بسم الله الرحمن الرحيم

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Prevalence and Risk Factors of Mastitis in Cattlein Eastern Nile Locality in KhartoumState – Sudan

معدل الإصابة وعوامل الخطر المرتبطة بإلتهاب الضرع في الأبقار في محلية شرق النيل ولاية الخرطوم ـ السودان

A thesis Submitted to the College of Graduate Studies, Sudan University of Science and Technology in partial fulfillment for the requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

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DEDICATION

I dedicate this work to my parents, my brothers and sisters, and to my teachers and friends, with my best wishes.

Acknowledgement

I consider it my utmost obligation to express my gratitude to Allah, the omnipresent, kind, and merciful who gave me the health, thoughts and theOpportunity to complete this task.

I offer my humble thanks from the core of my heart to the holy prophet Mohammed in the completion of this work.

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Abstract

A cross-sectional study was conducted on 399 lactating cows at East Nile Locality, during the period October-December 2015. The objectives of the study were to estimate the overall prevalence of clinical and subclinical mastitis and to investigate the association of potential risk factors associated with the disease. A total of 399lactating dairy cows comprising 58 local and 341 cross breed cows were randomly selected and screening using California Mastitis Test (CMT) for subclinical mastitis and clinically examined for clinical mastitis. The overall prevalence rate was found to be 51.9% (9.3% clinical and 42.6% subclinical). The highest prevalence of clinical mastitis was reported in Abu deleeg Administration Units (67%) and Wadi suba Administration Units showed the lowest prevalence of clinical mastitis (36%). The following risk factors: age (p-value = 0.002), breed (p-value = 0.044), body condition (p-value = 0.000), stage of lactation (p-value = 0.000), parity (p-value = 0.003), previous exposure to mastitis (p-value = 0.000), presence of tick (p-value = 0.001), teats injuries (p-value = 0.006), clean teats and udder (p-value = 0.017), wash hands (p-value = 0.001), sanitary practices (p-value = 0.003), floor disinfectant (p-value = 0.006), drainage system (p-value = 0.000), bedding removal (p-value = 0.000), herd size (p-value = 0.065), barn size (p-value = 0.544), types of fencing (p-value = 0.776), water source (p-value = 0.733),yielding milk (p-value = 0.000), milking technique (p-value = 0.554), education level of farmer (p-value = 0.001) and locality (p-value = 0.000) showed statistical significant association (p-value < 0.25) with the occurrence of mastitis in the Univariate analysis.The result of this study also showed that age (p-value = .000), body condition (p-value = .000), teat injuries (p-value = 0.029), yielding milk (p-value = .000) and locality (p-value = .000) had statistical significant association with mastitis (p-value = ≤ 0.05) in the multivariate analysis.

ملخص البحث

أجريت هذه الدراسة الوبائية قي مزارع أبقار اللبن بمحلية شرق النيل, في الفترة من أكتوبر – ديسمبر2015. هدفت الدراسة الي تقدير نسبة انتشارالتهاب الضرع (العياني ودون العياني), وتحديد عوامل الخطورة التي تساعد علي حدوث المرض تم اختيار مجمل 399 بقرة حلوب عشوائيا كالاتي: 58 محلي و 341 هجين وتم فحصها بواسطة (CMT) للأبقار دون العيانية وبواسطة العين للاصابات العيانية حيث سجلت النتائج التالية: نسبة إنتشار المرض في الولاية 51.9% (9.3%للفحص العياني و42.6% للفحص دون العياني). أعلي نسبة إنتشار للإصابات العيانية سجلت فيوحدة ادارية ابو دليق (67%) بينما سجلت وحدة ادارية وادي سوبا أدني نسبة إنتشار للإصابات العيانية (36%).

عند التحليل لكل عامل خطورة حددت الدراسة بعض عوامل الخطورة التي تساهم بشكل وثيق في حدوث المرض منها : العمر(p-value = 0.000), السلالة (p-value = 0.044), الحالة الصحية (p-value = 0.000), مراحل الحلابة (p-value = 0.000), عدد الولادات (p-value = 0.003), التعرض المسبق للإلتهاب (p-value = 0.000), وجود القراد علي الضراع (p-value = 0.001), الإصابات الموجودة علي الحلمات (p-value = 0.006), نظافة الضرع والحلمات (p-value = 0.017), غسل الأيادي (p-value = 0.001), الممارسة الصحية (p-value = 0.003),تطهير أرضية المزرعة (p-value = 0.006), نظام التصريف (p-value = 0.000), تردد إزالة الفرش (p-value = 0.000), حجم القطيع (p-value = 0.065),), حجم الحظيرة (p-value = 0.544), نوعية سور المزرعة(p-value = 0.776), مصدر الإمداد المائي (p-value = 0.733), كمية إنتاج الحليب (p-value = 0.000), تقنية إنتاج الحليب (p-value = 0.554), المستوي التعليمي للرعاة (p-value = 0.001), المحليات (p-value = 0.000) هي أكثر عوامل الخطورة التي تساهم في حدوث المرض وذلك في تحليل عوامل الخطورة مجتمعة عند القيمة (p-value = 0.025).

كذلك أظهرت نتائج هذه الدراسة بعض عوامل الخطورة التي تساهم بشكل وثيق في حدوث المرض منهاالعمر(p-value = 0.000), الحالة الصحية(p-value = 0.000), الإصابات الموجودة علي الحلمات (p-value= 0.029), كمية الحليب (p-value = 0.000) والمحليات (p-value = 0.000) هي أكثر عوامل الخطورة التي تساهم في حدوث المرض وذلك في تحليل عوامل الخطورة عند القيمة (p-value ≤0.05).

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Introduction

Milkis one of the most important foods of human being. It is universally recognized as a complete diet due to its essential compents (Javaid*et al.*, 2009).

Mastitis is defined as an inflammation of the udder resulting in an inflamed quarter or quarters with a change in the appearance of the milk. Mastitis can either be infectious, caused as a response to the presence of microbial organisms or non-infectious, as a result of physical injury to the mammary gland. The inflammatory response results in an increase in the blood proteins and white blood cells in the mammary tissue, which can then pass into the milk product. This response aims to destroy the irritant, repair the damaged mammarytissue and return the udder to its normal function (kudi*et al.*, 2009).

In terms of the sources of infection it has been established that bacteria are the most common cause of mastitis although other sources of infections including mycoplasm, algae and fungi are also prevalent. There are several species of such infectiousbacteria responsible for causing these infections including *Staphylococcus aureus, Streptococcus agalactaie, Streptococcus dysgalactiae* and the environmental bacteria, *E. coli* and *Streptococcus uberis*. The simple classification of mastitis recognizes two major groups; environmental mastitis and contagious mastitis. Environmental mastitis whichis caused by organisms such as *E. coli* and *Streptococcus uberis*that do not usually live upon the skin but enter the teat canal when the cow comes into contact with a contaminated environment. Further divisions between the two groups can be made including clinical, sub clinical and chronic mastitis. Therefore mastitis problems may be present within a herd despite no visible presence within the cows or the milk. Besides health disorders of the

mammary gland, mastitis can also cause significant losses in milk yield, alterations in its quality (impaired nutritive and technological properties of milk), fertility disorders and even systemic diseases.A Bovine mastitis is a large-scale infectious disease with significant impact on the economy. Moreover, causative agents of mastitis with zoonotic potential may represent a health risk for human populations via the food chain. In recent times, there is clear evidence for an increasing incidence of environmental mastitis while the incidence of contagious mastitis has decreased.There is a known relationship between particular pathogens and the form of the disease. For example: *S. uberis*, *Escherichia coli*, *Klebsiella*spp, *Pseudomonas aeruginosa*and pyogenic bacteria are mainly considered as causative agents of clinical mastitis. On the other hand, *S. agalactiae* and *Enterococcus* spp. are associated with subclinical mastitis. However, *S. aureus*has been designated as a causative agent of both clinical and subclinical mastitis. Unlike the clinical form of the disease, subclinical mastitis is hard to recognize, and for this reason it may cause significant losses in milk production. Moreover, sub clinically infected cows may represent a source of particular pathogens that can be spread via automatic milking system(Cervinkova*et al*., 2013*).*

1.2. Objectives of this study were:

1- To estimate the prevalence of subclinical and clinical mastitis in cattle in East Nile in Khartoum State of Sudan.

2- To investigate the potential risk factors that associated with the disease.

Chapter one

Literature Review

1.1. Definition:

Mastitis (Mast: breast, itis: inflammation) is an inflammatory reaction of udder tissue due to bacteria, chemical, thermal or mechanical injury (McDonal Campus., 2007).

Also it can be defined as an inflammation of the mammary gland resulting from the introduction and multiplication of pathogenic microorganisms in this gland (Osman *et al.,* 2009).

Mastitis is inflammation of parenchyma of mammary gland characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits*et al.*, 2000).

Mastitis may be caused by wide variety of microorganisms including bacteria, fungi, yeast, and mycoplasma. However, bacteria are the most frequent pathogens of these diseases (Koivala*et al.,* 2007 and Lim *et al.*, 2007).

1.2Epidemiology of mastitis:

A cross sectional study was conducted from November 2008 to April 2009 in Hawassa town, southern Ethiopia to determine the overall mastitis prevalence and identify the role of potential risk factors in 183 randomly selected small holder lactating dairy cows of 53 high grade Holstein Friesian, 113 Holstein-indigenous zebu cross and 17 indigenous zebu breeds. Of the total 183 lactating smallholder dairy cows examined for bovine mastitis 9 (4.9%) had clinical mastitis, while 56 (30.6%) subclinical mastitis. Out of 9 (4.9%) clinical mastitis, 9.43 and 3.53% occurred in high grade Holstein and Holstein-indigenous zebu, respectively but indigenous zebu breeds was found to be not affected. Among the potential risk factors considered, breed (2=

17.3, P< 0.05), presence of teat lesion and/or tick infestation (2= 7.73, P< 0.05), stage of' lactation (2=l3.8, P< 0.05) and parity number (2= l9.4, P< 0.05) had significant effect on the prevalence of subclinical mastitis (Moges*et al*., 2012*).*

A study was contemplated to find out the epidemiology of mastitis in lactating cattle and buffaloes in tehsil Burewala, Pakistan. A total of 673 animals (n=291 cattle, n=382 buffaloes) from 300 livestock farmers were tested using Surf Filed Mastitis Test (SFMT) for the presence of mastitis. A higher prevalence (24.60%) of clinical mastitis was found in buffaloes than cattle (18.21%). The prevalence of subclinical mastitis was 36.38% and 33.67% in buffaloes and cattle, respectively. Quarter based prevalence of clinical mastitis in buffaloes and cattle were 8.04% and 5.75%, respectively. Quarter based prevalence of subclinical mastitis was 16.04% in buffaloes and 14.47% in cattle. Risk factors of mastitis found were: age, lactation number, stage of pregnancy, stage of lactation, dry period length, hard milking, calf suckling, folded thumb milking technique, teat injury, backyard housing, bricks floor, uneven floor, poor drainage system and low frequency of dung removal (Hameed*et al.*, 2012).

A total of 669 individual cow milk samples originating from asymptomatic cows from 16 dairy farms were examined for the presence of microorganisms with the potential to cause mastitis. Coagulase-negative *staphylococci* clearly predominated (53.5% positive samples) followed by *streptococci* and *enterococci* (both occurring in 16.1% samples). Among *streptococci*, so-called mastitis streptococci (*S. uberis*, *S. dysgalactiae*and *S. agalactiae*) prevailed (11.7% positive samples). *Enterobacteriaceae*were found in 10.0% samples, most of which (6.6% samples) were positive for *Escherichia coli*. Yeasts (mainly *Candida* spp.) were found in 8.2% samples.

One of the major mastitis pathogens, *Staphylococcus aureus*subsp. *aureus*, was isolated from 9.0% of samples. *S. aureus*isolates were further characterized in terms of their capability to form biofilm, antimicrobial susceptibility and clonality (PFGE). All *S. aureus*isolates were capable of biofilm formation and were generally susceptible to the majority of tested antibiotics. The exception was ampicillin, resistance to which was observed in 27.7% isolates (Cervinkova*et al*., 2013).

Another study was conducted to determine the prevalence of subclinical and clinical mastitis and the associated factors in cows from selected smallholder dairy farms in Zimbabwe. Physical examinations were conducted on all lactating cows for evidence of signs of clinical mastitis. Composite milk samples were collected from all lactating cows for bacterial culture and somatic cell counting. Cows were categorized as clinical if they exhibited clinical features of mastitis, or subclinical if no apparent signs were present but they had a positive bacterial isolation and a somatic cell count of at least 300 x 103 cells/ml. Farm-level factors were obtained through a structured questionnaire. The association of mastitis and animal-and herd-level factors were analyzed using logistic regression. A total of 584 animals from 73 farms were tested. Overall, 21.1% (123/584) had mastitis, 16.3% (95/584) had subclinical mastitis and 4.8% (28/584) had clinical mastitis. Herd-level prevalence was 49.3%. Coagulase-negative staphylococci (27.6%), *Escherichia coli* (25.2%), *Staphylococcus aureus*(16.3%), *Klebsiella*spp. (15.5%) and *Streptococcus* spp. (1.6%) were the most common isolates. In individual cows, pure dairy herds (OR = 6.3) and dairy crosses (OR = 3.1) were more likely to have mastitis compared to Mashona cows. Farms that used pre-milking teat dipping were associated with reduced mastitis prevalence (Katsande*et al*.,2013).

