Evaluation and Comparison of Four Screening Tests against Milk Culture for Detection of Subclinical Mastitis in Lactating Cattle and Buffalo in Egypt

Ibrahim N. Alkhouly[1], Abdelmoneim M. Moustafa[2], Nahla A. Abou El Roos[1] and Sahar A. Kandeel[2*]

[1]Animal health Research Institute, Shibin El Koom branch, Agriculture Research center, Egypt
[2]Department of Animal Medicine (Infectious Diseases), College of Veterinary Medicine, Benha University, Kalyobiya, 13637, Egypt
***Corresponding Author:** Sahar A. Kandeel, **E-Mail:** saharkandeel@fvtm.bu.edu.eg

ABSTRACT

Subclinical mastitis (SCM) is an asymptomatic worldwide udder infection that results in substantial losses to the dairy industry. Our main objective was to evaluate and compare the clinical performance of 4 commercially available screening tests for diagnosing SCM. Foremilk samples were collected from 428 quarters of 107 apparently healthy lactating cows and buffaloes from El-Menofia governorate from 2020 to 2022. Quarter somatic cell count (SCC) was estimated using the FOSS-BacSomatic® counter and the California Mastitis Test (CMT), with SCM defined as SCC > 200,000 cells/mL. Milk pH was measured cow-side using an AD11®pH-meter and BOVIVET® indicator paper. Bacterial cultures of foremilk samples were used to diagnose SCM as a reference method based on the isolation of the causative pathogens. The tests' performance was evaluated by calculating test sensitivity (Se), specificity (Sp), and accuracy at the optimal-cut-point for each test. FOSS-BacSomatic® counter was the best-performing test for diagnosing SCM (Se = 0.967, Sp = 0.943, accuracy = 0.957) at an optimal-cut-point of >200,000 cells/mL. For comparison, CMT is the second best-performing test at an optimal-cut-point of a non-negative score (Se = 0.892, Sp = 0.878, accuracy = 0.887). The test performance of the AD11® pH-meter and BOVIVET® indicator paper was fair, however, the AD11® pH-meter performed better than the BOVIVET® indicator paper with Se = 0.807, Sp = 0.845, and accuracy = 0.822. We concluded that the FOSS-BacSomatic® counter and CMT are considered good tests for diagnosing SCM. On the other hand, milk pH doesn't provide a clinically useful method for diagnosing SCM. However, based on cost, availability and analysis time, there doesn't seem to be a persuasive reason to select the FOSS-BacSomatic® counter over the traditional CMT to diagnose SCM.

_____

INTRODUCTION

Mastitis is a common inflammatory condition of the mammary gland that results in physicochemical alterations in milk along with pathological changes in the glandular tissue **(Constable *et al.,* 2017).** Mastitis is classified into two types: clinical and subclinical. Clinical mastitis is identified by the presence of a visibly aberrant secretion from one or more glands; abnormal gland(s); or systemic alterations that may occur depending on the severity of the inflammation. In comparison, subclinical mastitis does not result in any visible changes, milk appears normal, and laboratory testing is required to identify the presence of inflammation. Mastitis, predominantly subclinical, is a global problem that results in substantial economic losses in the dairy sector **(Kader *et al.,* 2003; Hussein *et al.,* 2022);** where it was reported to be responsible for about 70% of the economic losses and is a critical factor limiting milk output **(Heleili *et al.,* 2012).**

Mastitis causes several compositional changes in milk as a result of local inflammation, glandular tissue damage, elevated leukocyte counts,

and serum components that enter milk due to the increase in blood-milk barrier permeability (**Kitchen, 1981; Pyorala, 2003**). These compositional changes reflect the extent of physical damage to the udder tissue. The damage to the blood-milk barrier leads to the escape of blood and extracellular fluid components into the alveolar lumen and reduces the rate of milk production (**Nguyen and Neville, 1998**). Blood and extracellular fluid components in inflamed quarters combine with released milk (**Zhao and Lacasse, 2008**), leading to the alteration of milk components, including an increase in milk SCC and pH, with the magnitude of the increase in these parameters positively correlated with the severity of the inflammatory process (**Batavani *et al.,* 2007; Qayyum *et al.,* 2016**). Milk SCC is the predominant indicator of udder health in lactating dairy cattle (**Constable *et al.,* 2017**). The SCM is defined by an SCC cut-point of >200,000 cells/mL and is widely used to identify the existence of inflammation in response to infection with a sensitivity of 0.75 and a specificity of 0.90 (**Schepers *et al.,* 1997; Breen *et al.,* 2009**).

