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Effect of some Risk Factor on Severity of Mastitis Disease in Autumn Season in Atbara, Sudan

A dissertation submitted to Sudan University of Science and Technology for partial fulfillment of the Requirement for Master Degree of Preventive Veterinary Medicine (M.P.V.M)

By

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Dedication

I devote my study to :

my father & mother

my husband

my kids Eyad & Awab

my sisters Ragda & Sara and my brother Abdalateef

Acknowledgments

First of all, my thanks and praise are due to almighty Allah , and then my sincere thank goes to my supervisor Dr. Isam Mahmoud Mohamed Adam Director of Atbara Animal Research Station, for his guidance help and kindness, also my thanks extended to Dr.Naser Mohamed Osman , Also I want to express my thanks to my Dear friend Dr Salma Mohyeldeen, for her continuous help and support. Faithfully thanks to husband Mardy Mohyeldeen for his tremendous my moral support , help and encouragement . Finally I would like to extend my warm gratitude towards all my colleagues who helped me.

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ABSTRACT

This study was conducted in Atbara for investigating the effect of some risk factor on prevalence of bovine mastitis infection ,the study was also investigated mastitis grade in different udder quarter. 200 sample were collected from 10 dairy farm examined with California mastitis test and then analyzed by using descriptive statistics and Chi-square. The results showed a strong positive correlation between influences factor and prevalence of mastitis infection. Whereas autumn season has a role in the prevalence of mastitis, as well the hygiene and animal cleaning had an effect, there was a positive relationship in case of the nutritional factor, that the infection was greater in the case of irregular feeding, there was a strong positive relationship between healthy milk and healthy animals,and healthy quarters of the udder.
The study also revealed what is the effect of using treatment, most of the breeders tended not to use treatments or uses of alternative treatment (cold water, semsem oil). also the study stated that the fore quarter are more sensitive and prone to infection than the hind quarter of the udder.

ملخص الاطروحة

اجريت هذه الدراسة في مدينة عطبرة لتقصي تأثير بعض عوامل الخطر في انتشار التهاب الضرع وشملت هذه الدراسة ايضا اثر هذه العوامل ودرجة المرض في الارباع الامامية والخلفية للضرع.

تم جمع 200عينة وتم اعتماد فحص كاليفورنيا ومن ثم تحليلها باستخدام الاحصاء الوصفي ومربع كأي. اظهرت النتائج علاقة ايجابية قوية بين العوامل المؤثرة وانتشار المرض ، فصل الخريف كان له اثر ضعيف في انتشار المرض، وكذلك النظافة كان لها اثر حيث اظهرت نسبة كبيرة من عدد الاصابات في حالة عدم النظافة.

وايضا كان هناك علاقة ايجابية في عامل التغذية حيث كانت نسبة الاصابة اكبر في حالة عدم الانتظام بالتغذية .

كذلك كانت العلاقة ايجابية في برنامج الحليب حيث كانت عدد الاصابات قليلة جدا عند بدء الحليب بالحيوانات السليمة والارباع السليمة. كذلك كشفت الدراسة عن اثر استخدام العلاجات (طبية او بديلة) في انتشار المرض حيث كان معظم المربين يميلون لعدم استخدام العلاجات او استخدام العلاجات البديلة (ماء بارد او زيت السمسم). اظهرت الدراسة ايضا اثر هذه العوامل في الارباع الامامية والخلفية للضرع كلا على حدا وجد ان الارباع الامامية اكثر حساسية وعرضه للإصابة من الارباع الخلفية.

Introduction:

The production of milk by dairy farmers is not only a business but also a way of life. Many of the decisions taken on the farm are consequently dictated by both financial constraints and job satisfaction.

River Nile State lies approximately between 22-35 longitude east and 16-22 latitude north, and extend from Elsabuloga falls on River Nile south toward Bayoda desert to the Egyptian boarder north. The River Nile passes through the state from south to north and Atbara ,river passes obliquely through the State from east to west where it meets the River Nile at Atbara town (Mohamed et. al, 1996).

In Sudan 92% percent of livestock population is possessed by nomads that follow extensive system of husbandry in eastern, western and southern part of the Sudan (Kamal, 1983). In Nahr EL-Nil State, the system of husbandry adopted is a semi-intensive one.

Among the Sudanese breeds of cattle, two breeds namely Kenana and Butana are known to show high potentiality for milk production (Alim, 1960; Osman, 1970; Osman and EL Amin, 1971).

Total Sudan cattle population in 2001 was 38.325 million heads. Butana cattle is the dominant breed. Friesian and Butana and Friesian crosses have been recently introduced in the State in order to improve milk productivity to meet the increasing demand for milk as high quality food. In the area where the study was conducted, milk production is so scanty and it is almost always sold by producers in unprocessed form.

Little information is available on diseases of cattle in Nahr EL-Nil State. however, mastitis, pneumonia and tropical theleriosis represent the major diseases of cattle in the State (Elghali and ELHussien, 1995).

Livestock kept or produced in small-scale farming systems are an important component of the agricultural economy in the developing world (McDermott et

al, 1999) and small-scale dairy development is a powerful tool for actively involving the poor in boosting rural economic growth, initiating a process of change and improving livelihoods (FAO, 2009).

The frequency of mastitis in the dairy cow population can be decreased by breeding for cows with better ability to resist udder disease. Udder health is unfavorably genetically correlated with milk yield and selecting only for increased production, which traditionally has been the focus of dairy cattle breeding in many countries, will therefore result in deterioration of udder health. This can be counteracted by applying a broad breeding goal which includes not only production traits, but functional traits such as mastitis resistance. The genetic progress in a trait is partly determined by the relative weight put on it in the total merit index of bulls. In order to assign proper economic weight to mastitis resistance, accurate estimates of the economic loss caused by mastitis are necessary(Carlén et al., 2004). Crossbred cows were more struck by subclinical mastitis than native one, youngest cows had the most sensitivity, hot weather increased frequency (Dar et al., 2014). Mastitic cows tend to have higher milk yield than non-mastitic cows before they develop clinical mastitis (Gröhn et al., 2004), indicating that high milk yield is a risk factor for clinical mastitis (CM). Previous occurrences of mastitis, CM or high somatic cell count (SCC), substantially increased the risk of a cow developing a new case of CM (Steeneveld *et al*., 2008). Cows of certain breeds are more prone to mastitis. Swedish Red cows have a lower incidence of mastitis than Swedish Holstein cows (Persson Waller *et al*.,2009).

prevalence of mastitis in dairy cattle :

Clinical mastitis can be defined as a farmer observed abnormality in the milk and/or the udder'.

