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Prevalence and Etiology of Mastitis in Cows in Berber Locality- River Nile State - Sudan

معدل الإصابة وأسباب إلتهاب الضرع في الأبقار في محلية بربر ولاية نهر النيل - السودان

A thesis Submitted to the College of Graduate Studies, Sudan University of Science and Technology in Partial Fulfillment of the Requirement for the Degree of Master in Preventive Veterinary Medicine (M.P.V.M)

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الإستهلال

قَالَ تَعَالَىٰ:

﴿ وَإِنَّ لَكُمْ فِي ٱلْأَنْعَٰمِ لَعِبْرَةً ۖ نُّسْقِيكُم مِّمَّا فِي بُطُونِهِۦ مِنۢ بَيْنِ فَرْثٍ وَدَمٍ لَّبَنًا

خَالِصًا سَآئِغًا لِّلشَّٰرِبِينَ ﴿٦٦﴾ ﴾

صدق الله العظيم

سورة النحل الاية 66

Dedication

I dedicate this work to my father soul and my mother, my husband and my two daughters, my brother and sister with my best wishes.

Acknowledgement

I consider it my utmost obligation to express my degree of master to Allah, kind and merciful who gave me the health, thoughts and the opportunity 3 to complete this task.

I was fortunate in having the generous advice and encouragement of my learned professor Mohamed Abd Asalam.

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Abstract

Across-sectional study was conducted on 100 lactating cows at Berber locality, in River Nile State in Sudan during the period October 2018 –November 2019. The objectives of the study were to estimate the overall prevalence of sub - clinical mastitis and to investigate the association of potential risk factors associated with the disease.

A total of 100 lactating dairy cows comprising 8 local and 92 cross breed cows were randomly selected and screening using California Mastitis Test (CMT) for subclinical mastitis. The overall prevalence rate was found to be 69%. The highest prevalence of sub clinical mastitis was reported in Albawga Administration units 83 % and Alabeedia Administration units showed the lowest prevalence of subc clinical mastitis 20 %, Berber town prevalence is 72%, South Berber prevalence is 42.9%, West Berber prevalence is 80%.

The result showed that some risk factors like age ,present of ticks on udder and previous exposures to mastitis had Asignificant association with mastitis .

In conclusion Mastitis is common in Lactating Cows in Berber Locality and Management Measures should be used for controlling the disease.

ملخص البحث

اجريت هذه الدراسة المقطعية في 100بقرة من ابقار الحليب بمحلية بربر ولاية نهر النيل في السودان في الفترة من اكتوبر للعام 2018 –نوفمبر 2019.هدفت الدراسة الي تقدير نسبة انتشار التهاب الضرع دون العياني والتى بلغت 69% وتحديد عوامل الخطورة التي تساعد علي حدوث المرض تم اختيار مجمل 100 بقرة حلوب عشوائايا كالاتي : 8محلي و92 هجين وتم فحصها بواسطة (CMT)للابقار دون العيان والتي بلغت 69%وحيث سجلت النتائج التالية : اعلى نسبة انتشار المرض في المحلية فى الباوقة 83 %للفحص دون العياني.ادنى نسبة انتشار للاصابات دون العيانية سجلت بوحدة العبيدية والتى بلغت 20%بينما سجلت وحدة مدينة بربر 72%نسبة انتشار للاصابات دون العيانية ،جنوب بربر سجلت نسبة 42.9 %بينما سجلت وحدة غرب بربر نسبة 80%.

عند التحليل لكل عامل خطورة حددت الدراسة بعض عوامل الخطورة التي تساهم بشك وثيق في حدوث المرض منها العمرووجود القراد على الضرع والتعرض للاصابات المسبقة لالتهاب الضرع .

في الخلاصة وجد ان مرض التهاب الضرع مرض شائع في الابقار في محلية بربر والمقاييس الادارية التي تم استخدامها للتحكم في المرض .

Introduction

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

Mastitis is defined as 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 response to the presence of microbial organisms or non – infectious, as a result of physical injury to the mammary glands. The inflammatory response result in an increase in the blood proteins and white blood cells in the mammary tissue, which can then pass into the milk product. The response aims to destroy the irritant repair the damaged mammary tissue and return the udder to its normal function **(Kudi *et al*., 2009)**. It is a single most common disease syndrome of adult dairy cows worldwide phenomenon with integrated economic component recognized mainly as clinical and sub clinical types. Bovine mastitis is persistent inflammatory reaction of the udder tissues due to physical trauma or microorganisms. clinical mastitis can present itself in wide degree of severity of symptoms and abnormalities in udder such as swelling /heat /hardness /redness or pain and other changes in the milk such as a watery appearance /flakes /clots or pus.in contrast the subclinical mastitis can be known only after laboratory examination.as there no gross inflammatory changes in the udder tissue. In almost all cows microorganisms are mainly involved in mastitis but some factors pendulous udder with long teats large size of teat orifice in high yielding cows traumatic injuries play important role as factor of mastitis as poor management and hygiene. Mastitis can occur after milking because the teat holes close after 15 minutes if the animal sits in a dairy place with feces and urine. In term of economic loss mastitis is undoubtedly the most important disease with which the dairy industry has to contend once a cow suffers from mastitis it will never return to its normal milk production.

The mode of transmission of mastitis causing bacteria including:

(1) Contagious mastitis which is generally spread from cow to cow so the primary habitat of bacteria on the udder and teat lesion chronic or sub clinical mastitis is type of this mode transmission.