Various screening approaches based on physical and chemical milk alterations are used to diagnose SCM during lactation (**Sharma *et al.,* 2010**). One such approach is the CMT, a quick, affordable, semi-quantitative screening widely used cow-side test that has been used for over 60 years to locate quarters with SCM (**Schalm and Noorlander, 1957; Barnum and Newbould, 1961**). The test estimates the quantity of inflammatory (somatic) cells in the milk, with higher scores being linked to an increased probability and severity of SCM (**Sargeant *et al.,* 2001; Dingwell *et al.,* 2003a; Sharma *et al.,* 2010**). Automated technologies for quickly assessing milk SCC have recently become available. Counting technology advancements have resulted in the common use of high-capacity flow cytometric counters with significantly enhanced performance in modern milk testing facilities. One of the high-capacity tools is Fossomatic, which uses a fluorescent dye to stain cells before counting the number of fluorescing particles. It can quickly determine the SCC in large numbers of samples (**Gonzalo *et al.,* 2003**). Results for somatic cells are delivered within 1.5 to 2 minutes. Measuring milk pH may also offer a useful diagnostic method for detecting quarters with SCM. Currently, there are several low-cost point-of-care devices available for measuring pH in different biological fluids that can be used in milk for on-farm or cow-side use (**Kandeel *et al.,* 2019**). The relatively low cost and portability of pH meters make them potentially beneficial for on-farm use. Likewise, a low-cost cellulose-based card test impregnated with bromothymol blue is commercially available in the Middle East as an SCM screening test. It is inexpensive and contains patented indicator dyes that change colour according to the pH.

The microbiological milk culture is the gold standard approach for identifying intramammary infection (**Sumon *et al.,* 2017**). However, the results are not available for at least 24 – 48 hours. More than a hundred different microbes have been isolated and identified from bovine mastitis; *Streptococci, Staphylococci,* and Gram-negative bacteria are the most frequently isolated pathogens (**Hussain *et al.,* 2013**). Considering the pathogen source, mastitis can also be categorized as either contagious, where the pathogen primarily spreads between animals and through milking equipment, or environmental, which is caused by common environmental pathogens, that are mostly found in faeces, dust, bedding material, and flies such as *E.* coli (**Wenz *et al.,* 2006**).

We hypothesized that measuring milk SCC and pH using commercially available diagnostic tools would provide clinically useful methods that help in predicting the presence of Intramammary infection (IMI) in dairy cows and buffaloes. We were interested in addressing this hypothesis for two reasons. First, the dairy industry coupled with a societal desire aims to reduce the use of intramammary antibiotics in food-producing animals to prevent the emergence of antibiotic-resistant bacteria. Therefore, finding a clinically useful test to accurately identify and treat infected cows is a crucial objective of mastitis control programmes. However, identifying a practical, precise, objective, and affordable method to determine the udder health status of cows is still a challenge (**Kandeel *et al.,* 2018**). Second, WHO has issued guidelines for developing diagnostic tests for infectious pathogens in resource-constrained environments such as dairy farms. Such tests must meet specific criteria, including affordability, specificity, sensitivity, user-friendliness, rapidity, robustness, equipment-free delivery, and delivery anywhere, creating the acronym "ASSURED" (**Urdea *et al.,* 2006**). Consequently, such diagnostic tests in such settings should be both accurate and cost-effective while having an immediate clinical impact. Therefore, our study aims mainly to evaluate the clinical performance of four commercially available screening tests for diagnosing SCM. In addition, we investigate the prevalence of SCM in dairy cows and buffalo in El-Menofia Governorate, Egypt.

MATERIALS AND METHODS

1. Animals, Housing, Feeding, and Milking

This study was conducted using convenient milk samples from 107 native dairy cows and buffaloes of different age groups (2 to 11 years old), parties (1 to 8), and different stages of lactation

(early, mid, and late). The study was performed on cows and buffaloes in moderate and small animal aggregations in the El-Menofia governorate in Egypt during the period from 2020 to 2022. Most of the animals under study were in tie-stall barn houses, and all were hand milked twice daily. Most hand milkers have no routine for udder disinfection before and after milking. Data including age, days in milk (DIM), milk production, and parity were retrieved from the farm or the animal owner.

2- Ethical approval

All methods were evaluated and approved by the Benha University Institutional Animal Care and Use Committee Research Ethical Board (BUFVTM10-02-23).

3. Sample collection

A total of 428 foremilk samples from each quarter were collected separately from 57 and 50 apparently healthy cows and buffaloes, respectively. Disposable gloves were worn before touching the udder. The teat end of each quarter was cleaned with water and then disinfected with alcohol 70% (NMC, 1999), and 15-mL sterile milk samples were collected in sterile polyethylene screw-capped, wide-mouth falcon tubes by hand stripping after discarding the first few streams of milk. The CMT and measuring the milk pH using AD11® pH-meter and BOVIVET® indicator paper were performed cow-side.

The milk samples were then stored in an ice box and transported to the laboratory at Animal Health Research Institute (AHRI) in El-Menofia governorate, where it is kept in a refrigerator (4 °C) for performing other tests. About 7.5 mL was used for further automatic somatic cell count (SCC) by (FOSS-BacSomatic®) apparatus, while 7.5 mL was brought to the laboratory within 2 hours after collection for further bacteriological examination.

Each animal was examined physically (including pulse, Resp. rate, body temp., superficial lymph nodes, and ruminal movement) to determine the presence of any systemic disorders. Udder and milk were inspected and palpated for the detection of any abnormalities, such as the cardinal signs of inflammation or abnormal milk.