In thestudy conducted on 453 lactating cattle of various breed at21 farms. The milk samples from these animals were collected to diagnose mastitisusing California Mastitis Test (CMT). The epidemiological data related to animalsand management was collected and analyzed to draw conclusions. The results of testrevealed significant association between body weight, udder depth, and lowerteat end to floor distance ($P<0.01$) with mastitis. The bivariate frequency analysisrevealed significant association for lactation stage ($P<0.0001$), teat end to floordistance, parity, udder shape, teat shape, live body weight, teat and/or udderpathology, use of oxytocin, feeding system and milk leakage with mastitis. Theresults of logistic regression analysis revealed significant negative associationbetween teat lengths, frequency of culling and number of attendants, while positiveassociation between mastitis teats involved, teat diameter (apex, mid and base), milkleakage, udder shape, pendulous udder, feeding system, udder depth, teat shape, calfsuckling, milk yield, teat and/or udder pathology and live body weight (Hussain*et al.,* 2012).

The overall herd-level prevalence rate for SCM was 85.33% (256/300 heads of cows) while the quarter-level prevalence rate of SCM was 43.25% (519/1,200 quarters). The prevalence of SCM was 50.67%, 43.67%, 39.67% and 39.13% for the left fore-quarter, right hind-quarter, left hind-quarter and right fore-quarter, respectively. The Rahaji breed had the highest prevalence of SCM with 65.91% (29/44), while the White Fulani breed had the least with 32.39% (57/176). A total of 32.33% (97/300) had only one mammary quarter affected, 30.33% (91/300) had two quarters affected, and 16.0% (48/300) had three quarters affected while 6.67% (20/300) had all the four quarters affected. A total of 53.00% had SCM in multiple quarters (159/300). The risk of SCM

decreased significantly among young lactating cows compared to older animals (OR = 0.283; P < 0.001; 95% CI = 0.155; 0.516). The Rahaji breed had significantly higher risk compared with the White Fulani breed (OR = 8.205; P = 0.013; 95% CI = 1.557; 43.226). Improved sanitation (washing hands before milking) will decrease the risk of SCM (OR = 0.173; P = 0.003; 95% CI = 0.054; 0.554). SCM is prevalent among lactating cows in the Nigerian Savannah; and this is associated with both animal characteristics (age, breed and individual milk quarters) and milking practices (hand washing) (Shittu*et al.,* 2012).

Twenty-nine dairy farms were selected to determine the incidence of clinical mastitis, prevalence of sub-clinical mastitis and bacterial etiology in the West Littoral Region of Uruguay. In samples taken by the owner and frozen at -20ºC during a week the incidence rate of clinical mastitis was determined as 1.2 cases per 100 cow-months at risk. *Staphylococcus aureus*was the most common isolated pathogen in 37.5% of 40 milk samples from clinical cases obtained in 1 month. No bacteria grew in the 32.5% of the total samples. A sub-sample including 1077 dairy cows from randomly selected farms was used to determine the prevalence of subclinical mastitis. These samples were taken on one visit to each farm. The prevalence was 52.4% on a cow basis and 26.7% on an udder quarter basis. In 55.1% of the quarters of the selected animals with more than 300 000 cells/ml there was no growth. The isolated pathogens from sub-clinical cases and their relative frequencies were: *Staphylococcus aureus*62.8%, *Streptococcus agalactiae*11.3%, *Enterococcussp.* 8%, coagulase-negative staphylococci 7.4%, *Streptococcus uberis*6.4%, *Streptococcus dysgalactiae*1.8%,

Escherichia coli 1.5% and *Staphylococcus hyicus*coagulase- positive 0.6% (Gianneechini *et al*., 2002).

Data from the national dairy cow recording systems during 1997 were used to calculate lactation-specific cumulative risk of mastitis treatments and cumulative risk of removal from the herds in Denmark, Finland Norway and Sweden. Sweden had the lowest risk of recorded mastitis treatments during 305 days of lactation and Norway had the highest risk. The incidence risk of recorded mastitis treatments during 305 days of lactation in Denmark, Finland, Norway and Sweden was 0.177, 0.139, 0.215 and 0.127 for first parity cows and 0.228, 0.215, 0.358 and 0.204 for parities higher than three, respectively. The risk of a first parity cow being treated for mastitis was almost 3 times higher at calving in Norway than in Sweden. The period with the highest risk for mastitis treatments was from 2 days before calving until 14 days after calving and the highest risk for removal was from calving to 10 days after calving in all countries. The study clearly demonstrated differences in bovine mastitis treatment patterns among the Nordic countries. The most important findings were the differences in treatment risks during different lactations within each country, as well as differences in strategies with respect to the time during lactation mastitis was treated (Valde *et al*., 2007).

1.3The causative agent:

Research findings have proved that buffalo is as susceptible to mastitis as cow. The causative organisms of mastitis in buffaloes have been reported to be *Staphylococci, Streptococci, Escherichia coli, Pseudomonas spp., Corynebacterium, Mycoplasma, Streptococcus dysaglactiae*, and *Mycobacterium tuberculosis*. Among all the pathogens of bovine mastitis,

*Staphylococcus aureus*is the predominant organism. Etiological agents of mastitis in buffaloes have been reported to be *Staphylococcusaureus*, *Staphylococcus hyicus*, *Staphylococcusepidermidis*, *Staphylococcus capotus*, *Streptococcusdysaglactiae, Streptococcus agalactiae*, *Streptococcuspyogenes and Corynebacteriumbovis*. The most common mastitis pathogens are found either in the udder as contagious pathogens or in the animal surroundings such as bedding and manure soil as environmental pathogens. Among the contagious pathogens, the most common are *Staphylococcusaureus*and *Streptococcus agalactiae*. These are spread from infected to clean udders during the milking process through contaminated milker's hands, cloth towels used to wash or dry udder of more than one animal and possibly by flies. Reviewing the incidence of mastitis in buffaloes and cows from India and Pakistan concluded that contagious organisms are responsible for most of the clinical cases and *Staph. Aureus*is at the top of the list in both the species of animals. Among environmental pathogens, the most common bacteria are *Streptococcus uberis*, *Str. dysgalactiae*, Coliforms such as *E. coli* and *Klebsiella.* Transmission of these pathogens may occur during milking but primarily between milking Coliform infections are usually associated with unsanitary environment, while *Klebsiella* are found in sawdust that contains bark or soil. Approximately 70- 80% of Coliform infections are manifested by abnormal milk, udder swelling and systemic disturbances such as high fever, swollen quarters, watery milk and depressed appetite. Environmental pathogens are most often responsible for the clinical cases.About 50% of environmental streptococci infections display clinical symptoms. Sixty to 70% environmental pathogen infections exist for less than 30 days and are not easily detected. The dry period is the time of greatest susceptibility to new environmental streptococci infections, especially the

first 1-2 weeks and the last 7- 10 days before calving or early lactation.The incidence at calving is twice than at drying off. Infections during early dry period are controllable by dry animal antibiotic therapy but this treatment in the late dry period is not as effective as early dry period. Dry period therapy can eliminate 70% of environmental streptococcal infections. It is unfortunate that dry period antibiotic therapy is not being practiced anywhere in Pakistan (Khan*et al.*, 2006).

It is known that the prevalence of contagiouspathogens causing mastitis is decreasing and environmentalcausative agents are becoming dominantpathogens for the mammary gland. Reports from United States ofAmerica and from Great Britain indicate creatingrole of environmental mastitis pathogens. Coliformsand streptococci other than *S. agalactiae*accountedfor 94% of the major pathogen infections. Contagious pathogens were isolated onlyin 3.4% of clinical mastitis cases in well-managedherds. It has been estimatedthat contagious mastitis pathogens represent lessthan one third of all mastitis cases compared to> 75% of all cases 20 years ago.Environmentalstreptococci,coliformsandcoagulase-negative*Staphylococcus* spp. were themost commonly isolated pathogens(Rysanek*et al.*, 2007).

1.4 Types of mastitis:

There are several ways of classifying mastitis; a simple classification recognizes mastitis as two major groups:

1.4.1Contagious mastitis:

The udder and teats are the reservoir of infection. It is caused by bacteria that live on the skin of the teat and inside the udder. Contagious mastitis can be transmitted from one cow to another during milking (Awale*et al*., 2012).

Infection establishes on the surface of the teat and teat canal. Bacteria may be penetrating the mammary gland. Most infections are subclinical and result in raised cell counts. Control majors include post milking teat disinfection, dry cow therapy and culling of cow showing contagious bacteria include *staphylococcus aureus, streptococcus agalactiae* and *streptococcus dysaglactiae*. If a herd somatic cell count is over 200000/ml then this indicates that there is a problem with contagious mastitis (Andrews *et al*., 2004).

1.4.1 Contagious mastitis can be divided into three types:

1.4.1.1 Clinical mastitis:

The clinical mastitis (CM) is diagnosed by gross abnormalities in the milk such as flakes, clots, or a watery appearance, and also by inflammatory symptoms such as swelling, edema of the mammary gland, fever and rapid heart rate (Abdel Hameed*et al*., 2006).

The detection of the clinical mastitis depends upon the examination of the mammary gland and its secretion**.** The affected gland may show swelling, heat, pain and hardness. The secretion may be clotted, serous or occasionally bloodstained (Andrews *et al*., 2004).

1.4.1.1 Types of clinical mastitis:

1.4.1.1.1 Per-acute: This form of mastitis is fairly uncommon and includes depression, raised pulse and respiratory rates, loss of muscle coordination, cold extremities, reduced papillary reflex dehydration and diarrhea (Philpot and Nickerson, 2000).

It is characterized also by gross inflammation, reduction in milk yield and changes in milk composition. Systematic signs like fever, depression, shivering and loss of appetite and loss of weight (Awale*et al*., 2012).

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

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

1.4.1.2Subclinical mastitis:

Subclinical mastitis (SCM) is of great economic importance to dairy farmers because it results in reductions in milk yield and undesirable change in the milk composition (Brightling*et al*., 2010 and Seegers*et al*., 2003), as well as increased costs associated with control strategies (Halasa*et al*., 2009).

It cannot be detected by visual observation though it can be identified by conducting tests to detect the presence of infecting microorganism or the product of inflammation such as somatic cell count (Philpot and Nickerson,2000).

Diagnosed when somatic cell count was ≥ 400 000 cells/ml of milk. It is classified into contagious when there were ≥500 cfu (colony forming units)/ml of *S. aureus*or *Str. agalactiae*. The environmental subclinical mastitiswas diagnosed when there were ≥2000 cfu/ml of CNS, *Str. dysgalactiae, E. coli*, other coli forms (*Klebsiella*and *Enterobacter),* and other bacterial species (environmental *streptococci*, *Bacillus cereus*, *Corynebacterium species*, *Pseudomonas species*) (Abdel Hameed*et al*., 2006).

1.4.1.3 Chronic mastitis:

The chronic form may begin as any clinicalform or assub clinical mastitis and may be evidenced by intermittent signs of clinical mastitis. There is usually a progressive development of scar tissue and a change in size and

shape of the effected gland, accompanied by reduced milk yield (Philpot and Nickerson, 2000).

1.4.2Environmental mastitis:

The environment is a reservoir of infection. Infections are transmitted into teats between milking or during udder preparation. Organisms are forced up through the teat canal during the milking processor or after milking if cows are allowed to lie down immediately following milking. Most infections cause clinical mastitis. The sub clinical infections are less common with *E.coli*, but frequently occur with *streptococcus uberis*. Environmental mastitis is controlled by clean environment, adequate accommodation for cows, milking through correctly functioning machine, good udder preparation. Environmental organisms include *E.coli, streptococcus uberis* (straw bedding), *Klebsiella spp.* (sawdust and shavings). Although there is potential for inter quarter transfer at milking time it appears not to be the predominant infection mechanism. Post milking teat disinfection does not prevent infection. Antibiotic therapy has some beneficial effects on Coliform mastitis prevention but does reduce the rate of new dry-period infection with *Streptococcus uberis*. Environmental mastitis is controlled through good environmental management, a good milking routine, an efficient milking machine, vaccination against Coliform mastitis and pre milking teat disinfection (Andrews *et al*., 2004).

1.5. Pathogenesis:

Pathogenic organisms in milk can be derived from the cow itself, human hand and utensils or the environment (Adane*et al*., 2012).

