2	1Acute +1 Chronic
2	38	9	0	-
3	53	8	2	2 Chronic
4	64	7	1	Chronic
5	73	18	1	Chronic
6	51	12	1	Acute
7	46	16	1	Chronic
8	33	6	0	-
9	58	15	3	3Chronic
10	45	8	2	2Chronic
11	56	10	0	-
12	41	7	0	-
13	37	13	2	2Chronic
14	55	11	0	-
15	47	8	1	Acute
Total	758	159(20.98%)	16(10.06%)	4A + 12C

A: Acute mastitis.

C: Chronic mastitis.

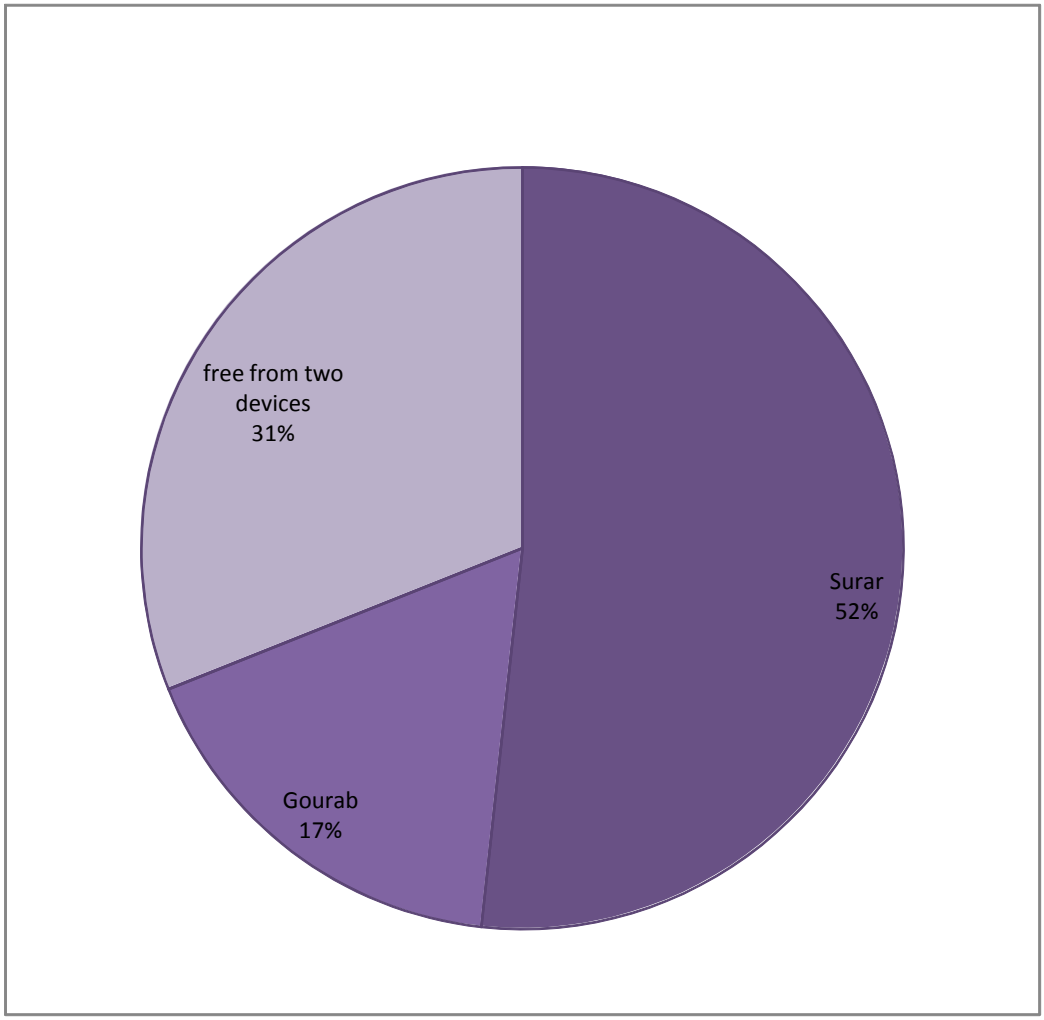


Figure (5) Effect of anti-suckling devices on the occurrence of clinical mastitis