(2) Environmental mastitis which referred to as environment to cow mastitis in this case the bacteria are found in the cow's environment (feces –soil –bedding or water

(3) Teat skin to udder. The pathogens which case streak canal infection. Germicidal teat dip and dry cow antibiotic treat are effective control measures.

(4) Mouth to udder. Calves suckling results in opening the teat canal –predisposing to intern mammary infusion، feeding calves with milk from infected cow.

(5) Flies sometime can transmit what is called summer mastitis with caused by antinomies pyogenic cause's abscesses and purulent mastitis in dry cow and heifers

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

the cow will indicate the severity of infection serve as a guide for the course of treatment. **(Muhammed *et al*; 2011)**.

Objectives

-To provide appalled estimate of the prevalence of overall, clinical and sub clinical mastitis in dairy cattle in Berber locality.

-To evaluate some risk factors related to management practices and production characteristics that influenced clinical mastitis.

Chapter one

Literature Review

1.1 Definition

Mastitis (Mast: breast, it is: inflammation) is an inflammatory reaction of udder tissue due to bacteria, chemical, thermal or mechanical injury. 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 tissue. **(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. **(Lim *et al*; 2007)**.

1.2 Epidemiology of mastitis

Across 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 breeds. Out of 183lactating small holder dairy cows examined for bovine mastitis 9 - 4.9 % had clinical mastitis , about 9.43% and 3.53% occurred in high grade Holstein and Holstein – indigenous zebu, respectively, but indigenous zebu breeds were 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 more 0.05) stage of lactation (2=13.8, p more 0.05) and parity number (2=19.4, p more 0.05) had significant effect on the prevalence of sub clinical 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 cattle (n=291 buffaloes (n =382) 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 sub clinical 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 sub clinical 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 a symptomatic cows from 16 dairy farms were examined for the presence of microorganisms with the potential to cause mastitis. Coagulase – negative S*taphylococci* clearly predominated (53.5%) followed by *Streptococci* and *Enterococci* (both occurring in 16.1% samples). Among *Streptococci*, so called mastitis *Streptococci* (*S. uberis*, *S. dysgalactiae* and *S.agalactiae*) is prevailed (11.7% positive samples). *Entero bacteria* were found in 10.0% samples, most of them (6.6% samples) were positive for *Escherichia coli*. And *Yeasts* (mainly *Candida spp.*) were found in 8.2% samples. One of the major mastitis pathogens, *Staphylococcus aureus sub sp. 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 clonally (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. In the study conducted on 453 lactating cattle of various breed at 21 farms. The milk samples from these animals were

collected to diagnose mastitis using California Mastitis Test (CMT). The epidemiological data related to animals and management was collected and analyzed to draw conclusions. The results of test revealed significant association between body weight, udder depth and lower teat end to floor distance (p more 0.01) with mastitis. The bivariate frequency analysis revealed significant association for lactation stage (p more 0.0001), teat end to floor distance, parity udder shape, teat shape, live body weight, teat and or udder pathology, use of oxytocin, feeding system and milk leakage with mastitis. The results of logistic regression analysis revealed significant negative association between teat lengths frequency of culling and number of attendants, while positive association between mastitis teats involved teat diameter (apex, mid and base), milk leakage, udder shape, pendulous udder, feeding system udder depth, teat shape, calf suckling, 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 quarter affected, and 16.0%(48/300) had three quarter affected while 6.67%(20/300) had all the four quarter affected. A total of 53.00% had SCM in multiple quarter (159/300) .The risk of SCM decreased significantly among young lactating cows compared to older animals (OR=0.283: p more 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 prevalence 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).

1.3 The causative agent

Research finding have proved that buffalo is as susceptible to mastitis as cow. The causative organisms of mastitis in buffaloes *Mycobacterium tuberculosis* have been reported to be *Staphylococci Streptococci, Escherichia coli ,Pseudomonas spp.*, *Corynebacterium, Mycoplasma ,Streptococcus dysaglactiae,* and among all the pathogens of bovine mastitis, Staphylococcus *aureusis* the predominant organism. Etiological agents of mastitis in buffaloes have been reported to be *Staphylococcus aureus, Streptococcus dysaglactiae, Streptococcus agalactiae,* and *Streptococcus Spyogenes* and *Corynebacterium bovis.* **(Khan *et al*; 2006)**. It is known that the prevalence of contagious pathogens causing mastitis is decreasing and environmental causative agents are becoming dominant pathogens for the mammary gland. Reports from United States of America and from Great Britain indicate creating role of environmental mastitis pathogens. *Coliforms* and *Streptococci* other than *S. agalactiaea* counted for 94%of the major pathogen infections. Contagious pathogens were isolated only in 3.4% of clinical mastitis cases in well managed herds. It has be estimated that contagious mastitis pathogens represent less than one third of all mastitis cases compared to less 75% of all cases 20 years ago. Environmental *Streptococci, Coliforms* and coagulase negative *Staphylococcus spp* were the most commonly isolated pathogens.

1.4 Types of mastitis

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

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

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. **(Abd Hameed *et al*; 2006)** .The detection of clinical mastitis depends upon the examination of the mammary gland and its secretion. The affected gland may show swelling, heat, pain and hardness. The secretion may be clotted, serous or occasionally blood stained **(Andrews *et al*; 2004)**.