Clinical mastitis then, is an observable disease. Cows are visibly sick, or the milk is visibly abnormal.

In most studies the median incidence is around 20-25 cases per 100 cows per year. Clinical mastitis occurs in all dairy herds. Even well-managed herds, as judged by somatic cell count level and a high level of milk production, may be suffering from a high incidence of clinical mastitis (Schukken *et al*. 1989).

Studies conducted in different parts of sub-Saharan Africa (SSA) reveal that mastitis, SCM and CM are widespread in the small-scale dairy cow sector. In fact, in numerous studies prevalence exceeds 50%, and it is clearly a threat for small-scale producers. In East and West Africa, most milk is produced by smallscale dairy units, so it is evident that the disease not only has a negative impact at farm level, but also more globally in the dairy sector in general.. Similarly, in a range of East African countries attention has been given to this disease, studying the prevalence, the risks and also the awareness of farmers. All studies reported that a high prevalence (16–80%) of sub-clinical mastitis, implying that it could be among the major constraints limiting optimum productivity in small-scale dairy cattle operations (Girma *et al*., 2012).

From the annual report of veterinary services of the River Nile State , Sudan, mastitis was the third disease after external parasites and pneumonia in cases which brought to the veterinary clinics of the state (Annual report, 2016). The prevalence of clinical and sub-clinical mastitis at cow level was 10.5% and 72% respectively in Sudan (Albagir *et al*., 2015).

Objectives of the Study

This study is aiming at studing the effect of some risk factors on severity of mastitis disease in Autumn season in Atbara.

Chapter one

Literature Review

Aspect concerned mastitis as general :

1.1 Definition and identification of mastitis:

During the last two decades milk production has grown into a specialized business. economic pressures make it vital for farmers to increase the profitability of their dairy herds; a goal only to be achieved by the intelligent application such as good husbandry based on the scientific principles involved in breeding management and feeding of cow (Leaver,1982) .it is well established that bovine mastitis is of great economic importance to the dairy industry (Blosser,1979; Meyer,1980; Miller,*et. al.*,1984).

The infection of the udder with microorganisms capable of causing mastitis. Hurley and Morin (1996) define mastitis as intra mammary infection, primarily bacterial infection, but also Mycoplasmal, Mycotic, . Whereas Jones and Bailey (1998) consider mastitis when the udder becomes inflamed because leukocytes are released into the mammary gland in response to invasion of the teat canal usually by bacteria. These bacteria multiply and produce toxins that cause injury to milk secreting tissues and various ducts throughout the mammary gland.

Elevated leukocytes, or somatic cells cause a reduction in milk production and alter milk composition (Jones, and Bailey,1998).

Mastitis is also defined by Cole (1962) as inflammation of the mammary gland caused by microbial infection or undue stress or both. Some investigators believe that the mere presence of pathogens in milk is not indicative of mastitis. The increase in somatic cell count and the finding of pathogens in milk samples give positive diagnosis (McDonald, 1977). The two criteria, somatic cell count and isolation of pathogens are necessary to differentiate actual cases of mastitis from mere contamination (McDonald, 1977). According to National Mastitis

Council's (1996): mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying or neutralizing the infectious agents and to prepare the way for healing and return to normal function.

Inflammation can be caused by many types of injury irritants. In the dairy cow, mastitis is nearly always caused by microorganisms, usually bacteria that invade the udder, multiply in the milk producing tissues and produce toxins that are immediate cause of injury.

1.2 Classification of mastitis:-

Craplet (1963) classified the disease on the following criteria:

A- By symptoms differentiates between :-

- Acute mastitis that develops rapidly with severe congestion and rapidly but is of infrequent occurrence, easily diagnosed and of reduced economic importance
- chronic mastitis, the development of which is more and less obvious clinically with a high frequency in occurrence.

B- By clinical characteristics which consider the mastitis condition as gangrenous, catarrhal, parenchymatous or suppurative.

C- By stage of lactation :-

Beginning of lactation, drying off and dry period .

D-By the causal organism :-

Contagious or chronic mastitis caused by *Streptococcus agalactia* and common mastitis caused by streptococcus groups, staphylococcus groups, *Corynebacterium pyogenes, Escherichia.coli Pseudomonas, Proteus*, yeast and bacteria species. Radostitis, Blood and Gay (1994) classified mastitis into two forms, clinical and sub clinical mastitis. Clinical mastitis is characterized by apparent changes of both milk and mammary gland and subclinical mastitis in which there are no apparent changes.

clinical mastitis are classified in to:

a- Peracute mastitis:

In this type there is marked abnormality of milk and udder with severe systemic reaction.

b- Acute mastitis:

Characterized by severe inflammation of the udder without marked systemic reaction.

c- Sub acute mastitis:

There is mild inflammation of the mammary gland and
abnormal milk secretion.

d- Chronic mastitis:

Most changes are detected in the milk with recurrent attacks of inflammation.

In subclinical mastitis there are no visible abnormalities of milk or udder.

Were subclinical mastitis is characterized by an increase in somatic cell and/ or leukocyte count (Radostitis, Blood and Gay, 1994). It is a problem of the herd rather than individual animals.

1-3. Signs of mastitis:

Michel (2000) described the signs of mastitis according to the mastitis form. In clinical mastitis, the infected quarter often becomes swollen, some times painful to touch and the milk is visibly altered by the presence of clots, flakes or discolored serum and some times blood.

