بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Appraisal of Mastitis in Dairy Cattle in Khartoum North-Sudan

تقييم إنتشار مرض التهاب الضرع في أبقار اللبن بالخرطوم بحرى- السودان

By

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الآية

بسم الله الرحمن الرحيم

قال تعالى :

(وإِنَّ لَكُم فِي الأَنعَامِ لَعِبرَةً نَسقِيكُم ممَّا فِي بُطُونِهِ مِن بَينِ فَرثٍ وَدَمٍ لَّبَناً خَالِصاً سَآئِغاً للشَارِبِين).

صدق الله العظيم

النحل ..... الأية (66)

Dedication

To my parents' soul, who's lighted my life.

To my lovely husband Mossaddege Mohamed,

who stood beside me. Through thick and thin.

Acknowledgements

All the thanks to *Allah* who gave me the way and power to do this work ……

It is my pleasure to express my sincere thanks to my professor Dr. Mohamed Hassan Musa Tabidi the main supervisor for his supervision and full support to this study…

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Abstract

The present study was conducted foe detected of mastitis disease by using California Mastitis Test (CMT). The study side was at Khartoum North – Khartoum State – Sudan. A number of ten farms were selected at random, and ten lactating cows from each farm were selected to be performed by (CMT). Positive (CMT) cases were (3-10%) due to lack of bio-security measures.

The study also included sensitivity test, bacteria detected were Staphylococcus and streptococcus, and the antibiotic sensitivity test showed positive result for Gentamicin, Cloxacillin, Roxithromycin and Lincomycin.

A questionnaire was performed and revealed that the farms had lack of health measures and absent of hygiene scored high incidence rate of mastitis, while low rate of mastitis were recorded in hygiene farms.

ملخص البحث

أجريت هذه الدراسة لمعرفة وجود مرض التهاب الضرع باستخدام اختبار كاليفورنيا لإلتهاب الضرع (CMT) بمنطقة الخرطوم بحري - ولاية الخرطوم – السودان. تم إختيار عدد عشرة مزارع للأبقار إختيارا عشوائيا، وأختيرت من كل مزرعة عدد عشرة أبقار حلوب لإجراء الإختبار. الحالات الموجبة لإختبار (CMT) بلغت مابين (3-10%) من إجمالي الحالات التي أجري عليها الإختبار، حيث ظهرت الحالات نتيجة لإفتقار المزارع معايير الأمن الحيوي.

أظهر إختبار الحساسية أن الميكروبات المسببة للإصابة هي البكتريا السبحية والعنقودية، وسجلت البكتريا المسببة لإختبار الضرع حساسية للمضادات الحيوية الاتية: (الجنتاميسين Gentamicin والكلوكساسيلين Cloxacillin والروكسيثرومايسين Roxithromycin واللينكوميسين Lincomycin).

أجري إستبيان للمزارع يوضح البحث، ووجدت علاقة طردية بين نسبة الإصابة والحالة الصحية للمزرعة، و كانت النتيجة بأن المزارع التي تقل فيها الرعاية الصحية الجيدة و الاهتمام بالنظافة سجلت حالات إصابة عالية والتي توفرت فيها الرعاية الصحية الجيدة كانت نسبة الإصابة بها متدنية.

CHAPTER ONE

INTRODUCTION

Milk is a white liquid produced naturally by the mammary glands of healthy mammals. It is the primary source of nutrition for infant mammals before they are able to digest other types of food. Early-lactation milk contains colostrums, which carries the mother's antibodies to its young and can reduce the risk of many diseases. It contains many other nutrients including protein and lactose (*Pehrsson et al., 2000).*

Mastitis remains the most economically destructive imminent disease for consumers irrespective of many years of research worldwide with different levels of economic losses identified by different countries (Winkles *et al., 2005).* Milk production is reduced considerably in the affected animals and estimated loss of milk yield may range from 100 to 500 kg per cow per lactation in Holstein Friesian (NAAS, 2013). The predictable loss causing clinical mastitis in cows is nearly 700kg in first lactation and 1,200kg in the second lactation in commercial Holstein dairy farms in New York State participated in the study (Wilson *et al, 2004).*

In any herd, where there were poor hygienic and milking practices, the incidence of *Staphylococcus* and *Streptococcus* species was higher that other organisms as these organisms exist in the mammary gland of the cow and the major root of transmission for these organisms occur during milking through hand milker’s hands, teat cup liners and udder clothes (Paape, 1992).

Economic consequences of mastitis, clinical or sub-clinical, include reduced milk yield, poorer quality milk, increased culling rate, increased cost of veterinary services and_medicine and increase labour cost for the farmers (Leach *et al., 2009).* Bacterial contaminated milk from mastitis infected cows

can impede with food manufacturing process or can cause food poisoning; may render unsuited for human consumption, and in some circumstances may have the possibility to transmit disease to humans (Tiwari *et al.*, 2013*)*. Enormous economic losses by mastitis are also experienced due to unmarketable milk or milk-products adulterated with antibiotic residues originating from treatment in the developing nations along with usage of antibiotics as growth promoters predominantly in dairy feedlots in the advanced world (Zafalon *et al.*, 2007). The long time use of antibiotics in the treatment of mastitis has directed further problem of occurrence of antibiotic resistant strains, therefore there is continual worry about treatment failure and the resistant strains entering the food chain (Collins *et al.*, 2010).

The disease should be studied as it causes financial losses as a result of reduced milk yield and quality, discarded milk following antibiotic therapy, veterinary expense and culling mastitis cow (Hundera *et al.*, 2005). Therefore, the objective behind this assessment is to provide an overview to find out about the burden and impact of mastitis on milking dairy cattle in Khartoum North, Sudan, which considered as a large centre for dairy production.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mastitis

Mastitis is considered to be the most frequent and most costly production disease in dairy herds of developed countries (Espeche *et al*., 2009). The assessment of the economic worthiness of control programme for mastitis has to be supported by a reliable evaluation of the economic losses caused by the disease and the knowledge of the costs of the implementation of the programme.

Mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying and neutralizing the infectious agents and to prepare the way for healing and return to normal function. Inflammation can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants (Jones and Bailey, 1998). Mastitis is one of the most common dairy diseases (Rajala *et al*., 1999) because of its high incidence (Seegers *et al*., 1997). The economic consequences of mastitis either clinical or sub-clinical include loss of milk production, loss of milk sales, increased culling rates, and cost for veterinary treatments (Schukken et al., 1997).

2.1.1 The Somatic Cell Count (SCC)

The Somatic Cell Count is a main indicator of milk quality. The majority of somatic cells are leukocytes (white blood cells) - which become present in increasing numbers in milk usually as an immune response to a mastitis causing pathogen - and a small number of epithelial cells, which are milk

producing cells shed from inside of the udder when an infection occurs (Schukken et al., 2003).

Milk cell count has been used extensively as an indicator of the infection status of the mammary gland (Hillerton, 1999), in addition to that high SCC in milk affect the price of milk in many payment systems that are based on milk quality (Schukken et al., 1997).

2.1.2 somatic cell scores (SCS)

As the somatic cell count (SCC) measurements from individual test milk data are not normally distributed, this data cannot be used directly for the breeding value estimation. The SCC measurements are transformed in order to comply with the condition of normal distribution into somatic cell scores (SCS) for the breeding value estimation. The transformation is as follows **(**Harmann, 1993): $SCS = 1000 + 100*(^{2}\log(SCC/1000))$

2.1.3 Intra-mammary infection (IMI)

In most countries, dairy cattle breeding programs are directed toward milk production traits. Although these traits are of primary economic importance, functional traits such as longevity fertility and udder health are of increased interest to producers to improve herd profitability. Mastitis is defined as an infection of the udder, caused by bacteria entering the quarter through the teat current concepts of bovine mastitis.

The German Veterinary Medicine Association (DVG, 1994) categorized the udder health status as shown in table 1. Table 1: Categorization of udder health status (DVG, 1994) The legal maximum bulk tank SCC is lower in other dairy exporting countries than USA (Smith and Hogan, 1995). Canada has a limit of $500x10^{3}$ cells/ml, in the European community, Norway, Switzerland, Australia and New Zealand the maximum bulk tank SCC is

400x10³ cells/ml. In those countries, SCC is calculated as a geometric mean of Cell count per ml milk Pathogenic organisms Negative Positive < 100x10³. Normal secretion latent infection > 100x10³. Non-specific mastitis mastitis 3 successive milk shipments over several weeks, therefore, it is expected to be lower than arithmetic mean (Shook and Ruegg, 1999).

2.2 Classes of mastitis

2.2.1 Clinical mastitis

Clinical mastitis is defined as an infection of the udder that results in visible changes in the udder quarter and milk (Rodenburg, 1990), may it be acute, sub acute or chronic. The development of clinical mastitis in dairy cows can be detected with high sensitivity and specificity in advance of visible changes in foremilk or udder tissue by determining the electrical conductivity of the foremilk (Milner et al., 1997). The genetic correlations between clinical mastitis and SCS among different lactations were positive and moderate to high varied from 0.37 for the first lactation to 0.68 for the third lactation Weller et al., 1992). Where as Mrode and Swanson (1996) estimated a genetic correlation between SCC and incidence of mastitis of 0.7. Peeler et al. (2000) in a study to assess the level of clinical mastitis and to quantify risk factors associated with the incidence rate of clinical mastitis in U.K, found a mean incidence rate of clinical mastitis of 22.8 cases per 100 cows/year. They also reported that the incidence rate of clinical mastitis increased when farmers reported that they had straw yard housing for milking cows (compared with cubicle housing), mucked out the calving area less frequently than once per month, when they had greater than 50% replacement rate and when always practiced post-milking teat disinfection. Barkema et al. (1999) attributed the increase in the incidence rate of clinical mastitis in herds practicing post-milking teat disinfection to *E. coli* infections. While Wilson and Kingwill (1975) and Wilesmith et al. (1986) claimed that the incidence rate of clinical

mastitis in Great Britain has declined from an estimated 12 cases per 100 cows/year in 1960 to approximately 40 cases per 100 cows/year in 1986 due to a reduction in mastitis caused by contagious pathogens particularly *S. aureus, St. agalactia* and *St. dysgalactia* through the introduction of improved control measures.

(Booth, 1988) reported that the reduction in the prevalence of contagious pathogens resulted in a decrease of the average bulk milk SCC from $573x10^3$ cells/ml to $352x10^3$ cells/ml. But (Barkema *et al*., 1998) showed in a recent study that there was no association between bulk milk SCC and incidence rate of clinical mastitis. (Aarestrup and Jensen , 1997) found that the presence of bacteria in a quarter before parturition increased the risk of IMI for the lactating cow and the variability in the prevalence and the duration of intra mammary infection occurred around the first parturition. (Schukken *et al*, 1997) reported that mastitis has many economic consequences among which are loss of milk production, loss of milk sales, increased culling rates and cost for veterinary treatments, in addition to that high SCC in milk affects the price of milk.

2.2.1.1 Clinical mastitis on milk yield

(Rajala-Schultz et al., 1999) studied the effect of clinical mastitis on milk yield in dairy cows, they found that the daily loss during the first 2 weeks after the occurrence of mastitis varied from 1.0 kg to 2.5 kg and the total loss over the entire lactation varied from 110 kg to 352 kg; cows with mastitis did not reach their pre mastitis milk yields during the remainder of the lactation after onset of the disease. (Rupp and Boichard, 1999) indicated that SCC is a more accurate measure of udder health than records of clinical mastitis. Because SCC are generally routinely recorded in most milk recording systems, in the time that clinical mastitis events are not routinely recorded in

most countries except in Scandinavian countries and the field data may not be accurate, complete or standard. In addition to that the heritability of SCC is much greater (0.15) than that of clinical mastitis (0.02-0.03) and SCC also reflects incidence of sub-clinical infections.

(Trinidad *et al*., 1990) studied the prevalence of IMI in unbred and primigravid dairy heifers, they found that 97% had IMI and 29% showed clinical symptoms, 75% of the quarters were infected. Presence of mammary inflammation in young dairy animals could be deleterious to the future milk production as the mammary tissue development occurs to the large extent during the first gestation (Tucker, 1987). (Etherington *et al*., 1996) reported that 6.8% of the culling rate of cows in Ontario-Canada was due to mastitis. Mastitis also found to reduce both milk production (Fetrow *et al*., 1991) and reproductive performance in a lactating cow (Moore and O'Connor, 1993). (Barkema *et al*.,1998) demonstrated that cows with clinical mastitis during early lactation exhibited a prolonged interval until first service (94 days) compared with animals with no clinical mastitis (71 days). Additionally, cows with clinical mastitis between the first service and the establishment of pregnancy had increased number of days open and a two fold increase in services/conception.(Rupp and Aazin,2000) stated that without clinical signs of mastitis during the first month of lactation and with a first test day a SCC lower than $400x10^3$ cells/ml. they also claimed that the risk of first clinical mastitis was highest around the second calving in lactation starting in summer and for high-yielding cows.

2.2.1.2 Probability of clinical mastitis

The probability of clinical mastitis occurring increased continuously as initial SCC increased. They also concluded that cows with the lowest initial SCC had the lowest risk for clinical mastitis without any intermediate optimum.

(Emanuelson and oltenacu, 1998) reported that direct selection against clinical mastitis is difficult because in most countries other than the Nordic ones clinical mastitis event is not widely recorded because the corresponding heritability of the trait is very low close to 0.02, while (Heringstad *et al.*,1999) estimated heritability of clinical mastitis in Norwegian cattle to be 0.035.

2.2.2 Subclinical mastitis

(Rodenburg, 1990) showed that 97% of all cases of mastitis are sub-clinical which do not involve visible changes to the quarter or the milk it produces. While Reneau and Packard, 1991 reported that approximately 70 to 80% of the mastitis cases are sub-clinical. Sub-clinical mastitis is found to be associated with decreased milk yield; also a positive relationship clinical mastitis with milk yield has been found (Fetrow *et al.*, 1991). (Laevens *et al.*, 1997) indicated that the measurement of SCC from dairy herd improvement programs is used worldwide as indicators of sub-clinical mastitis. Harmon and Reneau, 1993 reported in different studies that IMI have been recognized as major factors that influence SCC. Milk from healthy udder quarters was found to have an average value of SCC between $23x10^3$-$50x10^3$ cells/ml depending on the breed and the physiological status of the animal (Klaas, 2000).

