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Evaluation of different methods used for diagnosis of bovine subclinical mastitis and determination of the udder immuno response against it

A thesis presented
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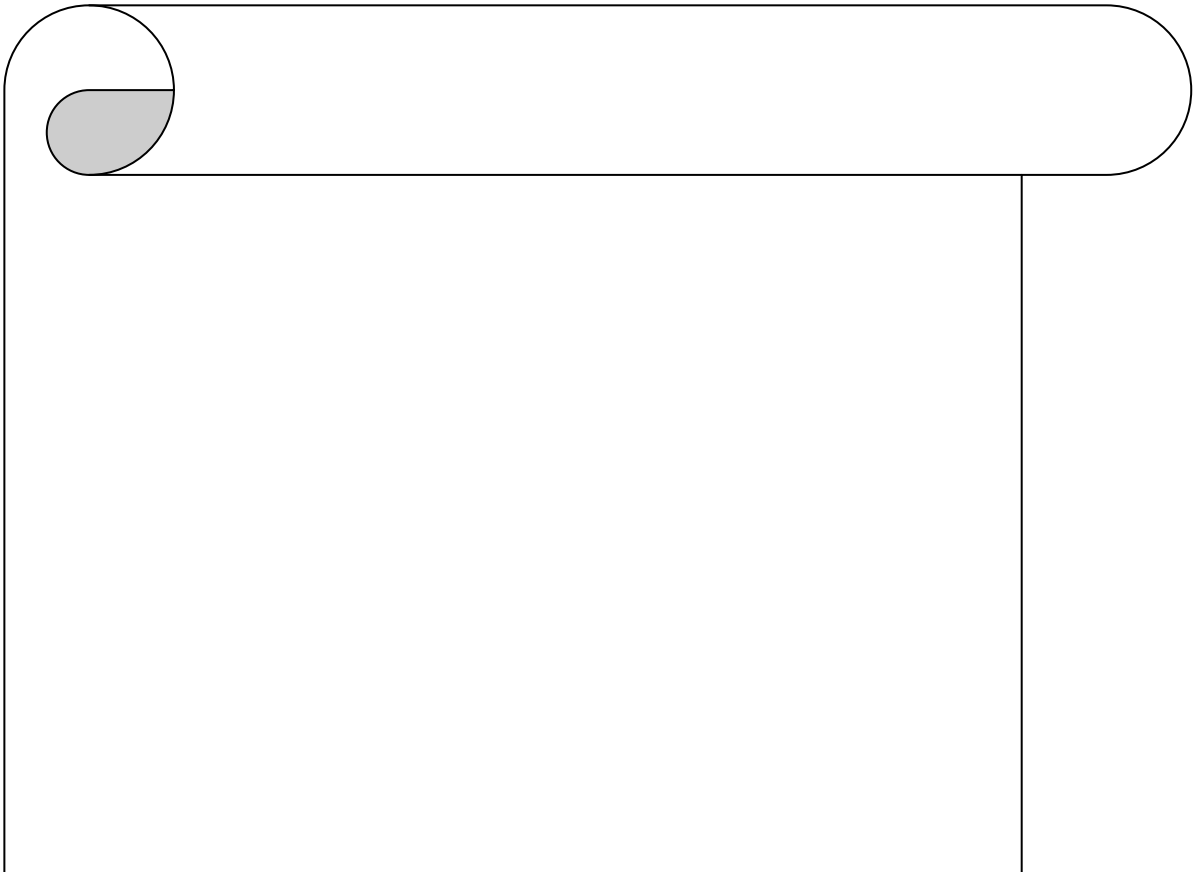
Abstract

Key words: Subclinical mastitis, Environmental mastitis, *E. coli***,** Streptococci, Antibiotic.