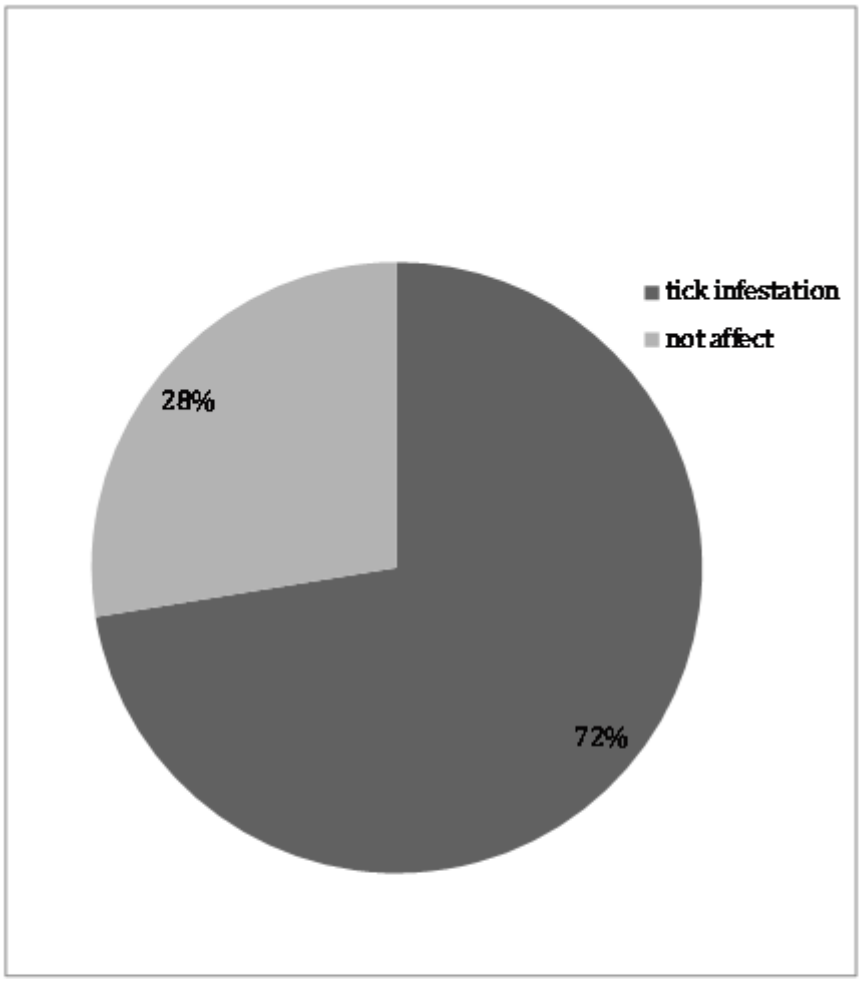


Figure (6) Effect of tick infestation on the occurrence of clinical mastitis

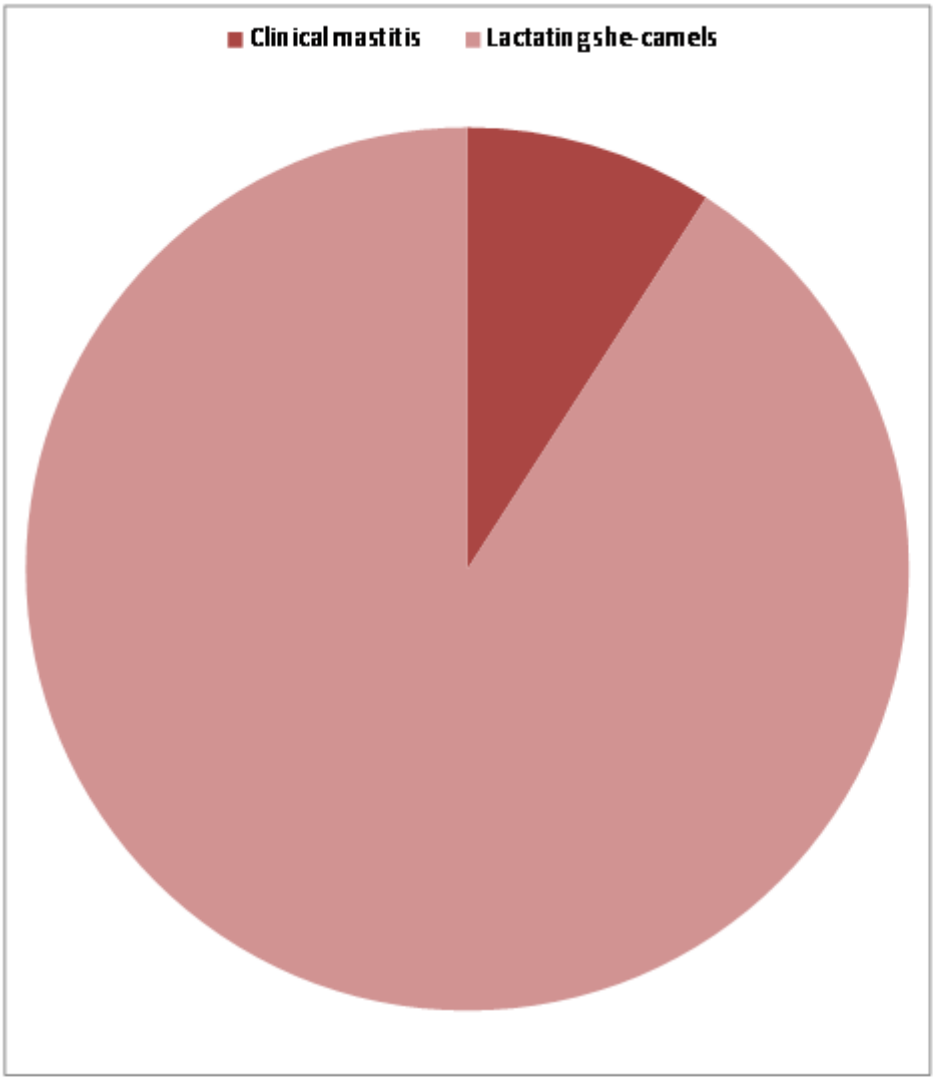


Figure (7) Prevalence of clinical mastitis among camel herds in Butana region

Table (4) Effect of clinical mastitis on temperature, pulse rate and respiratory rate among mastitic camels in Butana region

Clinical parameter / Mastitis	**Temperature C°**	**Pulse rate thrill/minute**	**Respiratory rate Murmur/minute**
Positive	37.9±1.44	53.97±6.64	16.03±2.57
Negative	36.97±1.24	48.22±4.95	14.63±2.42

Table (5) Percentage of the differed type of mastitis diagnosed in Butana region

Categories	**She-camels**	
	No	%
Acute mastitis	7	24.1
Chronic mastitis	21	72.4
Gangrenous mastitis	1	3. 5
Total	29	100

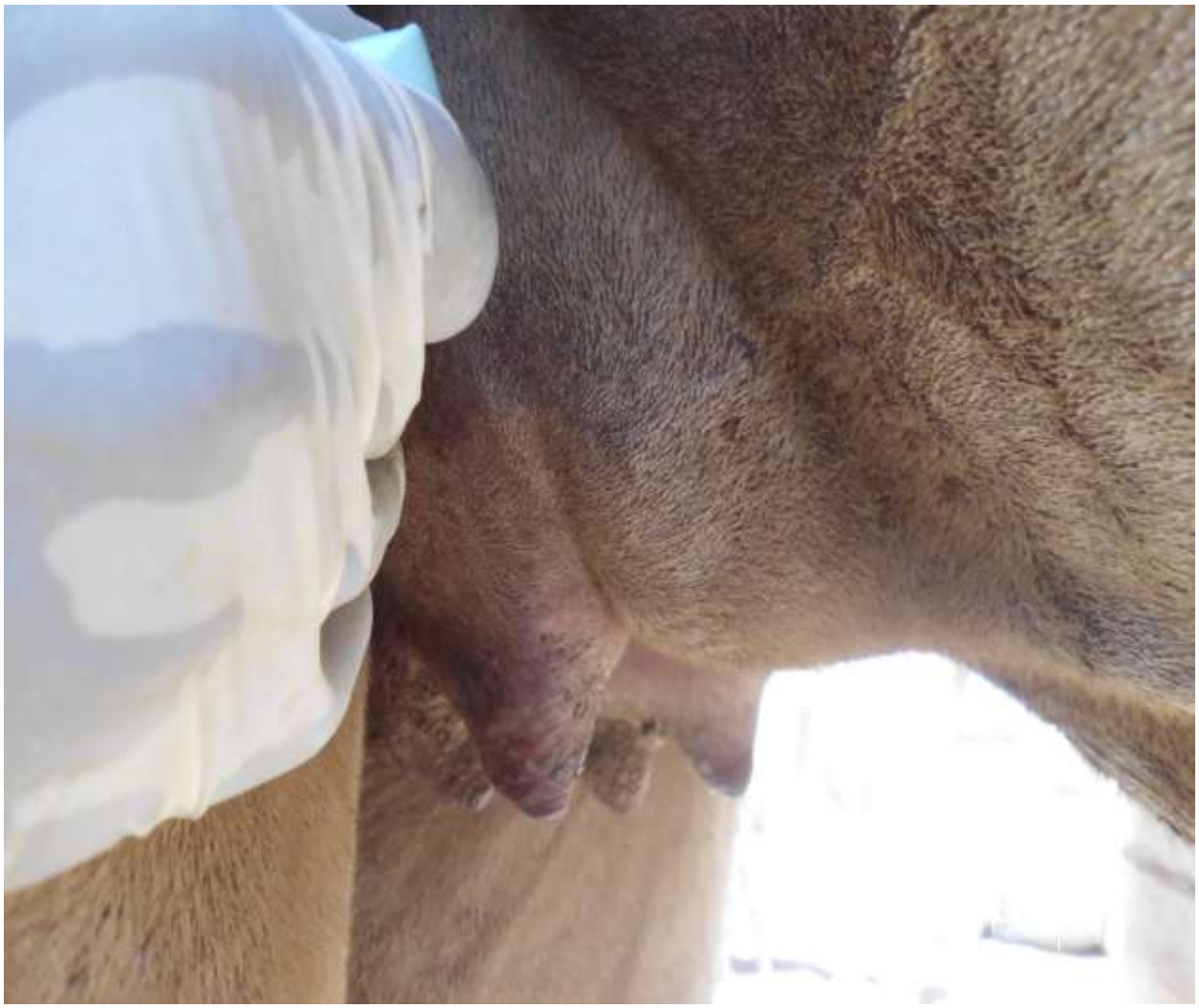


Figure (8) A case of acute mastitis (Redness of mammary gland and enlargement of supramummery lymph node)