1.4.1.1.1 Type of clinical mastitis

1.4.1.1.1.1 Per-acute

This form of mastitis is fairy uncommon and includes depression, raised pulse and respiratory rates, loss of muscle coordination, cold extremities, reduced papillary reflex, dehydration and diarrhea **(Philpot and Nickerson, 2000)**. It is characterized by gross inflammation, reduction milk yield and changes in milk composition. Systematic signs like fever, depression, shivering and loss of appetite and loss of weight. **(A wale *et al*; 2012)**.

1.4.1.1.1.2 Acute mastitis

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

1.4.1.1.1.3 Sub – acute mastitis

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

1.4.1.1.1.4 Sub clinical mastitis

Sub clinical mastitis (SCM) is the most prevalence and great economic importance to dairy farmers because it result in reductions in milk yield and undesirable change in the milk composition **(Brighting *et al*; 2010 and Seegers *et al*; 2003)**, as well as increased costs associated with control strategies **(Halasa *et al*; 2009)**.Sub clinical mastitis is the multimedia logical complex disease which consist of infection and noninfectious agent as potential risk factors. It cannot be detected by visual observation though it can be identified by conducting tests to detect the presence of infecting microorganism or the product of inflammation such as somatic cell count. **(Philpot and Nickerson, 2000)**.

1.4.1.1.1.5 Chronic mastitis

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

1.4.2 Environmental mastitis

The environment is a reservoir of infections 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 infection is less common with *E. coli*, but frequently occur with *Streptococcus uberis*. Environmental mastitis is controlled by clean environment, adequate accommodation or cows, milking through correctly functioning machine, good udder preparation. Environmental organisms include *E.coli*, *Streptococcus uberis* (Straw bedding), *Klebsiella spp*. (Saw dust 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. Mastitis in dairy animals occurs when the udder becomes inflamed and bacteria invade the teat canal and mammary gland. 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 cell in the milk, reducing its quantity and adversely affecting the quality of milk and milk by products. The teat end serves as the first line of defense against infection. From outside, a sphincter of smooth muscles surrounds the teat canal which their functions to keep the teat canal closed. It also prevent 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 waxy material composed of fatty acids and fibrous protein in the teat. During milking bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to 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. **(Khan and khan, 2006)**.

1.6 Diagnosis 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 it’s 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)**.

1.6.2 Physical examination of the udder

Signs of acute mastitis include quarters that are swollen, warm and painful to the touch. Change in size and presence of scar tissue may be detected more easily after milking, when the udder is empty **(Wattiaux, 2009)**.

1.6.3 Appearance of the milk

Observation of the first streams of milk (fore milk) permits the detection of abnormal milk that should be withheld. Abnormal milk may show discoloration (wateriness), flakes, or clots. Caution should be exercised during the removal of foremilk to avoid splashing of contaminated milk on the cow's limbs, tail or udder. In addition, the operator should not collect the foremilk in the palm of the hand because of the risk of transferring bacteria from one quarter to another and from one cow to the other. In stanchion barn, foremilk is typically drawn into a" strip cup "or plate. In a milking parlor, however, it may be drawn direct Lyon to the floor and flushed away immediately after observation **(Wattiaux, 2009)**.

1.6.4 California mastitis test

Strict aseptic 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 Samper 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.

*4 =Thick gel.

Egg white consistency with dark purple color (Subclinical infection). 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.

1.6.6. Bacteriological cultures

Each positive CMT milk sample was collected under septic conditions in a sterile screw caped bottle numbered to identify the particular quarter. All milk samples were sent directly to the laboratory with a minimum of delay for routine culture techniques. Milk samples were cultured into 10% sheep blood agar and Macon key agar plates. Suspected colonies were identified

morphologically, microscopically and biochemically **(Abdel-Rady and sayed, 2007)**.

1.6.7 Current approaches for diagnosis of mastitis

Currently assay often is used and include measurement of somatic count cells (SCCs), enzymatic analysis and the California milk clotting test. In Europe, elevated SCCs above 200000 cells/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 concentration of enzymes elevated in milk during mastitis (e.g. NAGase or LDH). Using of culturing techniques for the detection of mastitis – causing microorganisms is still the gold standard, although it is very labor – 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 *et al*; easy to detect, occur at a very early stage and that can be measured on site **(Viguier, 2009)**.

1.6.8 Other Current and alternative methods for detection of mastitis

1.6.8.1 Porta check

This assay uses an esterase – catalyzed enzymatic reaction to determine the SCCs in milk, this test is cost effective and rapid and low sensitivity at low SCC.

1.6.8.2 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 SCC in milk, this test is rapid and automated but it is expensive and complex to use.

1.6.8.3 Delavan cell counter

This counter operates on the principle of optical fluorescence, where by propodeum iodide is used to strain nuclear DNA to estimate the SCC in milk, this test is rapid and the device are easily transportable but it is relatively expensive.

1.6.8.4 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, this test can be used on site and non –mastitis –related variations in EC can present problems in diagnosis.

1.6.8.5 PH test

The rise in milk PH, due to mastitis is detected using bromothymol blue, this test is cost and rapid but it well not as sensitive as other tests.

1.6.8.6 Enzymes

Assay are used to detect enzymes, such as NAGase and LDH, assay is rapid but might be laboratory –based **(Viguier *et al*; 2009)**.