A total of 450 milk samples were collected from apparently normal of udder Friesian lactating cows were investigated for subclinical mastitis. These animals were subjected to California Mastitis (CMT), Electric conductivity (ECT) and pH indicator paper (pHI) tests. Milk whey were prepared from the positive reactors as well as another 50 samples from negative subclinical cases used as control were subjected to Catalase, Nitric oxide, Lysozyme and Electrophoresis determinations. Bacterial examinations of 166 quarter milk samples that reacted positively to different field screening tests used to evaluate the efficiency tests and detect its sensitivity. The results showed that 6.2%, of examined cows and 3.2 % of examined quarter were positive for CMT with different scores. While 8% of the examined cows and 3.8% of the quarters reacted positively to ECT with conductivity of 9 m/sc. or more. pH indicator paper test showed 3.8% cows and 2.1% of examined quarter were positively with variable degree of intensity of colour. Immunoanalysis for some components of the milk whey from the positive reactors were applied and compared with the normal milk whey values. Milk whey samples from the subclinical cases showed 15 folds, 2 folds and 3 folds significant increase in the main value of catalase, nitric oxide and lysozymes respectively as compared to control samples. Protein

fractions & profile of milk whey detected by electrophoresis revealed a significant increase in alpha lactoglobulin level. Bacterial examinations revealed that 62.7%, 75.4% and 68.4% were positive to the above screening tests respectively. *E. coli* was the more common environmental strain isolated in 37.3% of the examined samples as a single isolate and in 15.7% in combined with other bacteria. This is followed by *Streptococcus dysgalactiae* in 12.2% of the samples as a single strain and in 6.1% in a combined form. Other environmental pathogens isolated were *S. uberis* (8.7%), *Pseudomonas aeruginosa* (7.8%), *Klebsiella pneumoniae* (6.1%) and *Enterococcus faecalis* (5.2%) isolate and in 13.0%, 4.3%, 5.2% and 0.9% in combined form respectively with other strains. Antibiotic sensitivity test was carried out and the results showed that tetra delta, norfloxacin and gentamycin were more efficient in their antibiotic activity on most of the isolated strains. A field trial was done to treat the subclinical case detected by screening tests using non therapeutic method. This was applied by increased frequency of milking of the affected animals up to 6 frequencies milking daily for three consecutive weeks. The results showed that the percentage of cured animals after one week was 54.2% as detected by CMT, 66.7% by ECT and 63.2% by pH indicator paper. After two weeks these percentages were 23.8%, 29.0% and 26.3% respectively. After three weeks these percentages were 18.6%, 4.3%, and 10.5 respectively. Percentage of non cared animals after three weeks of frequent milking were 3.4% as detected by CMT only.



Dedication

To My Dear Family Husband Nabil,

To My Mother, Brothers Zaher and Micheal

To my sons Micheal, Marco, and My lovely daughter Maria.

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1-Introduction

Mastitis is the inflammation of the mammary gland. This term is derived from the Greek word *mastos*, meaning *"breast* "and *itis*, meaning *"inflammation of"*. Inflammation is the response of a tissue or organ to injury. The purposes of the inflammatory response in the udder are to destroy or even neutralize the infectious agents and their toxin and so allow the gland to return to normal function **(Bramely *et al*., 1996).**

Mastitis is multietioliogical complex disease which is characterized by physical, chemical and bacteriological change in milk and pathological change in glandular tissue **(Radostitis *et al*., 1995).**

Bovine mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production. Most countries including developed ones suffer large financial losses due to the disease. **(Sharma *et al*., 2004).**

The disease is usually classified into two forms, clinical in which the disease is diagnosed clinically and subclinical which is mainly diagnosed via assessment of somatic cell and bacterial examination. (**Belowey and Edmondson, 1995 and Blood *et al*. 1990).**

Subclinical form is considered a serious problem as infected quarter showed no symptoms either in udder or in milk for long time and the causative organisms act as invisible source for spreading infection in the herd **(Philpot and Nickerson 1991, and El-Kholy *et al*., 1994).**

Subclinical mastitis is a common and significant cause of loss. Losses to subclinical mastitis are approximately about 82% of estimated total financial loss caused by mastitis "this loss is as a result of reduced milk production and decreased milk quality". **(Robyn, 2012).** Reduced milk price, increased risk of subsequent clinical mastitis and increased risk of culling or death of the animal are encountered among infected animals **(Harmon, 1994)**. However, **DeGreaves and Fetrow (1993)** reported that total loss of subclinical mastitis to be 10-26% of the milk produced the affected quarter plus 1% of the total solids due to change in milk composition.