In severe cases (acute mastitis), the cow shows signs of
generalized reaction: fever, rapid pulse, loss of appetite and sharp decline in milk production. In contrast, subclinical mastitis is subtle and more difficult to detect.

The cow appears healthy, the udder does not show any signs of inflammation and the milk seems normal. However, microorganisms and white blood cells (somatic cells) that fight infections are found in elevated numbers in the milk.

Schroeder(1997) characterized subclinical mastitis by lack of consistent, visible and elevation of somatic cells count of the milk.

Bacteriological culturing of milk will detect bacteria in milk and this form causes the greatest loss in dairy farms through lowered milk production.

1-4. Etiology of mastitis:

Of the several causes of mastitis, only microbial infection is important. Although fungi, yeasts and possibly virus can cause udder infection.

2-4-1. Etiology of clinical mastitis:

Microbial causes of clinical mastitis include *Staphylococcus aureus* (Bryson, 1973), *Streptococcus agalactiae* (Gonzales *et. al*, 1988), *Escherchia coli* (Schukken *et. al*, 1989), *Pseudomonas aeruginosa* (Howel, 1972) *Klebsiella pneumoniae* and *Proteus* spp . (Park, 1979), *Streptococcus dysagalactiae* (Schaufuss *et. al*, 1968), *Streptococcus ubris*, *Streptococcus faecalis*, (Schaufuss *et. al*, 1968), *Streptococcus faecium, Streptococcus bovis* (Boutrel and Runiewiez, 1984*), Micrococcus spp*. (Jha *et al*, 1994), Coagulase negative *Staphylococci* (Gonzalez *et. al*, 1988), *Pasteurella multocida* (Pasco, 1960, and O'sullivan *et. al*, 1971),*Corynebacterium spp*. (*Costa et al*, 1987) *Mycobacterium spp* .(Oudar *et. al*, 1966), *Bacillus cereus* (Nielsen, 1972), *Yersinia pseudotuberculosis* (Messerli, 1972), *Serratia marcensce* (Kim and Kim, 1979), and *Mycoplama spp*. (Blood and Henderson, 1989).

However the predominant bacterium incriminated in acute bovine mastitis is *Staphylococcus aureus* (Bryson,1973; Pearson and Macki, 1979; Innes and Lynch, 1990) followed by *Streptococcus agalactiae*
(Gonzalez *et. al*,1988; Innes and Lynch,1990) or *Escherichia coli* (Jha *et. al*, 1994).

In the Sudan, *Staphylococcus auerus* was considered as the major bacteria isolated from bovine clinical mastitis (Mamoun and Bakheit, 1992) followed by *Streptococcus agalactiae*. Other organisms isolated include *Bacillus cereus* (Adlan *et. al*, 1980), *Escherichia coli* (Haghour and Ibrahim, 1980) ,*Klebsiella pneumoniae* (Bagadi, 1972) and *Staphylococcus epidermidis* .

1-4-2. Etiology of subclinical mastitis:

As in the case of clinical mastitis, many organisms have been isolated from cases of subclinical mastitis. These include *Streptococcus lactis*, and *Enterococcus faecalis* (Keskintepe *et. al,* 1992) *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae*, *Streptococcus uberis*, *Escherichia coli*, *Pseudomonas aeruginosa*, (Bozkir, 1986 and Aydin *et. al,* 1995), *Coryne-bacterium* spp., *Micrococcus* spp. (Costa *et. al,* 1987).

In the Sudan several bacteria have been isolated from cases of subclinical mastitis. These include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium spp., Pseudomonas spp. Streptococcus agalctia*, *Streptococcus dysagalactia* and *Micrococcus* spp.,(Shalallai *et. al,* 1992). High incidence of subclinical mastitis has been reported in Khartoum and the commonest species of bacteria isolated were *Enterococcus faecalis, Enterococcus faecium*, *Streptococcus bovis*, *Streptococcus equi*, *Streptococcus lactis* and *Streptococcus pyogenes* (Hashim *et. al,* 1991).

1-5. Pathogenesis of mastitis:

Infections begin when microorganisms penetrate the teat canal and multiply in the mammary gland. Irritation of the teat most often occurs during milking. Organisms present in the milk or at the teat end are propelled into the teat canal. After milking, the teat canal remains dilated for one to two hours. Organisms from the environment (Manure, bedding, etc.) or those found on injured skin at the tip of the teat may easily invade an open or partially open canal.

Some bacteria may proceed into the udder by attaching and colonizing new tissues; others my move around via milk current produced. Bacteria first damages the tissues lining the large milk collecting ducts. The bacteria may encounter leukocytes (white blood cells) present naturally in small numbers in milk. These cells are the cow's second line of defense because they can

engulf and destroy bacteria. If bacteria are not entirely destroyed, they continue to multiply and begin to invade smaller ducts and alveolar areas.

Milk secreting cells are damaged by toxins and other released irritants substances (chemotaxes factors) that lead to increased permeability of blood vessels. Additional leukocytes move to the site of infection . they enter the alveolar tissue in great numbers by squeezing between the damaged milk secreting cells . Fluid, minerals and clotting factors also leak into the affected area.

Clotted milk may close milk ducts. Sometimes the microorganisms are eliminated rapidly and the infection is cleared. In this case , the clogged ducts are opened and milk composition and production return to normal in several days. However as the infection persists and ducts remain clogged , the entrapped milk causes the secretory cells to revert to arresting (non-producing) state and the alveoli begin to shrink. Substances released by leukocytes lead to the complete destruction of alveolar structures, which are replaced by connective and scar tissues. Thus as the disease progresses the number of somatic cells in the milk becomes elevated and this are associated with a permanent reduction in milk yield (Michel,2000, Hurley and Morin,1996).

1-6. Factors affecting susceptibility to mastitis:

Hurley and Morin (1996) discussed the factors which lead to susceptibility of mammary gland to infection. Whether or not intramammary infection occurs depends on the interaction of host, agent and environmental factors. Host factors include, the presence or absence of natural resistance to mastitis, the state of defense mechanisms, the stage of lactation. Whereas agent factors include the number of organisms in the gland, the pathogencity of the organisms and the presence of other virulence factors. In addition the environmental factors include, the milking environment, the milking practices hygiene, the type of housing, bedding and the weather.