1.7 Mastitis Treatment

The first step in treating is to identify the causative agent. The presence of a pathogen and inflammatory response of the udder signify in an infection. The inflammatory response, which result in abnormal milk, is usually detected by the dairy man because mastitis is frequently sub clinical (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.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 **(Bramleya and 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 inframammary antimicrobial formulations are administered as an infusion.

Through the treat canal using single dose syringes with especially designed applicator tips. Appropriately selected systemic therapies may be as efficacious as intermammary preparations. In the United States, only infermammary antimicrobial infusion formulation are currently approved for treatment of either clinical or subclinical mastitis.

1.7.1 Antibiotic treatment

Typically, when clinical mastitis is detected, the cow is milked out and then given an inframammary 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 milked 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 . Prior to inframammary 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 aplastic tube with aplastic 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 teat cistem. After emptying the antibiotic tube, the teat is pinched off and the antibiotic fluid is palpated up into the gland. Because the cows udder then contains antibiotic which must be kept out of the food supply, that cow's milk tank for some specified number of milking after treatment (Tiwari *et al*;2013) .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 antibiotic 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 antibiotics **(Tiwari *et al*; 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 milk 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 **(Tiwari *et al*; 2013)**.

1.7.3. Non-responding cases

In spite of the natural resistance mechanisms of the cow, antibiotic treatment to help of the fight bacterial infection, and other method 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 **(Tiwari *et al*; 2013)**.

1.8 Prevention 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 agents 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)**.

1.8.1. Control of contagious mastitis

Contagious mastitis can be effectively controlled through air grouse program of teat dipping and dry cow antibiotic treatment. Teat must be dipped in germicide after each milking (this decrease incidence of the disease).Each quarter must be treated with dry cow antibiotics at end of lactation (this decrease prevalence of the disease). Cows with contagious mastitis should be milked last or a separate milking claw used for the infected cows. Milking cows should be flushed with hot water or germicide after milking infected cows (Called back flushing). Individual cloth /paper towels should be used to wash /dry teats. Milked should have clean hands and wear latex gloves. New addition to the herd should be cultured and persistently infected cows should be culled. Teat lesions should be minimized (from chapping, bite, stepped on teats, lacerations, or machine damage). Heifers can be given dry cow antibiotic treatment during gestation if *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. Udder can be clipped to minimize the amount of manure clinging to the glands, only clean dry teats should be milked. Teat should be pre-dipped with germicide before milking Cow should be kept standing after milking (offer them feed). Sterile single – dose infusion products should be used and sterile infusion techniques (alcohol swab) should be used. The milking parlor should be kept clean. The teat dipper should be kept clean; organisms survive in many germicide. Pipelines /water heater may need to be replaced in cases of pseudomonas contamination **(Awale *et al*; 2012)**.

1.8.3 Nutrition

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

1.8.4 Vaccines

Development of potential vaccines to prevent or control mastitis to be an important goal. Excellent progress has been made toward Coliform mastitis control with 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 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 lyphnode. Vaccination has been unsuccessful in reducing the number of new cases of mastitis, some vaccines have been effective in improving spontaneous cure rate and reducing severity of infection, these vaccine 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 Staph aureus vaccine is an ongoing objective of much research **(Hurley, 2009)**. The use of vaccination particularly with autogenously killed whole cell vaccines to control infectious disease 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, but few have claimed satisfactory outcomes neither in the field nor on backyard farms. **(Tiwari *et al*; 2013)**.

1.8.5 Culling

Culling a chronically infected cow with mastitis achieves both a reduction in herd prevalence and also a reduction in the risk of subsequent spread of infection. However, it comes with accost a current net loss of around 600 per cow culled, the decision to cull is unfortunately complex and depend on the herd status in terms of somatic cell count and clinical mastitis and the ability with in the herd to prevent the spread of infection . The cost of a cull need to be tempered by the cost and likely success after treatment as well as by the potential for spread with the herd position in mind and knowledge of the cow factors . An old cow with chronic high SCC, CMT positive in three quarters and fibrosis mammary tissue is clearly more eligible for culling than a young cow

with a recently increased medium SCC, one quarter positive on CMT. However, decisions are not always clear –cut and quantification of these decisions in a subject of current research, it is important to remember at culling alone is not the answer to high SCC problem, in the absence of institution of appropriate measures to control spread the end result is likely to be just more culls **(Green *et al*; 2004)**.

1.8.6 Crying off a quarter

This is a useful compromise measure, an alternative to culling the cow or treating infected quarters ceased for the remainder of that lactation. Antibiotic dry cow therapy is only used when the other quarter are infused at drying off. This technique works particularly well for high SCC infected quarters but not during a clinical episode. It is important to mark the quarter clearly to prevent accidental milking. Research studies report the use of (Povidone iodine or Chlorhexidine) to stop the offending quarter from lactation but these should only be considered when permanent cessation of milking in that quarter is acceptable if these measures are adopted it is important to consider the welfare aspects of this procedure and consideration should be given to using appropriate analgesia. Cessation of milking in a quarter for one-part lactation essentially gives that quarter a prolonged dry period and is often association with cure rates of over 50% (Green *et al*;2004).