4. Milk Analysis for SCC
4.1. California Mastitis Test (CMT)

The CMT was performed beside the animal according to Schalm and Noorlander (1957). In a plastic vessel, two milliliters of milk sample from each quarter was mixed with equal amount of CMT (Schalm) reagent (KERBL, Germany) by gently moving the paddle in a circular motion. The CMT

reaction was visually scored using a 4-point scale (negative, 1 positive, 2 positive, 3 positive) where we considered the trace score as 1 positive, and the non-negative score ($\geq$+ve) was used as a cut-point for identifying infected quarter.

4.2. FOSS-BacSomatic® automatic counter

The *FOSS-BacSomatic®* automatic cell counter (FOSS, Denmark) was performed at the laboratory of the Animal Health Research Institute (AHRI) in El-Menofia governorate according to the manufacturer's manual and instructions (www.FOSSanalytics.com). An optimal-cut-point of >200,000 cells/mL was used to identify SCM.

5. Milk Analysis for pH
5.1. AD11® pH-meter

In this study, the milk pH was measured cow-side using AD11® waterproof, pH-meter (Adwa, Romania). The meter was calibrated at two pH points with auto-buffer recognition and against five buffer values. The AD11® pH-meter range is from 2 to 16 with a resolution 0.1 to 0.01 according to the technical data of the manufacturer (www.adwainstruments.com). The pH electrode was dipped in the milk sample without exceeding the maximum immersion level. The pH was recorded after the reading had stabilized. An optimal-cut-point of 6.6 was used to identify SCM **(Kandeel *et al.*, 2019).**

5.2. BOVIVET® Indicator paper (Card test)

The milk pH for each sample was determined cow-side using the BOVIVET® Indicator Paper (Kruuse, Denmark) according to manufacturer instructions (www.kruuse.com). Milk from infected quarters changes the color of the spot from yellow (normal) into green or bluish-green. The change in color was correlated with pH as follows; pH 6.6-6.7 pale-green, pH 6.8 moderate green, pH 7.1 green, pH 7.4 dark blue-green.

6. Milk culturing for pathogens isolation`

Milk culturing was carried out in accordance with National Mastitis Council (NMC, 1999) recommendations except using a 100 μL aliquot instead of the suggested 10 μL aliquot for better identification of infected animals with low levels of pathogen shedding. Milk samples were initially drawn from freeze and incubated for 24 hours at 37°C. A 100 μL milk sample from each quarter was streaked on Blood agar (Remel®, Lenexa, KS) and MacConkey agar (Remel®) plates separately in a manner that allowed the growth of separated bacterial colonies. All plates were then incubated in an inverted position at 37°C for 24 - 48 hr. The microbiological results were recorded as colony-

forming units (cfu) per mL of milk and the isolated pathogens were identified using colony morphological appearance, the pattern of hemolysis, biochemical testing, including catalase and coagulase tests, Gram staining reaction, and cell morphology according to **NMC (1999)** recommendations.

7. Statistical Analysis

The study's data were analyzed using ANOVA and Turkey-Kramer HSD post hoc test using SPSS statistical software. P < 0.05 was considered significant. Subclinical mastitis was defined as a milk culture of ≥ 10 colonies/plate from the 100-µL inoculum as gold standard test **(Dohoo *et al.,* 2011)**. A somatic cell count of >200,000 cells/mL and a milk pH of 6.6 were used as cut-off points for defining SCM **(Kandeel *et al.*, 2019).** Sensitivity, Specificity, Tests accuracy, True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) were also calculated **(Jensen and Kjelgaard-Hansen, 2006).** The quarter served as the analytical unit and test performance was predicated on the assumption that sensitivity and specificity were equally important.

RESULTS

The quarter prevalence of SCM in the examined milk samples using CMT is shown in Table (1). The non-negative CMT reaction (one positive or higher) was the optimal cut-point used where the "trace" score was considered with the first positive score as we were more interested in a higher test sensitivity than specificity. The prevalence of SCM using the CMT test was 65.5% (n=280). The HR quarter showed the highest SCM prevalence of 40% (n=112), and the most frequent CMT score reported was two positives "++ve" (42.9%; n= 120).

Table 1: Quarter-wise prevalence of subclinical mastitis using the California mastitis test

Test	Quarter	Positive (%)			
		Total	+	++	+++
CMT	HR	112 (40%)[a]	25 (22.3%)	48 (42.8%)	39 (34.8%)
	HL	85 (30.5%)[b]	16 (18.8%)	41 (48.2%)	28 (33%)
	FR	47 (16.8%)[c]	10 (21.3%)	19 (40.4%)	18 (38.3%)
	FL	36 (12.7%)[c]	9 (25%)	12 (33.4%)	15 (41.6%)
	Total	280 (65.5%)	60 (21.4%)	120 (42.9%)	100 (35.7%)

a,b,c: Different letters in the same column are significantly different (P ≤ 0.001)

The quarter prevalence of SCM in the examined quarter milk samples using the FOSS-Bacsomatic® automatic counter is stated in table (2)**.** The prevalence of SCM using the FOSS-Bacsomatic® automatic counter was 63% (n=269) distributed among different quarters with the HR quarter showing the highest prevalence (33.5%; n=90). The SCC ranging from 400,000 – 750,000 cell/ml milk was the most frequently reported (40.8%; n=110).