The milk yield starts to drop with an increase in SCC over $100x10^3$ cells/ml (Korhonen and Kaartinen, 1995). They also showed that the increase in SCC to a level more than $100x10^3$ cells/ml resulted in 18% reduction in milk yield. (De Graaf and Dwinger ,1996) estimated the crude milk production losses per cow with sub-clinical mastitis as 1.56 kg/day for daily milk yield, and the milk production loss per affected quarter due to sub-clinical mastitis was estimated to be 17.6% on average. They concluded that the decrease in milk

production in heifers with sub-clinical mastitis did not differ significantly from the decrease in production in older cows. Sub-clinical mastitis is also known to affect the reproductive performance of the animals. (Schrick *et al*., 2001) found that cows with subclinical mastitis before the first service had an increase of days to first service (74.8±2.7d**)**, days open (107.7±6.9d) and services per conception (2.1±0.2) compared with the control (67.8±2.2d, 85.4±5.8d and 1.6±0.2; $p<0.05$).

2.3 Etiology and Epidemiology

Mastitis is known to be established as a result of the reaction of three bio-systems namely the causative agent, the animal and the environment in which the animal lives. Sandholm and Korhonen, 1995 reported that the primary and secondary body defence mechanisms prevent the pathogenic microbes from entering the mammary gland through the teat canal orifice. They also indicated that the concentrations of the antibacterial factors in the udder secretion are under genetic control and depend on the lactation stage and udder health. The environmental factors such as management, feeding, hygienic status, bedding, milking and the virulence of the organism contribute to the disease.

Lesile, 1996 reported that stress factors such as isolation of an individual and mixing groups of cows have been shown to increase somatic cells count in the absence of mastitis, moreover it has been reported that there was no increase in SCC.

2.4 Causative agents

2.4.1 Classes of mastitis pathogens

Several researchers (Smith and Hogan, 1995) concluded that mastitis causing organisms can be classified into two main groups: Contagious pathogens

which spread by means of hands, milking units and include *S. aureus, St. agalactiae,* and Mycoplasma. Environmental organisms which live in the cow's environment ansd are always present, they include *E. coli, St. dysg., St. ubris.* (Buzalski and Seuna , 1995) stated that contagious mastitis is mainly caused by Staphylococci and shows high cell count in bulk milk whereas environmental mastitis results in a high number of clinical cases, but the cell count in the bulk milk is usually not high. Another group of mastitis causing organisms called minor pathogens (Keown, 1997) and include *C. bovis* and Coagulase-negative *staphylococci* (CNS). (Buzalski and Seuna,1995) reviewed the results of the microbiological examinations of milk samples that were done in Finish milk inspection laboratories in 1991and reported the frequency of mastitis causing organisms as given in table 2. 7 Table 2: Frequency of mastitis causing organisms (FMI, 1991).

2.4.2 Mode of transmission

Several research studies concluded that the contagious organisms spread during the milking process (Bray and Shearer, 1996) causing an infection of the udder as a result of entering the teat canal (Rodenburg, 1990). The former authors also showed that scar or connective tissue replacing the destructed milk secreting tissues and result in a permanent loss of the productive ability. (Sandholm and Korhonen,1995) reported that the udder becomes infected through the teat canal which represents a physical barrier to the penetration of bacteria. They also added that when the udder is dilated the risk of infection is high. An infected mammary gland can act as a reservoir for mastitis microbes (Barnes *et al*., 1987). Pre-partum heifer infections have been attributed to the feeding of mastitis milk to heifer calves and allowing heifers to suckle each other (Mc Donald, 1982), however, in another studies it was found that feeding contaminated milk did not increase the prevalence of IMI at parturition over control heifers fed milk free from contagious organisms

(Barto et al., 1982) Bushnell, Bacterial species No. of samples % *St. agalactia* 13890.63 *St. dysg.* 93974.29 *St. ubris* 107674.91 *β-haemolytic streptococci* 15530.71 *S. aureus* 4254619.42 CNS 3041713.88 *E. coli* 31781.42 *Klebsiella* 7220.33 *Pseudomonas aeruginosa* 1440.07 *Actinomyces pyogenes* 12720.58 Yeast, moulds and fungi 12240.56 Other 106154.84 Total 11322451.67 No growth 10589248.33 All samples 219116100.008 1989). (Kirk *et al* ., 1996) presented that the high risk of contagious organisms can be from the movement of animals onto the dairy herd as they may carry in a pathogen which did not exist or they may themselves not have immunity to pathogens already exist.

Chrystal *et al*., 1999 stated that nearly all IMI occur as a result of micro organisms passing through the teat canal, and that wider teat diameters were associated with higher SCS. On the other hand, (David and Shearer ,1996) reported that the environmental organisms mainly live in the animal's environment like rumen and udder. The organism can also be found in feces, polluted water and bedding material. The inflammation results from the cow's reaction to the bacterial irritation and the progress of the infection depends on the ability of bacteria to adapt to milk environment and on various virulence factors (Ali-Vehmas and Sandholm, 1995).

2.5 Contagious pathogens

2.5.1 *Staphylococcus aureus*

Bray and Shearer, 1996 reported that *S. aureus* Lives in the udder and on the skin surfaces of an infected cow. Ali-Vehmas and Sandholm,1995showed that the organism can produce capsular material, hemolysin and β-lactamase when incubated in mastitis milk and are transmitted from infected quarters to uninfected quarters during the milking process (Risco *et al*.,1999).

Bray and Shearer, 1996 found that *S. aureus* is one of the organisms responsible for about 95% of IMI..(Roberson *et al.*,1994) found that the mean prevalence of *S. aureus* IMI in high prevalence herds (>10%) to be 30% where as the mean prevalence of *S. aureus* IMI in a low prevalence (<5%) herds was 2%. (Trinidad *et al*.,1990) isolated *S. aureus* from 37% of all cases and 14.9% of the quarters. (Pankey *et al*.,1991) reported that the prevalence of *S. aureus* IMI in primiparous cows at parturition to range from 2-50%. The prevalence of *S.aureus* IMI in pre-partum heifers varied considerably among different regions and herds, (Pankey *et al*.,1991) found a very low prevalence of *S. aureus* IMI. While Aarestrup and Jensen, 1991 found no evidence of *S. aureus* infection at all. Other researchers (Nickerson *et al*., 1995) reported a relatively high prevalence. (Waage *et al*.,1999) in a study of dairy heifers found that *S. aureus* was most frequently isolated organism from quarters (44.3%). (Trinidad *et al*., 1990) reported 20% of all infected quarters were *S. aureus.*

In Latvia a study was conducted by (Jemeljanovs *et al*., 1999) showed that 55.17% of all cases of udder inflammation of 439 cows udder secretion were caused by *S. aureus.* (Gentilini *et al*.,1994) discovered that *S. aureus* is considered one of the most etiologic agents in Argentina. Jones and Ward ,1989 found that of 20% Staphylococci isolated, 14 were *S. aureus,* and that cows immunization by *S. aureus* experimental vaccine increased their resistance and decreased SCC in comparison with the control groups Jemeljanovs and Bluzmanis, 2000). (Firat, 1993) reported that *S. aureus* IMI reduced milk yield 230 Kg, while the somatic cells count found to be $900x10^3$/ml compared to $200x10^3$/ml of non- *S. aureus* infection (Buelow, unpublished thesis,1993 cited by(Zepeda et al., 2000). (Barkema et al. , 1999) presented that the incidence rate of mastitis caused by *S. aureus* was mostly related to factors associated with bulk milk SCC.

2.5.2 *Streptococcus agalactiae*

St. agalactiae belongs to the group of pyogenic hemolytic streptococci and serologically to Lancefield's group B (Buzalski and Seuna, 1995). *St. agalactiae* is an obligatory organism of the cow's udder, mastitis caused by it spreads particularly during the milking through the equipment, and is highly contagious, either chronic or recurrent, often the cell count of the milk remains quite low (Pyörälä, 1995). (Morin and Hurley, 1999) stated that *St. agalactiae* inhibits ducts anssd cisterns of the mammary gland. It causes an inflammation which blocks the ducts, leading to decreased milk production and increased SCC. (Barkema *et al*., 1999) reported a 0.004 incidence rate of mastitis of *St. agalactiae* and as was associated with management practices.(The US national Mastitis Council 1996) published that *St. agalactiae* as a contagious bacteria is transmitted from infected quarters to uninfected quarters during the milking process. (Jemeljanovs and Bluzmani, 2000) showed that 14.85% of the mastitis cases in Latvia was *St. agalactiae*.

The organism was reported to have the highest interclass correlation within a cow for natural logarithm SCC (Barkema *et al*.,1997). In the forties of the last century it was reported that feeding milk containing *St. agalactiae* to heifers calves and subsequent suckling among heifers would result in IMI by this major contagious pathogen at first parturition (Roberson *et al*., 1994).(Ma et al., 2000) found that in milk collected from Holstein cows after IMI with *St. agalactiae,* post infection milk had significantly higher somatic cells count ($849x10^3$/ml) than pre-infection milk ($45x10^3$/ml). In a study for mastitis control (Bray and Shearer ,1996) found that *St. agalactiae* lives in the udder and cannot exit outside the gland for a long period, it is 10 susceptible to penicillin and once eliminated usually does not return to the herd unless infected cows are purchased.

2.6 Environmental pathogens

2.6.1 *Streptococcus dysgalactiae*

St. dysg. is one of the major pathogens belongs to the Lancefield's group C, *St. dysg.* is no longer included in the Streptococci group, but retained the name in the mastitis field (Buzalski and Seuna, 1995). The organism lives almost anywhere: in the udder, rumen and feces and in the barn, its spread can be stopped by dipping the whole teat to the base of the udder (Bray and Shearer, 1996). The pathogen is most prevalent in the examined quarter milk samples from 1500 heifers with clinical mastitis before or within 14d after parturition (Jonsson *et al*., 1991). (Pyörälä ,1995) stated that the identification of the organism is based primarily on a biochemical reaction and can be isolated from summer mastitis. (Sansdholm and Payörälä,1995) found that the incidence of *St. dysg.* increases in herds where teat dipping and dry cow therapy are applied. Whereas, (Payörälä and Buzlski, 1995) reported that the organism is highly susceptible to Penicillin and its derivatives. On the other hand (Buzalski and Payörälä , 1995) showed that herds infected with *St. dysg.* appears as high cell counts in the bulk milk.

Payörälä and Buzlski , 1995 found that the organism is found to be associated with teat lesions. In the study conducted by (Barkema *et al*., 1997) it was shown that a lower intra-class correlation within herd (0.03) was detected between the frequency of the organism and SCC (log).(Waage *et al*., 1999) found that the frequency of *St. dysg.* was 18.2% of 1040 heifer's quarters samples affected with clinical mastitis and that was collected prior or within 14 d after parturition. (Aarestrup and Jensen, 1997) discovered a strong association between IMI with *St. dysg.* before parturition and IMI with *St. dysg.* after parturition.

Whereas (Barkema *et al*., 1999) found a strong positive correla tion between the incidence rate of clinical mastitis caused by *St. dysg.* and that caused by *S. aureus.* They also added that the incidence rate of mastitis caused by *St. dysg.* was related to nutrition, milking technique and machine milking . (Ósterås *et al*., 1999) stated that a cow had an infection or identification of a major pathogen 45+32 days prior to drying off and a series of composite milk SCC>100x10^3/ml before sampling.

2.6.2 *Escherichia coli*

E. coli is an environmental pollutant organism, transmit by flies and digestive feed, found in the lower intestine of warm-blooded organisms, lives in feces, polluted water and bedding materials, it is not susceptible to antibiotics (Bray and Shearer, 1986). The organism belongs to the family Enterobacterioceae. The injury of the teat canal often leads to acute mastitis caused by *E. coli* (Buzalski and Pyörälä, 1995), and hence it is considered to be an environmental pathogen (Radostits *et al*.,1994). (Hogan and Smith, 1987) found that the microorganisms may be eliminated before or shortly after onset of clinical symptoms, therefore the host defense system appears to eliminate *E. coli* efficiently (Hill *et al*., 1978) especially when IMI occurs late in lactation (Hill and Shears, 1979). Recurrent clinical episodes were found in 9.1% of quarters with mastitis caused by *E. coli* (Lam *et al*., 1996). Whereas (Waage *et al*., 1999) found the frequency of *E. coli* to be 6.4% from infected quarters. *E. coli* was one of the most prevalent pathogens in the study of (Jonsson *et al*., 1991). (Döpfer *et al*., 1999) discovered that in 4.77% of all episodes of clinical mastitis caused by *E. coli*, persistent IMI caused by the same *E. coli* strain. (Jones and Ward , 1989) reported that *E. coli* was the predominant cause of mastitis in early and late lactation. (Barkema *et al*., 1999) stated that the incidence rate of clinical mastitis caused by *E. coli* was mostly related to housing, hygienic measures and machine milking.

2.7 Minor pathogens

2.7.1 Coagulase-negative *staphylococci* (CNS)

CNS were previously called micrococci, species most often isolated from CNS mastitis are *S. hyicus, S. simulans, S. epidermidis, S. warners, S.xylosus, S. hominis, S.haemolyticus* and *S. chromogens* (Buzalski and Seuna, 1995). Mastitis caused by them occurs at all stages of lactation but is most common during drying-off and soon after calving and considered milder than *S .aureus* mastitis because they possess less virulence factors than *S. Aureus* (Bramley, 1991).

CNS bacteria can often cause teat infection which cause only a slight increase in milk cells count, mastitis occurs particularly in heifers.(Jones and Ward , 1989) found that of 20 Staphylococci isolated four were CNS, which were seen in cows soon after parturition and caused 14% cases of mastitis.

A similar finding was reported by (Pankey *et al*., 1996), they stated that CNS were isolated from 21.8% of the heifers in Waikato. Studies in USA have reported that up to 90% of heifers quarters are infected before parturition and 70% were infected with CNS (Trinidad *et al*.,1990).(Aarestrup and 12 Jensen, 1997) found that *S. chromogenes* was the bacterial species isolated most often before parturition (15% of quarters). Whereas(Waage *et al*., 1999) found that of the most prevalent isolates of the CNS were *S. simulans* (53.7%), *S. hyicus* (14.8%) and *S. chromogenes* (14.8%). They also concluded that CNS were the main cause of sub-clinical IMI. (Laevens *et al*., 1997) concluded in a study that a single isolation of CNS was resulted in statistically increase in SCC with least square mean SCC (loge-transformed) as 3.97.

2.7.2 *Corynebacterium bovis*

C. bovis is a relatively common causal agent of a mild mastitis, it requires oleic acid present in milk to grow (Buzalski and Pyörälä,1995). This organism is considered to be a typical contaminant of milk flowing from the udder (Mantere-Alhonen, 1995). Classified as environmental pathogen that usually causes considerably less somatic cells count elevation (Keown, 1997).(Laevens *et al*., 1997) indicated that a single isolation of *C. bovis* was associated with a numerical increase in somatic cells count. However, (Rainard *et al*., 1990) in different studies concluded that a single isolation of *C.bovis* considered to be a false-positive result. (Barkema *et al*., 1997) found in a study that *C. bovis* had the highest intra-class correlation within herd (0.11) with the natural logarithm of SCC.