1.9 Economic Implication of Mastitis

Mastitis in dairy cows and its economic implication in the river nile state (Sudan), this study was conducted to investigate the prevalence of mastitis in dairy cows and the economical implication of bovine mastitis, through the reduction of milk yield, milk dumped after antibiotic treatment and the cost of drug and other inferred with mastitis. California mastitis test was performed in dairy farms to identify the infected quarters, the revealed that about 85.5% of

herd individuals were mastitis. The bacteria isolated from milk samples were *Staphylococci*, *Streptococci*, *Micrococci*, *Coliform* bacteria together with my colic infection. The economical implication of mastitis was evaluated through calculation of milk yield reduction, reduction which represented 67.2% from the total loss had been assessed through the direct and indirect estimation of somatic cell count it represented more than 12% of milk yield for one infected quarter. Also the reduction percentage had been assessed through comparison between infected and healthy quarter was about 12.5%, also the reduction percentage had been assessed yield for one infected and healthy quarter for the same cow. The reduction percentage of one infected quarter, the milk reduction cost reduction as average was 14.2% (234 Kg /cow/season), and the milk reduction cost was 27325 Sudanese Dinars, and the discarded milk due to treatment with antibiotic was14.5% of the total loss, with total cost 5970 Sudanese Dinars ,Drug cost was estimated as 5167 Sudanese Dinars . The effect of mastitis on milk yield for the mastitis cows statistically and the milk yield was confirmed statistically and the milk curve verified the difference in milk yield between mastitis and healthy cows **(Isam; 2007)**.

Chapter Two

Material and method

2.1 The Study Area

The study was conducted from October 2018 - November 2019 in fives administration unite of Berber Locality Berber town /Albawga /Alabedia /South Berber /Western Berber .The study area lies between latitudes 39 -18 and 42-17 N and longitudes 52-34 and 34 - 42 E It is stretches 100 Km from the Atbara locality border south to Abuhamed locality border to the north and is bordered to the east by the Red Sea state and from the west by the Northern state. It has a total of spaces 14711 square Km. Berber is a town in the River Nile state of north Sudan /50 Kh north of Atbara /near the junction of Atbara river and the Nile.

Coordinates of Berber Locality is 18 01 50 N 33 95.36E. Population 16.650 (1989).

The resident livestock system of animal breeding in Berber is various breed of cattle's kennana/butane /cross breed Frisian .The population of animals in the River Nile State is estimated as 106822 cattle's, 1654450 goats,1369619 sheep ,82723 camels ,184000 birds ,216276 equines . In Berber locality the population estimated as 8546 cattles,177026 goats ,194486 sheep ,5791 camels ,10000 bird ,40444 equines (General Directorate of Livestock and pasture River Nile State – Sudan Annual report ,2016).

2.2 Study Design

Across sectional study design in which all the study animals were tested for subclinical mastitis by California mastitis test (CMT). Information regarding the

potential risk factors was collected by questionnaire survey and by the observation and multistage random sampling was carried out in Berber locality as 5 administration unites included (Berber town farms /south Berber farms / western Berber farms /Albawga farms /Alabeedia farms). Association of farms were selected randomly and finally appropriate herds were selected from each farms followed by sampling lactating cows from each randomly selected herd

2.3 Sample Size Determination

A total 100 milk samples were collected from 100 lactating cow's health or mastitis from small holder dairy herd from fives units in Berber locality. These 100 samples were collected during the milking processing to test directly in the farms.

2.4 California Mastitis Test (CMT)

was used to detect subclinical mastitis per/two (ml) of milk from milk of each udder quarter in a plate that had four separated cup (buddle) two (ml) CMT liquid was added to each cup and mixed gently by rotating the plate . The reaction was then visually scored amount of gel formation. The result was classified into four scores: 0 = Negative or traces (no change in consistency)

1= Slightly positive (+) (infection light gel disappearing after stirring or purplish gray color).

2= Positive (++) (subclinical light persistent gel crumbly filaments or purple gray).

3 = Highly positive (+++) (subclinical immediate thickening viscous cluster at the bottom of the wall).

-4=Thick gel, egg white consistency with dark purple color (subclinical infection).

Scores 1 /2 /3/-4 depend on the degree of gelatin that were indicated by gelatinous mas. The reaction developed almost immediately with milk containing a high concentration of somatic cells.

2.5 Data collection

A semi structured questionnaire was prepared and filled to evaluate the effect of potential risk factors on the occurrence of mastitis. All the dairy cows in the farms which were selected were examined and the questionnaire was filled out by asking the owner. It is included age(dentition), breed, body condition ,stage of lactation ,exposure to mastitis,, teat injuries, present of ticks on udder, milk yield, housing and hygienic measures.

2.6 Data Management and Statistical Analysis

The risk factors data collected during the study periods were entered into MS excel and analyzed using SPSS version (18) the statistical analysis and used included comparison of proportions and chi square test which applied statistically significant association existed between predisposing some risk factors with mastitis positively like Age, Presence of tick on udder and previous exposure to mastitis. For all the analysis performed $P \leq 0.05$ was taken as statistically significant.

Chapter Three

Result

California Mastitis Test (CMT) performed on the milk samples of 100 cross and local breed dairy cows in Berber Locality. Table 3.1 showed the result as the following: -

Table 3.1 Distribution of sub-clinical mastitis in 100 examined cows in Berber Locality- River Nile State.