Table 2: Quarter-wise prevalence of subclinical mastitis using FOSS-Bacsomatic® automatic counter

Test	Quarter	Positive (%)			
		Total	200,000 – 400,000 cell/ml	400,000 – 750,000 cell/ml	> 750,000 cell/ml
FOSS-Bacsomatic® automatic counter	HR	90 (33.5%)[a]	25 (27.8%)	37 (41.1%)	28 (31.1%)
	HL	72 (26.8%)[b]	20 (27.7%)	29 (40.3%)	23 (32%)
	FR	60 (22.3%)[b]	16 (26.6%)	25 (41.6%)	19 (31.6%)
	FL	47 (17.5%)[c]	13 (27.6%)	19 (40.4%)	15 (32%)
	Total	269 (62.9%)	74 (27.6%)	110 (40.8%)	85 (31.6%)

a,b,c: Different letters in the same column are significantly different (P ≤ 0.001)

The quarter prevalence of SCM in the examined quarter milk samples using AD11® pH-meter is shown in table (3). The prevalence of SCM using AD11® pH-meter was 60.7% (n=260), and the HR quarter showed the highest prevalence (38.8%; n=101) and the highest reported pH range was 6.8 – 6.9 (40.3%; n=105).

Table 3: Quarter-wise prevalence of subclinical mastitis using AD11® pH-meter

Test	Quarter	Positive (%)			
		Total	pH 6.6 – 6.7	pH 6.8 – 6.9	pH > 6.9
AD11® pH-meter	HR	101 (38.8%)[a]	25 (24.7%)	40 (39.7%)	36 (35.6%)
	HL	75 (28.8%)[b]	20 (26.7%)	30 (40%)	25 (33.3%)
	FR	45 (17.3%)[c]	10 (22.2%)	20 (44.4%)	15 (33.4%)
	FL	39 (15%)[c]	10 (25.6%)	15 (38.5%)	14 (35.9%)
	Total	260 (60.7%)	65 (25%)	105 (40.3%)	90 (34.6%)

The quarter prevalence of SCM in the examined quarter milk samples using BOVIVET® Indicator Paper is shown in table (4). The prevalence of SCM for examined quarters was 58.2% (n=249) with the HR quarters showing the highest prevalence (34.1%; n=85). The most frequently reported pH color was the green color "pH 7.1" (42.5%; n=106).

Comparison between the prevalence of SCM on a quarterly basis using different tests of interest, sensitivity, specificity, and accuracy of each test are summarized in Table (5). Bacsomatic® automatic counter showed the highest Se (96.7%) and Sp (94.3%) with a test accuracy of 95.7%. On the other hand, BOVIVET® Indicator Paper showed the lowest Se (79.5%) and Sp (82.6%) with an accuracy of 80.8%.

Table 4: Quarter-wise prevalence of subclinical mastitis using BOVIVET® indicator paper

Test	Quarter	Positive (%)			
		Total	Moderate green (pH 6.8)	Green (pH 7.1)	Dark bluish green (pH 7.4)
BOVIVET® indicator paper	HR	85 (34.1%)[a]	14 (16.5%)	44 (51.7%)	27 (31.8%)
	HL	72 (28.9%)[b]	21 (29.1%)	28 (38.9%)	23 (32%)
	FR	50 (20.1%)[c]	12 (24%)	18 (36%)	20 (40%)
	FL	42 (16.86%)[d]	11 (26.2%)	16 (38%)	15 (35.8%)
	Total	249 (58.2%)	58 (23.3%)	106 (42.5%)	85 (34.2%)

a,b,c: Different letters in the same column are significantly different (P ≤ 0.001)

Table 5: Summary of the results analysis of the ability of California Mastitis Test (CMT), FOSS-BacSomatic® automatic counter, AD11® pH-meter, and BOVIVET® indicator paper to predict subclinical mastitis.

Test	Prevalence	TP	FP	TN	FN	Se	Sp	Accuracy %
FOSS-BacSomatic® automatic counter	269 (62.9%)	260 (96.7%)	9 (3.3%)	150 (94.3%)	9 (5.7%)	96.7%	94.3%	95.7%
CMT	280 (65.4%)	250 (89.3%)	30 (10.7%)	130 (87.8%)	18 (12.2%)	89.2%	87.8%	88.7%
AD11® pH-meter	260 (60.7%)	210 (80.7%)	50 (19.3%)	142 (84.5%)	26 (15.5%)	80.7%	84.5%	82.2%
BOVIVET® indicator paper	249 (58.2%)	198 (79.5%)	51 (20.5%)	148 (82.6%)	31 (17.4%)	79.5%	82.6%	80.8%

Sensitivity = True positive/ (True positive+ false negative) x100
Specificity= true negative/ (true negative+ false positive) x100

The prevalence of SCM based on milk culture on a quarterly basis was 60.7% (n=260). The frequency of isolated microorganisms in relation to positive samples was shown in table (6). Organisms were isolated in 260 of 428 (60.7%) quarter samples with *E. coli*, and *Klebsiella Spp* being the most commonly isolated organisms (28.4% and 23.1%, respectively). Of the 428 quarter samples, contagious pathogens primarily *Staphylococcus aureus*, were identified in 40 samples (9% of the quarter samples and 15.4% from the isolated pathogens), and environmental pathogens were identified in 220 samples (51.4% of the quarter samples and 84.6% from the isolated pathogens) and *E. coli* is the most isolated environmental pathogens (17.3% of the quarter samples and 33.6% from the isolated environmental pathogens).