Mastitis in dairy animals occurs when the udder becomes inflamed and bacteria invade the teat canal and mammary glands. These bacteria multiply and produce toxins that cause injury to the milk secreting tissue, besides, physical trauma and chemical irritants. These cause increase in the number of leukocytes, or somatic cells in the milk, reducing its quantity and adversely affecting the quality of milk and milk byproducts. The teat end serves as the first line of defense against infection. From outside, a sphincter of smooth muscles surrounds the teat canal which functions to keep the teat canal closed. It also prevents milk from escaping, and bacteria from entering into the teat. From inside, the teat canal is lined with keratin derived from stratified squamous epithelium. Damage to keratin has been reported to cause increased susceptibility of teat canal to bacterial invasion and colonization. The keratin is a waxy material composed of fatty acids and fibrous proteins in the teat. The fatty acids are both esterified and non-esterified, representing myristic acid, palmitoleic acid and linolinic acid which are bacteriostatic. The fibrous proteins of keratin in the teat canal bind electrostatically to mastitis pathogens, which alter the bacterial cell wall, rendering it more susceptible to osmotic pressure. Inability to maintain osmotic pressure causes lyses and death of invading pathogens. The keratin structure thus enables trapping of invading bacteria and prevents their migration into the gland cistern. During milking, bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus. The canal of a teat may remain partially open for 1-2 hour after milking and during this period the pathogens may freely enter into the teat canal. Bacterial pathogens which are able to traverse the opening of teat end by escaping antibacterial activities establish the disease process in the mammary gland which is the second line of defense of the host. In dairy

animals, the mammary gland has a simple system consisting of teats and udder, where the bacteria multiply and produce toxins, enzymes and cell-wall components which stimulate the production of inflammatory mediators attracting phagocytes. The severity of inflammatory response, however, is dependent upon both the host and pathogen factors. The pathogen factors include the species, virulence, strain and the size of inoculums of bacteria, whereas the host factors include parity, the stage of lactation, age and immune status of the animal, as well as the somatic cell count. Neutrophils are the predominant cells found in the mammary tissue and mammary secretions during early stage of mastitis and constitute >90% of the total leukocytes. The phagocytes move from the bone marrow toward the invading bacteria in large numbers attracted by chemical messengers or chemotactic agents such as cytokines, complement and prostaglandins released by damaged tissues (Khan and Khan, 2006). The Neutrophils exert their bactericidal effect through a respiratory burst and produce hydroxyl and oxygen radicals that kill the bacteria. During phagocytosis, bacteria are also exposed to several oxygen-independent reactants such as peroxidases, lysozymes, hydrolytic enzymes and lactoferrin. In addition to their phagocytic activities, neutrophils are a source of antibacterial peptides called defenses, killing a variety of pathogens that cause mastitis. Masses of neutrophils pass between the milk producing cells into the lumen of the alveoli, thus increasing the somatic cell counts and also damaging the secretary cells. Increased number of leukocytes in milk causes increase in the number of somatic cells. Clots are formed by aggregation of leukocytes and blood clotting factors which may block the ducts and prevent complete milk removal, resulting in scar formation with proliferation of connective tissue elements. This results in a permanent loss of function of that portion of the gland. The milk ducts remain clogged, secretary

cells revert to non-producing state, and alveoli begin to shrink and are replaced by scar tissue. This helps in formation of small pockets making difficult for antibiotics to reach there and also prevents complete removal of milk. Macrophages are the predominant cells found in milk and tissue of healthy involutes and lactating mammary glands. Macrophages ingest bacteria, cellular debris and accumulated milk components. The phagocytic activity of macrophages can be increased in the presence of opsonic antibody for specific pathogens. Because of indiscriminate ingestion of fat, casein and milk components, the mammary gland macrophages are less effective at phagocytosis than blood leukocytes. Macrophages also play a role in antigen processing and presentation. Conditions which contribute to trauma of mammary gland include: incorrect use of udder washes, wet teats and failure to use teat dips, failure to prepare milking animals or pre-milking stimulation for milk ejection, over milking, insertion of mastitis tubes or teat canulae, injury caused by infectious agents and their toxins and physical trauma (Khan and Khan, 2006).

1.6Diagnosis of mastitis:

1.6.1 Traditional Detection Methods:

The time served methods rely on the quality of the milkier and animal husbandry. These involve use of hands, ears, taste, smell and memory.

- **Smell:** Occasionally used to detect purulent odors.
- **Taste:** many older milers still taste milk, if suspicious, to determine if itis ‘salty.
- **Ears:** used to assist when the cow is in discomfort or pain.
- **Hands:** frequently used to assess pain, swelling and local temperature.

- **Eyes:** first information about the cow, the udder and the normality of the milk, its color and integrity (Hillerton, 2000).

1.6.2Physical examination of the udder:

Signs of acute mastitis includequartersthat are swollen, warm and painful to thetouch. Changes in size and presence of scartissue may be detected more easily aftermilking, when the udder is empty (Wattiaux, 2009).

1.6.3Appearance of the milk:

Observation of the first streams of milk(foremilk) permits the detection ofabnormal milk that should be with held. Abnormal milk may show discoloration(wateriness), flakes, or clots. Cautionshould be exercised during the removal offoremilk to avoid splashing ofcontaminated milk on the cow's limbs, tail or udder. In addition, the operator shouldnot collect the foremilk in the palm of thehand because of the risk of transferringbacteria from one quarter to another andfrom one cow to the other. In a stanchionbarn, foremilk is typically drawn into a"strip cup" or plate. In amilking parlor, however, itmay be drawn directlyonto the floor and flushedaway immediately afterobservation (Wattiaux, 2009).

1.6.4California mastitis test:

Strictaseptic procedures should be used when collecting milk samples in order to prevent contamination with microorganism present on the skin of cows, flanks, udder and teat, on the hands of the sampler and in the environment. Udder and especially teats should be cleaned with 70% ethyl alcohol and dried before sample collection. The California mastitis test carries

out as screening test for somatic cell count to detect sub-clinical mastitis. A small amount of milk from each quarter squire into shallow cups in the CMT paddle, an equal amount of the commercial CMT reagent is added to each cup. A gentle circular motion is applied to the mixture in horizontal plane for 15 seconds. Finally, the reaction is interpreted (Delelesse, 2010).

The reaction depends upon the amount of gel formation as follows:

- Negative = no reaction
- Trace = appearance of streak can be made visible during rotation of the plate.
- 1+ = distinct thickening during
- 2+ = slight formation of gel which follows the rotation plate very slowly.
- 3+ = solid formation of gel that adheres to the base of plate.

Quarters that scored negative and trace are assumed healthy, and the quarters with different positive scores are assumed infected (Hashemi*et al*., 2011).

1.6.5 Surf field mastitis test (SFMT):

The samples were subjected to surf test. For this purpose, 3% surf solution was prepared by addition of three grams of commonly used detergent powder in 100 ml of water. Milk samples and surf solution were then mixed in equal quantities in Petri dishes. The formation of gel depicted the positive samples (Muhammad *et al.*, 1995).

1.6.6Bacteriological cultures:

Each positive CMT milksample was collected under septic conditions in asterile screw caped bottle numbered to identify the particular quarter. All milk samples were sent directlyto thelaboratory with a minimum of delay for routineculture techniques. Milk samples were cultured into 10% sheepblood agar and MacConkey agar plates. Suspected colonies wereidentified morphologically, microscopically and biochemically (Abdel-Rady and sayed, 2007).

Cultures of bacteria in the milk may be useful to quantify bacteria and identify the organisms causing mastitis and high somatic cell counts. Most often, a mixture of different types of bacteria are found, butat times, a bacterial species may predominate (e.g., *Strep. agalactiae*). Thepresence (or absence) of specific organisms help formulate recommendations to prevent the spread of organisms found inthe herd. Well-managed herds have bacterial counts less than 1,000 per ml. usually: This test is performed on selected cows for which somatic cell counts of composite samples reveal a serious andpersistent problem. Cultures of anindividual cow's milk identify the bacterialspecies, so this is the most reliable way todecide on the optimum antibiotic treatmentfor a particular cow (Wattiaux, 2009).

1.6.7Current approaches for diagnosis of mastitis:

Currently assays often in use include measurement of somatic count cells (SCCs), enzymatic analysis and the California milk clotting test. In Europe, elevated SCCs above 200000cells/ml are widely used as an indicator of mastitis and are determined using haemocytometers or cell counters. Colorimetric and fluorometric assays have been developed for measuring the concentrations of enzymes elevated in milk during mastitis (e.g. NAGase or

LDH). Use of culturingtechniques for the detection of mastitis-causing microorganisms is still the gold standard, although it is very labour-intensive and therefore expensive. Mastitis can also be detected using 'cow-side' or 'on-site' tests, which can be used by both farmers and veterinarians and which require relatively little training. There is a major need for new biomarkers that are specific for mastitis, easy to detect, occur at a very early stage and that can be measured 'on-site' (Viguier*et al.*, 2009*).*

1.6.8Other Current and alternative methods for detection of mastitis:

(A)Portacheck: This assay uses an esterase-catalyzed enzymatic reaction to determine the SCC in milk.

- Advantages: cost effective (_US$3 per test) and rapid.
- Disadvantage: low sensitivity at low SCCs.

(B)Fossomatic(SCC): This counter operates on the principle of optical fluorescence. Ethidium bromide penetrates and intercalates with nuclear DNA, and the fluorescent signal generated is used to estimate the SCC in milk.

- Advantages: rapid and automated.
- Disadvantages: the device is expensive (_US$7000) and complex to use.

(C)Delaval cell counter: This counter operates on the principle of optical fluorescence, whereby propidium iodide is used to stain nuclear DNA to estimate the SCC in milk.

- Advantages: rapid and the device are easily transportable.
- Disadvantage: relatively expensive.

(D) Electrical conductivity (EC) test: This test measures the increase in conductance in milk caused by the elevation in levels of ions such as sodium, potassium, calcium, magnesium and chloride during inflammation.

- Advantage: can be used ‘on-site’.
- Disadvantage: non-mastitis-related variations in EC can present problems in diagnosis.

(E) PH test: The rise in milk pH, due to mastitis, is detected using bromothymol blue.

- Advantages: cost effective and rapid.
- Disadvantage: not as sensitive as other tests.

(F) Enzymes: Assays are used to detect enzymes, such as NAGase and LDH.

- Advantage: assays are rapid.
- Disadvantage: assays might be laboratory-based (Viguier*et al.*, 2009).

1.7Mastitis Treatment:

The firststep in treating mastitis is to identify the causative agent. The presence of a pathogen and the inflammatory response of the udder signify an infection. The inflammatory response, which results in abnormal milk, is usually detected by the dairyman. Because mastitis is frequently subclinical “hidden”, a number of tests have been developed for detecting mastitis. Most tests estimate the Somatic Cell Count (SCC) of a milk sample. There is no one somatic cell count at which a cow is free from mastitis. A level of 50,000 cell/ml of milk is usually used as a beginning point for closer observation. A variety of tests are available to determine the presence or absence of clinical and subclinical mastitis (McDonal Campus., 2007).

Treatment of mastitis accounts for a major use of antimicrobials in dairy cattle and many current protocols for clinical mastitis may be effective (Hillerton and Kliem, 2002 and sawant*et al.*, 2005).

Clinical mastitis is readily observed, and is frequently treated with the goal of returning milk to a normal marketable consistency (clinical cure) but often treatment is given without specific information in the cause of infection (Bramleyand Dodd, 1984).

Appropriate antimicrobial selection based on pharmacokinetic and pharmacodynamics principles must be considered when selecting drug, dose concentration, and dosing frequency to achieve minimum inhibitory concentration at the side of infection. Commercially available intramammary antimicrobial formulations are administered as an infusion through the teat canal using single dose syringes with especially designed applicator tips. Appropriately selected systemic therapies may be as efficacious as intramammary preparations. In the United States, only intramammary antimicrobial infusion formulations are currently approved for treatment of either clinical or sub clinical mastitis (Barlow *et al*., 2008).

1.7.1 Antibiotic treatment:

Typically when clinical mastitis is detected, the cow is milked out and then given an intramammary infusion of antibiotic, i.e. Infused directly into the infected gland. Clinical mastitis symptoms are indicated in the Mastitis Clinical Syndromes resource, but most often are recognized by the milkier from detection of clots or flakes in the milk, from a cow that has a quarter sensitive to the touch, a quarter that is swollen or hot to the touch. Priorto intramammary infusion, the teat is cleaned well and the tip of the teat is swabbed with an alcohol swab and allowed to dry for a number of seconds. The antibiotic comes in a plastic tube with a plastic infusion canulae on the end. Historically these have been long canulae and the canulae was

inserted completely through the streak canal (called full insertion). However, it was realized that this could be carrying bacteria into the teat cistern. More recently a shorter infusion canulae has been used in what is called a partial insertion method where the canulae only goes about half-way up into the streak canal and the antibiotic is expelled from the tube into the teat cistern. After emptying the antibiotic tube, the teat is pinched off and the antibiotic fluid is palpated up into the gland. Because the cow's udder then contains antibiotics which must be kept out of the food supply, that cow's milk must not be put into the milk tank for some specified number of milking after treatment (Tiwari*et al.*,). Typically this milk is either dumped down the drain or used to feed calves. Clear identification of the treated cow is critical to be sure the cow's milk is not inadvertently put into the milk tank. Shipping milk contaminated with antibiotics can lead the producer to lose their permit to ship Grade milk that is they are out of business. Use of leg-bands or some other physical markers on the cow, as well as clear records of antibiotic administration are essential for this process. It is common for a cow to be treated by multiple milking with the antibiotics (Tiwari*etal.*, 2013).

1.7.2 Oxytocin treatment:

A key contributing factor to duration of mastitis is the frequency and completeness of milk removal from the infected quarter. In some cases, cows are stripped between normal milking times, sometimes with injection of oxytocin to stimulate an effective milk let down. Clearly removal of the primary growth medium of the

bacteria, the milk, more often should enhance rate of recovery from infection (Tiwari1 *etal.*, 2013).

1.7.3 Non-responding cases:

In spite of the natural resistance mechanisms of the cow, antibiotic treatment to help her fight bacterial infection, and other methods such as frequently stripping out the milk, some cows are unable to eliminate the infection. These are often considered to be chronically infected cows, typically with *Staph. Aureus*, and remain a constant source of infection for other cows. Culling of chronically infected cows sometimes is the only way to effectively control spread of mastitis in the herd (Tiwari1 *etal.*, 2013).

1.8Prevention and Control:

Awareness of the economic losses associated with mastitis is resulting in a desire for mastitis control programs. Control programs are focused on detection of mastitis, identification of the causative agent(s) and prevention of transmission by removing the source of the agent (milk contaminated vomits, bedding, persistently infected cows, etc.). Knowledge of mammary anatomy and physiology, mammary defense mechanism, microbial habitats, microbial virulence factors, milking machine function, and antibiotics/germicides is important in achieving effective mastitis control (Awale*et al.*, 2012).