Administration Units	No of sample	Negative	Frequency	Sub Clinical	Frequency
Berber town	77	21	27.3%	56	72.7%
Albawga	6	1	16.7%	5	83%
Alabeedia	5	4	80%	1	20%
South Berber	7	4	57%	3	42.9%
West Berber	5	1	20%	4	80%
Total	100	31	31%	69	69%

S/N	Test Used	No Of Cows Tested	Negative	Average Of Positive	Total
	CMT	100	31	59.8	100

Positive Result: -

1+	2+	3+	4-
30	17	10	12

The Average 30+17+10+12/4=60

Table (3.2): Distribution of mastitis infection among 100 cows examined in

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	+ve	69	69.0	69.0	69.0
	-ve	31	31.0	31.0	100.0
	Total	100	100.0	100.0	

Berber locality.

Overall prevalence of mastitis in 100 examined cows was 69 % (Table 3.1).

3.1 Summary of the Result

3.1.1 Age

The result showed that 77 77.9% were old cows and 23 39.1% of cows were young (Table 3.2).

The prevalence of mastitis within age was 69% in old and 31% in young cows, a Significant association (p-value =.001) was observed between age and mastitis.

3.1.2 Breed

The result showed that 8(87.5%) of the examined cows were local and 92 (67.4%) were cross breed.

The prevalence of mastitis within breed was 87.5% in local and 67.4% in cross breed, there was no association (p-value =.225) between breed and mastitis (Table 3.2).

3.1.3 Body condition

In table (3.2). 67(71.6%) of examined cows were in good body condition, 33(63.6%) in fair body condition.

The prevalence of mastitis was 71.6% in good condition, 63.6% in fair condition

, that was no association (p-value =.278) between body condition and mastitis.

3.1.4 Stage of lactation

Twenty one (76.2%) of examined cows in third stage of lactation and 44 (70.5%) in second stage of lactation and 35 (62.9%) in first stage of lactation. The prevalence of mastitis was 76.2% in third stage of lactation ,70.5% in second stage of lactation ,62.9% in the first stage of lactation, Statistically there was no association (p-value =.558) between stage of lactation and mastitis.

3.1.5 Previous exposures to mastitis

As shown in table3.2, 38(57.9%) of examined animals were not previously exposure to mastitis, but 62(75.8) of them were exposure to the disease (Table

3.2).There was Significant association (p –value=0.049) between previous exposure of the disease and not exposure.

3.1.6 Present of tick on udder

The result showed that 19(89.5%) of examined cows with present of tick on udder and 81(64.2%) with absent of tick on udder.

The prevalence of mastitis was 89.5% in cows with present of tick on udder and 64.2% in cows with absent of tick on udder, a significant association (p-value =.025) was observed between present of tick on udder and mastitis (Table3.2).

3.1.7 Teat injuries

The result showed that 14(71.4%) of examined cows with present of teat injuries and 86(68.6%) with absent of teat injuries. The prevalence of mastitis was 71.4% in cows with present of teat injuries and 68.6% in cows with absent of teat injuries, statistically there was association (p-value =.551) between teat injuries and mastitis

3.1.8 Milk yield

The examined cows with high milk yield were 53(60.4%) animals and 32(78.1%) were moderate and 15(80%) animals were low. The infection rate of these animals was 60%, 78.1%, 80% respectively (Table3.2).There was no association between milk yield and the disease (p-value=.067).

3.1.9 Housing

Sixty six (68.2%) of examined cows were put in healthy houses, but 34(70.6%) of them were put in badly houses (Table3.2).Statistically there was association between animals housing and the disease (p-value=.496).

3.1.10 Hygienic measures

The result showed that 44(72.7%) of examined cows with poor hygienic measures and 56(66.1%) with good hygienic measures. The prevalence rate of mastitis in 56(66.1%) examined cows with good hygienic measures was 66.1% (Table3.2), where as in the 44(72.7%) examined animals was 72.7%.

Statistically there was no association (p-value =.311) between hygienic measures and mastitis.

3.2 Summary of Multivariate Analysis

A significant association was observed between mastitis and some potential risk factors (p-value ≤ 0.25) using Logistic Regression as follows:

Age (p-value =.002), present of tick on udder (p-value =.097), previous exposures to mastitis (p-value =.311).

Chapter Four

Discussion

The present study was to determine the mastitis prevalence, association with important potential risk factors like Age, present of tick on udder and previous exposures to mastitis but other important risk factors it will not Significant due to sample size variation, in the present study the prevalence of subclinical mastitis is 69%, this type of mastitis receives little attention and efforts have been concentrated only on the treatment of clinical cases. The overall prevalence of mastitis was 69% in agreement with previous observation that subclinical mastitis is more prevalent than clinical mastitis with rate of 31.67%and 0.93% of subclinical and clinical mastitis respectively in Gonder /Ethiopia **(Moges *et al*; 2011)** ,23.0% and 1.9% 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 higher 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 .The prevalence is relatively higher than that reported by Biru *et al*;(1998) 35.7%, Biffa *et al*.,(2005), 38.65%, Darsema (1991), 39.5% and Getahun (2006), 36.9% . The difference in prevalence of mastitis in the present study and other reports could probably be due to difference in farms management practices, breed, geographic location ,level of production and study methods and instruments employed by the investigation **(Radostits *et al*;2009)**. The following risk factors showed Significant association with mastitis under a significant level of ≤ 0.05: age (p-value =.001), present of tick on udder (p-value.025 =), previous exposures to mastitis (p-value = 0.049).

Conclusion

Sub - clinical mastitis is the most prevalence 69%, so mastitis in Berber locality is common among herds this indicates that mastitis is serious problem across herds in this area. The main risk factors associated with the mastitis were age, present of ticks on udder, previous exposures to mastitis.