Table 6: The prevalence of pathogens isolated from 428 quarter milk samples obtained from 107 dairy cattle and buffalo.

Prevalence	Number	Percentage (%)
Negative growth	168	39.3%
Positive growth	260*	60.7%
E. coli	74	28.4%
Klebsiella Spp.	60	23.1%
Staphylococcus. aureus	40	15.38%
Staphylococcus. Vitulins	10	3.8%
Streptococcus spp.	22	8.4%
Proteus Spp.	19	7.3%
Coreanea	8	3.1%
Pasteurella Spp.	3	1.1%
Helcoccus kenzi	4	1.5%
Lactococcus spp.	6	2.3%
Aspergillus spp.	15	5.7%
Yeast	17	6.4%

* The table contains samples that have more than one type of isolated organisms (mixed infection) so the number of isolates may exceed the number of the total positive growth samples.

The correlation between SCC measured by Bacsomatic® automatic cell counter and different CMT scores and pH values is shown in Fig. 1 and Fig. 2, respectively, where there is a positive correlation between SCC and increase CMT scores (r = 0.85) and pH value (r = 0.328), where increased the probability of SCM is positively associated with an increased CMT score using a cut-point of score > negative and increased pH value. The t-test showed significance at $p \leq 0.001$.
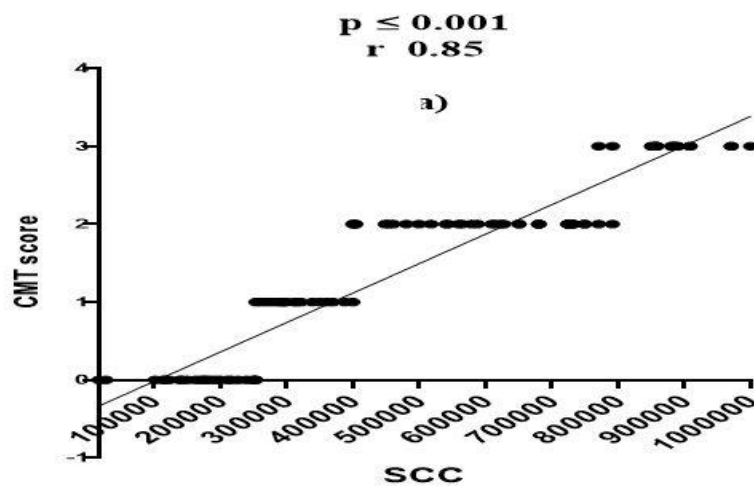

Fig 1: Scatterplot of the relationship between SCC measured by FOSS-Bacsomatic® automatic counter and CMT score for 428 quarter milk samples from 107 dairy cows and buffalo. The diagonal solid black line represents the line of identity.
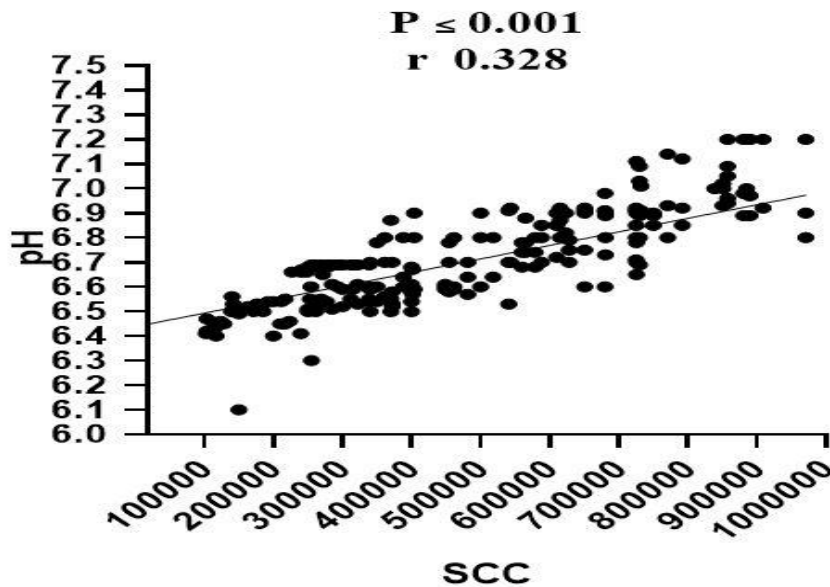
Fig 2: Scatterplot of the relationship between SCC measured by FOSS-Bacsomatic[®] automatic counter and pH value measured by AD11[®] pH-meter for 428 quarter milk samples from 107 dairy cows and buffalo. The diagonal solid black line represents the line of identity.