1.8.1Control of Contagious Mastitis:

Contagious mastitis can be effectively controlled through a rigorous program of teat dipping and dry cow antibiotic treatment. Teats must be dipped in germicide after

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

1.8.2 Control of Environmental Mastitis:

Environmental pathogens are more difficult to control than the contagious pathogens. Many of these organisms are resistant to germicides in teat dip and antibiotics in dry cow therapy. Identification of the source and removal (bedding, ponds, and mud) is the key to control. Udders can be clipped to minimize the amount of manure clinging to the glands. Only clean dry teats should be milked. Teats should be pre-dipped with germicide before milking. Cows should be kept standing after milking (offer them feed). Sterile single-dose infusion products should be used and sterile infusion techniques (alcohol swab) should be used. The milking parlor should be kept clean. The teat dipper should be kept clean; organisms survive in many germicides. Pipelines/water heater may need to be replaced in cases of Pseudomonas contamination (Awale*et al.,* 2012).

1.8.3Nutrition:

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

1.8.4 Vaccines:

Development of potential vaccines to prevent or control mastitis continues to be an important goal. Excellent progress has been made toward Coliform mastitis controlwith the development of mutant gram negative vaccines. The organisms used (E. coli and Salmonella) have lost the ability to synthesize outer polysaccharide antigens, resulting in exposure of common gram negative LPS (lipo-polysaccharide) antigens. Antibodies produced against these antigens are cross-reactive among gram negative pathogens. When used as directed, there is approximately a 70% decrease in clinical Coliform mastitis, as well as a decrease in severity of clinical signs. Cost benefit ratio is high in problem herds. Many attempts have been directed toward development of an effective vaccine for *Staphylococcus aureus*. Vaccines have been created (e.g. from Protein A) and injected intramuscularly or into the area of the supramammary lymph node. Vaccination has been unsuccessful in reducing the number of new cases of mastitis. Some vaccines have been effective in improving spontaneous cure rates and reducing severity of infection. These vaccines result in an increase in all types of leukocytes in the gland, thus improving defense. Overall, the success of vaccination has been minimal. Most of these vaccines have used bacteria cultured in-vitro, have been killed vaccines, and have stimulated production of IgG1. Development of a *Staph aureus* vaccine is an ongoing objective of much research (Hurley, 2009).

It is beyond the scope of this overview to describe detailed experimental approaches undertaken for the development of vaccines against bovine mastitis caused by the major bacterial pathogens thus far. In this overview, a brief description of the vaccines currently being formulated with the hope of reducing the incidence mastitis on-farm or backyard farming, and promising prototype vaccine candidates of the mastitis-associated pathogens, is presented. The use of vaccination particularly with autogenously killed whole cell vaccines to control infectious diseases on-farm in dairy cattle is common, and vaccination against mastitis pathogens is no exception. Several efforts have been made to develop a vaccine against mastitis, but few have claimed satisfactory outcomes, neither in the field nor on backyard farms. It is clear that a single vaccine will not prevent mastitis caused by the plethora of pathogens and their different mechanisms of pathogenesis (Tiwari1 *etal.*, 2013).

1.8.5 Culling:

Culling a chronically infected cow withmastitis achieves both a reduction in herd prevalence andalso a reduction in the risk of subsequent spread ofinfection. However, it comes with a cost, a current net lossof around £600 per cow culled. The decision to cull isunfortunately complex and depends on the herd status interms of somatic cell counts and clinical mastitis and theability within the herd to prevent the spread of infection. The cost of a cull needs to be tempered by thecost and likely success oftreatment as well as by thepotential for spread.With the herd position in mind andknowledge of the cow factors described above, a cull/treatdecision has to be made. An old cow with chronic highSCC, CMT positive in three quarters and fibrosismammary tissue is clearly more eligible for culling than ayoung cow with a recently increased medium SCC, onequarter positive on CMT.

However, decisions are notalways clear-cut and quantification of these decisions is asubject of current research. It is important to rememberat culling alone is not the answer to a high SCCproblem; in the absence of institution of appropriatemeasures to control spread the end result is likely to be justmore culls (Green *et al*., 2004).

1.8.6 Crying off a quarter:

This is a useful compromise measure, an alternative toculling the cow or treating infected quarters. Chronicallyinfected quarters are identified and milking of the quarteris ceased for the remainder of that lactation. Antibiotic drycow therapy is only used when the other quarters areinfused at drying off. This technique works particularlywell for high SCC infected quarters but not during aclinical episode. It is important to mark the quarter clearlyto prevent accidental milking (common now labour isminimized). Research studies report the use of povidineiodineor chlorhexidine to 'stop' the offending quarterfrom lactating but these should only be considered whenpermanent cessation of milking in that quarter isacceptable - if these measures are adopted it is importantto consider the welfare aspects of this procedure andconsideration should be given to using appropriateanalgesia. Cessation of milking in a quarter for one partlactation essentially gives that quarter a prolonged dryperiod and is often associated with cure rates of over 50% (Green., *et al*. 2004) .

1.9 Economic Impact of mastitis:

Clinical mastitis (CM) is a considerablecost to the dairy farmer and dairy industry.The costs associated with CM include:

1.9.1 Direct effects:

- Temporary or permanent loss in milk production.

- Poor milk quality, for example reduction in milk fat content, resulting in dairy products with less favorable organoleptic properties.
- Reduction in price due to high somatic cell count.
- Loss due to discarding of milk after the antibiotic treatment.
- Additional treatment costs related to, for example, drugs and veterinary care.
- Increased labour costs, for example extra labour required for husbandry of cattle and for application of preventive measures.
- Increased costs for surveillance of milk quality and disease status among rest of the herd.
- Premature culling or reduced productive-life of cattle.
- Lower value for culled cattle meat because the carcass yield and quality is reduced (Viguier*et al*., 2009).

An additional cost of inferior udder health is consumer perceptionregarding animal welfare as well as theimpact of using antibiotics in animals ontheir efficacy for human health (Berry and Meanoy, 2005).

1.9.2 Financial costs:

- In the US, the projected annual losses caused by mastitis are US$2 billion.
- In the UK, mastitis causes an annual loss of approximately £300 million to dairy farmers.
- In Northern Ireland, the cost of clinical mastitis for an average 100- cow herd is £5000 per year, with total mastitis infections costing £14 million annually.

- In the Republic of Ireland, the cost of clinical mastitis is approximately s693 per year for every infected cow.
- In the Netherlands, the average cost per infected cow varies between s164 and s235 (Viguier*et al.*, 2009).

Chapter two

2. Materials and Methods

2.1 The study area:

The study was undertaken in East Nile Locality of Khartoum State. This is boarded by the River Nile State in the north, Gezeira State in the South, ELGadarif State in the East and North Kordofan State in the West. Khartoum State is dominated by the semi desert climate which is characterized by very hot /dry summer and cold in winter. The average air temperature ranges

between 21.6 c° and 37.7c°. The mean annual evaporation rate is 7.7 mm/day, daily average relative humidity ranges between 21%-38%. The summer extends from March – October and ends with three month rainy season (mid July- mid October). On the other hand winter extends from November to February. The rainfall ranges between 75-300mm per year with the peak being experienced during August. The natural vegetation cover consists of annual (75%) and perennial plants (25%), shrubs and some tree. The main water sources are River Nile, tributaries, seasonal water courses (widens) and ground water mainly away from River Nile, Man had work in the Khartoum state in agriculture and animal husbandry since ancient times and was to force hit will in cultivation of wheat, corn, vegetables and fruits, and specialized in the breeding of cow, camel, goat and sheep for milk and meat production. Khartoum State is composed of seven localities namely Khartoum, Khartoum North, East Nile, Omdurman, Ombeda, Karare and Jebelawlia (Agricultural Census Report, 2009).

2.2 Study populations:

The population of animals in Khartoum is estimated as 6300 birds, 51300 for sheep, 1900 goats, 6585 camels and 240003 cattle. Cattle distribution in Khartoum State is 138067 in East Nile, 28016 in Bahry, 13578 in Ombeda, 13901 in Karari, 20455 in Omdurman, 20360 in JabalAwlia (Agricultural Census Report, 2009).

2.3 Study type:

A cross-sectional study was performed which involved the selection of sample of individuals from a large population and then determination from

each individual of the simultaneous presence of disease and hypothesized risk factors association were investigated (Thrusfield, 2007).

In this study, multistage random sampling was carried out in East Nile locality.

2.4 Sampling method:

Usethe probability sampling methods to select the animals. First the multistage sampling was used; four administration units were selected from the eight Administration units of East Nile Locality. Then from each administration unit was selected the farms according to density of population. Finally, animals were selected by using simple random sampling from each administration unit. From simple farm cluster sample was used (all animals). The prevalence was calculated by the formula described by Martin (Martin, *et al.,* 1987) as follow:

Prevalence rate = $\frac{No.of\ cows\ with\ mastitis}{Total\ No.of\ cows\ at\ a\ particular\ point\ in\ time}$

2.5 Sample size determination:

The sample size was calculated by the formula:-

$$N = \frac{4*P*Q}{L2}$$

N= sample size

P= expected prevalence

L= desired absolute precision

Q= (1-P) (Martin *et al.*, 1987).

From the previous studies the samples size was calculated, this was from the Khartoum state (Kundu., 2013) according to this study on prevalence

of mastitis (local, cross, pure breed) was estimated about 51.9%, then the sample size was be :-

$$N = \frac{4*(0.519)*(0.481)}{(0.0025)} = 399 \text{ animals}$$

2.6 Questionnaire execution:

A pre-tested structured questionnaire with the primary objective of elucidating the multifactorial background of the disease was conduct in an interactive manner at every farm visited. All the dairy cows in the farm which were selected examined and the questionnaire was filled out by asking the owner. The individual risk factors attributes included breed, age, previous history of the mastitis, body condition, appearance signs of disease. The farm attributes included herd size, manure disposal, farm hygiene, and hygienic practices before, during and after milking, type of milking, stoking density, use treatments and presence of other animalsin the same house. Then was divided these risk factors to categories

2.7 Diagnostic techniques:

2.7.1Clinical features inspection:

Clinical findings like abnormalities of secretions, abnormalities of size, consistency and temperature of mammary gland were examined by visual inspection and palpation. Pain reaction upon palpation, change in the milk (blood tinged milk, watery secretions, clots, pus) and change in consistency of udder were considered as indications of thepresence of clinical mastitis. Cows which did not had clinical mastitis, were subjected to further

investigation for subclinical mastitis using California Mastitis Test as screening test.

2.7.2Milk sample collection:

Milk samples were collected according to the National Mastitis Council (NMC. 1990). In a clean environment, thoroughlywiping the teats with 70% ethyl alcohol with paying extraattention to teat orifice was applied. After discardingthe first few milk squirts, individual quartermilk samples were subjected to the CMT.

2.7.3 California mastitis test:

Each 3 ml of milk sample was drawn from quarters in each of the 4 shallowcups in the CMT paddle thenapproximately equal volume of 3 ml of the commercialavailable CMT reagents was added to each cup and mixedtogether through swirling the paddle in a circular motionfor few seconds. According to the visible reaction of the CMT, theresults were classified into four scores: 0 = negativeor traces (no change in consistency), 1 = slightlypositive (+), 2 = positive (++) and 3 = highly positive (+++). Scores 1, 2 and 3 depend on the degree ofgelatin that were indicated by gelatinous mass.

2.8Statistical analysis:

All data collected about the risk factors and the results, cases was categorized as either positive or negative. For analysis of the data by SPSS program version 16 were used. Simple descriptive (mean – graphic polygon – frequency table). Univariate analysis: chi-square test was used to description

the variable, number of tested animals and degree of freedoms, chi-square and p-value.

Multivariable logistic regression model: described the risk factor, number of positive cases, odds ratio, confidence intervals and p-value.

Chapter three

3. Results

3.1 Descriptive statistical analysis frequency table, cross tabulation, and association table between the disease and risk factors:

A total of 399 lactating cow (58 local and 341 cross) were examined in 40 dairy farms in East Nile locality during the study period from October to

December, to determine the prevalence of mastitis by clinical inspection and California Mastitis Test (CMT) 207 (51.9%) animals were positive, 170 (42.6%) animals were subclinical and 37 (9.3%) animals were clinically affected (Table 3.2).

Table3.2: prevalence of clinical and subclinical mastitis in 399 cattle examined in East Nile locality.

Result		Frequency	Percent	Valid percent	Cumulative percent
Valid	Negative	192	48.1	48.1	48.1
	Subclinical	170	42.6	42.6	90.7
	Clinical	37	9.3	9.3	100.0
	Total	399	100.0	100.0	

3.3Summary of the results:

1. Age:

The result showed that 144 (36.1%) of the examined cows were young and 255 (63.9%) were old cows (Table3.4). The prevalence of mastitis within ages was 41.7% in young and 57.6% in old cows (Table3.5). A significant association (p-value = .002) was observed between age and mastitis (Table3.6).

2. Breed:

The results showed that 58 (14.5%) of the examined cows were local and 341 (85.5%) were cross (Table3.4). The prevalence of mastitis within breeds is 39.7% in local and 54% in cross cows (Table3.5). A significant association (P-value = .044) was not observed between breeds and mastitis (Table3.6).