2.8 Risk factors influencing determinants of intra-mammary infection

There are plenty of predisposing factors that can influence emergence of mastitis at individual and herd level in dairy cattle. The factors may be physiological, genetic, pathological or environmental (Sordillo, 2005) which are given below:

Age of cows has significant affects in occurring mastitis. It has been shown that manifestation of mastitis in infected quarters increases with advancement of age in cows (Sharma, 2010)the highest occurrence are being observed in cows of more than 7 years of age, (Schukken *et al*.,1989). This may be due to more dilated teat canals in older age, permanent udder tissue damage resulting from the primary infection or due to an increased cellular response to intra mammary infection (Rahman, 2009). Another reason may be effective innate host defence mechanism that makes the younger animals less susceptible to infection (Dulin, 1988). Moreover, cow's parity has significant influence of on prevalence of mastitis in farms. Cows in parity number more than 3 have

considerably higher mastitis prevalence than those of parity 2-3 and primipa rous one (Sarker *et al*.,2013). Primiparous cows have stronger defence mechanism than multifarious cows that make them less susceptible to mastitis.

2.8.1 Genetic Factors

Several genetic traits may also have substantial effect upon susceptibility to mastitis in bovine. These genetic traits are natural resistance of cows, comparative distance between teats, teat shape and conformation, position of udders and milk yield and fat content of milk. High yielding dairy cows with high fat contents are reported to be more vulnerable to mastitis (Rajala and Groh"n ,1998).

Udder conformation and shape of the teat are significant genetic traits that may also affect susceptibility to mastitis. Cows with extended teats are more susceptible to mastitis infection compared to cows with reversed teat ends (Ranjan *et al* ., 1976). An additional important influencing factor for mastitis is super numerous teats, which may be responsible for extra reservoirs for potential pathogens leading to manifestation of mastitis.

Lactation has a highly significant consequence on prevalence of mastitis. In bovine, there is possibility of increased oxidative stress and reduced antioxidant defence mechanisms immediately after parturition, early lactation and during the dry period and the incidence of mastitis is reported to be higher during these times (Sharma *et al*., 2011).

2.8.2 Environment Factors

The incidence of mastitis is obviously influenced by the weather and climatic conditions. A greater percentage of mastitis has been observed to arise mostly during summer and rainy months (Reneau, 2012) .Bacterial propagation as

well as the load of pathogens increases as heat and humidity increases in the environment (Godden *et al*., 2003). On the contrary, another study has reported a greater occurrence of coli form mastitis during the cold months when the temperature was recorded to be less than 21°C (Ranjan *et al* ., 2011) in the environment.

2.8.3 Nutritional Factors

Plan of nutrition is another important factor that influences clinical manifestation of mastitis in heifers and cows (Heinrichs *et al*., 2009). Vitamin E has been described to boost the immune response of cows by enhancing the phagocytic properties of neutrophils after parturition (Spears and Weiss, 2008). Vitamin E combined with selenium acts as an anti-oxidant substance by preventing oxidative stress (Mustacicn and Powis, 2000). Numerous investigations have proved that neutrophils of selenium fed cows are more active at killing mastitis triggering microorganisms than those not complemented with selenium (Underwood and Suttle, 1999). Beta-carotene and Vitamin A have also been recognized to be active in inhibiting the manifestation of mastitis, most possibly owing to their antioxidant and immune-enhancing properties to influence mucosal surface reliability of the mammary gland (Sordillo *et al*., 1997). Dairy feed supplemented with Zinc and copper contribute in mammary gland health by promoting cellular repair, wound healing and reduction in SCC (Prasad *et al*., 2004) assisted by rises inmetallothionein synthesis with antioxidant potential.

2.9 Herd management and milk hygiene

Herd management and milk hygiene are also considered significant risk factors for mastitis (Sarker *et al*., 2013). The occurrence of bovine mastitis is remarkably higher in less clean udder in contrast to clean one. The farms have followed pre and post teat dipping practices for milking have been less

affected by mastitis infection. Several studies have found that left front quarter (LFQ) are more affected by mastitis than the other quarters and may be due to contaminations from the operators left hands without proper washing and disinfection (Abdulahis *et al* ., 2012) . In farms where milking machines are used for milking, it is essential to conserve ideal pressure like 50kPa for most machines, because extra pressure may lead to incomplete milking and tissue damage in the teat (Blood and Radostits, 1989) .These may cause increased risk of both contagious and environmental mastitis. Therefore, it is better to use those milking machines that can achieve complete milking as well as fewer incidences of teat injuries (Mein and Schuring, 2003).

2.10 Factors influencing frequency of pathogens and infection rate

Infectious mastitis is present when the pathogen and the inflammatory changes were detected in the secretion, whereas non specific mastitis is present when there were inflammatory changes but no pathogen in the secretion and a latent infection is present when the secretion contained pathogens but had normal cell count (IDF, 1987). (Waage *et al*.,1999) analyzing data of 1122 infected quarters that were clinically affected found that after treatment the re-examination results showed 22% non functional quarters, 14% still affected by clinical mastitis and 12% affected by sub-clinical mastitis.

Hogan and Smith, 1989 stated that the percentage of quarters infected with environmental streptococci is low and seldom exceeds 10% of quarters. A group of researchers (Woodward et al.,1988) concluded that in herds in which post-milking teat antisepsis is not practiced, it is not unusual for *C. bovis* to be isolated from more than 60% of quarter milk samples and the new infection rate of such organism was nearly 30 times higher than that of *St. agalactiae* which is attributed to 13 teat colonization and subsequent contamination of

milk samples., (Peeler *et al*.,2000) stated that the reduction in the incidence rate of mastitis in Great Britain is attributed to the reduction in mastitis caused by contagious pathogens through the introduction of improved control measures. (Shoshani and Berman, 1998) assessed sub-clinical mastitis by deviation in milk yield and suggested that there are episodic aggravations in mammary health that do no evolve into mastitis but may induce significant losses in milk yield and quality.

2.10.1 Herd size

It was earlier suggested that there was a relation between the farm performance and the farm structure (Van Asseldonk *et al*., 1998). Herd size was observed as a risk factor for mastitis with a significant influence (Waage *et al*., 1998). Although herd size was found to have no significant effect on the occurrence of mastitis in the study of (Costa *et al*., 1998), but(Smith *et al*., 2000) stated that small herds reported more cows leaving for mastitis than high medium and low medium herd size.(Wilesmith *et al*., 1986) claimed that the incidence of mastitis declined with increasing herd size.

2.10.2 Year-Season

Waage *et al*., 1999 in their study of the bacteria associated with mastitis in dairy heifers found that the proportion of *S. aureus* and *Actinomyces pyogenes* were highest and the proportion of CNS were lowest in late autumn and early winter. The proportion of *E. coli* was highest in summer; they concluded that the relative percentage was significantly affected by season. (Jonsson *et al.,* 1991) who examined quarter milk samples of 1500 heifers with mastitis before or 14d after parturition, stated that the relative percentages of some organisms were significantly affected by season.(Jones and Ward, 1989) in their study of the cause of mastitis in dairy cows in

Wisconsin, detected mastitis with approximately equal frequency throughout the year.

Hogan *et al*., 1989 in their field survey of clinical mastitis in low SCC herds showed that the rate of infection was different among seasons of the year. Shpigel *et al*., 1998reported that the incidence of mastitis in Israeli dairy herds was lower in summer months.

2.10.3 Lactation number

The US national mastitis council (1997) showed that the rate of streptococcal infection increases progressively as the lactation number increases. Schaeffer and Solbu, 1987 who investigated the Norwegian red cattle, reported that a first lactation cows had a 10% 14 probability of having mastitis, which was roughly the same for second, third and fourth lactation, provided that they did not have mastitis in the previous lactations. While cows that had mastitis in the immediately previous lactation, had doubled this probability of having mastitis again. A fourth lactation cow that had mastitis in the three previous lactations had a 62% probability of having mastitis in the fourth lactation.

They also concluded that there does not seem to be an age effect on the probability of mastitis occurrence and any cow that has not had mastitis previously has a 10-11% chance of having mastitis in the current lactation regardless of parity number. Analogous findings were reported by(Firat, 1993) who analyzed data dealing with susceptibility of clinical mastitis in successive lactations and indicated that cows with mastitis in the preceding lactation were almost twice susceptible to mastitis in the current lactation than those without mastitis in the preceding lactation with probabilities of 0.46 and 0.29, respectively. (Fetrow *et al*., 1991) reported that the carry-over effect of mastitis from one lactation to the next found to be statistically significant but small. (Nickerson *et al*., 1995) found in a Louisiana study of 116 pregnant and

unbred Jersey heifers with collected samples from four herds that the bacterial infection were present in 97% of heifers and 75% of quarters, and there were 2.8 infected quarters per animal. (Shpigel *et al*., 1998) observed an increase in the incidence of mastitis as the lactation number increases till the fifth lactation then start to decrease.

Hogan *et al*., 1989 stated that the incidence of mastitis caused by environmental bacteria in the first and second lactation is greater than in older cows. Different from the result that obtained by (Zadoks *et al*., 2001) who found that the rate of infection with *St.uberis* was lower in first and second parity cows than in older cows and was depending on the stage of lactation in one herd.

Fleischer *et al*., 2001 found a significant relationship between the previous 305 days milk yield and the incidence of mastitis.

2.10.4 Stage of lactation

It is known that the risk of environmental mastitis infection is highest during early lactation and decreases as the lactation advances. The US national mastitis council 1997 stated that the rate of IMI is higher during the dry period than during lactation, and during the first 75 days postpartum the rate of infection is higher than it is during the remainder of lactation.

The percentage of infected quarters with environmental streptococci at any one point is generally low and seldom exceeds 10% of quarters. In an early study,(Munch- Petersen, 1970) stated that 22% of all quarters in heifers were already infected by the first 15 day of lactation, and by the end of the first week of the lactation the infection decreased to 9.4%. (Trinidad *et al*., 1990), reporting a US study, found that up to 90% of heifers had quarters infected before parturition, while other researchers in the USA and Europe (Matthews *et al*.,1992) claimed that the IMI rate in heifers was moderate

(13 to39%) (Jones *et al*., 1998) stated that the last 7-10 days before calving or early lactation is the time of greatest susceptibility to new environmental streptococci infections.

2.10.5 Farm management factors

The US national mastitis council's (Fact sheet, 1997) states that housed cows are at greater risk for environmental mastitis compared to cows on pasture. And that post milking teat barrier dips reduce new coli form IMI but their efficacy against the environmental streptococci and contagious pathogens appears to be lower than that of germicidal preparations. They showed also that back flushing of the milking unit does not control environmental mastitis. Additionally, malfunctioning milking machines which result in frequent liner slips and teat impacts can increase cases of environmental mastitis.

Washburn *et al*., 2002 compared seasonally calved Holstein and Jersey cows in confinement or pasture systems and found that cows in confinement had 1.8 times more cases of clinical mastitis and 8 times the culling rate for mastitis than did cows on pasture.(Jones and Bailey, 1998) reported that purchased heifers from another source could harbour mastitis pathogens and should be sampled for bacteriological culture after calving and should be isolated from the other milking animals until tested negative. In the past decade, hygiene and management practices have been provided as standard program to control IMI (Neave *et al*., 1969). (Radostits *et al*., 1994) summarized the control measures of mastitis among which pre-milking udder hygiene, post-milking teat dipping and environmental control during the dry and calving periods are to be mentioned. Each of these control measures is aimed at the management of specific pathogen types. (Malinowski, 2001) concluded that pre-milking udder hygiene and teat dipping are aimed at reducing infections mainly caused by contagious pathogens and preventing new infections and to a lesser extent at preventing infections that might be

caused by environmental pathogens. (Todhunter *et al*., 1995) showed that the environmental management during the transition and calving periods is targeted primarily at preventing new infection with environmental streptococcal species and Coli form bacteria e.g. *E. coli, Klebsiella* spp. Over half of the environmental 16 pathogens acquired during the dry period persist to lactation. (Sargeant *et al*., 2001) claimed that producing high quality milk will require effective udder health programs at the herd level.

Management practices at the time of dry-off and during the dry period are essential in this respect. Peeler *et al*., 2000 in their study of risk factors associated with clinical mastitis in low SCC British dairy herds found that the incidence of mastitis increases when milking cows were housed in straw yard, cows were standing in the yard after milking, which always practiced post-milking teat disinfection and had greater than 50% replacement rate.

They discovered also that the incidence of mastitis was lower when the gathering yard used before milking was scraped at least twice a day. (Oliver *et al*., 2001) demonstrated that pre-and post-milking teat disinfections with phenolic combination were significantly more effective in preventing new IMI than was post-milking teat disinfections only. They also added that pre-milks sing teat disinfections with phenol combination in association with good udder preparation and post-milking teat disinfections can further reduce the occurrence of new IMI by numerous mastitis pathogens during lactation.

A similar conclusion was reported by (Saloniemi and Kulkas, 2001) who described the mastitis control in Finland. They recommended post-milking teat dipping as control tool in herds with contagious udder pathogen problem. (Hogan and Smith, 1987) in their practical look at environmental mastitis concluded that no single uniform management procedure effectively prevents environmental mastitis under controlled conditions. (Rodenburg ,1990).

Claimed that high energy or high protein diets do not increase or decrease the number of new mastitis infections, however, feeding high producing cows for maximum production does increase stress on the udder and may cause infected cows to flare-up. Rodenburg also showed that too small stalls subjected animals to teat injury. In free-stall barns cows are less likely to lie in the dirt and the lying area is always of adequate size.

2.11 Factors influencing levels of SCC

The measurement of SCC from dairy improvement programs is used worldwide as an indicator of sub-clinical mastitis (Ostensson, 1993) because of its relatively high genetic correlation with mastitis which was estimated to be ~0.7 (Mrode and Swanson, 1996) and an important criterion of quality payment systems. As an indicator for the hygienic quality of milk and for the mastitis status in a given herd (DVG, 1989), cow SCC is used to trace sub-clinically infected cows (Laevens *et al*., 1997), is relatively easy to record and has a higher heritability (h2=0.11) than mastitis incidence (h2~0.04) (Mrode and Swanson, 1996). (Philipsson *et al*., 1995) concluded that it is possible to improve resistance to mastitis by 17 selecting for a low SCC, due to the higher heritability of the SCC. Philipsson added that selection based on the heritability of the SCC was more efficient than selection directly on mastitis. Results of several studies indicated that SCC is a more accurate measure of the udder health, as it is routinely recorded in most milk recording systems (Rupp and Boichard, 1999). (Ma *et al*., 2000) stated that post-infection milk had a significantly higher SCC (849X10³cells/ml) than pre-infection milk (45x10³cells/ml) in experimentally intramammary infected Holstein cows.