1-6-1. The Peripartum period:-

In the peripartum period, several defense mechanisms are compromised just prior to and after parturition which predispose the gland to mastitis.

Fluid volume in the gland increases resulting in increased intra mammary pressure and dilatation of the teat canal and sometimes leakage of colostrums.

Citrate concentration rises and lactoferrin is low. Phagocytic cells are not efficient in engulfing and killing bacteria in colostrum contained in the gland at this time.

High immunoglobulin concentration in the gland at this time is not effective in preventing new intra mammary infection. IgG1, the major immunoglobulin isotype in cow colostrum, is not normally an effective opsonin in the mammary gland.

Antibiotic concentration from dry cow antibiotic therapy is too low to combat infection and teat dipping during this period is not particularly effective in mastitis prevention.

1-6-2.Deficiencies of vitamins and minerals:

Deficiencies shown to be related to increased incidence of clinical or subclinical mastitis increased severity of infection , or elevated somatic cell counts, include Selenium, Vitamin E, Vitamin A, Zinc, Cobalt and some others deficiencies.(Hurley and Morin,1996).

1-7. Mastitis caused by different organisms:-

1-7-1. *Staphylococcal mastitis*:-

Staphylococci are widely distributed in nature where they make up the normal bacterial flora of the mucous membranes and the skin (Zingeser *et. al*, 1991).

Staphylococci are gram positive, catalase positive and ferment glucose. they are classified according to the coagulation of human or rabbit plasma in to coagulase postive staphylococci represented by *Staphylococcus aureus* and coagulase negative *Staphylococci* such as *Staphylococcus epidermidis* (Baird and Parker,1962). Staphylococci were found to be the most frequent causative

agents of mastitis among cattle (Cargil and Bootas, 1970; Kapur and Singh, 1978).

Staphylococcus aureus is known to cause peracute, sub acute and chronic mastitis, in addition to gangerinous mastitis (Radostitis and Gay, 1994). it also acts as contaminant of milk (VanDijk and Swanberge, 1963; Zingeser *et. al*, 1991; Lafont and Lafont, 1985).

On the other hand coagulase negative *Staphylococci* were identified as primary caustive agent of cattle mastitis during first lactation (Timms and Schultz, 1987; Derieze and Keyser, 1980).

1-7-2. *Streptococcal mastitis*:-

Streptococci are most common upon skin, mucous

membranes and intestine of man and animals (Garge and Mital,1990). they are classified according to precipitation reaction of specific carbohydrate antigens into 12 groups (Merchant and Packer,1967). Streptococci are the second most common pathogens isolated from cows' milk (Sharma and Pasker, 1970; Ahmed *et. al,* 1991).*streptococcus agalactiae* represents the major streptococcus species that cause mastitis in cattle (Costa *et. al*, 1991). however that is known as environmental mastitis. Enterococci that include *Enterococcus faecalis, Enterococcus faecium* and *Streptococcus bovis* gain entry into milk and milk products through water supply,

equipments and insanitary and unhygienic conditions of production and handling (Hashim *et. al,* 1991), They have been incriminated as direct or indirect causes of the disease (Garge and Mital, 1991).

1-7-3. Coliform mastitis:-

Coliform mastitis is an udder infection of cows caused by *Escherichia coli, klebsiela pneumoniae* and *Enterobacter aerogenes* (Odongo and Ambani, 1989) . They are gram – negative bacilli, that inhabit water and soil and transmitted by flies , contaminated water and feed (Merchant and Packer, 1967).

Escherichia coli was defined as the most common Gram

-negative bacilli associated with clinical and subclinical mastitis (Jha *et. al*,1994; Elliot et al,1976) and causes sudden sharp drop in production of milk (Mustafa *et al* ,1977).

Escherichia coli was also isolated from udders of cattle at calving and during dry period (Timms and Schultz, 1987) .

Escherich coli may cause acute and peracute form of clinical mastitis (Radostitis and gay, 1994).

It has been isolated from milk, teat canal and teat wall puncture milk samples as contaminants (Preeze, 1988; Mammoun ,1981). *Klebsiella* is the second most common Gram-negative bacillus isolated from cattle milk infected with mastitis (McDonald et al 1970; Howel et al, 1972). *Enterobacter* spp. were found to cause bovine mastitis (Park,1979; Haghour and Ibrahim ,1980).

1-8. Diagnosis of mastitis:-

Identification of cows infected with mastitis is necessary to make decisions regarding treatment,culling or isolation of infected animals. Common methods used to identify infected cows include: milk microbiology ("cultures"), the California Mastitis Test (CMT),. Microbiologic exam of milk samples may be used for control programs (such as segregation plans) or for detection of new pathogens. Culturing is also used to determine antibiotic susceptibility of mastitis pathogens. Microbiologic examination of milk samples is often considered to be the gold standard for identification of infected quarters. Negative results (no growth of bacteria from samples of animals suspected to be infected) are a common outcome. No bacteria were isolated from approximately one-third of milk samples submitted to a major mastitis laboratory in Wisconsin between 1994 and 2001 (Makovec and Ruegg, 2003). To ensure that meaningful data will be obtained from milk samples taken from cows with suspected subclinical infections, at least 25 quarter samples should be submitted for culture.

1-8-1. Clinical mastitis:-

Diagnosis of clinical mastitis can be achieved by visual examination of both milk and mammary gland where the abnormality could be detected easily (Blood and Henderson , 1989) Confirmation of diagnosis is usually done by the isolation of causative agent (Emanuelson et al,1986).

1-8-2. Subclinical mastitis:-

Diagnosis of subclinical mastitis presents a problem due to unapparent signs, however several screening tests have been developed beside culturing methods. Culturing method is not suitable for large scale monitoring of udder health (Emanuelson *et. al*, 1986).