3. Body condition:

The results showed that 230 (57.6%) of examined cows were in good body condition, 111 (27.8%) in fair and 58 (14.5%) in poor body condition (Table3.4). The prevalence of mastitis was 43.5% in good condition, 63% in fair and 63.8% in poor body condition (table3.5). A significant association (P-value = 0.000) was observed between body condition and mastitis (Table3.6).

4. Stage of lactation:

The results showed that 192 (48.1%) of the examined cows in late stage of lactation and 207 (51.9%) in early stage (Table3.4). The prevalence of mastitis was 42.7% in late stage of lactation and 60.4% in early stage of lactation (Table3.5). A significant association (P-value =0.440) was not observed between stage of lactation and mastitis (Table3.6).

5. Parity:

The results showed that 185 (46.4%) of the examined cows had a few (<5) parity and 214 (53.6%) had many (>5) parity (Table3.4). The prevalence of mastitis was 43.8% in a few parity, and 58.9% in many parity (Table3.5). A significant association (P-value = 0.003) was observed between parity and mastitis (Table3.6).

6. Previous exposure to mastitis:

The results showed that 177 (44.4%) of the examined cows with not previous exposure to mastitis and 222 (55.6%) with previous exposure to mastitis (Table3.4). The prevalence of mastitis was 41.2% in cows with not previous exposure to mastitis and 60.3% with previous exposure to mastitis (Table3.5). A significant association (P-value = 0.000) was observed between previous exposure to mastitis and mastitis (Table3.6).

7. Present of ticks on udder:

The results showed that 288 (72.2%) of examined cows with absent of ticks on udder and 111 (27.8%) With present of ticks on udder (Table3.4).The prevalence of mastitis was 46.5% in cows with absent of ticks on udder and 65.8% in cows with present ticks on udder (Table3.5). A significant association (P-value = 0.001) was observed between present of ticks on udder and mastitis (Table3.6).

8. Teat injuries:

The results showed that 295 (73.9%) of examined cows with absent of teat injuries and 104 (26.1%) with present of teat injuries (Table3.4). The prevalence of mastitis was 47.8% in cows with absent teat injuries and 63.4% in cows with present of teat injuries (Table3.5). A significant association (P-value = 0.006) was observed between teat injuries and mastitis (Table3.6).

9. Clean teats and udder:

The results showed that 101(25.3%) of the farms their milkman was clean teats and udder and 298 (74.7%) was not clean teats and udder (Table3.4). The prevalence of mastitis was 41.6% in the farms their milkman was clean teats and udder and 55.3% in the farms their milkman was not clean teats and udder (Table3.5). A significant association (P-value = 0.017) was observed between clean teats and udder and mastitis (Table3.6).

10. Washing hands:

The results showed that 249 (62.4%) of the farms their milkman washed their hand before milking and 150 (37.6%) of the farms their milkman did not wash their hand before milking (Table3.4). The prevalence of mastitis was 45.4% in farms their milkman washed their hand before milking and 62.7% in farms their milkman did not wash their hand before milking (Table3.5). A

significant association (P-value = 0.001) was observed between washing hands and mastitis (Table3.6).

11. Sanitary practices:

The results showed that 190 (47.6%) of the farms with good sanitary practices and 209 (52.4%) with poor sanitary practices (Table3.4). The prevalence of mastitis was 44.2% of cows in good sanitary practice and 58.6% of cows in poor sanitary practices (Table3.5). A significant association (P-value = 0.003) was observed between sanitary practices and mastitis (Table3.6).

12. Floor disinfectant:

The results showed that 79 (19.8%) of the farms used floor disinfectant and 320 (80.2%) of the farms did not use floor disinfectant (Table3.4). The prevalence of mastitis was 38%in cows their owners used floor disinfectant and 55.3% in cows their owners did not use floor disinfectant (table3.5). A significant association (P-value = 0.006) was observed between floor disinfectant and mastitis (Table3.6).

13. Drainage system:

The results showed that 188 (47.1%) of the farms with good drainage system and 211 (52.9%) of farms with poor drainage system (Table3.4). The prevalence of mastitis was 42.2% in good drainage system and 59.8% in poor drainage system (Table3.5). A significant association (P-value = 0.000) was observed between drainage system and mastitis (Table3.6).

14. Frequency of bedding removal:

The results showed that 179 (44.9%) of the farms were frequencies of bedding removal and 220 (55.1%) of the farm did not frequency of bedding removal (Table3.4). The prevalence of mastitis was 36.9% in farms with frequency bedding removal and 64% in farms did not frequency of bedding

removal (Table3.5). A significant association (P-value = 0.000) was observed between frequency of bedding removal and mastitis (Table3.6).

15. Herd size:

The results showed that 90 (22.6%) of herd were small herd size cows and 309 (77.4%) was large herd size cows (Table3.4). The prevalence of mastitis was 43.3% in cows within small herd size and 54.4% in cows within large herd size (Table3.5). A significant association (P-value = 0.065) was not observed between herd size and mastitis (Table3.6).

16. Barn size:

The results showed that 260 (65.2%) of the farm was barn size adequate and `139(34.8%) of the farm was non-adequate barn size (Table3.4). The prevalence of mastitis was 50.8% in cows within adequate barn size and 54% in cows within non-adequate barn size (Table3.5). A significant association (P-value = 0.544) was not observed between barn size and mastitis (Table3.6).

17. Type of fencing:

The results showed that 50 (12.5%) of the farms fencing by walls and 349 (87.5%) of the farms fencing by iron (Table3.4). The prevalence of mastitis was 50% in farms fencing by walls and 52.1% in farms fencing by iron (Table3.5). A significant association (P-value = 0.776) was not observed between type of fencing and mastitis (Table3.6).

18. Water source:

The results showed that 331 (83.0%) of the farms were used pipeline water and 68 (17.0%) of the farms were used wells water (table3.4). The prevalence of mastitis was 52.2% in farms used pipeline water and 50% in farms used wells water (Table3.5). A significant association (P-value = 0.733) was not observed between water source and mastitis (Table3.6).

19. Yielding milk:

The results showed that 231 (57.9%) of examined cows with low yielding milk and 168 (42.1%) of cows with low yielding milk (Table3.4). The prevalence of mastitis was 41.1% in cows with low yielding milk and 66.7% in cows with high yielding milk (Table3.5). A significant association (P-value = 0.000) was observed between yielding milk and mastitis (Table3.6).

20. Milking technique:

The results showed that 210 (52.6%) of the farms their milkman used their stripes in milking and 189 (47.4%) of the farms their milkman used all fingers in milking (Table3.4). The prevalence of mastitis was 50.5% in farms their milkman used stripes in milking and 53.4% in farms their milkman used all fingers in milking (Table3.5). A significant association (p-value = 0.554) was not observed between milkingtechniqueand mastitis (Table3.6).

21. Education level:

The results showed that 219 (54.9%) of the farms their owners were educated and 180 (45.1%) of the farms their owners were illiterate (Table3.4). The prevalence of mastitis was 44.3% among farms their owners were educated and 61.1% in farms their owners were illiterate (Table3.5). A significant association (P-value = 0.001) was observed between education level and mastitis (Table3.6).

22. Locality:

The result showed that 100 (25.1%) of the examined animals were from Abu deleeg (A), 100 (25.1%) from Elhajyousif (B), 100(25.1%) from Eliseelat(C) and99(24.8%) from Wadisuba (D) Administration units(Table3.4). The prevalence of mastitis was 67%, 42%, 36% and 62.6% in A, B, C and D respectively (Table3.5). A significant association (P-value =.000) was observed between locality and mastitis (Table3.6).

Table 3.4: Frequency distribution of 399 dairy cows in East Nile locality examined for mastitis according to potential risk factors investigated.

Risk factors	Frequency	Relative frequency (%)	Cumulative frequency (%)
Age			
young (<6years)	144	36.1%	36.1%
old (> 6 years)	255	63.9%	100.0%
Breed			
Local	58	14.5%	14.5%
Cross	341	85.55%	100.0%
Body condition			
Good	230	57.6%	57.6%
Fair	111	27.8%	85.5%
Bad	58	14.5%	100.0%
Stage of lactation			
Late	192	48.1%	48.1%
Early	207	51.9%	100.0%
Parity			

Few (< 5 calves)	185	46.4%	46.4%
Many (> 5 calves)	214	53.6%	100.0%
Previous exposure to mastitis			
Not exposed	177	44.4%	44.4%
Exposed	222	55.6%	100.0%
Present of ticks			
Absent	288	72.2%	72.2%
Present	111	27.9%	100.0%
Teats injuries			
Absent	295	73.9%	73.9%
Present	104	26.1%	100.0%
Clean teats and udder			
Yes	101	25.3%	25.3%
No	298	74.7%	100.0%
Risk factors	**Frequency**	**Relative frequency (%)**	**Cumulative frequency (%)**
Wash hands			
Yes	249	62.4%	62.4%
No	150	37.6%	100.0%
Sanitary practices			
Good	190	47.6%	47.6%
Bad	209	52.4%	100.0%
Floor disinfectant			
Yes	79	19.8%	19.8%
No	320	80.2%	100.0%
Drainage system			
Good	180	45.1%	45.1%
Bad	219	54.9%	100.0%
Bedding removal			
Yes	179	44.9%	44.9%
No	220	55.1%	100.0%
Herd size			
Small	90	22.6%	22.6%
Large	309	77.4%	100.0%
Barn size			
Adequate	260	65.2%	65.2%

Inadequate	139	34.8%	100.0%
Type of fencing			
Walls	50	12.5%	12.5%
Iron	349	87.5%	100.0%
Water source			
Pipeline	331	83.0%	83.0%
Wells	68	17.0%	100.0%
Yielding milk			
Low	231	57.9%	57.9%
High	168	42.1%	100.0%
Milking techniques			
Stripes	210	52.6%	52.6%
Fingers	189	47.4%	100.0%
Risk factors	**Frequency**	**Relative frequency (%)**	**Cumulative frequency (%)**
Level education			
Educated	219	54.9%	54.9%
Illiterate	180	45.1%	100.0%
Localities			
Abu deleeg	100	25.1%	25.1%
Elhajyousif	100	25.1%	50.1%
Eliseelat	100	25.1%	75.2%
Wadisuba	99	24.8%	100.0%

Table 3.5: Cross tabulation of mastitis infection in 399 dairy cows from East Nile locality with potential risk factors investigated.

Risk Factors	No. Tested	No. Positive	Positive (%)
Age			
Young (< 6years)	144	60	41.7%
Old (> 6 years)	255	147	57.6%
Breed			
Local	58	23	39.7%
Cross	341	184	54.0%
Body condition			
Good	230	100	43.5%
Fair	111	70	63.0%
Bad	58	37	
Stage of lactation			

Late	192	82	42.7%
Early	207	125	60.4%
Parity			
Few (< 5 calves)	185	81	43.8%
Many (> 5 calves)	214	126	58.9%
Previous exposure to mastitis			
Not exposed	177	73	41.2%
Exposed	222	134	60.3%
Risk Factors	**No. Tested**	**No. Positive**	**Positive (%)**
Present of ticks			
Absent	288	134	46.5%
Present	111	73	65.8%
Teats injuries			
Absent	295	141	47.8%
Present	104	66	63.4%
Clean teats and udder			
Yes	101	42	41.6%
No	298	165	55.3%
Wash hands			
Yes	249	113	45.4%
No	150	94	62.7%
Sanitary practices			
Good	190	84	44.2%

Bad	209	123	58.9%
Floor disinfectant			
Yes	79	30	38.0%
No	320	177	55.3%
Drainage system			
Good	180	76	42.2%
Bad	219	131	59.8%
Risk Factors	**No. Tested**	**No. Positive**	**Positive (%)**
Bedding removal			
Yes	179	66	36.9%
No	220	141	64.0%
Herd size			
Small	90	39	43.3%
Large	309	168	54.4%
Barn size			
adequate	260	132	50.8%
Inadequate	139	75	54.0%
Type of fencing			
Walls	50	25	50.0%
Iron	349	182	52.1%
Water source			
Pipeline	331	173	52.2%
Wells	68	34	50.0%
Yielding milk			

Low	231	95	41.1%
High	168	112	66.7%
Milking techniques			
Stripes	210	106	50.0%
Finger	189	101	66.7%
Risk Factors	**No. Tested**	**No. Positive**	**Positive (%)**
Level education			
Educated	219	97	44.3%
Illiterate	180	110	61.1%
Localities			
Abu deleeg	100	67	67.0%
Elhajyousif	100	42	42.0%
Wadisuba	99	36	36.0%
Eliseelat	100	62	62.6%

Table 3.6: Univeriable analysis of differenceassociated potential risk factors with mastitis using the Chi-square (χ^2) test.