A high SCC was found to decrease the value of milk intended for manufacturing, has adverse effects in cheese making, reduces curd firmness

and decreases cheese yield, and increases fat and casein loss in whey (Klei *et al*., 1998).

2.11.1 Herd size

Herd size and SCC were declared to be negatively related, and larger herds had lower SCC than smaller herds(Van Schaik *et al*., 2002). (Lafi *et al*., 1994) found that the mean value of SCC was negatively associated with herd size. (Norman *et al*., 2000) added that herd size and SCC were negatively related and large herds had a lower SCC. (Peeler *et al*., 2000) stated that herds with greater than 50% replacement rate indicate that herd size was increasing culling for some reasons including high individual cow SCC.

2.11.2 Year-season

Season of calving is reported to have a significant effect on milk SCC and SCS (Rodriguez et al., 2000). However,(Liebe *et al*., 1996) reported no influence of season on SCC of German brown cows.(Leslie, 1996) found that SCC were lowest during winter and highest during the summer months of July and August, he attributed the seasonal variations to the effect of housing and temperature changes on infection status. (Kelly *et al*., 2000) found a significant seasonal influence on milk SCC, with cows calving in spring having a SCC>160x10^3 cells/ml with higher proportions of polymorph nuclear leukocytes in the total milk SCC than milk from autumn calving cows.(Norman *et al*., 2000) estimated the mean herd SCC to be lower during October through January (280x10^3 to 300x10^3 cells/ml) than during July and August (340x10^3 cells/ml).(Rupp *et al*., 2000) illustrated that regardless of the lactation stage, SCC were higher in summer and lower in autumn of the milk SCC in French dairy breeds. Whereas (Allore *et al*., 1997) found that SCC were significantly higher in spring than in fall. However, (Jemeljanovs and Bluzmanis, 2000).

Determined a seasonal effect on SCC. They claimed that SCC/ml milk was less in summer, a little more in autumn and more high in spring and most SCC encountered in winter. Season was suggested to have no significant influence on SCC in healthy mammary glands (Malinowski, 2001).

2.11.3 Lactation number

Several studies revealed a significant effect of the cow age and the lactation number on the level of milk SCC(Haile-Mariam *et al*.,2001).(Kiiman and Saveli, 2000) studied the factors affecting milk SCC and reported that milk SCC increased with increasing lactation number, in the first lactation SCC was $285x10^3$ whereas in the second, third and fourth lactations were $321x10^3$, $461x10^3$ and $477x10^3$, respectively.(Godollo and Tanszek, 2000) reviewed 98 scientific publications related to physiological and environmental factors influencing SCC. They reported that the number of lactation significantly affect the SCC in milk. A similar conclusion was realized by (Labohm *et al*., 1998) who found that lactation number influence the SCC in a statistically reliable extent. But attributed the rise in SCC above $100x10^3$ to infected quarter. (Leslie, 1996) reported that higher SCC have been found in the milk of older cows. (Hortet and Seegers, 1998) investigated the relationship between SCC and variation in milk production at the cow level, they indicated that at the test-day level an average loss of 0.4 kg milk in primiparous cows and 0.6 kg in multifarious by each 2-fold increase of SCC above $50x10^3$ cells/ml. At the lactation level, the average trend was a loss of 80 kg of milk in primiparous and 120 kg in multiparous by each 2-fold increase of the geometric mean of SCC above $50x10^3$ cells/ml. Similar results were published by (Hortet *et al*., 1999) who found that the reduction in milk yield in kg increased with parity and with days in milk to the extent that the reduction in milk yield was 0.32 kg per $100x10^3$ cells/ml increase in SCC, 0.63 kg per $200x10^3$ cells/ml SCC and 1.13 kg decrease in milk per $600x10^3$ cells/ml

increase in SCC. This result is in joint agreement to that of (Jemeljanovs and Bluzmanis, 2000) in their study of somatic cell and micro-organisms contents in milk. They revealed that SCC in milk increased in clinically healthy cows with the increase in the age. The further interpretation of these findings is that: if 90% of the 2nd lactation cows had up to $200x10^3$ cells/ml, then only 63.4% of the older than the 4th lactation cows had such level of SCC and 18.1% had more than $500x10^3$ cells/ml SCC. These findings supported the results published earlier by(Tyler *et al*., 1989) who stated that primiparous and multiparous cows were similarly showed production losses due to the increase in SCC. In primiparous cattle 19 with SCC range $403x10^3$-$665x10^3$ had 5.22 kg decrease in test-day milk yield whereas multiparous cows with the same range had 3.01 kg reduction in milk yield. (Koldeweij *et al*., 1999) found a geometric mean for SCC of 63.1 in the first lactation and 107.2 in the later lactations. They also found an individual milk yield loss of 1.29 kg/day for each unit increase in log10(SCC) for cows in the first lactation and 2.04 kg/day milk yield decrease per unit log10(SCC) for cows in the later lactations. (Kiiman and Saveli, 2000) found a significant ($p<0.001$) effect of lactation number on milk SCC, they found that in the first lactation the milk SCC was $285x10^3$/ml, in the second and third lactation $321x10^3$/ml and $461x10^3$/ml respectively. (Laevens *et al*., 1997) stated no significant effect of lactation number on SCC when cows were bacteriological negative and the least square mean of SCC for first, second and third lactations were 3.80, 3.93 and 3.97, respectively. (Schepers *et al*., 1997) estimated the variance components for SCC, they illustrated the shape of the SCC curve which was flat for the first lactation cows compared with the shape of the SCC curve for cows in the subsequent lactations.

2.11.4 Stage of Lactation

A group of researchers reported that SCC and milk yield traits vary the stage of lactation (Kelly *et al*., 2000) (Rupp *et al*., 2000) and with test-day (Haile-Mariam *et al*., 2001).(Schepers *et al.*, 1997) showed that stage of lactation affected the SCC, since the logarithm SCC was high at the beginning of the lactation, dropped to a minimum between 40 and 80 days postpartum and then steadily increased until the end of lactation. (Carnier *et al*., 1997) stated that from a genetic view point, SCS in early lactation behaves differently from those in later stages of lactation.

Williams *et al*., 1991 claimed that stage of lactation had a pronounced effect on milk SCC, with the level being high in early lactation, low in mid-lactation and high again in late lactation. However, (Rodriguez *et al*., 2000) stated that milk SCS typically reaches a minimum early in lactation and then rises, but lactations starting between October and December had the highest fall of SCS at the beginning of lactation, and smallest increase thereafter. Early results were obtained by (Emanuelson *et al*., 1988) who found a significant effect of the stage of lactation on SCC of morning milk samples from cows over 18 months and concluded that stage of lactation must be taken into account when establishing normal values for ATP as an indicator of mastitis.

Seker *et al*., 2000 found that a positive CMT score increased in Brown-Swiss cows with higher yield and at the 4th and 6th month of lactation.

Kirk *et al*., 1996 indicated that sub-clinical infection with minor pathogens 20 (primarily CNS.) had no significant effect on average SCC during early and mid lactation. Laevens *et al*., 1997 obtained least squares mean SCC for first, second and third parity bacteriological negative cows as 3.80, 3.93 and 3.97 respectively, with no significant effect of parity, stage of lactation and parity,

stage of lactation interaction, however the effect was significant when including the data of both infected and bacterial free cows.

2.11.5 Farm management factors

In the past decade, the standard mastitis control program has provided hygienic and management practices to control IMI (Neave *et al*., 1969), a decrease in bulk milk SCC is an indicator of the success of the control program (Suriyasathaporn *et al*., 2000). (Yalcin *et al*., 1999) studied the impact of mastitis control procedures in Scottish dairy herds, and concluded that udder preparation involving washing was associated with higher SCC and had detrimental effects on the efficacy of post-milking teat disinfections. Smith and Ely, 1997 reported that free-stall bedding did not significantly affect milk quality, with no difference in linear SCS among the herds studied.

They also showed that herds fed inside the free-stall barn or under covered roof had higher milk production and lower SCS than those fed outside. However, Bewley *et al*., 2001 stated in a comparison of free-stall barns used by modernized Wisconsin dairies that herds with four-row free-stall barns had higher production than herds with six-row barns and that the average linear was SCS significantly ($p<0.05$) lower in new four-row barns than six-row barns (2.71 vs. 2.95).(Omore *et al*., 1999) assessed the impact of a clinical trial of three mastitis control strategies among which improved udder hygiene in smallholder dairy farms in Kenya, they concluded that the trial had some impact in lowering the prevalence of contagious pathogens by 18%, but found no significant increase in milk yield or lowered SCC.

Barkema *et al*., 1998 reported about post-milking teat disinfections and good milking management as important factors for the prevention of a high bulk milk SCC. (Godollo and Tanszek, 2000) indicated that technological

environment, feeding and milking are known to interfere with changes in SCC.

Mazzucchelli *et al*., 2000 gave an account of the changes in the management of a Spanish herd of cows affected by mastitis by making a dietary adjustment, an improvement of the housing management and improving the design of milking parlours and management of milking. These changes resulted in a reduction of the milk SCC from $380x10^3$ cells/ml to $200x10^3$ cells/ml.

Kiiman, 2001 indicated that the adequate pre-milking cow preparation was essential to milk SCC as well as over-milking ($p<0.001$). He 21 also stated that the effect of milking equipment was not statistically significant for milk SCC.

2.12 High milk yield

Gröhn, 2000 studied the relationship between disease and milk production, he found that high milk yield predisposed a cow to certain diseases particularly and mastitis. (Whitaker *et al*., 2000) found that there was a positive association between bulk milk SCC and mastitis rate.

Haile-Mariam *et al*., 2001 estimated the correlation between test-day yield and SCC, they stated that genetic correlations between yield and log SCC were positive at the beginning and negative at the end of the first lactation, in the second and third lactations genetic correlations were nearly zero at the beginning of the lactation but negative at the end, however, environmental correlations were always negative.

The authors attributed the positive correlations to the fact that high producers are more susceptible to mastitis than cows with average or low production whereas the negative correlations in the second half of the first parity and

later parities due to the mastitis cause high SCC and udder damage resulting in reduced milk yield. These findings support results presented by (Gröhn *et al.*,1995) who claimed that cows with mastitis are often higher yielding cows, which produce more milk even having contracted the disease, compared to their healthy and generally lower yielding herd-mates.

The herd-level SCC is a result of many factors such as cow factors, management practices, and seasonal fluctuations. The pathogen distribution among the also influences the level of hard SCC.

For instance, Staph aurous-positive herds have higher bulk milk SCC than Staph. aurous-negative herds .To continuously monitor and interpret SCC on the herd level and to detect an increase in the trend over time would be ideal .Bonuses programs are applied in many countries based on a SCC threshold value varying from 150,000 to 250,000 cells/ml "Five point plan" proposed for the National mastitis Council (Auldist *et al* .,1996) Summarizes Several strategies for controlling herd mastitis , based upon adoption of preventive and control strategies including diagnosis , segregation 0f the animal and the use of improved hygiene and therapeutic protocols .

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study has been conducted in Khartoum North (Bahri), Khartoum state. The number of the farms covered by this study were ten in this area (Appendix 1), both government and private farms (owned by local inhabitants). The total number of subjected cows in these farms was 100, ten from each one. Most of the cows were hybrid cross breed (*Friesians* + local). Random test was applied using California mastitis detector, because it is one of the most important diagnostic techniques of udder inflammation. Samples were taken at the time of the second milking which is always done between 2 and 3 o'clock pm local time. California mastitis test was performed in the cows milked in the milking place or in the fence. The udder was sterilized by potassium permanganates solution then samples of milk were taken from each teat to be tested.

3-2 Materials used

3-2-1 Collection of samples

Collection of samples needed a four circled plastic plate, a plastic dropper and ice bag.

3-2-2 In laboratory

Devices and materials used in culturing: Samples of milk, loop, Petri dishes, stove, Incubator, antiseptic and microscope.

3-3 Methods of diagnostic techniques for mastitis

3-3-1 The California mastitis test (CMT)

California mastitis test is a simple cow-side indicator of the somatic cell count (SCC) of milk. It operates by disrupting the cell membrane of any cells present in the milk sample, allowing the DNA in those cells to react with the test reagent, forming a gel. It provides a useful technique for detecting subclinical cases of mastitis. Whiteside described a reaction between sodium hydroxide and milk that resulted in the thickening of mastitis milk. The utility of this reaction as a field test was limited by the fact that the reaction was sometimes difficult to observe, and would eventually occur even in normal milk. A refined version of the test, which enhanced its sensitivity, and eliminated the confounding effect of milk fat, uses an anionic surfactant, which forms a gel with the DNA in somatic cells in the milk (David *et al.*, 2005).

Use: A four-well plastic paddle is used, one well being for each quarter of the cow to be tested. Foremilk is discarded, and then a little milk drawn into each well. An equal volume of test reagent is added, and gently agitated. The reaction is scored on a scale of 0 (mixture remains unchanged) to 3 (almost-solid gel forms), with a score of 2 or 3 being considered a positive result. A special reagent for the test is marketed under the name 'CMT-Test', but domestic detergents are frequently used instead, being cheaper and more readily available. Fairy Liquid is as suitable as a reagent for the CMT, although many cheaper detergents are not (Leach *et al.*, 2008).

3-3-2 Antibiotic sensitivity test (AST)

Antibiotic sensitivity or antibiotic susceptibility is the susceptibility of bacteria to antibiotics. Susceptibility can vary even within a species (with some strains being more resistant than others), antibiotic susceptibility testing is usually carried out to determine which antibiotic will be most successful in

treating a bacterial infection in vivo. Small wafers containing antibiotics are placed onto a plate upon which bacteria are growing. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth.[1] Other methods to test antimicrobial susceptibility include the Stokes method, E-test (also based on antibiotic diffusion), Agar and Broth dilution methods for minimum inhibitory concentration (MIC) determination. The results of the test are reported on the antibiogramma. An antibiogramma is the result of an antibiotic sensitivity test. It is by definition an in vitro sensitivity. Once a culture is established, there are two possible ways used to get an anti-biogramma:

- A semi-quantitative way based on diffusion. Small discs containing different antibiotics are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lyses will become visible. Since the concentration of the antibiotic was the highest at the centre, and the lowest at the edge of this zone, the diameter is suggestive for the Minimum Inhibitory Concentration, or MIC, (conversion of the diameter in millimetre to the MIC, in µg/ml, is based on known linear regression curves).