Figure (9) A case of acute mastitis (Redness - watery and yellowish secretions)

Figure (10) A case of acute mastitis (swelling in affected quarter, with tick infestation)

Figure (11) A case of chronic mastitis with clear thick and white pus

Figure (12) A case of chronic mastitis(Obstruction of teat canal and hypertrophy on affected quarter)

Figure (13) A case of Chronic mastitis (firm and hypertrophy of all affected quarters)

Figure (14) A case of gangrenous mastitis (Bluish discoloration with injury due to the anti-suckling device (Surar))

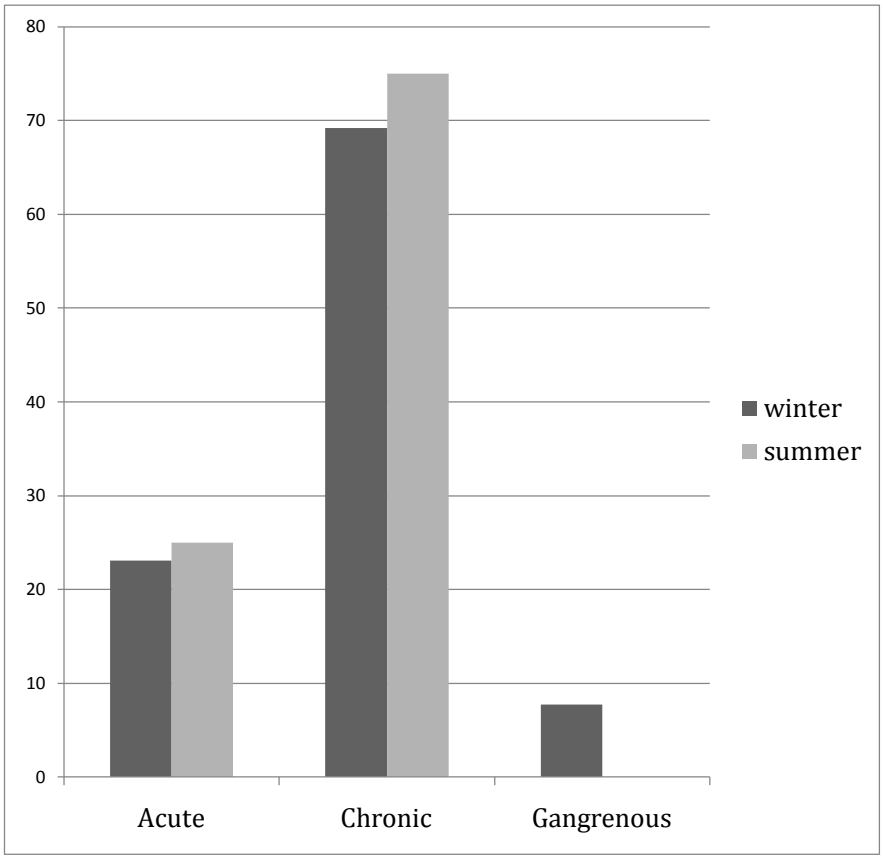


Figure (15) Effect of season on the occurrence of clinical mastitis

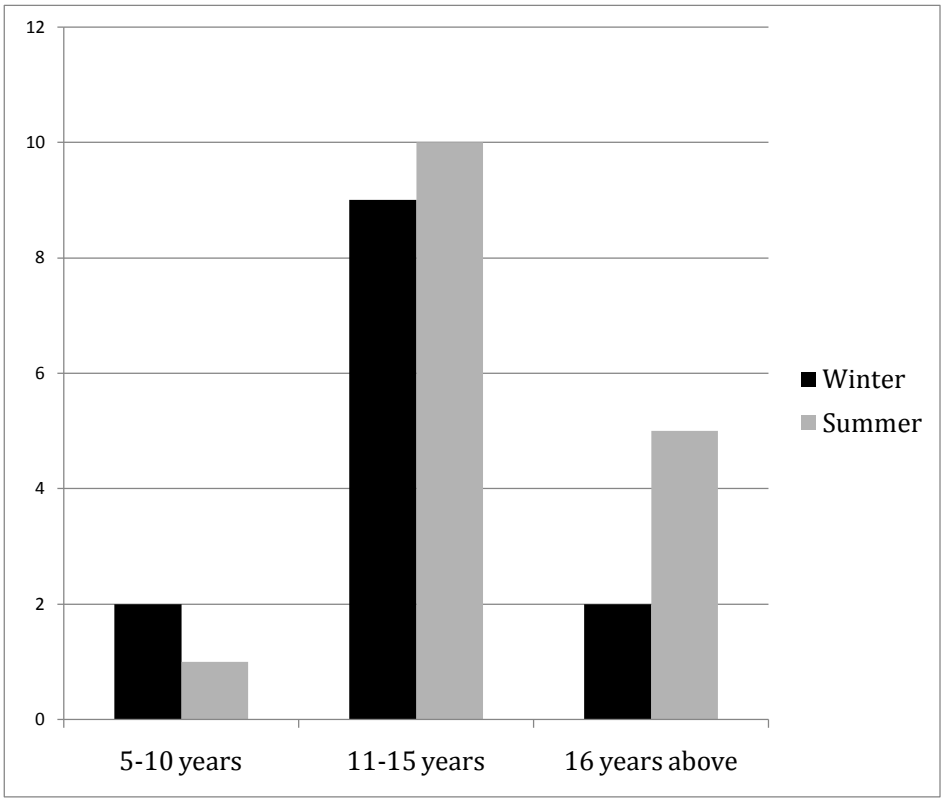


Figure (16) Effect of age on the occurrence of clinical mastitis

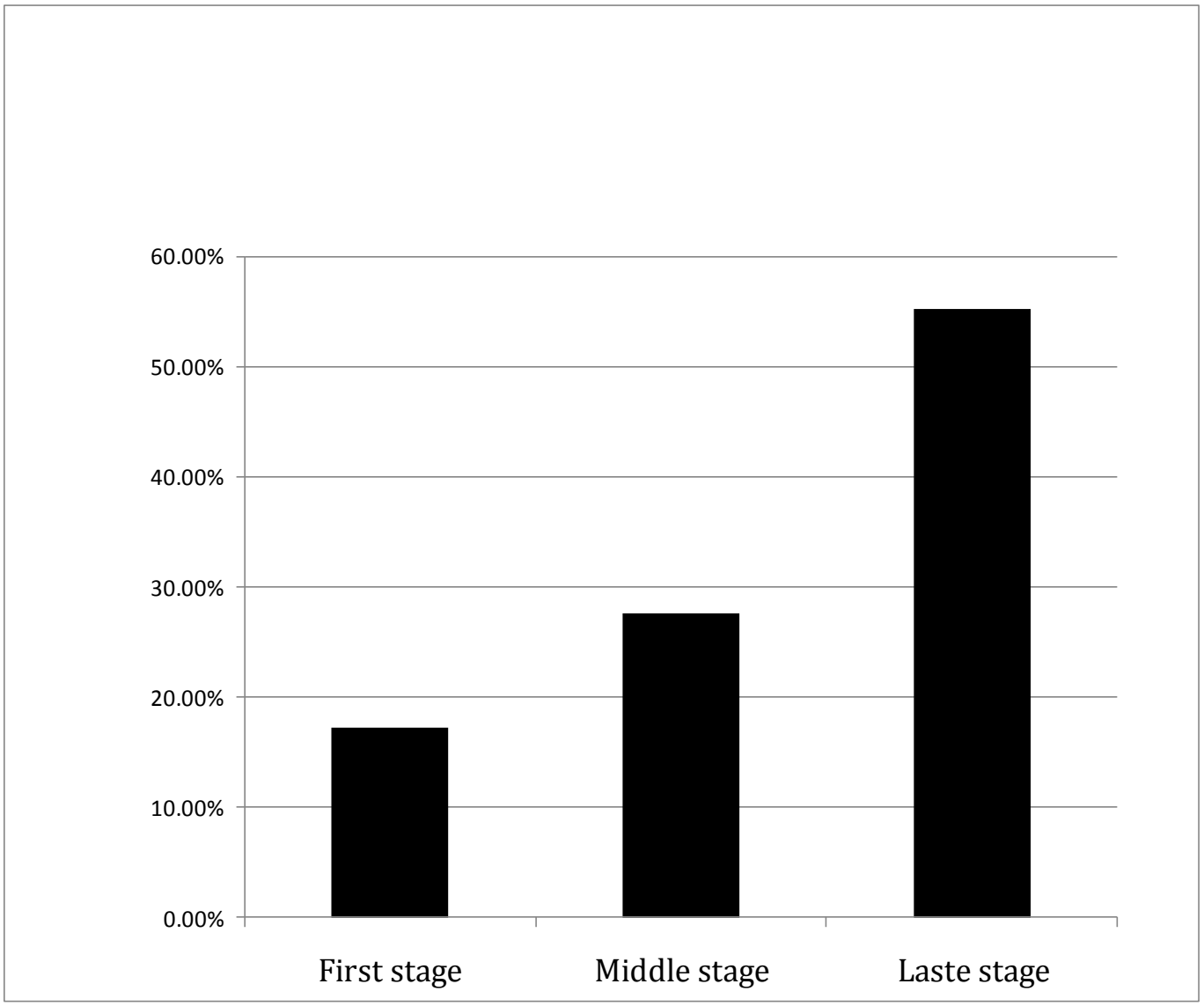


Figure (17) Effect of stage of lactation on the occurrence of clinical mastitis