DISCUSSION

To our knowledge, this is the first study comparing the clinical utility of the FOSS-BacSomatic[®] automatic counter, CMT, AD11[®] pH-meter, and BOVIVET[®] Indicator Paper as rapid diagnostic tests for identifying SCM defined by the isolation of the causative pathogens from quarter milk samples of lactating cows and buffaloes in Egypt. The primary findings of our investigation were that the FOSS-BacSomatic[®] automatic counter showed the highest sensitivity, specificity, and test accuracy among the tested methods for diagnosing SCM followed by the CMT. The clinical utility of using the AD11[®] pH-meter for diagnosing SCM was only fair as a clinically useful test. We therefore, concluded that measuring SCC using the FOSS-BacSomatic[®] automatic counter and CMT is a clinically useful method for identifying subclinically infected quarters, while determining milk pH is not a sufficiently sensitive or specific method for detecting quarters with SCM in lactating cows and buffaloes in Egypt.

The measurement of SCC in milk is widely utilized as a means of assessing milk quality and overall udder health. This is due to the positive correlation between the number of inflammatory cells present in milk and the existence of IMI **(Schukken et al., 2003; Green et al., 2004).** As defined by **Berry and his colleagues (2007)**, SCC refers to the concentration of leukocytes in milk that facilitate the elimination of invading mastitis pathogens, as well as the epithelial cells that are frequently shed from alveolar tissue into milk and are described as "cells per mL of milk".

Our study showed that the *FOSS-BacSomatic[®]* automatic counter is the most sensitive, specific, and accurate method followed by CMT. Similar results were published in an earlier study **(Badiuzzaman et al., 2015)** where the automatic cell counter was more sensitive (86.60%), followed by CMT (80.08%). In another earlier study, the sensitivity of the automatic cell counter was 88.60%, the specificity was 97.76%, and the accuracy was 91.94% **(Sharma et al., 2010).** Lower results were recorded in another study, as the sensitivity of CMT and the automatic cell counter were 71% and 65.2%, respectively, while, the specificity was 75.75% and 78.78%, respectively **(Reddy et al., 2014).**

The California mastitis test is a semiquantitative screening cow-side test that has been used for more than 60 years and offers results within one minute, despite the significant variation in SCC within each CMT score. Our study showed good Se (89.2%) and Sp (87.8%) of CMT using a threshold reaction ≥ +ve (i.e., any non-negative CMT score). To achieve the highest sensitivity with acceptable specificity, interpreting the CMT as negative or positive can optimize its clinical usefulness in diagnosing SCM. Throughout our entire study, the CMT results were read by only one investigator. However, the subjectivity involved in interpreting the CMT results can cause different estimations of test sensitivity and specificity when used by other investigators **(Kandeel et al., 2018).** Several studies have reported varying values for Se and Sp when diagnosing mastitis or IMI at different lactation

stages using the CMT. For example, **Middleton *et al.,* (2004)** found a Se of 50% and a Sp of 73% in a US herd with a high bulk tank SCC, while **Bhutto *et al.,* (2012)** reported a Se of 87% and a Sp of 26% in two English dairy herds. **Kandeel *et al.* (2018a)** found a Se of 45% and a Sp of 56% in cows admitted to a US Veterinary Teaching Hospital, while **Sargeant *et al.,* (2001)** reported a Se of 57% and a Sp of 56% on day 3 of lactation in three North American herds. **Dingwell *et al.,* (2003)** found a Se of 82% and a Sp of 81% for detecting IMI caused by a major mastitis pathogen on day 4 of lactation. **Godden *et al.,* (2017)** reported a Se of 52% and a Sp of 71% in early-lactation quarters and a Se of 63% and a Sp of 53% in late-lactation quarters.

However, our study showed results quite similar to those of **Brito *et al.,* (1997),** who used a SCC of 200,000 cells/mL as a reference to indicate IMI and found a Se of 79% and a Sp of 90%. More recently, **Kandeel *et al.,* (2018)** found a sensitivity of 95% at dry-off and 79% in fresh cows and a specificity of 81% at dry-off and 95% in fresh cows when using a CMT cut-point score of trace or higher. The CMT test has several benefits, including high sensitivity, accuracy, and simplicity. Moreover, the presence of foreign substances, for example, hair or other debris, does not affect the test's results **(Mohammed *et al.,* 2019).** The high SCC levels recorded here may explain differences in management systems between farms and intramammary infection that are in agreement with other previous studies **(Haltia *et al.,* 2006; Almaw *et al.,* 2008; Minnat and Hammadi, 2015).** These variations might be assigned to the complex nature of mastitis, which involves the interactions of various factors such as management practises, environmental factors, animal-related factors, and the causative pathogen **(Constable *et al.,* 2017).**