- A quantitative way based on dilution: a dilution series of antibiotics is established (this is a series of reaction vials with progressively lower concentrations of antibiotic substance). The last vial in which no bacteria grow contains the antibiotic at the Minimal Inhibiting Concentration. Once the MIC is calculated, it can be compared to known values for a given bacterium and antibiotic (International Organization for Standardization, 2007).

3-4 Questionnaires

The survey of the study was accompanied by questionnaire form (Appendix 2), included questions to have general information about the farm breeds, location, number of animals, disease history, type of treatment, control program, type of milking and bio-security procedures.

3-5 Statistical analysis

The obtained data was analysed statistically by using analysis of variance ANOVA SPSS Program (V16).

CHAPTER FOUR

RESULTS

4-1 Detection of mastitis by California Mastitis Test

The present study showed the milk samples were taken from different dairy farms which applied on (100) Cows from 10 farms, from each10 milk samples, results shown on (Table 4-1) (Figure4-1) represent positive and negative cases of (CMT). The highest positive case was 10% and the lowest positive case was 3%. Test result of the targeted farms in this study shown as followed respectively:

- They respective examination of Mastitis Test (CMT) were recorded low infection by mastitis in Sudan University of Science and Technology farm (Shambat) , South Selate (B) , South Selate (C), and Mustafa Ibrahim farm. Which show lower infection by mastitis disease in percentage rate 3%.

- The following farms were recorded moderate infection by mastitis as follow in Khartoum University farm ,in Sudan University KuKu farm, Ali Mohammed farm ,Halfaya farm ,and South Selate (A) farm ,which recorded (3-5-8-9%).

- Highly infection by mastitis on South Selate (D), which showed that ten percentage rate as which consider highly infection by mastitis due to low bio-security measures applied in this farms.

The present study indicate that four farms were recorded a low infection rate by mastitis (Selate B, Selate C, Shambat and private sector Mustafa), which was 3%. Farm were recorded moderate infection rate (Halfaya, Selate A, Sudan kuku, University of Khartoum and private sector Ali Mohamed). The

percentage rate between 5% - 9%. High infection rate recorded in Selate D farm was 10%as show (Table 1).(Figure 1).

Table 4.1: Result of Infected farms by Mastitis using (CMT)

Farms	Number of cows	Number of teats		Number Total teats	Infection rate %
		Infected	Non infected		
Ali Mohamed	10	3	35	38	9%
Halfaya	10	2	36	38	5%
South Selate (A)	10	3	36	39	8%
South Selate (B)	10	1	38	39	3%
South Selate (C)	10	1	38	39	3%
South Selate (D)	10	4	36	40	10%
Mustafa Ibrahim	10	1	39	40	3%
University of Sudan kuku	10	3	36	39	8%
University of Sudan Shambet	10	1	39	40	3%
University of Khartoum	10	3	37	40	3%

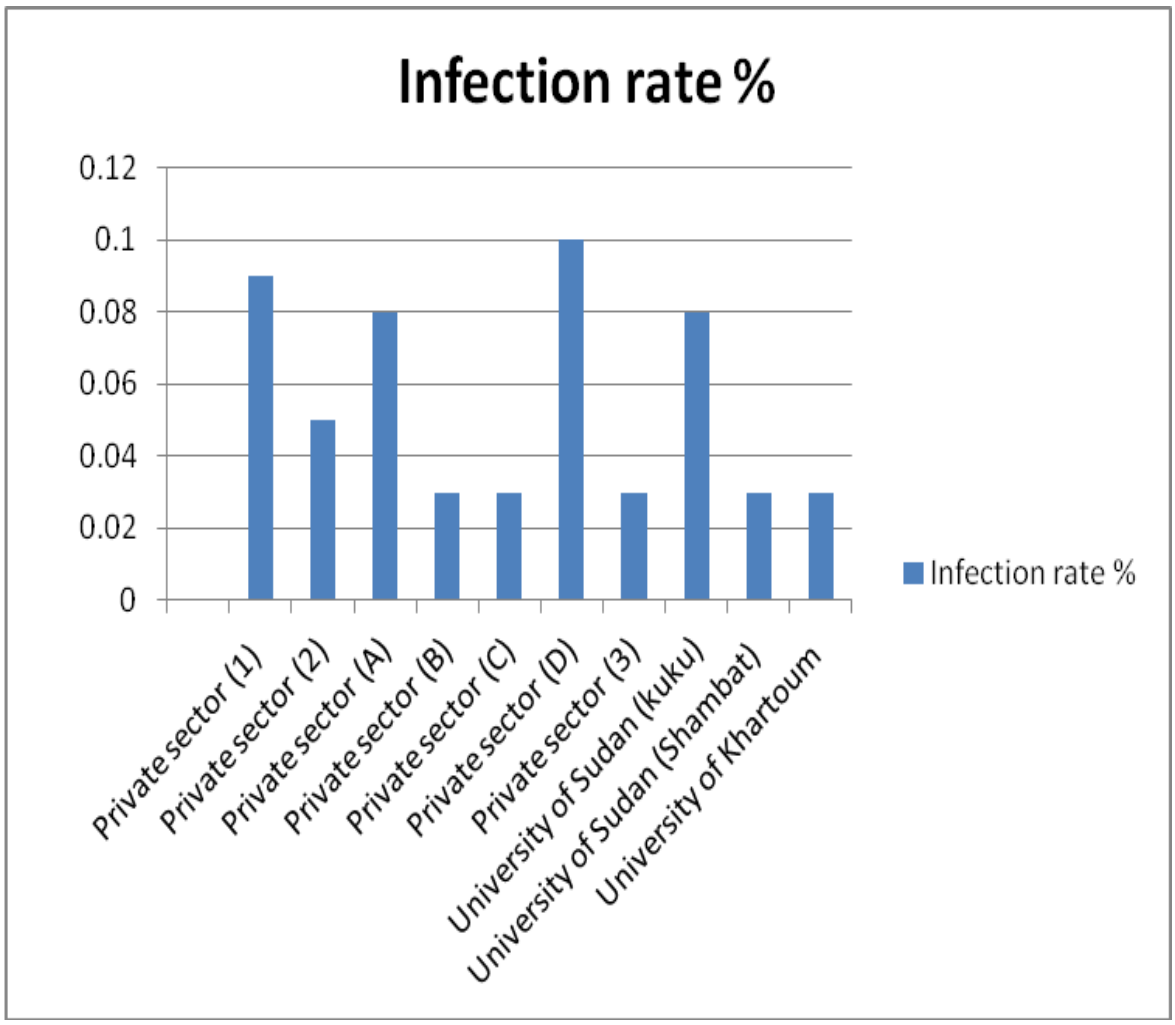


Figure 4.1 Show infected farms by Mastitis using (CMT)

Table 4.2: The questionnaire data used to evaluate the management of dairy farms

Survey	Option	Number of farm	Percentage obtained %
Production system	Modern	3	30%
	Traditional	7	7%
Type of breed	Cross	10	100%
	Local	-	-
Bio-Security procedures	Yes	10	100%
	No	-	-
Cows hygiene	Yes	4	40%
	No	6	60%
Milkers hygiene	Yes	5	50%
	No	5	50%
Place of milking	Inside pen	10	100%
	Milking private	10	100%
Private milkers in farms	Yes	10	100%
	No	-	-
Udder hygiene before milking	Yes	5	50%
	No	5	50%
the udder hygiene after milking	Yes	1	10%
	No	9	90%
Mastitis detection	Yes	7	70%
	No	3	30%
Common diseases	Theillreia	2	20%
	CBPP	1	10%
	FMD	4	40%
	Gastritis	2	20%

4.2 Laboratory examination

Laboratory examination of pathogenic slides revealed that the pathogens were Streptococcus and Staphylococcus is more sensitive antibiotics like erythromycin, penicillin and cephoxitin, show that in (Table. 2).

4-3 Data collected from questionnaire

- The study was accompanied by questionnaire. The collected data was shown in (Table 4-2).
- The breeds found in the selected farms were (cross breeds), 65%frogien blood and 35 % local blood.
- Four farms out of ten were applied hygiene measures.
- Labours (milkers) personal hygiene recorded 50%.
- Place of milking was shown that there were private places of milking in 4 farms and inside pen 6 farms.
- Washing of udder before milking registered 50% equal.
- The study found that 10% of the farms washing udders after milking 90%.
- All 10 farms milkers work only in their farms.
- Periodic inspection of mastitis in the farm were 70% and 30% did not.
- Other diseases were found in the farms as follow:
    - Theillreia 20%.
    - Contagious bovine pleura pneumonia 10%.
    - Foot and mouth disease (FMD) was recorded 40%

Table 4.3: Antibiotic sensitivity of different microbial pathogens

Antibiotics	Bacteria Percentage %	
	S.aureus	Streptococcus
Gentamicin	97.50	87.50
Cloxacillin	100	87.50
Roxithromycin	100	87.50
Lincomycin	100	87.50
Cefotaxime	87.50	75
Ciprofloxacin	90	75
Lincomycin	85	75
Linezolid	87.50	75
Ampicllin	85	62.50
Co-Trimoxazole	85	62.50
Cephalexin	82.50	62.50
Tetracycline	83.33	50

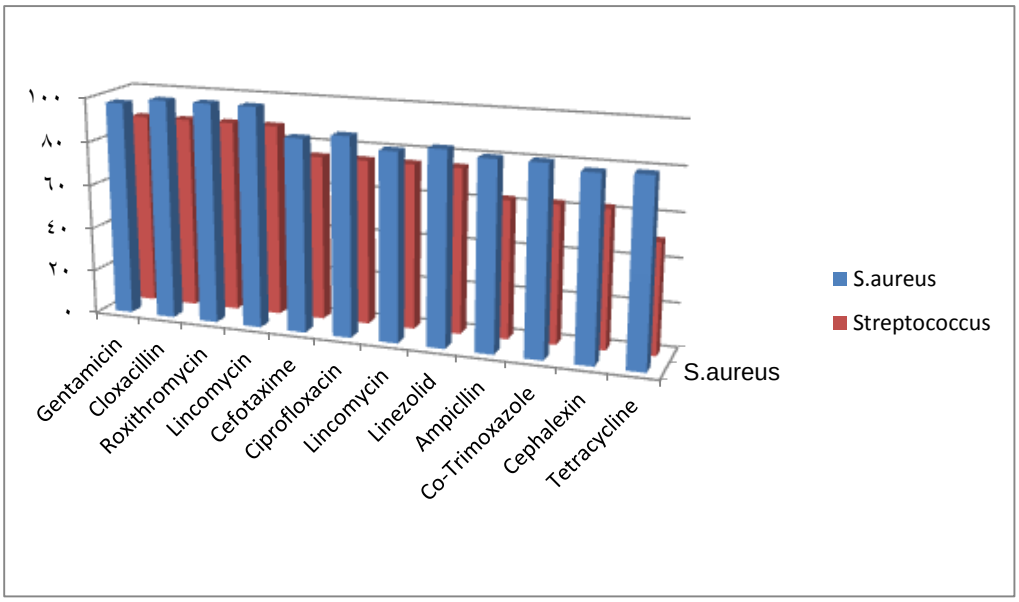


Figure 4.2: Antibiotic Sensitivity of different microbial pathogens

CHAPTER FIVE

DISCUSSION

In the present study on application the California Mastitis Test (CMT) was applied to detect mastitis in ten farms in Khartoum north state. According to history records, 8 out of 400 quarters from the 100 cows of the study had been damaged and lost due to trauma, congenital atrophy, mastitis or other diseases.

Result obtained showed that some of the farms which they are south slate B and C (private sectors),Mustafa Ibrahim (private sector) , Sudan University (Shambat farm) and University of Khartoum farm were reported an infection rate of (3%) by mastitis, which can be easily controlled.

Only one farms was moderately infected in the private sector (Halfaya) in percentage (5%). the Highly infected farms by mastitis in private sector (Ali Mohammed), South Salate A ,D and Sudan University Kuku were recorded a percentage of mastitis infection (8-9-10%) which is considered an indicator of bad hygiene in these farms. The problem was due to Lack of udder Hygiene, isolation and treatment of infected cows in early stage was not applicable.

Incidence of mastitis in Khartoum North of study area the reasons of the disease presents subclinical mastitis this study agree with (Batavian *et al* . , 2003). Accordingly from results obtained Sudan University Shambat and Khartoum University and private sector Salate B. C recorded lower incidences of bovine mastitis due to applying bio security programs. The result of the test is not affected by external factors. This test needs skilled personnel to perform it. Laboratory examination of pathogenic slides revealed

that the pathogens were Streptococcus and Staphylococcus and they are more sensitive antibiotics like Gentamicin, Cloxacillin, Roxithromycin and Lincomycin. The results are in agreement with the findings of (Radostits *et al*., 1994). The study revealed that the farms with cross breeds cows showed the highest incidence of mastitis due to high milk producing trait. It was observed that in farms of manual milking the spread of mastitis was higher. Previous studies showed that heredity has influence on mastitis incidence especially in Holstein Friesian. The mastitis incidence varied between 9-22% which considered very high in compare with other animal diseases. The studies also showed that the correlation between heredity and environmental interaction in the highest mastitis incidence. The environmental impact varies according to different environments; it is online with (Amin and Gere, 2000).

Conclusion and Recommendations

Conclusion

CMT is considered as a dependable test which can diagnose and reflects the incidence of mastitis in dairy cattle and suitable for farm conditions in Khartoum state.

There is high correlation between the hygiene measures and mastitis incidence in the selected farms.

Antibiotics to be used for mastitis treatment is Gentamicin, Cloxacillin, Roxithromycin and Lincomycin.

Recommendations

A proposal of protective measures that could improve the general udder health and milk production:

• improve hygienic measures during milking by:

- Proper washing of hands before milking.
- Only using clean water and separate towels for cleaning the udders.
- Implementing the use of teat-dip after milking.
- Keeping animals from lying down immediately after milking

• Divide herd into groups according to udder status.

• Implement correct and gentle milking technique.

• Apply correct treatment of mastitis based on bacteriological culturing by consulting a veterinarian.

• Avoid zero-grazing systems.

• Do not keep high milk producing cows with a poor udder health.

• Improve feeding routines.

• Improve record keeping at farms and for practicing veterinarians in order to gain statistics on health status of dairy cattle in herds and on a national level.