Risk factors	No. tested	No.+ve (%)	d.f	χ^2	p-value
Age					
Young (< 6years)	144	41.7%	1	9.414	0.002
Old (> 6 years)	255	57.6%			
Breed					
Local	58	39.7%	1	4.062	0.044
Cross	341	54.0%			
Body condition					
Good	230	43.5%	2	15.361	0.000
Bad	111	63.0%			
Stage of lactation					
Late	192	42.7%	1	12.469	0.000
Early	207	60.4%			
Parity					
Few (< 5 calves)	185	43.8%	1	9.056	0.003
Many (> 5 calves)	214	58.9%			
Previous exposure to mastitis	177		1	14.417	0 .000
Not exposed	222	41.2%			

Exposed		60.4%			

Risk factors	No. tested	No. +ve (%)	d.f	χ^2	p-value
Present of ticks					
Absent	288	46.5%	1	11.878	0.001
Present	111	65.8%			
Teat injuries					
Absent	295	47.8%	1	7.558	0.006
Present	104	63.5%			
Clean tests and udder					
Yes	101	41.6%	1	5.742	0.017
No	298	55.4%			
Wash hands					
Yes	249	45.4%	1	11.203	0.001
No	150	62.7%			
Sanitary practices					
Good	190	49.1%	1	8.546	0.003
Bad	209	50.9%			
Floor disinfectant					
Yes	79	38.0%	1	7.629	0.006
No	320	55.3%			

Drainage system					
Good	180	42.2%	1	12.252	0.000
Bad	219	59.8%			

Risk factors	**No. tested**	**No. +ve (%)**	**d.f**	**χ^2**	**p-value**
Bedding removal					
Yes	179	36.9%	1	29.291	0.000
No	220	64.0%			
Herd size					
Small	90	43.3%	1	3.400	0.065
Large	309	54.4%			
Barn size					
Adequate	260	50.8%	1	.369	0.544
Inadequate	139	53.4%			
Type of fencing					
Walls	50	50%	1	.081	0.776
Iron	349	52.1%			
Water source					
Pipeline	331	52.2%	1	.116	0.733
Wells	68	50.0%			
Yielding milk					
Low	231	41.1%	1	25.416	0.000
High	168	66.7%			
Milking techniques					

Stripes	210	50.0%	1	.350	0.554
Fingers	189	53.4%			
Risk factors	**No. tested**	**No. +ve (%)**	**d.f**	**χ^2**	**p-value**
Level education					
Educated	219	44.3%	1	11.195	0.001
Illiterate	180	61.1%			
Locality					
Abu deleeg	100	67.0%	3	27.748	0.000
Elhajyousif	100	42.0%			
Wadisuba	99	36.0%			
Eliseelat	100	62.6%			

3.7: Summary of multivariate analysis:

A significant association was observed between mastitis and potential risk factors(p-Value ≤ 0.05) using Logistic Regression as follows:
Age (p-value = 0.000), body condition (p-value = 0.000), teat injuries (p-value = 0.029), yielding milk (p-value = 0.000) and locality (p-value = 0.000).

Table 3.8: Multivariable analysis of the difference associated potential Risk Factors with mastitis using Logistic Regression.

Risk factors	No. tested	No. +ve (%)	Exp-B	95% CI	p-value
				Lower-upper	
Age					
Young (< 6years)	240	60.2%	.066	.018-.236	.000
Old (> 6 years)	159	39.8%	Ref		
Body condition					
Good	276	69.2%	.0439	.056-3.461	.000
Bad	123	30.8%	Ref		
Teat injuries					
Absent	295	47.8%	Ref		.029
Present	104	63.5%	.246	.070-.866	
Yielding milk					
Low	235	58.9%	.164	.083-.324	.000
High	164	41.1%	Ref		
Locality					
Abu deleeg	100	67.0%	1.003	101.797-9.88	.000
Elhajyousif	100	42.0%	7.586		
Eliseelat	99	36.0%	Ref		
Wadisuba	100	62.6%	.299		

Chapter Four

Discussion

Mastitis is one of the most important diseases causing enormous economic losses to the dairy farms. This disease is the outcome of the interaction of many risk factors associated with host, pathogen(s), and environment. This information is imperative for planning an intervention strategy for this costly disease, without knowing the epidemiology; it is very difficult and rather impossible to control the disease. The present study was to determine the mastitis prevalence, association with important potential risk factors and the major causative agent. It is anticipated that deduced from this study would help the farmers, veterinarian and other concerned authorities in the control of this disease.

The epidemiological studies in this investigation were applied through combination of the CMT and udder inspection.

Subclinical mastitis was defined as, when mammary glands without clinical abnormalities giving apparently normal milk but was bacteriological positive and with positive CMT (Stefanakis*et al.,* 1995).

In the present study, the prevalence of subclinical mastitis was higher than that of clinical mastitis. This could be due to the reason that in Khartoum state subclinical mastitis receives little attention and efforts have been concentrated only on the treatment of clinical cases.

The prevalence of clinical and subclinical mastitis was 37 (9.3%) and 170 (42.6%) respectively. The overall prevalence of mastitis was (51.9%).

The high prevalence(42.6%)of subclinical mastitis and low prevalence (9.3%)of clinical mastitis are in agreement with previous observation that sub

clinical mastitis more prevalent than clinical mastitis with a rate of (31.67%)and (0.93%) of subclinical and clinical mastitis respectively in Gonder, Ethiopia (Moges*et al.*, 2011), 23.0% and11.9% in Southern Ethiopia (Biffa *et al.*, 2005), and 38.2% and 21.5% also in Ethiopia (Workineh*et al.*, 2002).

The overall prevalence in the present study is lower than those reported in some previous study by Zerihun(1996), (61.11%), Tadesse and Chanie, (2012) in Addis Ababa which was 65.3%, Matios*et al.*, (2009) in Asella, Ethiopia, which was 64.5%, and Abdurrahman *et al.*, (1998), reported (68.1%) in different parts of Ethiopia. This prevalence is relatively higher than that reported by Biru*et al.*, (1998), (35.7%), Biffa *et al.*, (2005), (38.9%), Fekadu (1995), (38.65%), Darsema (1991), (39.5%), and Getahun (2006), (36.9%). However, it is similar to three previous studies conducted by Sori*et al.*, (2005) in Sebeta, Ethiopia, Hashemi*et al.*, (2011) and Junaidu *et al.*, (2011)in Sokoto, which was 52.78% , 44.7% and 52.0% respectively.

The difference in prevalence of mastitis in the present study and other reports could probably be due to differences in farms management practices, breeds, geographic location, level of production and study methods and instruments employed by the investigators (Radostits*et al.*, 2009).

The following risk factors showed significant association with mastitis under a significant level of ≤ 0.05: age (p-value = 0.000), Body condition (p-value = 0.000), teat injuries (p-value = 0.029), yielding milk (p-value = 0.000) and locality (p-value = 0.000).

In this study there is a difference in mastitis prevalence among four localities that were selected randomlyAbu deleeg, Elhajyousif, Eliseelat and Wadisuba this might be due to different management practices that were applied in farms in different localities. This significant statistical association

of mastitis infection with locality (p-value = 0.000), is supported by previous study conducted in Southern Ethiopia by Biffa *et al.*, (2005) (p-value =0 .0001).

Body condition was based on palpation of back bones and lumber processes. In our study body score showed a significant statistical association with mastitis (p-value = 0.000). This result is in agreement with the finding of previous work conducted in Tanzania by Kivaria, (2006) (p-value = 0.02) and by Uddin, (2009) in Mymunsingh, Bangladesh (p-value = 0.05). On the other hand, Bedacha and Manghistu (2011) in Batu, Ethiopia did not observe significant statistical association with mastitis were not fully explained by authors, but it is well established that poor body condition usually may associated with depilating disease which may produce high somatic cell count (SCC) reflect intramammary infection and have negative effect on milk quality and milk production (Kivaria*et al.*, 2004).

About teat injuries and lesions predispose the udder to infection that might be the reason of higher prevalence of mastitis in injured teat, the finding of the present study supports by previous studies conducted in Batu, Ethiopia by Bedacha and Manghistu, (2011) (p-value = 0.000), in Dar Esalam, Tanzania by Kivaria*et al.*, (2006) (p-value = 0.000) and Matios*et al.*, (2009) in Asella, Ethiopia (p-value = 0.000), it was explained by Uddin*et al.*, (2009), that teat injuries provide a medium for the growth of the pathogenic bacteria, which affect the udder, so that, in case of injuries the risk of an infection increases. It could be also due to traditional diary husbandry practices, whereby, calves are kept away from their dam over a long period and are only allowed to suckle for a short period, as well as inadequate milk supply which lead to calves suckling vigorously inducing teat injuries and subsequent infection of the mammary gland (Junaidu*et al.*, 2011).

Concerning yield milk as risk factor, our study showed significant statistical significant association with prevalence of mastitis (p-value = 0.000), higher yielding cows were more susceptible to mastitis than low yielding ones. This may be due to the case with which injuries are sustained in large udders, so that foci for the entrance of pathogens are created and stress associated with a high milking cow upset the defense system of the animal (Kerro and Tarek, 2003).

Conclusion

-Mastitis is prevalent in East Nile Locality dairy farms.

-Subclinical mastitis is the most prevalent 42.6%

-Individual risk factors such as age, body condition, teat injuries, yielding milk and locality influenced the prevalence of mastitis.

Recommendations

Therefore based on the above conclusion the following paints are forwarded as recommendations:

1.Using California Mastitis Test (CMT) in all farms for discovering the disease early.

2.Program for the controlling and eradication of bovine mastitis should be implemented as soon as possible by veterinary authority in East Nile Locality.

3.Extension service and training programs aiming at creation of awareness about the importance and prevention of subclinical mastitis among dairy farms should be done by local veterinary authority.

4. To reduce prevalence of the disease, different epidemiological factors that interplay in mastitis occurrence should be well studied.

References

Abdel-RadyA.and M. Sayed, (2007). Epidemiological studies on subclinical mastitis in dairy cows in assist governorate. Veterinary world, Vol.2 (10):373-380(2010).

Abdel Hameed K. G., G. Sender and A. Korwin,(2006). Public Health Hazard Due to Mastitisin Dairy Cows. Animal science papers and reports

Abdurrahman, O., Agab, H., Abbas, B and Aston, G., (1998). Relation between udder infection and somatic cells in camel (*Camelus dromedaries*) milk.Acta. Vet. Scand. 36: 424-431.

Agricultural Sunsus Report, (2009). Ministry of agriculture, animal wealth and irrigation Khartoum State.

Andrews, A. H., Blowey, R. W., Boyd, H. and Eddy, R. G.(2004). Bovine medicine disease and husbandry of cattle, second edition, black well science.

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

Barlow, J. W., White, L. J., Zadoks, R. N., and Schukken, Y. H., (2008). A mathematical model demonstrating indirect and overall effects of lactation therapy targeting subclinical mastitis in dairy herds.Veterinary research, 39, 23-31.

Bedacha,B. D., and Manghistu, H. T., (2011). Study prevalence of mastitis and its association risk factors in lactating dairy cows in Batu and its Environs, Ethiopia. Global veterinaria, 4 (3): pp 299-302.

Berry, D. P. and W. J. Meaney (2005). Cow factors affecting the risk of clinical mastitis.Irish journal of agricultural and food research (2005).

Biffa, D., Etana, D and Fekadu, B., (2005). Factors associated with udder infections in lactating cows in Southern Ethiopia. Inter. J. Appl. Res. Vet. Med., 3: 189-198.

Biru, G., (1989). Major bacterialcausing bovine mastitis and their sensitively to common antibiotics. Eth J. Agri. Sc., 11: 47-54.

Bramley, A. J., Dodd, F. H., (1984). Reviews of the progress of dairy science: mastitis control-progress and prospects. J. dairy res., 51, 458-512.

Brightling, P., Mein, G. A., Malmo, J., (2010). Countdown down under: farm guidelines for mastitis control. In: countdown. Dairy research and development corporation, 1998.
http://www.countdown.org.an/farm.htm. Accessed October 2010.

Cervinkova, D., H. Vlkova, I. Borodacova, J. Makovcova, V. Babak, A. Lorencova, I. Vrtkova, D. Marosevic and Z. Jagli, (2013). Prevalence of mastitis pathogens in milk from clinically healthy cows. Veterinarian medicine, 58, 2013(11): 567-575(2013).

Darsema, G., (1991). A survey of bovine mastitis in different dairy farms: Dire Dawa autonomous and East Harerge administrative Regions. DVM thesis: Faculty of veterinary medicine, Addis.

Delelesse, G. D., (2010). Study on prevalence of bovine mastitis on cross breed dairy cow around Holeta areas, West Shewa Zone of Oromia, Ethiopia, global veterinarian, Vol. 5(6):pp 318-323.

Fekadu, K., (1995). Survey on the prevalenceof bovine mastitis and the predominant causative agents in Chaffa Valley.In: proceeding of the 9th conference Ethiopia association, Addis Ababa Ethiopia, pp: 101-111.

Getahun, K., (2006). Bovine mastitis and antibiotic resistance patterns of major pathogens in small holder dairy forms in central high land of Ethiopia. MSC thesis: Debre-Zeit: Factory of veterinary medicine, Addis Ababa university, Ethiopia.

Gibson, A. Paxman1, E. Morgan1, P. Costantino, R.Sunagar, S.Isloor and T. Mukkur (2013). Trends in therapeutic and prevention strategies for management of bovine mastitis (2013).

Green, M. A., Bradley, S. Borsberry, J. Harwood, C. Penny, C. Watson and A. White, (2004). Clinical forum - *Staphylococcus aureus*mastitis in cattle. UK Vet - Volume 9 No 4 July.

Halasa, T., Niclen, M., Whist, A. C., (2009). Meta-analysis of dry cow management for dairy cattle. Part 2: cure of existing intramammary infections. J of dairy Sci: 92: 3150-3157.

Hameed, S., Arshad, M., Ashraf, M., Avias, M. and Shahid, M. A., (2012). Cross sectional epidemiological studies on mastitis cattle and buffaloes of TehselBurewalaPakistan. The J. animal and plant Sci, 22(3 Suppl.) pp: 371-376 ISSN: 10181.

Hashemi,M., Kafi, M., Safdarian, M., (2011). The prevalence of clinical and subclinical mastitis in dairy cows in the Central Region of Fars Province, South ofIran. Iranian J. vet Res, Shifaz University, Vol. 12, No.3, and Ser. No. 36.