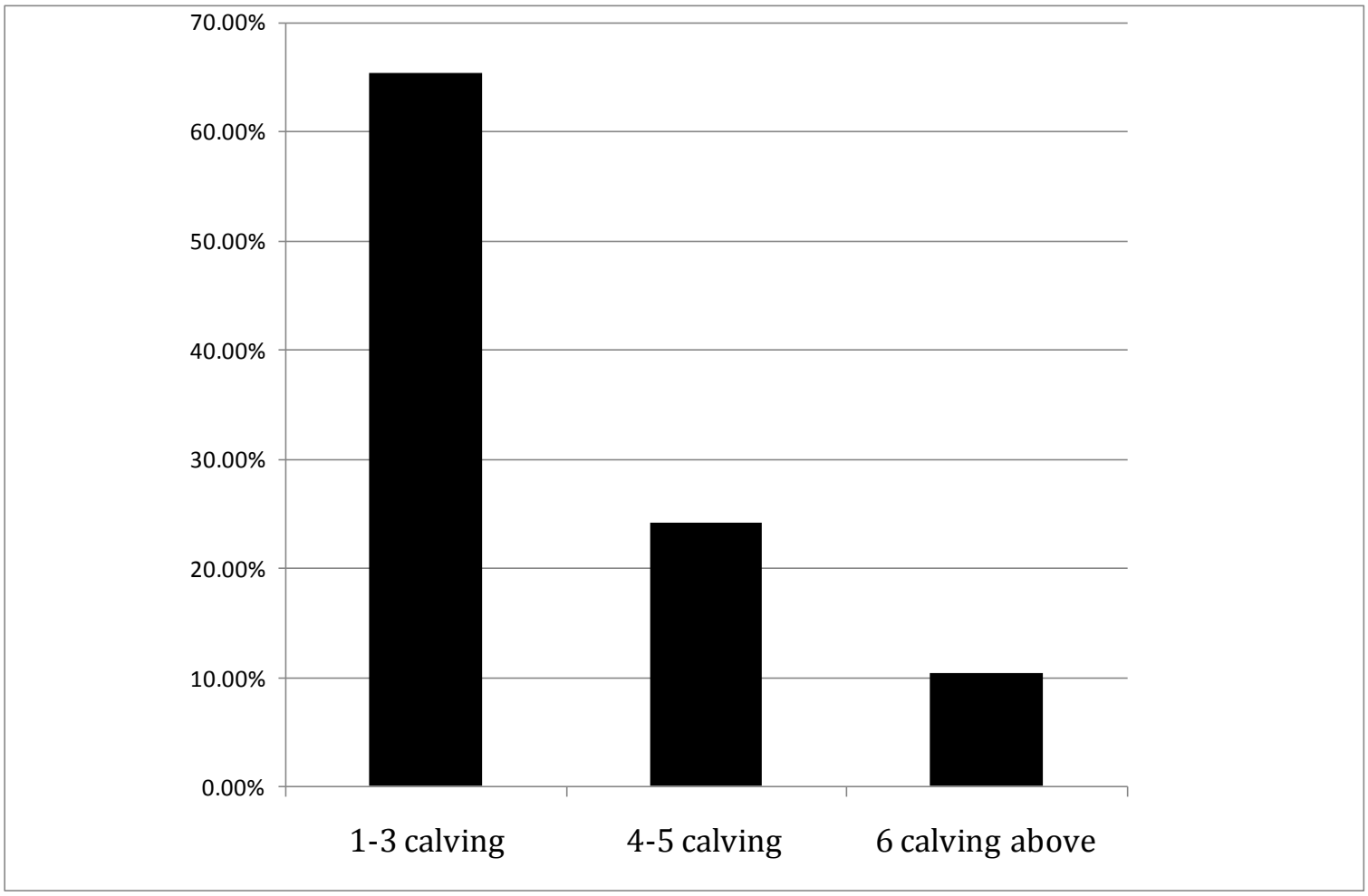


Figure (18) Effect of number of calving on the occurrence of clinical mastitis

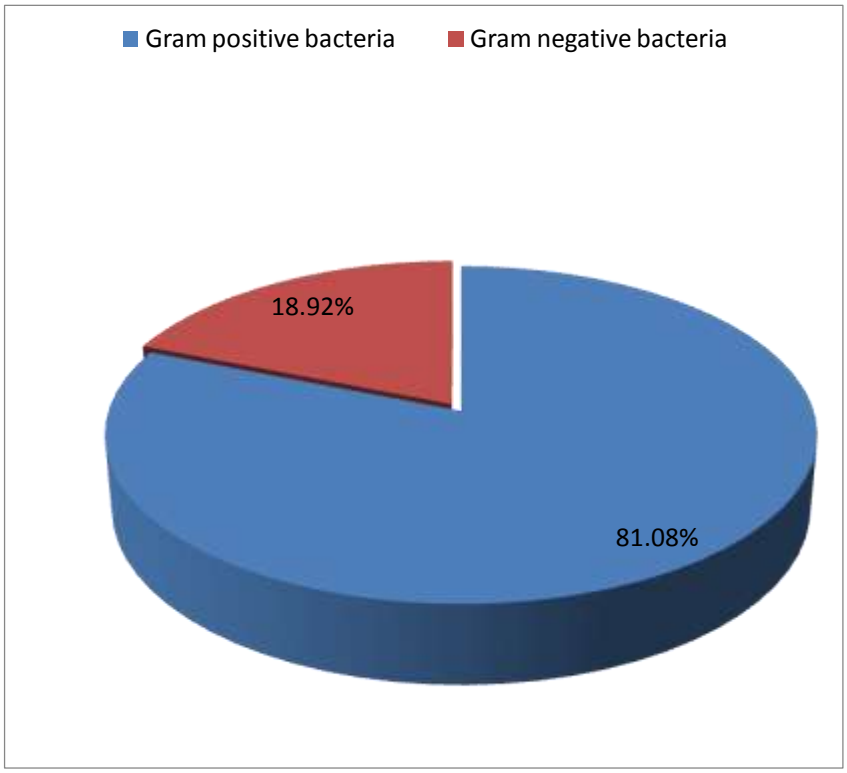


Figure (19) The percentage of microorganisms isolated form clinical mastitis in she-camel in Butana region

Table (6) Biochemical tests for identification to the genus level of the isolated of bacteria

Bacteria genus	**Shape**	**Gram stain**	**Motility**	**Growth in air**	**Catalse**	**Oxidase**	**Glucose**	**OF**	**Anaerobic growth**
Staphylococcus	sphere	+	-	+	+	-	+	F	+
Streptococcus	sphere	+	-	+	-	+	+	F	w
Micrococcus	sphere	+	-	+	-	+\-	+	F	-
Bacillus	Spore	+	-	+	+	-	+	F	D
Corynebacterium	Rods	+	-	+	+	-	+	F	+
E.coli	Rods	-	-\+	+	+	-	+	F	+
Salmonella	Rods	-	-	+	+	-	-	F	+

+ Positive

- Negative

Table (7) Biochemical tests for differentiation of various types of the isolated Staphylococcus bacteria.