References

Aarestrup, F.M. and Jensen, N.E. (1997): prevalence and duration of intra mammary infection in Danish heifers the pre partum period. J. dairy sci. 80:307-312.

AbdullahiSJ. Jibril A, Mohammed AA, Fascinator 2012. SUB-clinical mastitis and associated risk factors on lactating cows in the savannah Region of Nigeria. BMC Vet Res. 2012,8:134.

Ali- Vehmas,T.and Sandholm, M. (1995): The bovine udder and mastitis: Balance between bacteria and host-The bacteria's point of view, Univ. of Helsinki, Factulty of vet.Med.ISSBN: 951-834-047-1pp49-74.

Amin, A.A.; Gere, T. 2000. Genetic parameters of udder, mastitis and milk traits in two different climactic areas using animal model analysis. Czech J. Anim. Sci. 45, 193–199.

Auldist M.J., Coats S., Sutherland B.J., Mayes J.J., McDowell G.H., Rogers G.L. 1996, Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese, J. Dairy Res. 63 (1996) 269-280 .

Barenes-Pallesen,F.D.;Blachmer,P.;Britten, A.;Bushnell,R.B.; Van Damme, D.M. and welcome,F. (1987): Laboratory and field handbook on bovine mastitis. Natl.Mastitis Counc.,Arlington, VA.

Barkema H.W.; Schukken, Y.H.;Lam,T.J.G.M.;Beiboer,M.L.;wilmink, H.;Benedictus,G.and Brand, A. (1998): Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell count . J.dairy sci.81(2):411-419.

Barkema, H.W.; Schukken, Y.H.; Lam, T.J.G.M.; Galligan, D.T.; Beibore, ,A. (1997): Estimation of interdependence among quarters of the bovine under with sub- clinical mastitis and implication for analysis. J. dairy Sci. 80:1592-1599.

Barkema, H.W.; Schukken, Y.H.; Lam,T.J.G.M.; Beiboer, M.L.; Benedictus, G. and Brand,A. (1999): Management practices associated with the incidence rate of clinical mastitis .J.Dairy sci.82:1643-1654.

Barkema, H.W.; Schukken, Y.H.; Lam. T.J.GM.; Beiboer, M.L.; Benedictus, G.and Brand, A. (1998): Management practices associated with low, mid and high bulk milk somatic cell count.J. dairy sci.81:1917-1927.

-Barkema,H.W.; Schukken, Y.H.; Lam, T.J.G.M.; Beiboer, M.L.; Benedictus, G. and Brand, A. (1999): Management practices associated with the incidence rate of clinical mastitis .J. dairy sci. 82:1643-1654.

Bewley, J.; palmer, R.W. and Jackson-Smith, D.B. (2001): Acomparison of free-stall barns used by modernized Wisconsin dairies.J. dairy sci.84:528-541.

-Blood DC, Radostits OM. Veterinary medicine. 1989. In: textbook ED of the diseases of cattle. sheep, pigs. Goats and horses. (7^{th} end).WB Saunders Co. Philadelohia.USA. 1989;501.

Bluzmanis,J. (2000): somatic cells and microorganinsms content in milk and it effecting factors. 51th Annual meeting of the European association for animal production, the Hague,the Netherlands21-24 August 2000. Poster no. 530.

Booth, J.M.(1988):Progress in controlling mastitis in England and Wales.Vet.Res. 122:299-302.

Bradley A ,Green MJ 2005. Use and interpretation of somatic cell count data in dairy cows. In Pract. 2005; 27: 310-315.

Bramley, A.J. (1991): Mastitis physiology or pathology ? Flem.Vet.J. 62(1):3-11.

Bramley,A.J. and Dodd, F.H. (1984): Reviews of the progress of dairy science : mastitis control-progress and prospects.J. dairy res.51:481-

Bray, D.R. and shearer,J.K. (1996): Mastitis control .Uni. Florida, dept. dairy and poultry, institute of food and agric.Sci.

Buzalski, T.H. and Pyörälä, S. (1995): The bovine udder and mastitits: Monitring and management of health at the farm. Uvin. Of Helsinki, Facalty of vet . Med . ISBN: 951-834-047-1 :252-260 ..

Buzalski, T.H. and Seuna, E. (1995): the bovine udder and mastitis: Isolation and identification of pathogens from milk Univ. of Helsinki, Faculty of vet.Med.ISBN: 951-834-047-1. 121-141.

Buzalski, T.H.and Pyörälä, S.(1995): The bovine udder and mastitis : Monitring and management of udder health at the farm . Univ . of Helsinki, Faculty of vet. Med.ISBN: 951-834-047-1:252-260.

Carnier, P.; Bettela, R.; Cassandro, M.; Gallo, L.; Mantovani, R. and Bittante, G. (1997) : Genetic Parameters for test day somatic cell count in Italian Holstein Friesian cows. 48th Annu. Meet. Europ. Assoc. Anima. Prod. Vienna-Austria-August 25-28,1997 Session 1V: Mastitis control Programmes.1-5.

Collins NA. Moses M. Moneoang SM. Comeleus B.2010. Antibiotic Resistance in Staphylococcus aureus isolated from milk in the Mafikeng Area. North West province. South Africa South Afr g Sci 2010. 106: 1-6.

David White, Michael Walmsley, Alvin Liew, Rod Claycomb and Graeme Mein (2005): "Chemical and rheological aspects of gel formation in the California Mastitis Test", Journal of Dairy Research, 72:115-121

David, R.B and Shearer,J.K.(1996): Mastitis control. Co-operative extension service, institute of food and agricultural sciences, university of Florida. DS-7.

De Graaf,T.and Dwinger,R.H.(1996):Estimation of milk production Losses due to sub-clinical mastitis in dairy cattle in Costa Rica.Prev.vet.Med. 26(3-4):215-222.

Deutsche Veterina"rmedizinische Gesellschaft(DVG,1994), Leitlinien zur Beka"mpfung der Mastitis des Rindes als Bestandsproblem. Sachversta"adigenausschuss (subklinische Mastitis) des Arbeitskreises Euterjesundheit der Fachgruppe Milchhygiene des Arbeitsgebietes Lebensmittelhygiene, 3.Aufl.Kiel.

Diseases, Pregnancy status and milk yield on culling in Finnish Ayrshire cows .Prev. Vet. Med. 41(4): 295 -309.

Do''pfer, D.; Barkema, H.W.; Lam, T.J.G.M.; Schukken,Y.H. and Gaastra, omastitis caused by Escherichia coli in dairy cows. J.dairy sci.82:80-85.

Dulin AM, Paape M J, Nickerson sci 1988. Comparison of phagocytosis and chemiluminescence by Blood and mammary gland neutrophils

from multiparous and nulliparous cows. A m J vet Res. 1988;49:172-177.

DVG, Fachgruppe Milchhygiene des Arbeitsgebietes Lebensmittelhygiene, Kiel. Deutsche Veterina''rmedizinische Gesellschaft (DVG,1980): leitlinien zur Beka"mpfung der Mastitis des Rindes als Bestandsproblem.2.Aufl. Kiel.

Emanelson,U.and Oltenacu,P.A.(1998): Incidence and effects of diseases on the performance of Swedish dairy herds stratified by production. J. dairy sci. 81(9):2376-2382.

Emanuelson, Ulf; Thomas, Olsson; Tiina, Mastitis; Goran, Astrom; and Olof, -Holmberg (1988): Effects of parity and stage of lactation on adenosine triphophate,somatic cell count and antitrypsin content in cows milk,J. dairy res.55(1):49-55.

Espeche C, Otero M, Sesma F, Nader-Macias M Screening of surface 2009, properties and antagonistic substances production by lactic acid bacterial isolated from the mammary gland of healthy and mastitis cows. Vet Microbial. 2009; 135:346-357.

ETHerington,W.G.; Kinsel,M.L. and Marsh,W.E.(1996): Relationship of production to reproductive performance in Ontario dairy cows:Herd Level and individual animal descriptive statistics.Theriogenology 46:935-959.

Fetrow,J.; Mann,D.; Butsche,K. and McDanicl,B.(1991):production losses from mastitis :carry over from the previous lactation. J. dairy sc.74:833-839.

Firat, M.Z. (1993): Suscptibility of clinical mastitis in successive Lactation. Livestock prod. Sci. 34(1-2):175-180.

Fleischer, P., Metzner, M., Beyerbach, M., Hoedemaker, M. and Klee, W. (2001): the relationship between milk yield and the incidence of some disease in dairy cows . J.dairy sci . 84,2025-2035.

Gentilini, E.; Denamiel, G.; Perez Monti, H.; Marco, C. and Lopez Amoedo, M. (1994): Mastitis Bivina: perfiles de sensibilidad de cepas de Staphylococcus spp.Por elm'etodo be antibiograma semicuantitativo en agar frente a 10 antimicrobianos. Vet. Arg. Vol. 10:314-321.

Godden S, Rapnicki P, Stewart S, Fetrow J, Johnson A, et al 2003, Effectivenss of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. J Dairy Sci. 2003; 86: 389911.

Godollo, S.I.E. and Tanszek, S.J. (2000): Factors influencing somatic cell count in milk 2-physilogical and environmental factors. Tejgazdasag.60:16-25.

Gro''hn, Y.T.; Eicker, S.W. and Hertl, J.A. (1995): The association between 305-day milk yield and disease in New York dairy cows . J. dairy sci.78:1693-1702.

Haile- Mariam, M.; Goddard, M.E. and Bowman, P.J.(2001): Estimates of genetic parameters for dairy somatic cell count of Australian dairy cattle. J.dairy sci.84,1255-1264.

Harmann,R.J. and Reneau,J.K.(1993):Factors affecting SCSs in milk Proc.Annu.Mtg.Natl .Mastitis Counc.,Kansas City .MO.Natl.Mastitis Counc.,Inc., Arlingyon,VA. 48-54.

Heinrichs AJ, Costello SS, Jones CM 2009. Control of heifer mastitis by nutrition. Vet Microbiol.2009; 134;127-176.

Heringstad,B.G;Klemetsdal,G.and Ruane,J.(1999): Clincal mastitis in Norwegian cattle :Frequency,variance components and genetic correlation with protein yield .J.dairy sci.82:1325-1330.

Hill, A.W. and Shears, A.L. (1979): Recurrent coliform mastitis in the dairy cow. Vet. Resci. 105:299-301.

Hill, A.W.; shears, A.L. and Hibbit, K.G. (1978): The elimination of serum-resistant Escherichia coli from experimentally infected single mammary glands of healthy cows. Res. Vet. Sci. 25:89-93.

Hillerton ,J.E.(1999), Redefining mastitis based on somatic cell count. Inter.Dairy Fed . Bullet.No.345/1999,4-6.

Hogan JS, Smith KL, Hoblet KH, Todhunter DH, Schoenberger PS, et al 1989..Bacteria counts in bedding materials used on nine commercial dairies. J dairy sci. 1989; 72:250-258.

Hogan, J.s., Smith, K.L., Hoblet, K.H., Schoenbrger, P.S., Todhunter, D.A., Hueston,W.D., Pritchard, D.E., Bowman, G.L., Heider, L.E., Brockett, B.L. and Conral, H.R. (1989) : Field survey of clinical mastiris in low somatic cell count herd .J.dairy sci .72:1547-1556.

Hogan,J.S. and Smith,K.L. (1987): A practical look at environmental mastitis . Comp. Cont.Educ. pract. VET. 9:F341.

Hortet, P. and Seegers, H. (1998): Calculated milk Production Losses associated with elevated somatic cell counts in dairy cows:review and critical discussion . Vet. Res.29(6):497-510.

Hortet, P.; Beaudeau, F.; Seegers, H. and Fourichon, C. 1999): Rrduction in milk yield associated with somatic cell counts up to 600X10 cells/ml in French Holstein cows without clinical mastitis. Livestock prod. Sci.61:330-42.

Hundera, S., Z. Ademe and A. Sintayehu, (2005): Dairy cattle mastitis in and around Sebeta, Ethiopia. Intern. J. Appl. Vet. Med., 3(4): 1525-1530.

International Organization for Standardization 2007, Clinical laboratory testing and in vitro diagnostic test systems—susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices. Part 2: evaluation of performance of antimicrobial susceptibility test devices. ISO 20776-2 , 2007 Geneva.

Jemeljanovs, A. and Bluzmanis,J. (2000): Somatic cells and microorganisms content in milk and it effecting factors. 51th Annual meeting of the European association for animal production, the Hague, the Netherlands 21-24 August 2000. Poster no.530.

Jemeljanovs, A. and Bluzmanis,J. (2000): Somatic cells and microorganisms content in milk and it effecting factors.15th Annual meeting of the European association for animal production, the Hague, the Netherland 21-24 August 2000. Poster no.530.

Jemeljanovs, A.; Bluzmanis, J.; Mozgis, v. and Reine, A.(1999): The evaluation of mastitis pathogenic agents and its possible influence on consumers health.50th Annual meeting of the European association for animal production, Zurich, Switzerland,22-26.

Jones, F.G., ward, (1989) : Cause, occurrence and clinical signs of mastitis and anorexia in cows in a Wisconsin, JAVMA.195(8) 1108-1113.

Jones, G.M.and Bailey, Jr. (1989), Mastitis control in heifers and first lactation. Virginia cooperative extension, publication number: 404/281.

Jonsson, P.; Olsson, S.O.;Olofson, A.S.; Fa''lth, C.; Holmberg,O.and Funke, H.(1991): Bacteriological investigations of clinical mastitis in heifers in Sweden.J.dairy Res.58:179-185.

Kelly, A.L.; Tiernan, C.O'Sullivan and Joyce,P.(2000): correlation between bovine millk somatic cell count and polymorphnuclear leuckocyte level for samples of bulk milk and milk from individual cows. J.dairy sci.83:300-304.

Keown,J.F.(1997): Dairy 10-point quality control program –Mastitis treatment records.Institute of Agric.Nat .Resour.Uni. Nebraska-Lincoln.G92-1101-A.

Kiiman, H. (2001): The analysis of the milk somatic cell count reducing possibilities. J.agric.sci.12(3):162-174.

Kiiman, H. and Saveli ,O.(2000): On the factors affecting milk somatic cell count in dairy cattle in Estonia. European Association for animal production 51the Meeting-the Hague- The Netherlands.

Kiiman, H. and Saveli, O. (2000): On the factors affecting milk somatic cell counts in dairy cattle in Estonia European Association for animal Production 51the Meeting-The Hague-The Netherlands.

Kirk, J.H.;Wright, J.C.; Betty, S.L.;Reynolds, J.P.; Maas, J.P. and Ahmadi, A.(1996): Relationships of milk culture status at calving with somatic cell counts and milk production of dairy heifers during early lactation on a Californian dairy.Prev. Vet Med-28(3):187-198.