Hillerton, J. E., (2000). Detecting cow-side.National mastitis council annual meeting proceedings.

Hillerton, J. E., and Kliem, K. E. (2002) Effective treatment of *Streptococcus uberis* clinical mastitis to minimize the use of antibiotics J. dairy Sci. 85, 1009-1014.

Hurley, W. L.,(2009). Mastitis treatment and control.

Hussain, S. A., (2012). Prevalence and bacterial etiology of subclinical mastitis in dairy cows in Al Sulaimaniyah District.Kufa journal for veterinary medical sciences Vol. (3) No. (1).

Javaid, S., Gadahi, B., Khaskeli, M., Bhutto, M. B., Kumbher, S. and Panhwar, A.H., (2009). Physical and chemical quality of market milk sold at Tandogem, Pakistan. Pakistan Vet J, 29: pp27-31.

Junaidu, A. U., Salihu, M. D., Tambuwal, F. M., Magaji, A. A. and Jaafaru, S., (2011). Prevalence of mastitis in lactating cows in some selected commercial dairy farms in Sokoto Metropolis. Advances in applied science research, 2011, 2 (2): pp 290-294.

Kerro,O. and Tarekegn, F., (2003). Bovine mastitis in selected areas of Southern Ethiopia. Journal of tropical animal health and production, pp: 197-205.

Khan, M. Z., and A. Khan, (2006). Basic facts of mastitis in dairy animals: A review. Pakistan vet**.**

Kivaria, F. M., Noordhuizen, J. P. T. M. and Kapaga, A. M., (2004). Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. Trop anim health and prod 36 (6): pp 581-592.

Kivaria, F. M., Noordhuizen, J. P. T. and Kapaga, A. M., (2006).samples of smallholder dairy cows in Tanzania. Journal research in veterinary science, pp: 25-36.

Kivaria, M. F., (2006). Epidemiological studies on bovine mastitis in smallholder dairy herd in the Dar Esalam Region, Tanzania, population studies animal diseases research institute, P. O. Box, 9254, Dar Esalam, Tanzania.

Koivula, M., Pitkala, A., Pyorala, S., Mantysaari, E., (2007). Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland: Acta Agric. Scand. A. in press.

Kudi A. C., M. P. Bray, and A. Y.Niba (2009). Mastitis causing pathogens within the dairy cattle environment.International journal of biology Vol. 1, No (2009).

Lim, G. H., Leslie, K. E., Kelton, D. F., Duffield, T. F., Timms, L. L. and Dingwell, R. T., (2007). Adherence and efficacy of an external sealant to prevent new intramammary infections in the dry period. J dairy Sci. 90: pp: 289-1300.

Martin, S. Wayne, M. Alan, H. WillebergPreben, (1987) Veterinary epidemiology principal and methods. (Iowa State University Press/Ames, IA), 32

Matios, L., Tadele, T. and Tigre, W., (2009). Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. Trop. Anim. Health and prod, 41: pp1526-1530.

Mc Donald Compus of McGill, (2007). University department of animal science http://animsci,agrenv.mcgill,ca/courses/450/topics/13:pdf

Moges, N, Asfaw, Y. and Belihu, K., (2011). A cross sectional study on the prevalence of subclinical mastitis and associated risk factors in and around Gondar, Northern Ethiopia. International journal of animal and veterinary advances Vol. 3, No.6, pp.455-459.

Moges, N., Hailemarian, T., Fentahun, T., Chanie, M., and Melaku, A., (2012). Bovine mastitis and associated risk factors in smallholder lactating dairy farms in Hawassa, Southern Ethiopia. Global veterinaria 9 (4): 441-446.

Muhammad, S., Naveed, S., Ishtiaq, A., Raj, w., Muhammad, I., Muhammad, R. and Sajjad A., (1995). Diagnosis of subclinical mastitis in bovine using conventional methods and electronic detector.ARPN journal of agricultural and biological science. Vol. 6, No. 11, pp. 18-22.

Nigo, K. L. S., (2013). *Epidemiological and bacteriological investigation of bovine mastitis in Khartoum State – Sudan*

NationalMastitisCouncil., (1999). Laboratory Handbook on Bovine Mastitis. National Mastitis Council Inc., Madison, WI.

Osman, K. M., El-Enbaawy, M. I., Ezzeldeen, N. A. and Hussein, H. M. G., (2009). Mastitis in dairy buffalo and cattle in Egypt due to clostridium perfringens: prevalence, incidence, risk factors and costs. Rev. Sci. Tech. Off. Int. Epiz. Vol. 28, No. (3), pp: 975-986.

Philpot, W. N. and Nickerson, S. N., (2000). Winning the fight against mastitis. info@hambydairysupply.com.

Radostits, O M., (2000). Bovine mastitis a textbook of Cattle, Sheep, Pigs, Goat and Horse. Veterinary medicine, 9thedn. pp. 563-618. ELBS and BailliereTindau.

Radostits, O M., Gay, C. C., Blood, D. C. and Hincheliff, (2000). Mastitis in veterinary medicine: A text book of disease of Cattle, Sheep, Pigs, Goat and Horses 9th ed.,(W. B. Saunder, London), pp: 603-700.

Sawant, AA.,Sordillo, LM and Jayarao, BM., (2005). A survey on antibiotic usage in dairy herds in Pennsylvania. J Dairy Sci. 88, 2991-2999.

Seegers, H., Fourichon, C., Beaudeau, F., (2003). Production effect related to mastitis and mastitis economics in dairy cattle herd. Vet Res:34, pp, 475-491.

Sori, H., Zerihun, A. and Abdicho, S., (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. International J. Appl. Res. Vet. Med. Vol. 3, No.4.

Stefanakis, A., Boseos, C., Alexopoulos, C., (1995). Frequency of subclinical mastitis and observations on somatic cell counts in ewes milk northern Greece. J AnimSci, 61: pp 69-73.

Tadesse, A. and Chanie, M., (2012). Study on the occurrence of bovine mastitis in Addis Ababa dairy farms and associated risk factors. Advances in biological research 6 (4): pp 151-158.

Thrusfield, M., (2007). Veterinary epidemiology, 3th Ed. Black well: Oxford. UK, ISBN: 978-1-405-15627.

Tiwari, J. G., C. Babra, H. K. Tiwari, V. Williams, S. De. Wet, J. Gibson, A. Paxman1, E. Morgan1, P. Costantino, R. Sunagar, S.Isloor and T.

Mukkur(2013). Trends In Therapeutic and Prevention Strategies for Management of Bovine Mastitis (2013).

Uddin, M. A., Kamal, M. M. and Haque, M. E., (2009). Epidemiological study of udder and teat disease in dairy cows. Bangladish J Vet Med. 17893/0187/09.

Valde, J. P., Lystad, M. I., Simensen, F., and Osteras, O., (2007). Comparison of feeding management and body condition of dairy cows in herds with low and high mastitis rates. J. Dairy Sci.90, pp: 4317-4324.babcock@calshp.cals.wisc.e.

Viguier, C. S.,Arora, N. Gilmartin, K. Welbeck and R. O'Kennedy, (2009). Mastitis detection: current trends and future perspectives.

Workineh, S., M. Bayleyegn, H. Mekonnen and L.N.D. Potgieter., (200). Prevalence and etiologyof mastitis in cows from two major Ethiopian Dairies.Kluwer academic publishers, Netherland.

Wattiaux, M. A.,(2009). Babcock institute for international diary research and development.Mastitis; prevention and detection.

Wattiaux, M. A. The disease and its transmission.Babcock institute for international dairy research and development.

Zerihun, T., (1996).A study on bovine subclinical mastitis at Stela dairy farm. Thesis, Debrezeit: Faculty of veterinary medicine, Addis Ababa University: Ethiopia. pp: 25-27.

Appendix 1

Distribution of 399 dairy cattle examined in mastitis (clinical and subclinical) in East Nile Locality according to potential risk factors.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	young	144	36.1	36.1	36.1
	old	255	63.9	63.9	100.0
	Total	399	100.0	100.0	

1.2Breed:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	local	58	14.5	14.5	14.5
	cross	341	85.5	85.5	100.0
	Total	399	100.0	100.0	

1.3Body condition:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	good	230	57.6	57.6	57.6
	fair	111	27.8	27.8	85.5
	bad	58	14.5	14.5	100.0
	Total	399	100.0	100.0	

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)

								Lower	Upper
Step 1[a]	age(1)	-2.725	.653	17.437	1	.000	.066	.018	.236
	Body			21.616	2	.000			
	body(1)	-.824	1.054	.611	1	.434	.439	.056	3.461
	body(2)	1.883	.704	7.162	1	.007	6.575	1.655	26.119
	stage(1)	-.823	1.065	.597	1	.440	.439	.054	3.541
	parity(1)	.597	.641	.865	1	.352	1.816	.517	6.381
	exposure(1)	-.829	.920	.812	1	.368	.436	.072	2.650
	tick(1)	-.305	.663	.211	1	.646	.737	.201	2.703
	injuries(1)	-1.402	.642	4.769	1	.029	.246	.070	.866
	udder(1)	-.377	.672	.314	1	.575	.686	.184	2.563
	hand(1)	.198	.662	.089	1	.765	1.219	.333	4.461
	yield(1)	-1.806	.346	27.233	1	.000	.164	.083	.324
	sanitary(1)	1.299	1.086	1.431	1	.232	3.665	.436	30.773
	floor(1)	-.646	.547	1.395	1	.238	.524	.179	1.531
	dranaige(1)	-1.488	1.289	1.333	1	.248	.226	.018	2.822
	bedding(1)	-2.096	1.156	3.288	1	.070	.123	.013	1.185
	education(1)	1.498	.825	3.298	1	.069	4.475	.888	22.546
	Locality			66.523	3	.000			
	locality(1)	6.911	1.167	35.051	1	.000	1.003E3	101.797	9.884E3
	locality(2)	2.026	.935	4.695	1	.030	7.586	1.213	47.425
	locality(3)	-1.209	.652	3.435	1	.064	.299	.083	1.072
	Constant	1.962	.440	19.914	1	.000	7.111		

a. Variable(s) entered on step 1: age, body, stage, parity, exposure, tick, injuries, udder, hand, yield, sanitary, floor, dranaige, bedding, education, locality.

1.4 Stage of lactation:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	late	192	48.1	48.1	48.1
	early	207	51.9	51.9	100.0
	Total	399	100.0	100.0	

1.5 Parity:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	few	185	46.4	46.4	46.4
	many	214	53.6	53.6	100.0
	Total	399	100.0	100.0	

1.6 Previous exposure to mastitis:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	not exposed	177	44.4	44.4	44.4
	exposed	222	55.6	55.6	100.0
	Total	399	100.0	100.0	

1.7 Presence of tick:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	288	72.2	72.2	72.2
	present	111	27.8	27.8	100.0
	Total	399	100.0	100.0	

1.8 Teat injuries:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	absent	295	73.9	73.9	73.9
	present	104	26.1	26.1	100.0
	Total	399	100.0	100.0	

1.9 Barn size:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	adequate	260	65.2	65.2	65.2
	non-adequate	139	34.8	34.8	100.0
	Total	399	100.0	100.0	

1.10 Clean udder:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	101	25.3	25.3	25.3
	no	298	74.7	74.7	100.0
	Total	399	100.0	100.0	

1.11 Wash hand:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	249	62.4	62.4	62.4
	no	150	37.6	37.6	100.0
	Total	399	100.0	100.0	

1.12 Herd size:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	small	90	22.6	22.6	22.6
	large	309	77.4	77.4	100.0
	Total	399	100.0	100.0	

1.13 Yielding milk:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	low	231	57.9	57.9	57.9
	high	168	42.1	42.1	100.0
	Total	399	100.0	100.0	

1.14 Sanitary practices:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	good	190	47.6	47.6	47.6
	bad	209	52.4	52.4	100.0
	Total	399	100.0	100.0	

1.15 Floor disinfectant:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	79	19.8	19.8	19.8
	no	320	80.2	80.2	100.0
	Total	399	100.0	100.0	

1.16 Drainage system:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	good	180	45.1	45.1	45.1
	bad	219	54.9	54.9	100.0
	Total	399	100.0	100.0	

1.17 Bedding removing:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	yes	179	44.9	44.9	44.9
	no	220	55.1	55.1	100.0
	Total	399	100.0	100.0	

1.18 Water source:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	pipeline	331	83.0	83.0	83.0
	wells	68	17.0	17.0	100.0
	Total	399	100.0	100.0	

1.19 Type of fencing:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	wall	50	12.5	12.5	12.5
	iron	349	87.5	87.5	100.0
	Total	399	100.0	100.0	

1.20 Milking technique:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	stripes	210	52.6	52.6	52.6
	all fingers	189	47.4	47.4	100.0
	Total	399	100.0	100.0	

1.21 Education level:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	educated	219	54.9	54.9	54.9
	illiterate	180	45.1	45.1	100.0
	Total	399	100.0	100.0	

1.22 Mastitis test:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	192	48.1	48.1	48.1
	+ve	207	51.9	51.9	100.0
	Total	399	100.0	100.0	

1.23 Locality:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	a	100	25.1	25.1	25.1
	b	100	25.1	25.1	50.1
	c	100	25.1	25.1	75.2
	d	99	24.8	24.8	100.0
	Total	399	100.0	100.0	

Appendix 2

Cross tabulation of mastitis prevalence among 399 dairy cows examined in East Nile Locality with potential risk factors.