Genus / **Test**	*Staphylococcus aurues*	*Staphylococcus hyicus*	*Staphylococcus lentus*
oxidase	-	-	w
VP	+	-	-
Coagulase	+	-	-
Nitrate	+	+	+
Arginine	+	+	-
Urea	-	+	+
Novobiocin	s	s	r
Mannitol	+	-	+
Lactose	+	+	+
Fructose	+	+	+
Sucrose	+	+	+
Maltose	+	-	+
Raffinose	-	-	+
Mannose	-	+	+

s: sensitive

r: resistant

w: weak

Table (8) The type of bacteria isolated from clinical mastitis in she-camel in Butana region

Bacteria	**No**	**%100**
Staphylococcus spp.	14	37.8
S.aureus	10	
S.hyicus	2	
S.lentu	2	
E. coli	7	18.9
Streptococcus spp.	5	13.5
St. agalactiae	4	
St. dysagalatiae	1	
Bacillus cereus	4	10.8
Micrococcus spp.	3	8.1
Corynebacterium spp.	2	5.4
Salmonella spp.	2	5.4
Total	**37**	**100%**

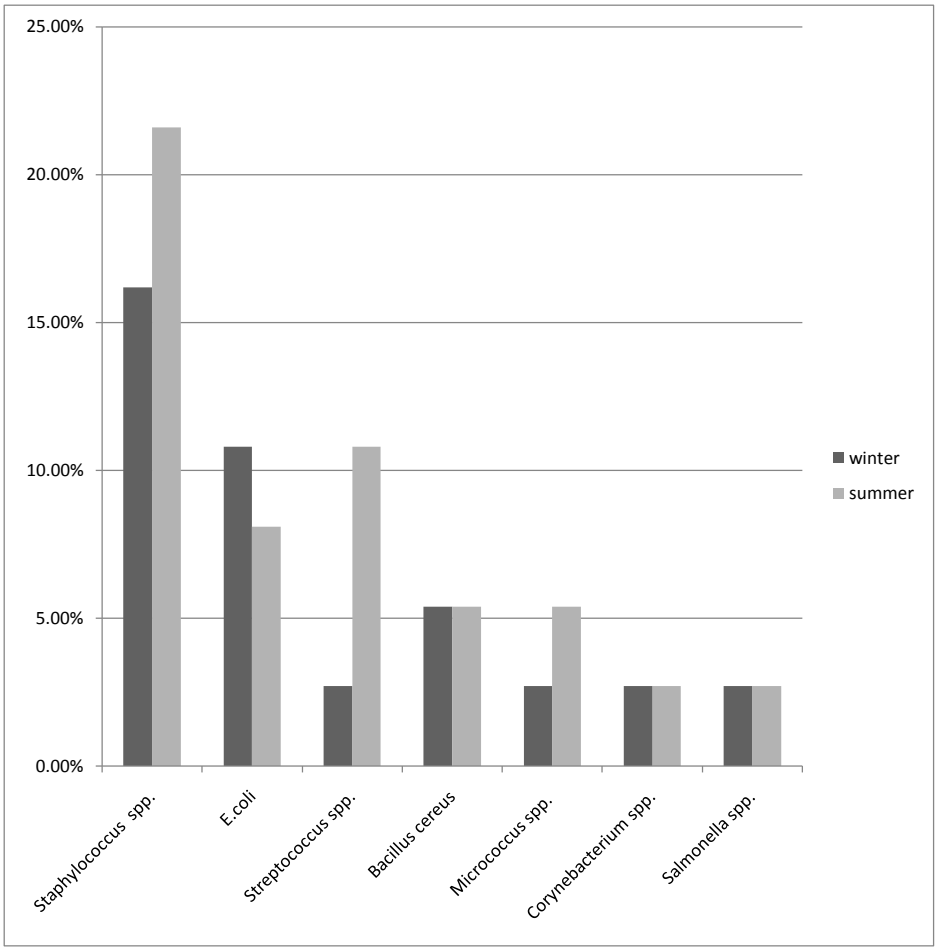


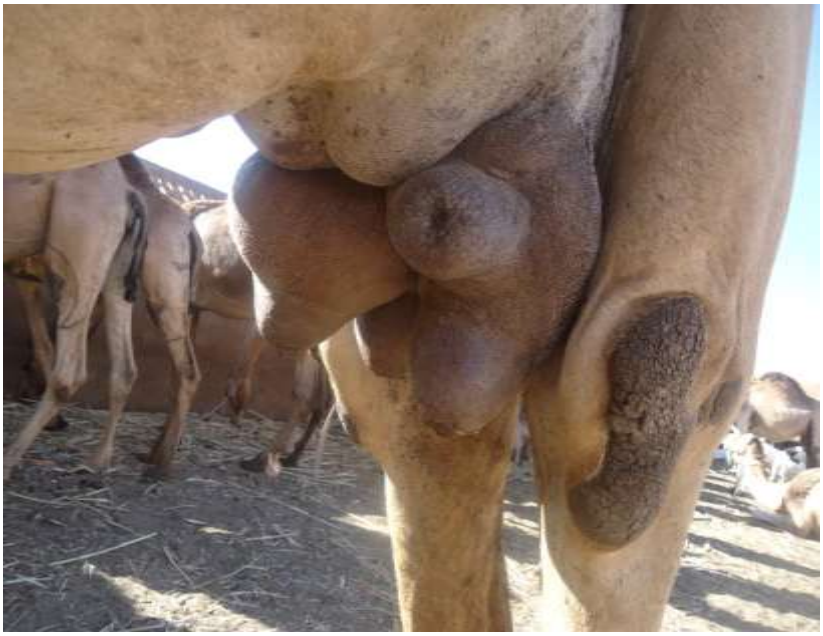
Figure (20) Effect of season on bacteria isolated from clinical mastitis