Recommendations

*To reduce the prevalence of disease by using different epidemiological methods.

*Factors that interplay in mastitis occurrence should well studies.

* A practical mastitis control strategy in the herd and national approach is needed.

*Do not keep high parity cows with poor udder health.

*Apply correct treatment of mastitis by consulting a veterinarians.

* Divide herd into group according to udder status in order to establish a milking order.

* Using California mastitis test (CMT) in all farms for early monitoring of the disease.

*Adequate housing with proper sanitation and regular screening for early detection and treatment follow up of chronic cases, culling of older cows with repeated attack are recommended to control the disease.

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Appendix I

Frequency table for the distribution of infection among 100 cows examined at Berber locality according to potential risk factors. A Frequency distribution of Age.

Age					
		Frequency	Percent	Valid Percent	Cumulative Percent
	Valid old	77	77.0	77.0	77.0
	Young	23	23.0	23.0	100.0
	Total	100	100.0	100.0	

B Frequency distribution of breed.

Breed					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Cross	92	92.0	92.0	92.0
	Local	8	8.0	8.0	100.0
	Total	100	100.0	100.0	

C Frequency distribution of body condition.

Body condition					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Fair	33	33.0	33.0	33.0
	Good	67	67.0	67.0	100.0
	Total	100	100.0	100.0	

D Frequency distribution of Stage of lactation.

Stage of lactation					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Third	21	21.0	21.0	21.0
	Second	44	44.0	44.0	65.0
	First	35	35.0	35.0	100.0
	Total	100	100.0	100.0	

E Frequency distribution of exposure of mastitis.

Exposure of mastitis					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	62	62.0	62.0	62.0
	No	38	38.0	38.0	100.0
	Total	100	100.0	100.0	

F Frequency distribution of teat injury.

Teat injury					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	14	14.0	14.0	14.0
	No	86	86.0	86.0	100.0
	Total	100	100.0	100.0	

G Frequency distribution of Present of tick.

Present of tick					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	19	19.0	19.0	19.0
	No	81	81.0	81.0	100.0
	Total	100	100.0	100.0	

H Frequency distribution of milk yield.

Milk yield					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	High	53	53.0	53.0	53.0
	Moderate	32	32.0	32.0	85.0
	Low	15	15.0	15.0	100.0
	Total	100	100.0	100.0	

I Frequency distribution of housing.

Housing					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Not Healthy	34	34.0	34.0	34.0
	Healthy	66	66.0	66.0	100.0
	Total	100	100.0	100.0	

J Frequency distribution of hygienic measures.

Hygienic Measures					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Poor	44	44.0	44.0	44.0
	Good	56	56.0	56.0	100.0
	Total	100	100.0	100.0	

Appendix II

Cross –tabulation for the distribution of infection among 100 cows examined at Berber locality according to potential risk factors.

A. Cows mastitis and age Cross –tabulation.

Count		California Mastitis Test		Total
		+ve	-ve	
Age	Old	60	17	77
	Young	9	14	23
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Breed	Cross	62	30	92
	Local	7	1	8
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Body condition	Fair	21	12	33
	Good	48	19	67
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Stage of lactation	Third	16	5	21
	Second	31	13	44
	First	22	13	35
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Exposure of mastitis	Yes	47	15	62
	No	22	16	38
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Teat injury	Yes	10	4	14
	No	59	27	86
Total		69	31	100

Count	California Mastitis Test		Total
	+ve	-ve	

Present of tick	Yes	17	2	19
	No	52	29	81
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Milk yield	High	32	21	53
	Mode rate	25	7	32
	Low	12	3	15
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Housing	Not Healthy	24	10	34
	Healthy	45	21	66
Total		69	31	100

Count		California Mastitis Test		Total
		+ve	-ve	
Hygienic Measures	Poor	32	12	44
	Good	37	19	56
Total		69	31	100

Appendix III

Univarate analysis for the association of cow's mastitis in 100 cows with potential risk factors using Chi-square test.

A. Age.

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12.459[a]	1	.000		
Likelihood Ratio	11.736	1	.001		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	12.334	1	.000		
N of Valid Cases	100				

B. breed

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.391[a]	1	.238		
Continuity Correction[b]	.610	1	.435		
Likelihood Ratio	1.619	1	.203		
Linear-by-Linear Association	1.377	1	.241		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.662[a]	1	.416		
Continuity Correction[b]	.341	1	.559		
Likelihood Ratio	.654	1	.419		
Linear-by-Linear Association	.656	1	.418		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.169[a]	2	.558
Likelihood Ratio	1.175	2	.556
Linear-by-Linear Association	1.147	1	.284
N of Valid Cases	100		

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.534[a]	1	.060		
Continuity Correction[b]	2.746	1	.097		
Likelihood Ratio	3.483	1	.062		
Linear-by-Linear Association	3.498	1	.061		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.045[a]	1	.832		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.045	1	.831		
Linear-by-Linear Association	.044	1	.833		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.597[a]	1	.032		
Continuity Correction[b]	3.491	1	.062		
Likelihood Ratio	5.365	1	.021		
Linear-by-Linear Association	4.551	1	.033		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.936[a]	2	.140
Likelihood Ratio	4.014	2	.134
Linear-by-Linear Association	3.343	1	.067
N of Valid Cases	100		

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.061[a]	1	.805		
Continuity Correction[b]	.000	1	.985		
Likelihood Ratio	.061	1	.805		
Linear-by-Linear Association	.060	1	.806		
N of Valid Cases	100				

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.510[a]	1	.475		
Continuity Correction[b]	.247	1	.619		
Likelihood Ratio	.514	1	.474		
Linear-by-Linear Association	.505	1	.477		
N of Valid Cases	100				

Appendix IV

Table (3.3) was Summaried frequency of all animals were Examined for Mastitis.