Screening tests commonly used include various inflammatory mediators such has somatic cell, cerum proteins,emzymes such as NAGas (N-acetyl-B-D Glucosaminidase), sodium and chloride concentration were conductivity test, directly and indirectly used for somatic cell count.(Kitchen *et. al*, 1978; Sandholm, 1983 and Mancini *et. al*, 1995).

1-9. Somatic cell count:-

Somatic cells are defined as epithelial cells or neutrophils derived from the blood (Schalm and lasmanis ,1968).

Normally milk contains somatic cells and the number of these cells in normal quarter milk is less than 10.000 cells / ml/,however , counts as greatas 250.000 cell/ml are found in normal milk samples (Blood and Henderson, 1989).

The preferable method used for indirect counting of somatic cell is the California Mastitis Test (C.M.T.) (Brook banks, 1966).

1-10. Economic importance of mastitis:

Mastitis in developed countries is considered as the most important and main disease of dairy cows (kaneen and Bandhard, 1990). It is of great economic importance due to reduction in milk yield, change in milk quality ,the possibility of permanent damage of a quarter or even the entire udder and death of the cow (ElTayeb and Habiballa,1978). the loss of milk yield due to clinical mastitis may reach 40% whereas in subclinical mastitis it may reach 60%

(Dijkhuizen and Stelwagen , 1981). In Sudan, clinical and sub clinical mastitis are lead to substantial (20%) drop in milk (Musstafa et al, 1977). schroeder (1997) estimated the economic loss from mastitis in the United States is estimated to be approximately $ 185/cow annually.

1-11.Treatment:

Generally, mastitis treatment choices are made on the basis of bacteriological culture and antimicrobial susceptibility testing when applicable. The choice of treatment is also made from clinical manifestation and prognosis, and depends on legislation and available drugs. The prognosis after treatment is defined by pathogen, antimicrobial susceptibility, chronicity of infection, infection load, age of the cow, breed, and number of quarters affected. Treatment during the dry-off period is an efficient method for the cure of subclinical mastitis and for control of somatic cell counting. Drugs should be administered for period of 5 to 10 days to allow efficacy of the product. The use of antibiotic is recommended in some cases. cure rates may vary from animal to animal and according to the severity of the case. After treating with antibiotic it is necessary to withdraw drug treatment to prevent antibiotic from building up in the milk and meat that can be hazardous to humans.(Samia Abdallah, 2012).

1-12. Prevention and control:

The best way to reduce prevalence of mastitis is to prevent new IMI. However, few intervention studies have been performed and preventive measures are generally suggested on the basis of risk factors associated with mastitis. mainly prevented through improvements in milking hygiene, use of post-milking teat disinfectants, blanket dry-cow therapy, and treatment, segregation, or culling of infected animals, more knowledge about the impact of udder pathogens and bacterial genotype on udder health and production, as well as a better understanding of herd variation in IMI occurrence in regards to species, genotypes of species, seasonal variations, and parities is needed. With more knowledge about spread of infections, and more knowledge about which infections have the highest impact on udder health, the best prevention methods,

motivated both economically and by animal welfare, can more easily be chosen. The ways available to protect livestock from infectious diseases are by increasing the host's defense and by preventing the animals from meeting the contagion. Through breeding regimes, animals have become more tolerant or even resistant to some diseases. Generally, by providing good hygienic conditions, the disease pressure can be diminished (Payne and Wilson, 1999). By proper management of the grazing environment, many parasitic diseases can be controlled. In a housed environment, other ways of spreading diseases has to be taken into consideration, many animals then have close contact in a limited area, the walls and floors may harbor infectious agents. Control of diseases also involves vaccination, vector control (e.g.dipping) and for young animals by securing colostral immunity (Bo Algers et al., 2009).

Chapter Two

Material and Methods

2.1 Study Area:

This investigation is conducted in Atbara Livestock Research Station. The station is situated in the River Nile State. It is located at 17/42° N Latitude and 33/58° E Longitude at an altitude of approximately 345 meter above sea level. The population was estimated in (2010) about 139.264 . it is located at the junction of the Nile and Atbara rivers. It is an important railway junction and railroad manufacturing centre and most employment in Atbara is related to the rail ways. The city also is home to one of sudan largest cement factories (Atbara cement corporation).

The state surface area is about 124.000 km2 and the climate is that of a dry hot desert where annual rainfall varies from zero in the northern part to150.m.m in the southern one. At Atbara there are three seasons winter (November – February) dry summer (March-June) and wet summer (July-October) (Hewiston,1945). The atmospheric temperature in this area varies from a maximum of 47.7°C recorded in April, to the lowest minimum of 4.5°C was registered in January .However Atbara falls in atypical semi-desert ecological zone with an average annual precipitation of 70m.m. Trees and grasses such as Acacia. Cherenbergiana and Aristida spp. Represent the natural pastures beside the irrigated land around the river blanks which is considerably small (Mohammed et al 1996).

2.2 Herd Management:-

Cows were fed on irrigated forage crops and grasses together with locally available concentrate feeds .In Atbara dairy Farm animals are allowed to graze sorghum grass and legumes twice daily for five hours whereas in the other farms animals were fed the same grasses in yard. The milking cows are fed a

concentrate mixture composed of 34.5% cotton seed cakes or peanut oil meal, 34.5% wheat bran and 30% sorghum grains where in Atbara dairy farm milking cows are fed 50% Molasses , 30% wheat bran, 10% sorghum grains ,5% peanut oil meal, 3% urea and 2% salt and vitamins. Some changes to the general pattern of feeding policy either as deliberate policy or due to circumstances prevailing at particular occasion . Only natural mating was practiced. Bulls run freely with the appropriate herd groups .However, breeding bulls were selected from the offspring of highest yielding dams in the herd. Calves in Atbara dairy farm are bucket fed on colostrum and fresh milk to an age of one month after which the amount is reduced gradually as the calf advances in age and substitution amount of grass and concentrates are given in small amounts. In the private sectors calves are reared by lactating the dam beside feeding the calf. All cows are milked is done by hand twice daily. Animals are vaccinated against the major infectious livestock disease in Sudan.