Subclinical mastitis is the most costly animal disease in the world; it's a mammary gland infection that robs milk producers of $10 billion annually around the globe due to productivity losses and treatment costs. This loss is about $2 billion annually in the US alone. Tangible costs of antibiotics, treatment expense and milk discarded after treatments are added to this cost **(John, 2013).**

The causative organism of mastitis will be broken down into contagious organisms that colonize the mammary gland and can be spread by milking machines and milkers to other quarters. These is caused primary by *Staph*. *aureus, Stept. agalactiae* and *Coryn. Bacterium bovis.* The environmental pathogens that do not normally infect the mammary gland but can do so when the cows environment, the teats and udder or the milking machine is contaminated with these organisms and they gain access to the teat cistern are caused by*E.coli* , *Klebsiella* , *Pseudomonas aeruginousa* , and *Strept.uberis* **(Bramley *et al.,* 1996 ; and Burvenich*et al* .,2003).**

California mastitis test (CMT) is the most widely test used for diagnosis of subclinical intra-mammary infection in cattle **(Radostitis *et al.,* 2000)**.

When the mammary epithelium is damaged as result of mastitis the electrical conductivity (EC) of milk changes **(Biggadike *et al.,* 2002).** These changes tend to occur before the development of visible clinical singe **(Hamann and Zecconi 1998).** Electrical conductivity during milking could be used for subclinical mastitis determination and control of udder health status **(Barth *et al*., 2000, Ferrero *et al* 2014).**

Determination of pH of the milk in subclinical mastitis is carried out to detect the change of pH of milk which tends to be more alkaline. The PH level more than 6.8 indicates the incidence of subclinical mastitis. **(Muhammad *et al.*, 2011)**.

Bovine milk is a live bioreactor, into which H_2O_2 and nitric oxide (NO) are being constantly surged from surrounding epithelial cell and milk leucocytes **(Silanikove *et al*., 2005 and 2009)** .Nitric oxide is formed of nitrogen and oxygen highly reactive compound, it only exists for six to ten seconds inside the body then it is converted by oxygen into other compounds of nitrogen called nitrites **(Talan 1993)**. Nitric oxide in milk of normal cows is significant by lower than that in mastitic cows **(Ahmed *et al*., 2009)**. Nitric oxide (NO) levels and oxidant capacity (TOC) in milk whey could be used as an alternative diagnostic tool to screen for subclinical mastitis **(Atakisi *et al*., 2010)**. Lysozyme level was significantly higher in case of the subclinical mastitis. Lysozyme protect from the ever present danger of bacterial infection **(Magda, 2006)**. Determination of lysozyme is used as a method for subclinical mastitis diagnosis.

Bacteriological culture of milk samples is the standard method for detection and evaluation of intramammary infection. **(National Mastitis Council, 1999).** It served as a gold standard for evaluation of different tests used for subclinical mastitis.

The present work was aimed mainly to:

- Evaluate the different methods for detection of subclinical mastitis in milking cows by California mastitis test (CMT), Electrical conductivity test (EC) and determination of pH of milk.

- Estimate of biochemical change in milk whey of subclinical mastitic cases.

- Carry out a bacteriological examination of positive subclinical mastitis cases for isolation and identification of bacterial causative organisms.

- Field trial treatment by non-therapeutic method for subclinical mastitis in milking cows.

2-Review of literature

2-1- Screening tests for detection of subclinical mastitis

2-1-1- California mastitis test (CMT):

El-Kholy and Hosein (1990) reported that 180 milk samples were collected from apparently health lactating cows by using Schalm test for