	Frequency	Relative Percent %	Cumulative Percent%
Age			
old	77	69.0	69.0
young	23	31.0	100.0
Breed			
cross	92	92.0	92.0
local	8	8.0	100.0
Body condition			
Fair	33	33.0	33.0
Good	67	67.0	100.0
Stage of lactation			
Third	21	21.0	21.0
Second	44	44.0	100.0
First	35	35.0	
Exposure of mastitis			
Yes	62	62.0	62.0
No	38	38	100.0
Teat injury			
Yes	14	14.0	14.0
No	86	86.0	100.0
Present of tick			
Yes	19	19.0	19
No	81	81.0	100.0
Milk yield			
High	53	53.0	53.0
Moderate	32	32.0	85.0
Low	15	15.0	100.0
Housing			
Not Healthy	34	34.0	34.0
Healthy	66	66.0	100.0
Hygienic Measures			
Poor	44	44.0	44.0
Good	56	56.0	100.0

Table : (3.4) Summary Cross – tabulation of Mastitis in 100 Cows at Berber locality.

Risk factor	Animals tested	Animals affected	Rate of infection %
Age			
Old	77	60	77.9
Young	23	9	39.1
Breed			
Cross	92	62	67.4
Local	8	7	87.5
Body condition			
Fair	33	21	63.6
Good	67	48	71.6
Stage of lactation			
Third	21	16	76.2
Second	44	31	70.5
First	35	22	62.9
Exposure of mastitis			
Yes	62	47	75.8
No	38	22	57.9
Teat injury			
Yes	14	10	71.4
No	86	59	68.6
Present of tick			
Yes	19	17	89.5
No	81	52	64.2

Milk yield			
High	53	32	60.4
Moderate	32	25	78.1
Low	15	12	80
Housing			
Not Healthy	34	24	70.6
Healthy	66	45	68.2
Hygienic Measures			
Poor	44	32	72.7
Good	56	37	66.1

Table (3.2): Summary of univariate analysis potential risk factors of mastitis in 100 cows examined in Berber Locality River Nile State using Chi-square test.

Risk factor	No. inspected	No. affected %	D f	X^2	P-value
Age					
Old	77	60 (77.9)			
Young	23	9(39.1)	1	12.459	.001
Breed					
Cross	92	62(67.4)	1	1.391	.225
Local	8	7(87.5)			
Body condition					
Fair	33	21(63.6)	1	.662	.278
Good	67	48(71.6)			

Stage of lactation					
Third	21	16(76.2)	1	1.169[a]	.558
Second	44	31(70.5)			
First	35	22(62.9)			
Exposure of mastitis					
Yes	62	47(75.5)	1	3.534[a]	0.049
No	38	22(57.9)			
Teat injury					
Yes	14	10(71.4)	1	.045[a]	.551
No	86	59(68.9)			
Present of tick					
Yes	19	17(89.5)	1	4.597[a]	.025
No	81	52(64.2)			
Milk yield					
High	53	32(60.4)	1	3.936[a]	.067
Moderate	32	25(78.1)			
Low	15	12(80)			
Housing					
Not Healthy	34	24(70.6)	1	.061[a]	.496
Healthy	66	45(68.2)			
Hygienic Measures					
Poor	44	32(72.7)	1	1	.311
Good	56	37(66.1)			

Means significant value. P value ≤ 0.05

Table (3.3) Multivariate analysis of Mastitis and potential risk factors in 100 cows examined at Berber locality River Nile State using logistic regression.

Risk factors	Animals affected %	Exp(B)	95% Confidence Interval for Exp (B)		P-value
			Lower Bound	Upper Bound	
Age					
Old	60 (77.9)	4.768	1.720	13.216	.002
Young	9(39.1)	.	.	.	
Exposure of mastitis					
Yes	47(75.5)	1.630	.634	4.188	.311
No	22(57.9)	.	.	.	
Present of tick					
Yes	17(89.5)	3.471	.690	17.467	.097
No	52(64.2)	.	.	.	

***Means significant value.p –value ≤ 0.25.**

Appendix V

Questionnaire for data collection for survey of Bovine Mastitis in Berber locality in River Nile State

Locality: Administration Unite:

Farm No: Date of investigation:

Investigator:

General Characteristics:

Owner :-

Name :

Address :

Telephone No:

Individual risk factors :

1.Age : Old (0) Youing (1)

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

3.Body condition : Fair (0) Good (1)

4.Stage of lactation :Third (0) Second (1) First (2)

5.Exposure to mastitis : Yes (0) No(1)

6.Teat injuries : Yes(0) No(1)

7.Present of tick on udder: Yes(0) No(1)

8.Milk yield : High (0) Moderate(1) Low(2)

9.Housing : Not healthy (0) Healthy (1)

(Housing risk factors inclouding: Type of fencing –Drainage system-Source of water)

10.Hygienic Measures: Poor(0) Good (1)

(Hygienic Measures risk factors inclouding : Sanitary practices-wash hand before milking –dung removing-milking techniques).