Our findings indicate that milk pH does not provide a practical and useful screening tool for identifying SCM in on-farm settings. The Se and Sp for the AD11[®] pH-meter and BOVIVET[®] pH Indicator Paper revealed moderate precision as diagnostic methods for SCM. Since the CMT was first introduced in 1957 **(Marschke and Kitchen, 1985),** the use of milk pH measurement as an on-farm diagnostic test for SCM has fallen out of favour. This is primarily due to the lower milk pH sensitivity for detecting SCM, as shown in our study. The wide range of milk pH values even in uninfected quarters, coupled with the relatively small pH increase in infected quarters, contributes to the lower sensitivity of the test **(Cherrington *et al.,* 1933).** This may be attributed to the fact that, unlike blood pH, milk pH is not tightly regulated, and subsequently, a greater

variance or a higher fluctuation in milk pH is expected even in healthy quarters. However, using milk pH in combination with other diagnostic methods, such as SCC, might improve its accuracy and clinical utility in detecting subclinical infected quarters. The exact physicochemical mechanism underlying the increase in milk pH in infected quarters has not been definitively determined yet, however, applying the strong ion difference theory to milk and taking into consideration the physicochemical models developed for plasma **(Constable, 1997)** and urine **(Constable *et al.,* 2009),** it may be assigned to an increase in the concentration differential between the primary strong cation (sodium) and the sum of the main strong anions (chloride and casein) in milk, which consequently increases the strong ion difference **(Bruckmaier *et al.,* 2004; Ogola *et al.,* 2007).**

The milk pH (excluding mastitic and colostrum milk) is typically claimed to range between 6.4 and 6.8 **(Schalm and Noorlander, 1957);** however, there was a positive correlation observed between the pH of quarter milk samples and the CMT score **(Ashworth *et al.,* 1967)** as an indirect estimation of milk SCC. Recommended pH cut-points of ≥ 6.8 **(Prouty, 1934)** have been suggested to diagnose SCM. However, another recent study revealed an optimal pH cutpoint of ≥ 6.67 for cows at dry-off and 6.52 for freshening cows for diagnosing SCM using a pH meter with adjusted milk temperature **(Kandeel *et al.,* 2019).**

The performance disparity between the AD11[®] pH-meter and the BOVIVET[®] pH Indicator Paper in identifying quarters with SCM was unexpected. The BOVIVET[®] pH Indicator Paper used in this study employs the pH indicator "bromothymol blue," which assigns a yellow colour as normal milk pH. A change in the colour of the indicator paper from yellow to green or bluish-green was considered a positive SCM. The darker the colour, the higher the milk pH which may be correlated to advanced SCM. Bromothymol blue provides certain benefits over CMT for diagnosing SCM, as it is considered more objective since the changes in colour are easier to notice than the changes in viscosity; it is not required to mix equal proportions of reagents; and the test result is accessible for recording even at a later time **(Marschke and Kitchen, 1985; Tawfik *et al.,* 2014).**

The prevalence of SCM (quarter basis) in our study was 60.7% based on milk culture, however, it varies from 65.4% to 58.2% according to the other screening tests reported here. Similar results using CMT were recorded by previous studies **(Wahba *et al.,* 2005; Enany *et al.,* 2007; Duguma *et al.,* 2014;**

Badiuzzaman *et al.,* 2015; Mureithi and Njuguna, 2016). A higher prevalence was obtained by other studies **(Hussein, 2012; Kamal *et al.,* 2014).** Other studies reported a lower prevalence **(Ayano *et al.,* 2013; Barua *et al.,* 2014; El-Kholy *et al.,* 2018).** Poor hygienic standards, suboptimal housing, inadequate bedding, malfunctioning or improper milking operations, and insufficient treatment approaches may all contribute to the higher prevalence of SCM among dairy animals **(Sri Balaji and Senthilkumar, 2017).**

Our study revealed that the right hind (RH) quarters showed the highest prevalence of SCM among the infected quarters in the studied population. On the other hand, the LF quarters showed the lowest prevalence of SCM. Similar results were obtained from earlier studies **(Badiuzzaman *et al.,* 2015; Mohammed *et al.,* 2019).** Our results disagree with another study that reported a higher prevalence of SCM in the examined milk samples of LF quarters, followed by RF, LH, and RH quarters, respectively **(Hussein *et al.,* 2022).** Other studies reported a higher prevalence of SCM in the forequarters than the hindquarters **(El- Kholy *et al.,* 2018; Mourya *et al.,* 2020).** The higher prevalence of SCM in the hind quarters may be linked to the anatomical structure of the udder **(Donagh and William, 2005)** and the higher milk production of the hind quarters compared to the forequarters **(Lancelot *et al.,* 1997).**

The bacteriological examination showed that the most predominant isolated bacteria are *E.coli* followed by *Klebsiella spp. and S.aureus*. A previous study reported that over 80% of cases of coliform mastitis are attributed to *E.coli* **(Fahim *et al.,* 2019).** However, our result is higher than that reported previously by **Abd El-Fattah *et al.,* (2023).** The frequency of *E.coli* infections could be linked to unsanitary practices at the farms, including inadequate cleaning, faulty drainage, manure disposal issues, insufficient washing of the udder, inadequate pre-milking drying, using unclean washing towels, and the failure to apply post-milking teat dipping **(Ayano *et al.,* 2013).**