Table (9) Effectiveness values of antibiotics used for bacterial isolates

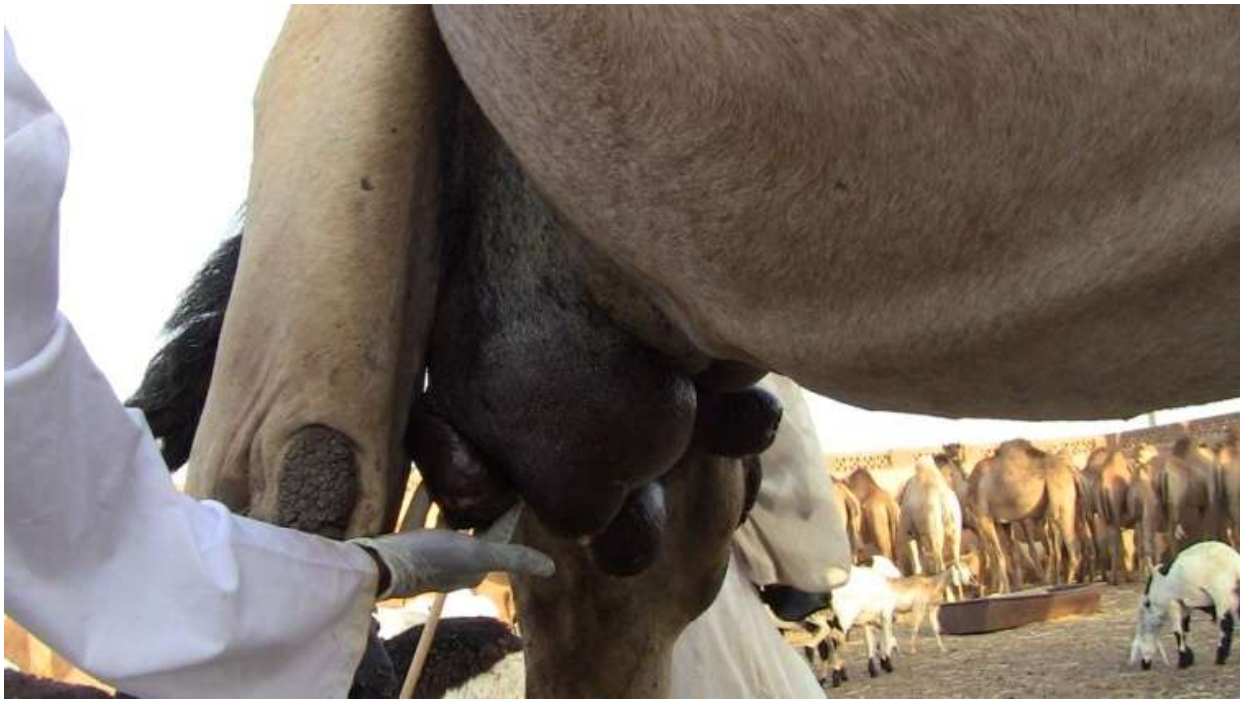
Antibiotic used	No. isolates examined	No. sensitive isolates	%. sensitive isolates	No. resistant isolates	%. sensitive isolates
AS	37	30	81.08	7	18.92
BA	37	28	75.68	9	24.32
CP	37	37	100	0	0
TE	37	15	40.54	22	59.46
GM	37	37	100	0	0
CF	37	15	40.50	22	59.50
LE	37	36	97.30	1	2.70
PR	28 Gram+ve	10	35.14	18	64.86
LZ	28 Gram+ve	22	78.38	6	21.62
CX	28 Gram+ve	26	94.60	2	5.40
RF	28 Gram+ve	27	97.30	1	2.70
LM	28 Gram+ve	15	54.05	13	45.95
TZP	9 Gram-ve	4	43.24	5	56.76
CH	9 Gram-ve	5	54.06	4	45.95
CL	9 Gram-ve	3	31.80	6	67.57
OF	9 Gram-ve	8	91.89	1	8.11
AK	9 Gram-ve	9	100	0	0

Table (10) Treatment trial on some cases of clinical mastitis in Tamboul area

No	**Type of mastitis**	**Test:** (CMT) and clinical mastitis	**Bacterial isolates**	**Treatment protocols**	**Observant**
1	Acute	+	*Staphylococcus spp. and E.coli*	Pen and strep ® 20 ml for 3 days, with adroit ® daily one time in the evening. After milked and washed by cool water.	-ve (CMT) -ve visual examination -ve bacterial growth
2	Chronic	+	*E.coli* and *Bacillus spp*.	Pen and strep ® 20 ml for 5days, with adroit ® twice (morning and evening) daily. After milked and washed by hot water.	-ve (CMT) -ve visual examination -ve bacterial growth
3	Acute	+	*Staphylococcus spp.*	Pen and strep ® 20 ml for 2 days without adroit ® and used adroit ®daily one time in the evening for two days after the udder be hard.	-ve (CMT) -ve visual examination -ve bacterial growth
4	Chronic	+	*Streptococcus spp.* and *Bacillus spp*.	Oxytetercicillin 5% for 5 days with adroit ® twice (morning and evening) daily. After milked and washed by hot water.	+ve (CMT) -ve visual examination +ve bacterial growth
5	Acute	+	*Staphylococcus aureus*	Amoxicillin 20ml for 4 days and washed by cool water daily.	Not treated. The form of mastitis changed to gangrenous form.



A. Acute mastitis



A. Gangrenous mastitis

Figure (21) Changed of acute mastitis from gangrenous mastitis in same case

Chapter four

Discussion

This study was performed to investigate the type of clinical mastitis among camel herds in Butana region. The effect of season, age, stage of lactation, number of calving on occurrence of mastitis the causative agent and antibiotic sensitivity of the bacterial isolates from mastitic she-camel were studied. The study further included treatment selected cases suffering from acute and chronic mastitis in Tamboul area. Common drugs used in Butana region was used for this trial of treatment.

The occurrence of clinical mastitis in she-camel herds in the present investigation was 9.09%. This is in agreement with reports by Hawari and Hassawi (2008), Sibtain *et al* (2012) and Abera *et al* (2010) these authors found an incidence in the range of 10.2%, 8% and 8.3% respectively. The prevalence in the present study was lower than that reported in previous studies where the prevalence of mastitis was 12.5% by Bakele and Molla (2001) in Ethiopia, Alamin *et al* (2013) in Kordofan state (25%) and AL-juboori *et al* (2013) in Abu Dhabi, United Arab Emirates (24.7%). And the prevalence in the present study was higher than that reported 5.22% in Iraq by AL-Tofaily and ALrodhan (2011).