2.3. Subjects and plan:-

A total of 200 sample were tested for mastitis collected from apparently healthy local and cross bred (Local X Friesian). The samples location of herds kept in dairy farm around Atbara town .I went every month to collect samples for a period of four consecutive months.

2.4 samples collection:-

Milk sample were a septically collected from apparently healthy tested cows. Milk sample were drawn after morning milking . after discarded the first few ml of milk the teat end of each udder half was thoroughly cleaned with cotton soaked in 70% ethanol.

2.5 Reagents used:-

California Mastitis Test. (C.M.T.)

C.M.T. was performed on milk samples collected from individual udder quarters to detect subclinical mastitis. Before collection of milk sample for bacteriological examination, 2ml of fore milk were squeezed from each quarter into the cup of the paddle where equal volume of California mastitis test reagent (Alvetera rapid mastitis test kit-Alvetera Gmbh-Germany) was added. The paddle is rotated to mix the contets .inapproximately 10 secnds the score should be read . while continuing to rotate the paddle . because the reaction disappears within 20 seconds the test must be read quicly . the reaction between them was interpreted according to Schalm and Noorlander (1957) as follows:

Negative (-ve):- The mixture remained liquid, homogenous with no evidence of thickening.

Trace (T):- There was a slight thickening that was seen best by tipping the paddle back and forth and observing the mixture as it Materials and Methods 30 flows over the bottom of the cup. Trace reactions tended to disappear with continued rotation of the paddle.

Weak positive (+1):- A distinct thickening of the liquid formed, but there was no tendency toward a gel formation.

Distinct positive (+2):- The mixture thickened immediately, and a gel formation was suggested.

Strong positive (+3):- A gel was formed, which caused the surface of the mixture to become elevated like a partially fried egg. (Duane, 1997).

The C.M.T. reaction scores.

C.M.T score	Average somatic count (cells / ml)
Negative	100,000
Trace	300,000
+1	900,000
+2	2,700,000
+3	8,100,000

Chapter Three

Results

3.1 Result of the field test:

3.1.1 Association potential risk factor with prevalence of mastitis infection.

Table(1) :

Results showed no association between season and prevalence of mastitis infection p.value (0.852) in(table1) while there was a high significant p.value 0.000 between animal cleaning and prevalence of mastitis . Mastitis prevalence was 47(52.0%) from 104 dirty cases. chi square test revealed positive association between nutrition and prevalence of mastitis p.value 0.000 (86.8%) positive cases that is changed nutrition. an association between milking non infected animal firstly and prevalence of mastitis 0.000 was observed . 47(80.8%) positive cases that is beginning milking with infected animal firstly. Also chi square test revealed an association between milking non infected udder quarter firstly and prevalence of mastitis 0.000 . 38(100.0%) of positive cases that is start milking with infected udder quarter firstly. and the result revealed an association between the use of treatments and prevalence of mastitis 17(100.0%) in use of intra mammary infusion and 9(5.9%) in non uses of treatment.

Factor	No. of cases	No. positive (%)	df	χ^2	P. value
Season					
Early autumn	50	13 (26.0)	2	0.320	0.852
Mid autumn	100	26 (26.0)			
Late autumn	50	11 (22.0)			
Animal cleaning					
Clean	96	3 (1.5)	1	47.115	0.000**
Dirty	104	47 (52.0)			
Nutrition					
Fixed	147	4 (2.7)	1	146.800	0.000**
Changed	53	46 (86.8)			
Milking non-infected animal firstly					
Yes	148	8 (5.4)	1	116.6	0.000**
NO	52	47 (80.8)			
Milking non-infected udder quarter firstly					
Yes	162	12 (81.0)	1	140.7	0.000**
NO	38	38 (100.0)			
Uses of treatments					
NO	152	9 (5.9)	3	128.9	0.000**
Cold water	23	16 (69.6)			
Sesame oil	8	8 (100.0)			
Intra mammary infusion	17	17 (100.0)			
**= significant at P<0.01, df = degree of freedom.					

3.1.2 Effect of different risk factor on mastitis grade of infection of front and hind udder part.

Table (2):

Result Showed there were no significant differences p.value 0.821 between the effect of different rainy season stage and mastitis grade of infection of front udder part it reach the highest level 70.0% in mid-Autumn stage and lowest level 10.0% in late Autumn .

3.2 Effect of different rainy season stage on mastitis grade of infection of front and hid udder part

Table 2. Effect of different rainy season stage on mastitis grade of infection of front udder part

Mastitis grades	Autumn stages						Total	
	Early		Mid		Late			
	n	%	n	%	n	%	n	%
Negative	43	25.4	83	49.1	43	25.4	169	84.5
Weak Positive	5	26.3	9	47.4	5	26.3	19	9.5
Distinct Positive	2	20.0	7	70.0	1	10.0	10	5.0
Strong positive	0	0.0	1	50.0	1	50.0	2	1.0
Total	50	50.0	100	50.0	50	25.0	200	100.0
χ^2 value=2.906, P. value=0.821 (no significant differences)								

Table (3):

Result showed there were no significant differences 0.519 between the effect of different rainy season stage and mastitis grade of infection of hind udder part it reach highest level 71.4% in mid-Autumn stage and lowest level 20.0% in late Autumn stage.

Table 3. Effect of different rainy season stage on mastitis grade of infection of hind udder part

Mastitis grades	**Autumn stages**						**Total**	
	Early		**Mid**		**Late**			
	n	%	n	%	n	%	n	%
Negative	41	24.1	86	50.6	43	25.3	170	85.0
Weak Positive	8	40.0	8	40.0	4	20.0	20	10.0
Distinct positive	0	0.0	5	71.4	2	28.6	7	3.5
Strong positive	1	33.3	1	33.3	1	33.4	3	1.5
Total	50	50.0	100	50.0	50	25.0	200	100.0
χ^2 value=5.196, P. value=0.519 (no significant differences)								

Table (4):

Results showed there was an association between barns cleaning and mastitis grade of infection of front udder part 0.000 .(94.7%) of not cleaned.