2.1 age:

		age		
		young	old	Total
Valid	-ve	84 58.3%	108 42.4%	192 48.1%
	+ve	60 41.7%	147 57.6%	207 51.9%
Total		144	255	399

2.2Breed:

		Breed		
		local	cross	Total
Valid	-ve	35 60.3%	157 46.0%	192 48.1%
	+ve	23 39.7%	184 54.0%	207 51.9%
Total		58	341	399

2.3Body condition:

		body condition			Total
		good	fair	Bad	
Valid	-ve	130 56.5%	41 36.9%	21 36.2%	192 48.1%
	+ve	100 43.5%	70 63.1%	37 63.8%	207 51.9%
Total		230	111	58	399

2.4Stage of lactation:

		stage of lactation		Total
		late	early	
Valid	-ve	110 57.3%	82 39.6%	192 48.1%
	+ve	82 42.7%	125 60.4%	207 51.9%
Total		192	207	399

2.5 Parity:

		parity		
		few	many	Total
Valid	-ve	104 56.2%	88 41.1%	192 48.1%
	+ve	81 43.8%	126 58.9%	207 51.9%
Total		185	214	399

2.6 Previous exposure of mastitis:

		previous exposure to mastitis		
		not exposed	exposed	Total
Valid	-ve	104 58.8%	88 39.6%	192 48.1%
	+ve	73 41.2%	134 60.4%	207 51.9%
Total		177	222	399

2.7 presence of tick:

		presence of tick		
		absent	present	Total
Valid	-ve	154	38	192
	+ve	134	73	207
Total		288	111	399

4.8.teats injuries

		teat injuries		
		absent	present	
Valid	-ve	154	38	192
	+ve	141	66	207
Total		295	104	399

2.9 Barn size:

		barn size		
		Adequate	non-adequate	Total
Valid	-ve	128	64	192
	+ve	132	75	207
Total		260	139	399

2.10 Clean teats and udders:

		clean teats and udder		
		yes	no	Total
Valid	-ve	59 58.4%	133 44.6%	192 48.1%
	+ve	42 41.6%	165 55.4%	207 51.9%
Total		101	298	399

2.1: Age:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9.414[a]	1	.002		
Continuity Correction[b]	8.785	1	.003		
Likelihood Ratio	9.443	1	.002		
Fisher's Exact Test				.002	.002
Linear-by-Linear Association	9.391	1	.002		
N of Valid Cases[b]	399				

2.2: Breed:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.062[a]	1	.044		
Continuity Correction[b]	3.510	1	.061		
Likelihood Ratio	4.077	1	.043		
Fisher's Exact Test				.047	.030
Linear-by-Linear Association	4.052	1	.044		
N of Valid Cases[b]	399				

2.3: Body condition:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.361[a]	2	.000
Likelihood Ratio	15.497	2	.000
Linear-by-Linear Association	12.858	1	.000
N of Valid Cases	399		

2.4: Stage of lactation:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12.469[a]	1	.000		
Continuity Correction[b]	11.771	1	.001		
Likelihood Ratio	12.532	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	12.438	1	.000		
N of Valid Cases[b]	399				

2.11 Wash hands:

		wash hand		
		yes	no	Total
Valid	-ve	136 54.6%	56 37.3%	192 48.1%
	+ve	113 45.4%	94 62.7%	207 51.9%
Total		249	150	399

2.12 Herd size:

		herd size		
		small	large	Total
Valid	-ve	51 56.7%	141 45.6%	192 48.1%
	+ve	39 43.3%	168 54.7%	51.9%
Total		90	309	399

2.13 Yielding milk:

		yielding milk		
		low	high	Total
Valid	-ve	136 58.9%	56 33.3%	192 48.1%
	+ve	95 41.1%	112 66.7%	207 51.9%
Total		231	168	399

2.14 Sanitary practices:

		Sanitary practices		
		good	bad	
Valid	-ve	106 55.8%	86 41.1%	192 48.1%
	+ve	84 44.2%	123 58.9%	207 51.9%
Total		190	209	399

Count				
		floor disinfectant		
		yes	no	Total
mastitis test	-ve	49	143	192
	+ve	30	177	207
Total		79	320	399

2.5: Parity:

2.15 Floor disinfectant:

		floor disinfectant		
		yes	no	Total
Value	-ve	49 62.0%	143 44.7%	192 48.1%
	+ve	30 38.0%	177 55.3%	207 51.9%
Total		79	320	399

2.16 Drainage system:

		drainage system		
		good	bad	Total
Valid	-ve	104 57.8%	88 40.2%	192 48.1%
	+ve	76 42.2%	131 59.8%	207 51.9%
Total		180	219	399

2.17 Bedding removing:

		bedding removing		
		yes	no	Total
Value	-ve	113 63.1%	79 35.9%	192 48.1%
	+ve	66 36.9%	141 64.1%	207 51.9%
Total		179	220	399

2.18 Water source:

		water source		
		pipeline	Wells	Total
Value	-ve	158 47.7%	34 50.0%	192 48.1%
	+ve	173 52.3%	34 50.0%	207 51.9%
Total		331	68	399

2.19 Type of fencing:

		type of fencing		
		wall	Iron	Total
Value	-ve	25 50.0%	167 47.9%	192 48.1%
	+ve	25 50.0%	182 52.1%	207 51.9%
Total		50	349	399

2.20 Milking technique:

		milking technique		
		stripes	all fingers	Total
Value	-ve	104 49.5%	88 46.6%	192 48.1%
	+ve	106 50.5%	101 53.4%	207 51.9%
Total		210	189	399

2.21 Education level:

		education level		
		educated	illiterate	Total
Value	-ve	122 55.7%	70 38.9%	192 48.1%
	+ve	97 44.3%	110 61.1%	207 51.9%
Total		219	180	399

2.22 Locality:

		locality				
		a	b	c	d	Total
Valid	-ve	33 33.0%	58 58.0%	64 64.0%	37 37.4%	192 48.1%
	+ve	67 67.0%	42 42.0%	36 36.0%	62 62.6%	207 51.9%
Total		100	100	100	99	399

Appendix 3

Association of different potential risk factors with mastitis using Chi Square test (χ2)

3.1 Age:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9.414[a]	1	.002		
Continuity Correction[b]	8.785	1	.003		
Likelihood Ratio	9.443	1	.002		
Fisher's Exact Test				.002	.002
Linear-by-Linear Association	9.391	1	.002		
N of Valid Cases[b]	399				

3.2 Breed:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.062[a]	1	.044		
Continuity Correction[b]	3.510	1	.061		
Likelihood Ratio	4.077	1	.043		
Fisher's Exact Test				.047	.030
Linear-by-Linear Association	4.052	1	.044		
N of Valid Cases[b]	399				

3.3 Body condition:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.361[a]	2	.000
Likelihood Ratio	15.497	2	.000
Linear-by-Linear Association	12.858	1	.000
N of Valid Cases	399		

3.4 Stage of lactation:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12.469[a]	1	.000		
Continuity Correction[b]	11.771	1	.001		
Likelihood Ratio	12.532	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	12.438	1	.000		
N of Valid Cases[b]	399				

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3.5 Parity:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9.056[a]	1	.003		
Continuity Correction[b]	8.461	1	.004		
Likelihood Ratio	9.086	1	.003		
Fisher's Exact Test				.004	.002
Linear-by-Linear Association	9.033	1	.003		
N of Valid Cases[b]	399				

3.6 Previous exposure to mastitis:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14.417[a]	1	.000		
Continuity Correction[b]	13.662	1	.000		
Likelihood Ratio	14.494	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	14.381	1	.000		
N of Valid Cases[b]	399				

3.7 Presence of tick:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11.878[a]	1	.001		
Continuity Correction[b]	11.120	1	.001		
Likelihood Ratio	12.053	1	.001		
Fisher's Exact Test				.001	.000
Linear-by-Linear Association	11.848	1	.001		
N of Valid Cases[b]	399				

3.8 Teats injuries:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.558[a]	1	.006		
Continuity Correction[b]	6.944	1	.008		
Likelihood Ratio	7.641	1	.006		
Fisher's Exact Test				.006	.004
Linear-by-Linear Association	7.539	1	.006		
N of Valid Cases[b]	399				

3.9 Barn size:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.369[a]	1	.544		
Continuity Correction[b]	.252	1	.616		
Likelihood Ratio	.369	1	.544		
Fisher's Exact Test				.599	.308
Linear-by-Linear Association	.368	1	.544		
N of Valid Cases[b]	399				

3.10 Clean teats and udder:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.742[a]	1	.017		
Continuity Correction[b]	5.203	1	.023		
Likelihood Ratio	5.754	1	.016		
Fisher's Exact Test				.021	.011
Linear-by-Linear Association	5.727	1	.017		
N of Valid Cases[b]	399				

3.11. Wash hands:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11.203[a]	1	.001		
Continuity Correction[b]	10.521	1	.001		
Likelihood Ratio	11.296	1	.001		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	11.175	1	.001		
N of Valid Cases[b]	399				

3.12 Herd size:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.400[a]	1	.065		
Continuity Correction[b]	2.972	1	.085		
Likelihood Ratio	3.403	1	.065		
Fisher's Exact Test				.073	.042
Linear-by-Linear Association	3.392	1	.066		
N of Valid Cases[b]	399				

3.13 Yielding milk:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	25.416[a]	1	.000		
Continuity Correction[b]	24.403	1	.000		
Likelihood Ratio	25.780	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	25.352	1	.000		
N of Valid Cases[b]	399				

3.14 Sanitary practice:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.546[a]	1	.003		
Continuity Correction[b]	7.969	1	.005		
Likelihood Ratio	8.574	1	.003		
Fisher's Exact Test				.004	.002
Linear-by-Linear Association	8.524	1	.004		
N of Valid Cases[b]	399				

3.15 Floor disinfectant:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.629[a]	1	.006		
Continuity Correction[b]	6.950	1	.008		
Likelihood Ratio	7.670	1	.006		
Fisher's Exact Test				.008	.004
Linear-by-Linear Association	7.610	1	.006		
N of Valid Cases[b]	399				

3.16 Drainage system:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12.252[a]	1	.000		
Continuity Correction[b]	11.557	1	.001		
Likelihood Ratio	12.307	1	.000		
Fisher's Exact Test				.001	.000
Linear-by-Linear Association	12.221	1	.000		
N of Valid Cases[b]	399				

3.17 Bedding removing:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	29.291[a]	1	.000		
Continuity Correction[b]	28.211	1	.000		
Likelihood Ratio	29.634	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	29.218	1	.000		
N of Valid Cases[b]	399				

3.18 Water source:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.116[a]	1	.733		
Continuity Correction[b]	.043	1	.836		
Likelihood Ratio	.116	1	.733		
Fisher's Exact Test				.790	.418
Linear-by-Linear Association	.116	1	.734		
N of Valid Cases[b]	399				

3.19 Type of fencing:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.081[a]	1	.776		
Continuity Correction[b]	.018	1	.894		
Likelihood Ratio	.081	1	.776		
Fisher's Exact Test				.880	.447
Linear-by-Linear Association	.081	1	.776		
N of Valid Cases[b]	399				

3.20 Milking technique:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.350[a]	1	.554		
Continuity Correction[b]	.241	1	.623		
Likelihood Ratio	.350	1	.554		
Fisher's Exact Test				.616	.312
Linear-by-Linear Association	.349	1	.555		
N of Valid Cases[b]	399				

3.21 Education level:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11.195[a]	1	.001		
Continuity Correction[b]	10.531	1	.001		
Likelihood Ratio	11.260	1	.001		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	11.167	1	.001		
N of Valid Cases[b]	399				

3.22 Locality:

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	27.748[a]	3	.000
Likelihood Ratio	28.129	3	.000
Linear-by-Linear Association	.759	1	.384
N of Valid Cases	399		

بسم الله الرحمن الرحيم

Appendix 4 Questionnaire

Questionnaire for Data Collection for Survey ofBovine mastitis in Eastern Nile Locality in Khartoum State

Locality……. Administration unit……
Farm No…… Date of Investigation…../……/……..
Investigator……. …….

General Characteristics

Owner:

Name…… Address……..

Age…….. Telephone No…….

Education level……..

Housing and codes:

1/ Herd size: Small (<=20) (0) ( ) Large (>20) (1) ( )

2/Barn size: Adequate (0) ( ) Not adequate (1) ( )

3/Frequency removingof bedding: Yes (0) ( ) No (1) ( )

4/Sanitary practice: Good (0) ( ) Bad (1) ( )

5/Floor disinfectant: Yes (0) ( ) No (1) ( )

6/Drainage system: Good (0) ( ) Bad (1) ( )

Cow and milking management:

1/Clean teats and udder: Yes (0) ( ) No (1) ( )

2/Wash hand: Yes (0) ( ) No (1) ( )

3/Milking technique: Stripes (0) ( ) All fingers (1) ( )

4/Water source: Pipeline (0) ( ) Wells (1) ( )

5/Type of fencing: Wall (0) ( ) Iron (1) ( )

Others comments:

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Individual risk factors:

1/age: Young (<=6) (0) ( ) Old (>6) ( )

2/Breed: Local (0) ( ) Cross (1) ( )

3/Body condition: Good (0) Fair (1) Poor (2)

4/Stage of lactation: Late (0) ( ) Early (1) ( )

5/Parity: Few (<=4) (0) ( ) Many (>4) (1) ( )

6/Previous exposure to mastitis: No (0) ( ) Yes (1) ( )

7/Teat injuries: Absent (0) ( ) Present (1) ( )

8/Present of tick on udder: No (0) ( ) Yes (1) ( )

9/Yielding milk: Low (0) ( ) Low (1) ( )

10/Result: -ve (0) ( ) +ve (1) ( )