The anti-suckling devices used for mastitic she-camels proved to be a risk factor for the occurrence of mastitis as 69% of camel with anti-suckling were mastitic. Tick infestation which causes teat lesions was also found to predispose to mastitis, these lesions or wounds together with poor udder hygiene facilitate bacterial entry and hence to infection of the udder. The results of anti-suckling devices found in this study is similar to the study done by Alamin *et al* (2013) in western Sudan who reported that mastitis spread between she-camels due to the bad milking habits and or the use of local anti-suckling devices which caused wound and invaded by the *Staphylococcus spp.*

than to mammary gland tissues. Megersa (2010), Husein *et al* (2013) reported that tick infestation causes lesions and is one of the potential risk factors for occurrence of mastitis though creating a conducive situation for the majority of mastitis causing microorganisms.

The clinical mastitis found in this study with its three forms which was diagnosed by the obvious clinical signs and the visible alteration of milk and udder shape is in agreement with reports by Tibary and Anouassi (2000) and AL-Tofaily and ALrodhan (2011). The high incidence of chronic mastitis followed by acute and at last is gangrenous form. This agrees with finding of Sanaa (2005) who reported that the acute and chronic mastitis were one of important diseases of she-camel in Eastern Sudan. AL-Tofaily and ALrodhan (2011) reported in Iraq that last incidence type of clinical mastitis was gangrenous mastitis.

The present results displayed that the age of she-camels at different seasons had no significant effect on the occurrences of clinical mastitis this is in accordance with the studies of Abdurahman (2006) and AL-Tofaily and ALrodhan, (2011). The highest occurrence of clinical mastitis was found in age between 11-15 years these confirmed the study of Ynte *et al* (2003) who demonstrated she-camels above 9years were most susceptible for clinical mastitis. Husein *et al* (2013) studied the incidence of mastitis influenced by age and the results significantly associated ($p < 0.05$) and the lowest prevalence of mastitis in she-camels in age between 5 to 7 years in Jijiga town, Ethiopia.

The present findings indicated that there was a correlation between stage of lactation and clinical mastitis, this agrees with some studies Salwa (1995) and Suheir (2004) who reported the percentage of mastitis increased with progress of lactation.

During this study it was found that at the first, second and third calving the incidence of clinical mastitis was not affected significantly by the occurrence of the clinical mastitis disease. these is in agreement with that study reported by AL -Tofaily and ALrodhan (2011) who recorded the high incidence of clinical mastitis at the first, second and third calving. Salwa (1995) reported at the first calving the incidence of mastitis reached 20.5%, where during the second and third calving the incidence decreased to 19.2% and then continued to decline till last calving where it reached a 1.3%. The present findings are in contrast to the study of Suheir (2004) who reported that, during fourth and fifth calving the high incidence of mastitis (43.8%) and Omer (1991) who reported the incidence of mastitis increased during fifth calving then continuous to decline till the last calving.

In the present study the isolated Gram positive bacteria was at the rate 81.08% this is in accordance with the findings of Hawari and Hassawi (2008), Husein *et al* (2013) and Wanjohi *et al* (2013) who reported that Gram positive cocci of genera: *Staphylococcus, Streptococcus* and *Micrococcus* were most dominant udder pathogens isolated and regarded as an impotent pathogens in camel. Also the Gram negative bacteria was found in the present study lower than reported by AL -Tofaily and ALrodhan (2011) who recorded that 23.8% were *Salmonella spp, Klebsialla pneumonia* and *Mannheimia haemolytica*.

The predominant isolated organism of clinical mastitis in this study was *Staphylococcus spp.*(37.8%). This result agrees with the studies of Kalla *et al* (2008) who reported the coagulase positive and coagulase negative *Staphylococci* (28.5%) were the main pathogenic bacteria occurring in camel mastitis. Saleh and Faye (2011) and Husein *et al* (2013) reported that the *Staphylococcus spp.* was 42.9% and 43.8% respectively in these animals. Alamin *et al* (2013) reported that 80.3% she-camels examined that suffering from wounds on teat caused by the pieces of wood and cloth used in the anti-

suckling devices and *Staphylococcus spp.* might spread between she-camels due to the anti- suckling devices. The difference of percentage may be due to the fact the number of samples were collected for other studies. In these study *Staphylococcus aureus* has been identified as highest bacteria as the causative agent of mastitis for two types clinical and sub-clinical mastitis. In the present study *Staphylococcus aureus* was isolated at a rate of 27.03%, this is similar to the finding obtained by Abdel Gader (2001) and Alamin *et al.*, (2013) who isolated *Staphylococcus aureus* at the rate of 24.7% and 22.75% respectively. In the present study the isolation rate was lower than that reported by AL-juboori *et al* (2013) (44.82%)**.**

The present result showed that the second isolated bacterium was *Escherichia coli* (18.92%). This is similar to that reported by Amel (2003) and Kalla *et al* (2008) who recorded the isolation percentage 18.18% and 18% respectively. Also the present results was lower than that isolated by Wanjohi *et al* (2013) (60%) from North-Eastern province, Kenya.

The isolation rate of *Streptococcus spp.* in this present study was 13.51%. This is similar to the findings of Sibtain *et al* (2012) (15.63%). The present result is lower than results of Suhier (2004) and AL-juboori *et al* (2013) where their findings were 22.22% and 21.67% respectively.

All the *Bacillus cereus* isolated were found in mixed culture with Staphylococcus *spp.* and *Streptococcus spp.* In mastitic she-camels considered as a contaminant. This present result is in agreement with the findings of Alamin *et al* (2013) (9.09%) and higher than that of Suhier (2004) (2.02%). This bacterium was reported by Salwa (1995) and Eyassa and Bekele (2010) as a cause of all types of she-camel mastitis.

Micrococcus spp. isolation rate in this study was 8.11% where the isolation rate by AL-juboori *et al* (2013) was 5%. Amel (2003) and Suhier (2004) also stated that these organisms are an important causative of the mastitis among camels.

Corynebacterium spp. isolation rate in present study was 5.4%. This is similar to isolated by Suhier (2004) (7.07%). It is lower than AL-juboori *et al* (2013) and Sanna (2005) whose findings were 10% and 30.7% respectively. It is higher than Alamin *et al* (2013) (3.03%).

Salmonella spp. isolated in present study was 2 isolates (5.4%). This is similar to number recorded by AL-Tofaily and ALrodhan (2011) 2(9.52%). in this present study the *Salmonella* strain were isolated from acute cases, mixed by S*taphylococcus aureus* and the affected udder was cover by anti-suckling device (Gourab).

Anti microbial susceptibility test against the isolated bacteria in the present study generally showed high susceptibility to most used antimicrobial agents. It was highly sensitive to Gentamicin, Ciprofloxacin, Cloxacillin and Amikacin, moderate sensitivity to Ampicillin/Sulbactam and Trimoxazole and showed greatest resistance to Tetracycline and Chloramphenicol. These results are in agreement with the finding of AL-Tofaily and ALrodhan (2011), Fazlani *et al* (2011) and Alqurashi *et al* (2013) and in contrast to the findings of Abdel Gadir (2001) who recorded Oxytetracycline, Tetracycline and Chloramphenicol were effective drugs against camel mastitis. This high sensitivity to antimicrobial agents may be due to uncommonly used of these antibacterial in camel's diseases.