Table 4. Association between barns cleaning and mastitis grade of infection of front udder part

Mastitis grades	**Barn cleaning**				**Total**	
	Not cleaned		**cleaned**			
	n	%	n	%	n	%
Negative	74	43.8	95	52.2	169	84.5
Weak Positive	18	94.7	1	5.3	19	9.5
Distinct positive	10	100.0	0	0.0	10	5.0
Strong positive	2	100.0	0	0.0	2	1.0
Total	104	52.0	96	48.0	200	100.0
χ^2 value=29.547, P. value=0.000						

Table (5):

Results showed that there was an association between barns cleaning and mastitis grade of infection of hind udder part 0.000 (90.0%) of not cleaned.

Table 5. Association between barns cleaning and mastitis grade of infection of hind udder part

Mastitis grades	**Barn cleaning**				**Total**	
	Not cleaned		**cleaned**			
	n	%	n	%	n	%
Negative	77z	45.3	93	54.7	170	85.0
Weak Positive	18	90.0	2	10.0	20	10.0
Distinct positive	7	100.0	0	0.0	7	3.5
Strong positive	3	100.0	0	0.0	3	1.5
Total	105	52.5	95	47.5	200	100.0
χ^2 value=24.152, P. value=0.000						

Table (6):

Result showed that there was a relationship between nutrition stability and mastitis grade of infection of front udder part by 0.000 (100.0%) of changed nutrition status.

Table 6. Relationship between nutrition stability and mastitis grade of infection of front udder part

Mastitis grades	Nutrition status				Total	
	Changed		Fixed			
	n	%	n	%	n	%
Negative	24	14.2	145	85.8	169	84.5
Weak Positive	17	89.5	2	10.5	19	9.5
Distinct positive	10	100.0	0	0.0	10	5.0
Strong positive	2	100.0	0	0.0	2	1.0
Total	53	26.5	147	73.5	200	100.0
χ^2 value=85.092, P. value=0.000						

Table (7):

Result showed that there was a relationship between nutrition stability and mastitis grade of infection in front udder part by 0.000 (100.0%) of changed nutrition status.

Table 7. Relationship between nutrition stability and mastitis grade of infection of hind udder part

Mastitis grades	**Nutrition status**				**Total**	
	Changed		**Fixed**			
	n	%	n	%	n	%
Negative	27	15.9	143	84.1	170	85.0
Weak Positive	17	85.0	3	15.0	20	10.0
Distinct positive	7	100.0	0	0.0	7	3.5
Strong positive	3	100.0	0	0.0	3	1.5
Total	54	27.0	147	73.0	200	100.0
χ^2 value=73.360, P. value=0.000						

Table (8):

Chi square test revealed a relationship between milking of non-infected animals and mastitis grade of infection in front udder part by 0.000 .(85.8%) beginning with non-infected animals and 100.0% beginning with infected animals.

Table 8. Relationship between milking of non-infected animals and mastitis grade of infection of front udder part

Mastitis grades	**Beginning with non-infected animals**				**Total**	
	Yes		**No**			
	n	%	n	%	n	%
Negative	145	85.8	24	14.2	169	84.5
Weak Positive	3	15.8	16	84.2	19	9.5
Distinctpositive	0	0.0	10	100.0	10	5.0
Strong positive	0	0.0	2	100.0	2	1.0
Total	148	74.0	52	26.0	200	100.0
χ^2 value=79.844, P. value=0.000						

Table (9):

Chi square test revealed the relationship between milking of non-infected animal and mastitis grade of infection in hind udder part by 0.000 . (84.1%) of beginning with non-infected animal and 100.0% beginning with infected animals.

Table 9. Relationship between milking of non-infected animals and mastitis grade of infection of hind udder part

Mastitis grades	**Beginning with non-infected animals**				**Total**	
	Yes		**No**			
	n	%	n	%	n	%
Negative	143	84.1	27	15.9	170	85.0
Weak Positive	5	25.0	15	75.0	20	10.0
Distinctpositive	0	0.0	7	100.0	7	3.5
Strong positive	0	0.0	3	100.0	3	1.5
Total	162	81.0	38	19.0	200	100.0
χ^2 value=62.042, P. value=0.000						

Table (10):

Results showed an association between milking of non-infected quarter and mastitis grade of infection of front udder part by 0.000 (92.3)beginning with non-infected quarter and 100.0% of beginning with infected quarter

Table 10. Association between milking of non-infected quarter and mastitis grade of infection of front udder part

Mastitis grades	**Beginning with non-infected quarter**				**Total**	
	Yes		**No**			
	n	%	n	%	n	%
Negative	156	92.3	13	7.7	169	84.5
Weak Positive	5	26.3	14	73.7	19	9.5
Distinctpositive	1	10.0	9	90.0	10	5.0
Strong positive	0	0.0	2	100.0	2	1.0
Total	162	81.0	38	19.0	200	100.0
χ^2 value=92.240, P. value=0.000						

Table (11):

Results showed an association between milking of non-infected quarter and mastitis grade of infection of front udder part by 0.000 (91.8%) of beginning with infected quarter

Table 11. Association between milking of non-infected quarter and mastitis grade of infection of hind udder part

Mastitis grades	**Beginning with non-infected quarter**				**Total**	
	Yes		**No**			
	n	%	n	%	n	%
Negative	156	91.8	14	8.2	170	85.0
Weak Positive	6	30.0	14	70.0	20	10.0
Distinctpositive	0	0.0	7	100.0	7	3.5
Strong positive	0	0.0	3	100.0	3	1.5
Total	162	81.0	38	19.0	200	100.0
χ^2 value=88.701, P. value=0.000						

Table(12)

Results showed an effect of used different curing treatment on mastitis grade of infection of front udder part and found high significant in curing treatment by medicament (100%).