Klaas,I.C. (2000): Untersuchungen zum Auftreten von Mastitiden und zur Tiergesundheit in 15 Milchviehbetrieben Schleswig-Holsteins.Dissertation ,Free university-Berlin.

Klei, L.; Yun, J.; Sapru, A.; Lyunch, J.; Barbano, D.; Sears, P. and Galton, D. (1998): Effects of milk somatic cell count on cottage cheese yield and quality .J. Dairy sci.81:120-1213.

Koldeweij ,E.; Emanuelson, U. and J. (1999): Relation of milk Production Loss to milk somatic cell count . Acta Veterinaria Scandinavica 40(1):47-56.

Korhonen,H. and Kaartinen ,L. (1995):The bovine udder and mastitis :Changes in the composition of milk induced by mastitis. Univ.of Helsinki,Faculty of vet.Med.ISBN:951-834-047-1:76-82.

Labohm, R.; Go"tz, E.; Luhofer, G.; Hess, R.G. and Bostedt, H. (1998): factors influencing the somatic milk-cell count in dairy cows.1-influence of bacteriological finding, stage and number of lactation Milchwissenschaft 53(2):63-69.

Laevens, H., Deluyker, H., Schukken, Y.H., De Meulemeester, L., Vandermeersch, R., DE Muelenaere, E .and De kruif, A.(1997): Influence of parity and stage of lactation on somatic cell count in bacteriologically negative dairy cows . J. dairy sci.80,3219-3226.

Lafi,-S.; AL- Rawashdeh,-O.; Na'was,-T. and Hailat, N. (1994): National cross-sectional study of mastitis in dairy cattle in Jordan. Trop. Anim. Health. Prod.26(3):168-174.

Lam, .J.G.M.; Lipman,L.J.A.; Schukken. Y.H.; Gaastra, w. and Brand , A. (1996): Epidemiological characteristics of bovine clinical mastitis

caused by Escherichia coli and Staphlococcus aureus studied by DNA fingerprinting. Am.J. Vet. Res.57:38-42.

Leach, KA; Green, MJ; Been ,Halasa T, Nielen M, De Roos APW, Van Hoorne R, de Jong G, Lam TJGM. *et al* 2009, Production loss due to new sub-clinical mastitis in Dutch dairy cows estimated with a test-day model. J Dairy sci.2009; 92: 599-606.

Leach, KA; Green, MJ; Breen, JE; Huxley, JN; Macaulay, R; Newton, HT; Bradley, AJ (8 November 2008). "Use of domestic detergents in the California mastitis test for high somatic cell counts in milk". The Veterinary record. 163 (19): 566–70. doi:10.1136/vr.163.19.566. PMID 18997186.

Leslie,K.E.,(1996):somatic cell count: Interpretation for individual cows. Ontario factsheet. Agdex:410/662.

Liebe, A.; Worstorff, H. and Schams, D. (1996): Changes in somatic cell count and plasma cortisol conrtisol concentration during three relocation trials in German Brown cows . Milchwissenschaft 51:423-426.

Ma, Y.; Ryan, C.; Barbano, D.M.; Galton, D.M.; Rudan, M.A. and boor, K.J (2000): Effects of somatic cell count on quality and shel-life of pasteurized fluid milk. J. dairy sci. 83:264-274.

Malinowski, E. (2001): Somatic cells in millk. Medycyna Weterynaryjna 57(1):13-17.

Mantere-Alhonen, S. (1995): The bovine udder and mastitis : Microbiology of normal milk. Univ. of Helsinki, Faculty of vet. Med.ISBN: 951-834-047-1pp115-141.

Matthews, K. R., Harmon, R.J. and Langlois, B.E.(1992): Prevalence of S. species during the periparturient period in primiparous and multiparous cows, J. dairy sci. 75:1835-1839.

Mazzucchelli, F.; Parrilla, G.; Blanco, F.J.; Martin, J.V. and Gonzalez, M. (2000): Bovine mastitis. An evaluation of problem on the farm. Mundo-Ganadero.11(118):44-46.

McDonald, J.S. (1982): Experimental infection of the bovine mammary glands during the dry period.Proc. 21st Annu. Meet. Nati. Mastitis Coun.,Louisville,KY. Natl. Mastitis Coun., Arlington,VA,pp 112.

Mein GA , Schuring N 2003. Lessons from scrapbooks and scrapbooks of history. Bulletin-International Federation. 2003 .

Milner,P.; page,K.L. and Hillerton , J.E.(1997), The effects of early antibiotic treatment following diagnosis of mastitis detected by a change in the electrical conductivity of milk .J. dairy sci .80:859-863.

Moore,D.A.and O'connor, M.L.(1993): Coliform mastitis: Its possible effect on reproduction in dairy cattle. Proc. 32nd Annu Meet. Natl . Mastitis Council:Kansas city, Mo. Natl. Mastits counc.:Inc. Arlington, VA. 162-166.

Morin, D.E. and Hurley, W.L. (1999): Mastitis lesson B. http:// classes. Aces. uiuc. edu/ AnSci308/mastitisb.html (22.12.1999)

Mrode ,R.A. and Swanson,G.J.T.(1996) : genetic and statistical properties of somatic cell count and its suitability as an indicator means of reducing the incidence of mastitis in dairy cattle. Anim .breed . Abstr.64: 847-857.

Munch- Petersen, E.; Mastitis in bovine primiparae. Vet .res.87:568-574.

Mustacich D, Powis G 2000. Thioredoxin reductase. Biochem J. 2000;346: 1-8.

NAAS. 2013,Mastitis Manage in Dairy Animals Policy Paper No.61, National Academy of Agricultural Science, New Delh, India .**2013**.

Neave; F.K., Dodd, F.H., Kingwill R.G., and Westgarth D.R. (1969): Control of mastitis in the dairy herd by hygiene and management. J. dairy sci.52:696-707.

Nicherson, S.C.; Owens,W.E. and Boddine, R.L. (1995): Mastitis in dairy heifer:initial studies on prevalence and control.J. dairy sci.78:1607-1618.

Norman, H.D.; Miller, R.H.; Wright, J.R. and Wiggans, G.R. (2000): Herd and state means for somatic cell count from dairy herd improvement. J. dairy sci.83:2782-2788.

Oliver, S.P., Gillespie, B.E., lewis, M.J., Ivey, S.J., Almeida, R.A., luther, D.A., Johnson, D.L., lamar, K.C., Moorehead, H.D. and D Dowlen, H.H.(2001): Efficacy of a new premilking teat disinfectant containing phenolic combination for the prevention of mastits . J. dairy sci. 84,1545-1549.

Omore, A.O.; McDermott, J.J.; Arimi, S.M. and Kyule, M.N. (1999): Impact of mastitis control measures on milk production and mastitis indicators in smallholder dairy farms in Kiambu district, Kenya. Trop. Anim. Helth. Prod.31(6):347-361.

Ostensson, K. (1993): trafficking of leukocytes and immunoglobulin isotypein the bovine udder. Ph.D.Diss.Swedish Univ. Agric. Sci. Uppsala, Sweden. Cited by Schepers et at. (1997).J. Dairy sci.80:1833-1840.

Osteras, O.; Edge , V.L. and Martin,S.W. (1999): Denterminants of success or failure in the elimination of major mastitis organisms in selective dry cow therapy . J. dairy sci .82:1221-1231.

Paape, M.J. and R.M. Miller, 1992. Influence of involution on intramammary phagocytic defence mechanism. J. Dairy. Sci., pp: 56.

Pankey, J.W.; Drechsler,P.A. and Wildman, E.E (1991): Mastitis prevalence in primigravid heifers at parturition.J. dairy sci. 74:1550 (quoted by Roberson et al .,1994.J. dairy sci.77:958-969).

Pankey, J.W.; pankey,P.B.; Barker, R.M.; Williamson, J.H. and Woolford, M.W. (1996): The prevalence of mastitis in primiparous heifers in eleven Waikato dairy herds. New Zealand Veterinary J.44(2): 41-44.

Peeler EJ, Green MJ Fizpatrick JL, Morgan KL, Green LE 2000. Risk factors associated with clinaical mastitis in Low somatic cell count British dairy herds.J Dairy Sci.2000; 83: 2464-2472.

Peeler,E.J., Green, M.J., Fitzpatrick, J.L., Morgen, K.L., and Green, L.E, (2000): Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. J. dairy sci. 83:2464-2472.

Pehrsson, P.R.; Haytowitz, D.B.; Holden, J.M.; Perry, C.R.; Beckler, D.G. (2000). "USDA's National Food and Nutrient Analysis Program: Food Sampling" *(PDF). Journal of Food Composition and Analysis.* ***13*** *(4): 379–389.* doi:10.1006/jfca.1999.0867. *Archived from* the original *(PDF) on April 7, 2003.*

Prasad AS, Bao B, Beck FW, o, Sakar FH 2004. Antioxidant effect of zinc in human. Free Radic Biol Med. 2004;37: 1182-1190.

Pyörälä (1995): The bovine udder and mastitis caused by different microbes, Staphylococcal and Streptococcal mastitis.Univ. of Helsinki, Faculty of vet. Med. ISBN:951-83-047-1PP143-160.

Radostits, O.M., Leslie K.E. and Fetrow, J. (1994): Herd health: Food animal production medicine 2nd ed. W.B.Saunders co., philadephia, PA.

Rahman OM, Gay CC, Blood DC, Rahman AM. Bhuiyan MU,Kamal MM, Shamsuddin M. 20009, The Bangadesh Veterinanan-2009;26:54-60.

Rainard, P.; Ducelliez, M. and Poutrel, B. (1990) : The contribution of mammary infection by coagulase- negative Staphylococci to the herd bulk milk somatic cell count . Vet. Res. Comm. 14: 193-198.

Rajala P J, Gro''hn Y T.1998. Disease occurrence and risk factor analysis in Finnish Ayrshire cows . Acta Vet Scand .1998; 39:1-13.

Rajala-Schultz, p .J.and Gro"hn,Y.T.(1999),Culling of dairy cows. Part 3. Effects of diseases.

Ranjan R, Gupta MK, Singh KK 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Vet World. 2011; 4: 205-208.

Reneau J 2012. Gear up for warm weather mastitis mangementt now . Dairy star.

Reneau, J.K. and Packard ,V.L.(1991):Monitoring mastitis , milk quality and economic losses in dairy fields.Dairy ,food, and Environ.Sanit.11:4-11.

Risco,C.A.; Donovan,G.A. and Hernandez, J. (1999): Clinical mastitis associated with abortion in dairy cows. J, dairy sci.82:1684-1689.

Roberson, J.R.; Fox, L.K.; Hancock, D.D.; Gay, J.M and Besser, T.E. (1994): Ecology of Staphylococcus aureus isolated from various sites on dairy farms. J. Dairy sci 77:3354-3364.

Rodenburg, J. (1990): Mastitis prevention: Environmental control: Ontario. Ministry of Agriculture and food Factsheet. AGDEX410/662.

Rodriguez, Zas. SI.; Gianola, D. and Shook, GE. (2000): An approximate Bayesian analysis of somatic cell score in Holsteins. Acra Agriculturae Scan dinavica- Section- A, Animal Science.50(4):291-299.

Rupp, R. and Bertrand, D. (1999): Genetic parameters for clinical mastitis , somatic cell score, production ,udder type traits, and milking ease in first Lactation Holsteins. J. dairy sci. 82.2198-2204.

Rupp, R.;Bertrand,C. and Bazin, S.(2000): Overview of milk Somatic cell counts in French dairy cattle breeds. Productions-Animales 13(4):257-267.

Sandholm,M. and Korhonen,H.(1995): The bovine udder and mastitis:Antibacterial defence mechanisms of udder .Univ.of Helsinki,Faculty of vet. Met .ISBN:951-834-047-1pp37-48.

Sargeant, J.M., leslie, K.E., Shirley, J.E., pulkrabek, B.J. and Lim, G.H (2001): Sensitivity and specificity of somatic cell count and California mastitis test for identifiying intramammary infection in early lactation .J. dairy sci.84:2018-2024.

Sarker SC, Parvin MS, Rahman A K M A and Islam M T 2013.Prevalence and risk factors of sub clinical mastitis in Lactating dairy cows in-north and south regions of Bangladesh, Troipcal Animal Health and production .2013;45:1171-1176.

Schaeffer,L.R and Solbu, H. (1987): Disease recording of dairy cows. Holstein J.(Sept., 1987).

Schepers, A.J.; Lam, T.J.G.M.; schukken, Y.H.; Wilmink, J.B.M. and Hanekamp, W.J.A. (1997): Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters.J. dairy sci .80:1833-1840.

Schrick,F.N.;Hockett,M.E.; Saxton,A.M.;Lewis,M.J.;Dowlen,H.H. and Oliver,S.P.(2001): Influence of sub-clinical mastitis during early lactation on reproductive parameters.J. dairy sci.84:1407-1412.

Schukken YH, Grommers FJ. Van do Geer D. Brand A 1989 . Incidence of clinical mastitis on farms with Low somatic cell counts in bulk milk. Vet Rec 1989;125:60-63.

Schukken, YH; Wilson, David J; Welcome, Francis; Garrison-Tikofsky, Linda; Gonzalez, Ruben N (2003). "Monitoring udder health and milk quality using somatic cell counts". Veterinary Research. EDP Sciences. **34** (5): 579–596. *doi*:*10.1051/vetres:2003028*. *PMID* *14556696*

Seeger,J:menardJL:,Dejan,O.and weber ,M.(1997b) , Call count evolution and clinical mastitis freguency in milk recording herds of the OpTLLALT are (SouthWest) .Rencontres Rech. Ruminents 4:279.

Seker, I.; Risvanli, A.; kul, S.; Bayraktar, M.and Kaygusuzoglu, E. (2000): Relationship between California mastitis test (CMT) scores and udder traits and milk yield in Brown-Swiss cows. Lalahan Hayvancilik Arastirma Enstitusu Dergisi 40(1):29-38.

Sharma N,Maiti sk 2010. Incidence, etiology and antibiogram of sub clinical mastitis in cows in drug. Chhattisgarth. Ind J Vet Res. 2010: 19:45-54.

Sharma N,Singh NK,Singh OP,Pandey V,Veerma PK. (2011). Oxidative stress and antioxidant Status during transition period in dairy cows. Asian-Aust J Anim sci.;24:479-484.

Shook, G.and Ruegg,P.(1999) ,Geometric mean somatic cell count :what are they and what do they do ?Proc . 38th Annu.Mtg. Natl. Mastitis Council,Arlington,VA. Natl.Mastitis Council,Inc., Maddison , WI.93-100.