The trial of treatment in present study showed that the most effective drugs against camel mastitis was Pen&Strep® (Procaine Penicillin, Dihydrostreptomycin Sulphare), these results are in agreement with Kalla *et al* (2008) who reported the most effective drug agents *Staphylococcus spp.* and *Streptococcus spp.* was Stretopen® (Procaine Penicillin, Dihydrostreptomycin Sulphare)in Kano,Nigeria. Their results may confirm that mastitis in camel is mainly caused by Gram +ve organism.

Conclusion:

The results of the study could be concluded in the following.

1- The occurrence of clinical mastitis during both summer and winter seasons were found at the rate of 9.09%.
2- Three forms of clinical mastitis were diagnosed. Chronic form was of the highest occurrence (72.41%).
3- The effect of season on the incidence of mastitis was not significant at (P. <0.05).
4- The highest occurrence of clinical mastitis was found at the age between 11-15 years (65.52%).
5- There was a direct relationship between stage of lactation and number of calving and clinical mastitis.
6- The isolated organisms were Staphylococcus *spp*. 37.8 %, *E.coli* 18.9%, *Streptococcus spp.* 13.5%, *Bacillus spp*. 10.8%, *Micrococcus spp.* 8.1%, *Corynebacterium spp*. 5.4% and *Salmonella spp.* 5.4%.
7- The effective antibiotics against the isolated organisms were Gentamicin, Ciprofloxacin, Cloxacillin and Amikacin.
8- The treatment trial for few cases of mastitis showed that the most effective antimicrobial drugs was Pen&Strep®(Procaine Penicillin, Dihydrostreptomycin Sulphare).

Recommendations:

1. Mastitis in she-camel is an important disease problem. The extensive investigation and researches of mastitis aetiology may be capable of helping to provide optimistic approach to control and reduce the incidence of camel mastitis.

2. There must be extension program for the camel herd owners. This will minimize and control camel mastitis and stopped it the use anti-suckling devices and control the tick infestation.
3. A national program to diagnose and combat this disease should be launched. More studies in trial of treatment are needed to prevent before protocols of therapeutic management.

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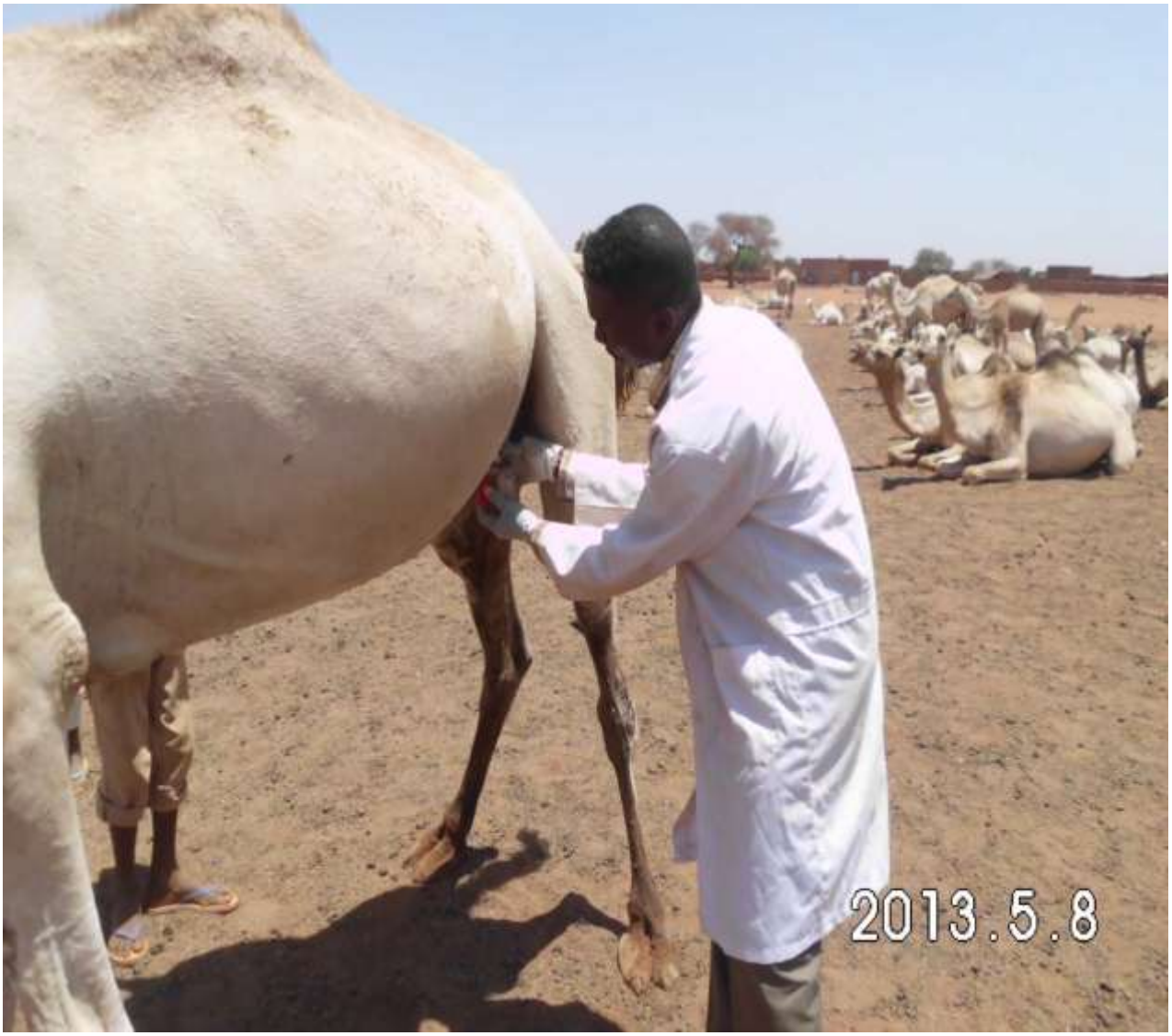
Appendix



Samples collection in Butana region (summer season)

Questionnaire

Sample number : Date: / /

Season.....… Location: ……

Number of the herd (size): …….. Breed: …….

Ratio of milking she- camels in the herd: …… Number of calving: ……

Respiratory rate: ……. Temperature: …..°C pulse rate: …….

Describe of Case: …….....

…….....

Type of mastitis: ……..

Do you use treatment for the disease? Yes [ ] No [ ]

Type of treatment: Medical [ ] Conventional drug [ ]

Name of drug: ……..

Respond to the treatment? Yes [ ] No [ ]

If don't response to treatment: …….

Are the tick infestations around infected udder?

Yes, there [ ] No, there [ ] There, same [ ]

Consumption of milk from mastitic udder? Yes [ ] No [ ]

The technique used to prevent the calves from suckling: ……

The age of infected animals: 5-10 [ ] 11-15 [ ] above 16 [ ]

Stage of lactation: first [ ] middle [ ] late [ ]

Milk production: high [ ] medial [ ] low [ ]

Other type of diseases in the herd: ……