Table 12. Effect of uses different curing treatment on mastitis grade of infection of front udder part

Mastitis grades	**Curing treatments**								**Total**	
	None		**Cold water**		**Sesame oil**		**Medicaments**			
	n	%	n	%	n	%	n	%	n	%
Negative	146	86.4	14	8.3	4	2.4	5	3.0	169	84.5
Weak Positive	6	31.6	7	36.8	0	0.0	6	31.6	19	9.5
Distinct positive	0	0.0	2	20.0	4	40.0	4	40.0	10	5.0
Strong positive	0	0.0	0	0.0	0	0.0	2	100.0	2	1.0
Total	152	76.0	23	11.5	8	4.0	17.0	8.5	200	100.0
χ^2 value=113.2, P. value=0.000										

Table(13)

Results showed an effect of used different curing treatment on mastitis grade of infection of hind udder part and found high significant in non-curing by treatment.

Table 13. Effect of uses different curing treatment on mastitis grade of infection of hind udder part

Mastitis grades	Curing treatments								Total	
	None		Cold water		Sesame oil		Medicaments			
	n	%	n	%	n	%	n	%	n	%
Negative	148	87.1	14	8.2	3	1.8	5	2.9	170	85.0
Weak Positive	3	15.0	9	45.0	1	5.0	7	35.0	20	10.0
Distinct positive	0	0.0	0	0.0	3	42.9	4	57.1	7	3.5
Strong positive	0	0.0	1	33.3	1	33.3	1	33.4	3	1.5
Total	151	75.5	24	12.0	8	4.0	17	8.5	200	100.0
χ^2 value=122.9, P. value=0.000										

Chapter Four

discussion

Mastitis is a multi-etiological and complex disease, which is defined as inflammation of parenchyma of mammary glands. It is characterized by physical, chemical and usually bacteriological changes in milk, and pathological changes in glandular tissues (Radostis *et al*., 2000). The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011). All studies reported that a high prevalence (16–80%) of sub-clinical mastitis, implying that it could be among the major constraints limiting optimum productivity in small-scale dairy cattle operations (Girma *et al*., 2012).

From the annual report of veterinary services of the River Nile State , Sudan, mastitis was the third disease after external parasites and pneumonia in cases which brought to the veterinary clinics of the state (Annual report, 2009). The prevalence of clinical and sub-clinical mastitis at cow level was 10.5% and 72% respectively in Sudan (Albagir *et al*, 2015). This study is aiming to investigate the effect of some risk factors on severity and prevalence of mastitis in bovine dairy farm in atbara as an example for River Nile State. sample were collected from bovine dairy farms. and Laboratory investigation to identify the prevalence of incidence of mastitis. Finally Statistical analysis of results.

In the present study CMT is used to detect the subclinical mastitis as a screening test. Because the CMT was positivelily correlated with SCC (S.Mcdougall *et al* ., 2010).

In this investigation, the California mastitis test had been conducted as the main tool to verify the prevalence of mastitis. In this study they is no association between Autumn season and prevalence of mastitis infection. with high percentage in early and mid-autumn was (26.0%) followed by (22.0%) in late autumn. This may be due to humidity cause the most growth of pathogenic

agent. Maede *et al*., (2014) reported in other study occurrence of clinical mastitis in the first and second parity cows increases in late lactation period in autumn and (Diego B *et al*., 2011) reported the frequency (IMI) cases was higher in the rainy season than in dry season.

In our study the hygiene factor influences the prevalence of mastitis with high percentage (52.0%) of mastitis infected of animal in dirty barn and just(1.5%) cases in the cleaning barn. Similar to study agreed with (Trevor *et al*., 2012) the poor dairy cow hygiene has been consistently associated with elevated somatic cell count and the risk of subclinical mastitis .

In this study the highest prevalence of mastitis infection (86.8%) in the state of change nutrition and very lower percentage cases in the fixed of nutrition (2.7%). This corresponds to (D O'Rourke et al., 2009) nutrition can influences the cows resistance to mastitis. This because good nutrition strengths the immunity and resist diseases.

In this study the factor of milking non infected animal firstly is effect in the decrease prevalence of mastitis (5.4%) in compare with milking by infected animal firstly (80.8%). This agree with (G.M.Jones *et al*., 2006) problem cows with clinical mastitis and those receiving antibiotic therapy must be milked last. In the our study the factor of milking non infected udder quarter firstly is effect in the decrease prevalence of mastitis by (81.0%) in compare with milking infected udder quarter firstly (100%). This measure is to prevent the disease from spreading to the rest of the herd.

In this study revealed that when uses of medicaments for treatment of mastitis by intra mammary infusion the rate of disease (100.0%). and when using alternative treatments (cold water & semsm oil) the rate percentage is (100.0%) and(69.0%). When not using medicaments the rate of disease (5.9%).(Steve Nickevson .2019) reported the infusion procedure used to administer intra mammary treatment are critical to achieving desired results.

Also the study revealed the effect of these factors on the fore and hind quarters of the udder separately, where it was found that the fore quarter are more sensitive and prone to infection than the hind quarter of the udder. Interpretation of this may be as a result of touching the fore quarter of the udder to the ground when the animal descends to sleep, or the back legs also cover the hind quarter of the udder.

Conclusions:

Multivariable analysis were used to assess the effect of different risk factors with prevalence of mastitis, the experiment was conducted during the autumn season and no significant different. On the other hand the factor of nutrition and hygiene and milk behavior (milking non infected animal firstly, non-infected udder quarter firstly) they have an effective role in prevalence of mastitis. also done the assessment of the mastitis grade of infection of fore and hind udder.

Recommendations:

•Farm management should be seriously considered in controlling mastitis.

• Hygiene measurements should be followed to reduce mastitis in dairy farms. use proper milking procedure as Wash only teats and lower udder surface using water and disinfectant and dry each teat thoroughly with individual paper towels and check quarters for abnormalities each milking by stripping also Dip or spray teats, especially post. And Milking with an effective teat dip**.**

• culling chronically infected cow will help significantly in the control of mastitis.

• The flock must be well fed and ensuring that the cow has a adequate energy (minerals and vitamins) that for the maintenance of udder health and immune status .

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