Shoshani, E. and Berman, A. (1998) : Subclinical mastitis assessed by deviation in milk yield and electrical resistance .dairy res. 65(1) :31-41.

Shpigel, N.Y.; Winkler, M.; Ziv, G. and saran, A. (1998): Clinical, bacteriological and epidemiological aspeets of clinical mastitis in Israeli dairy herds Prev. Vet. Med.35(1):1-9.

Smith, J.W and Ely, L.O. (1997): The influence of feeding and housing systems on production, reproduction and somatic cell count scores of southern Holstein herds. Prof. Anim. Sci.13:155-161.

Smith, K.L. and Hogon , J.S.(1995): Epidemiology of mastitis Proc. 3th IDF.International mastitis seminar,Tel-Aviv,Isreal,S6,3-13 (cited by Klaas,2000 : Untersuchungen zum Auftreten von Mastitiden und zur Tiergesundheit in 15 Milchviehbetrieben Schleswig-Holsteins. Dissertation , Free university –Berlin).

Smith,J.W.; Ely,L.O and Chapa, A.M. (2000): Effect of region , herd size and milk production on reasons cows leave the herd .J.dairy sci .83 (12):2980-2987.

Sordill LM, Shafer- Weaver K, DeRosa D. (1997) . Immunobiology of the mammary gland . J Dairy Sci. 1997; 80: 1851-1865.

Sordillo LM.2005, Factors affecting mammary gland imrnunlty and mastitis susceptibility. Liv Prod sci.2005: 98:89-99.

Spears JW Weiss WP 2008 . Role of antioxidants and trace elementnts in health and immunity of transition dairy cows. Vet J. 2008; 176:70-76.

Sshukken,y.H.;Lam,T.J.G.M. and Barkema,H.W.(1997), Biological basis for selection on udder health traits INTERBull Bull.No.15 Int Bull Eval. Serv., Uppsala ,Sweden.27-33.

Suriyasathaporn, W.; Schukken, Y.H.; Nielen, M.and Brand, A. (2000): Low somatic cell count: a risk for subsequent clinical mastitis in a dairy herd.J.dairy sci.83:1248-1255.

Swinkels JM, Hogeveen H, Zadoks RN 2005, A partial bulged model to estimate economic benefits of location al treatment of subclinical Staphylococcus aureus mastitis . J Dairy Sci. 2005; 88: 4273-4287.

Tiwari JG, Babra C, Tiwari Hk, Williams V,Sharon DW, Gibson J, *et al*. 2013, Trends in Therapeutic and prevention Strategies for Management of Bovine Mastitis ; An Overview. J Vaccin. 2013; 4:2.

Todhunter, D.A., Smith, K.L., and Hogan, J.S. (1995): Environmental Streptococcal intra mammary infection of the bovine mammary gland . J. dairy sci.78:3266-2374.

Trinidad, P.; Nickerson, S.C. and Alley, T.K. (1990): Prevalence of intra – mammary infection and teat canal colonization in unbred and prim gravid dairy heifers.J. Dairy sci.73:107-114.

Tucker,H.A.(1987): Quantitative estimates of mammary growth during various physiological states: a review.J.dairy sci 70:1958.

Tyler, J.W.; Thumond, M.C. and Lasslo, L. (1989): Relationship between test-day measures of somatic cell count and milk Production in California dairy cows . Cand. J. Vet. Res. 53(2):182-187.

Underwood FJ, Suttle NF . iIn 1999 : The Mineral Nutrition of Livestock. Underwood EJ and Suttle NF (eds), CABI Publishing, New York. 1999.

Van Schaik, G.; Lotem, M. and Schukken, Y.H. (2002): Trends in somatic cell count, bacterial count and antibiotic residue violations in New York State during 1999-2000. J. dairy sci. 85(4):782-789.

Waage ,S., MØrk, A., Aasland ,D .,Hunshamar , A . and Ødegaard, S.A . (1999) : bacteria associated with clinical mastitis in dairy heirers.J. dairy sci.82: 712-719.

Waage-Washburn, S.P.; white, S.L.; Green,JT. And Benson, G.A. (2002): Reproduction,mastitis pasture systems. J. Dairy sci.85:105-111.

Whitaker, D.A.; Kelly, J.M. and Smith, S. (2000): Disposal and disease rares in 340 British dairy herd. Vet. Record.146(13):363-367.

Wilesmith,J.W.; Francis,P.G. and Wilson,C.D.(1986):Inciddence of clinical mastitis in acohort of British dairy herd. Vet .Record. 118:199-204.

Williams, D.J.; Marschke, R.J.; Nottingham, S.M. and Kitchen, B.J. (1991): Effects of stage of lactation, number of lactation and dry period on N-acetyI-B-D-glucsaminidase levels and somatic cell count in bovine milk .Aust.J.dairy technol.46(1):43-45.

Wilson DJ, Gonzaiez RN, Hertl J,Schulte HF,Bennett GJ,Schukken YH, et al.2004, Effect of clinical mastitis on the lactation curve: Amixed

model estimation using Daily milk Weights. J. Dairy Sci. 2004; 87: 2073-2084.

Woodward, W.D.; ward , A.C.S.; Fox, L.K and Corbeil, L.A. (1988): Teat skin normal flora and colonization with mastitis pathogen inhibitors. Vet .Microbio.17:357-365.

Yalcin, C.; Stott, A.W.; Logue, D.N. and Gunn, J. (1999): The economic impact of mastitis-control procedures used in Scottish dairy herds with high bulk-tank somatic-cell counts. Prev. Vet. Med. 41(2-3):135-149.

Zadoks,R.N.; Allore , H.G.; Barkema, H.W.; Sampimon, O.C.; Gro''hn, Y.T. and Schukken, Y.H (2001): Analysis of an outbreak of Streptococcus ubris mastitis . J. dairy sci. 84:590-599.

Zafalon LF, Nader Filho A, Oilveira JV, Resende FD (2007). Subclinical mastitis caused by Staphyloco aureus; Cost benefit analysis of antibiotic therapy in lactation cows . Arq Bras Med Vet Zootec. 2007;59:577-585.

Zepeda,L.; Buelow,K.L.; Nordlund,K.V.; Thomas,C.B.; Collins, M.T. and Goodger, W.J. (2000): Corrigendum to A linear programming assrssment of the profit from strategies to reduce the prevalence of Staphylococcus aureus mastitis".Prev.Vet.Med.44:61-71.

APPENDICES

The questionnaire form of the study

جامعة السودان للعلوم و التكنولوجيا

كلية الدراسات العليا

كلية الدراسات الزراعية

قسم الإنتاج الحيواني

تقييم لانتشار مرض إلتهاب الضرع في ابقار اللبن بمحلية الخرطوم بحري

استمارة استبيان

التاريخ: .....

مساحة المزرعة : .....

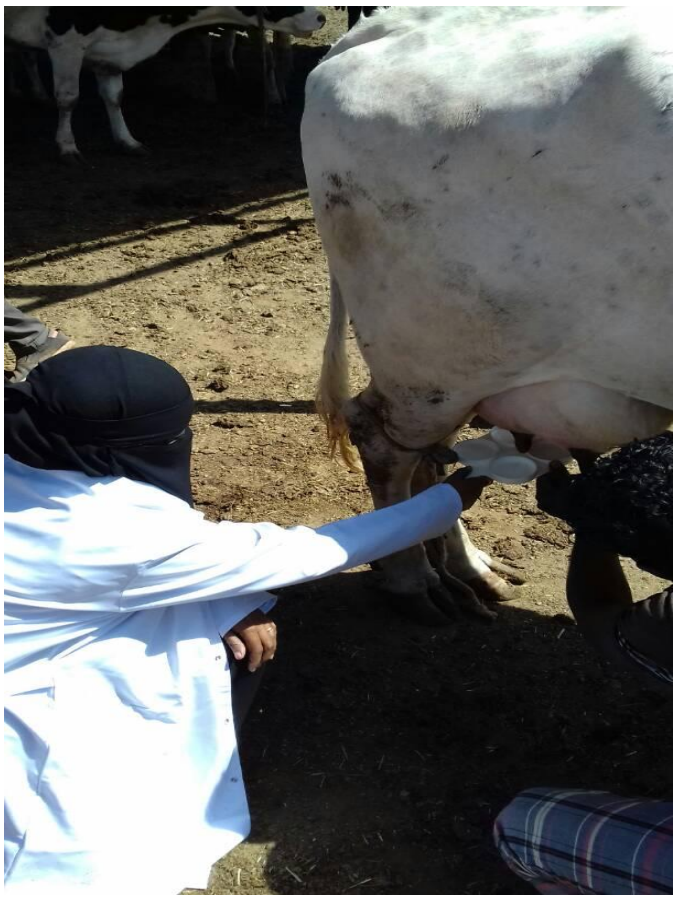
عدد الأبقار في المزرعة : .....

01	ما هو نظام التربية المستخدم بالمزرعة	حديثة		تقليدية
02	ما نوع السلالة المرباة	محلي	هجين	أجنبي
03	هل تتم نظافة الحظيرة	يومياً	اسبوعياً	شهرياً
04	هل يوجد اهتمام بالبقرة	نعم		لا
05	هل يوجد اهتمام بالحلابين	نعم		لا
06	ما هي طريقة الحلب المتبعة	آلي		يدوي
07	هل الحلابين خاصين بالمزرعة	نعم		لا

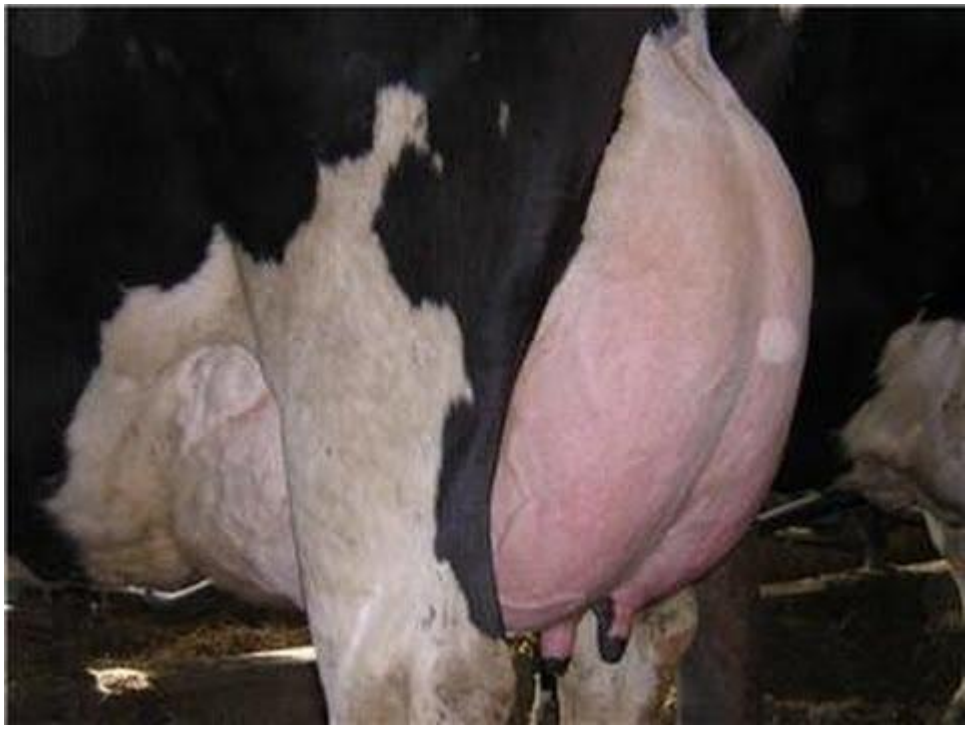
08	اين يتم الحلب	داخل الحظيرة	محلب خاص
09	هل يوجد اهتمام للضرع قبل الحلب	نعم	لا
010	هل يوجد اهتمام للضرع بعد الحلب	نعم	لا
011	هل يتم الكشف عن المرض دورياً	نعم	لا
012	ما هي أكثر الأمراض السائدة في المزرعة		

California Mastitis reagent

Preparing for CMT



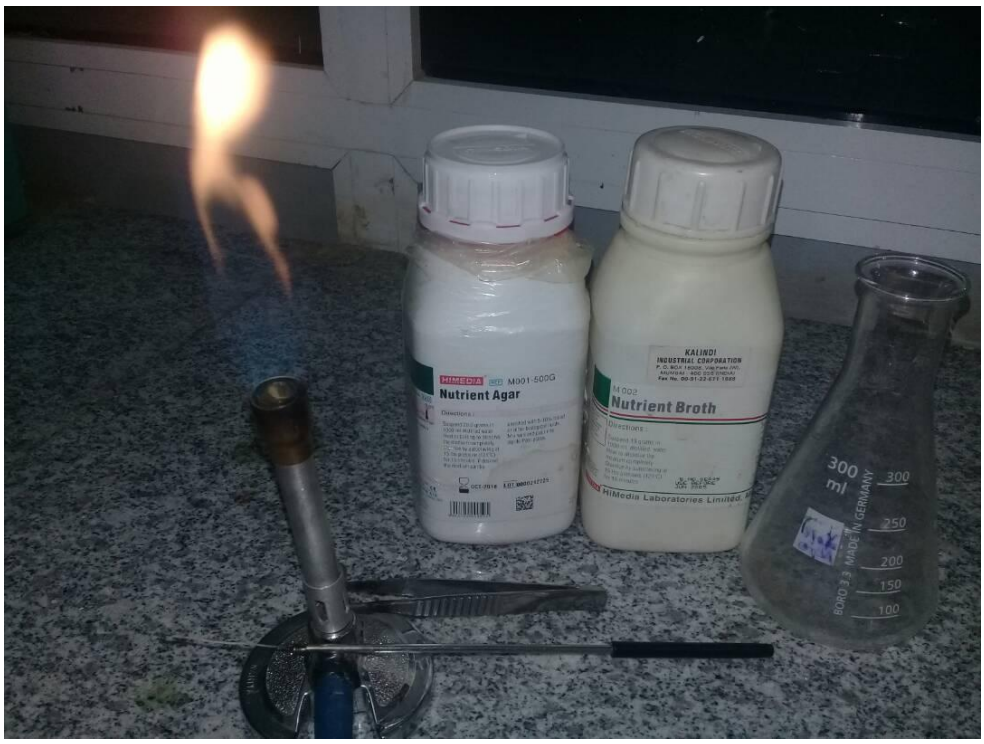
Taking simples



Inflammation of udder

Negative result

Positive result



Laboratory test