In our study, the primary isolated contagious pathogen was *S. aureus. Staphylococcus aureus* is the most significant contagious mastitis-causing pathogen, with a high level of penetration, that forms deep-seated foci in the infected glands **(Ranjan *et al.,* 2011).** This can lead to significant problems and a financial impact on dairy cattle **(Dego *et al.,* 2002).** The high prevalence of *S. aureus* in our study may be attributed to several factors, including poor hygienic practices (especially of milkers' hands) before and during milking, a lack of post-milking teat dipping,

failure to cull animals with chronic infections, and the lack of dry cow therapy in many dairy herds **(Abebe *et al.,* 2016).** Eradication programs that rely on treatment strategies using antimicrobial agents and appropriate herd management to limit the incidence of new infections can successfully reduce mastitis caused by *Streptococcus spp*. **(Reyes *et al.,* 2015).** These microorganisms pose a public health risk to humans, besides affecting animal health and the economy.

We reported here the most frequent scores/results of each of the investigated screening tests, however, we did not investigate if they is related to the most often isolated pathogens in our study. Therefore, further studies are required to investigate if there are associations between certain screening test results/scores and specific pathogens.

CONCLUSION

The obtained results showed a relatively high prevalence of SCM among dairy cows and buffalo in El-Menofia governorate, with *E. coli* as the predominant isolated organism. The *FOSS-BacSomatic*® automatic counter followed by CMT are reliable screening tests for detecting SCM. However, by comparison, the CMT test is most easily performed cow-side and on the farm and requires about 1 minute to obtain a result at a lower estimated cost. The CMT, when used cow-side with a cut-point greater than negative, had higher test sensitivity and specificity than milk pH.

Conflicts of interest

There is no conflict of interests of any sort between authors or elsewhere.

REFERENCES

ABD EL-FATTAH, O.A., KOTB, E.E.Z. IBRAHIM, H.S., EL-GOHARY, A.H., and MOHAMMED, A.A.E., 2023. Detection and Molecular Characterization of Some Virulence Genes of Escherichia Coli Isolated from Milk in Dairy Cow Farms. Journal of Applied Veterinary Sciences, 8(2): 01 – 09. 10.21608/javs.2023.173140.1190.

ABEBE, R., HATIYA, H., ABERA, M., MEGERSA, B., and ASMARE, K., 2016. Bovine mastitis. prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa

milk shed, South Ethiopia. BMC Veterinary Research, 12(1): 1-11. https://doi.org/10.1186/s12917-016-0905-3

ALMAW, G., ZERIHUN, A., and ASFAW, Y., 2008. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Tropical animal health and production, 40(6), pp.427-432. https://doi.org/10.1007/s11250-007-9115-0

ASHWORTH, U.S., FORSTER, T.L., and LUEDECKE, L.O., 1967. Relationship between California mastitis test reaction and composition of milk from opposite quarters. Journal of Dairy Science. 50:1078–1082. https://doi.org/10.3168/jds.S0022-0302(67)87568-4

AYANO, A.A., HIRIKO, F., SIMYALEW, A.M., and YOHANNES A., 2013. prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. Journal of Veterinary Medicine and Animal Health, 5(3): 67-72. https://doi.org/10.5897/JVMAH12.056

BADIUZZAMAN, M., SAMAD, M.A., SIDDIKI, S.H.M.F., ISLAM, M.T., and SAHA, S., 2015. Subclinical mastitis in lactating cows: Comparison of four screening tests and effect of animal factors on its occurrence. Bangladesh Journal of Veterinary Medici*e*, 13(2),41-50. 10.3329/bjvm.v13i2.26627

BARNUM, D. A., and NEWBOULD, F. H. S., 1961. The use of the California Mastitis Test for the detection of bovine mastitis. Candian Veterinary *Journal.* 2:83–90. PMC1585631

BARUA, M., PRODHAN, M.A., ISLAM, K., CHOWDHURY, S., HASANUZZAMAN, MD., IMTIAZ, M.A., and DAS, G.B., 2014. Sub-clinical mastitis prevalent in dairy cows in Chittagong district of Bangladesh: detection by different screening tests. Veterinary World, (7): 483-488. https://doi.org/10.14202/vetworld.2014.483-488

BATAVANI, R. A., ASRI, S., and NAEBZADEH, H., 2007. The effect of subclinical mastitis on milk composition in dairy cows. Iranian Journal of Veterinary Research. 8:205–211. 10.22099/IJVR.2007.925

BERRY, D.P., LEE, J.M., MACDONALD, K.A., STAFFORD, K., MATTHEWS, L., and ROCHE, J.R., 2007. Associations among body condition score, body weight, somatic cell count, and clinical mastitis in seasonally calving dairy cattle. Journal of Dairy Science. 90:637–648. 10.3168/jds.S0022-0302(07)71546-1

BHUTTO, A. L., MURRAY, R. D., and WOLDEHIWET Z., 2012. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. Research in Veterinary Science. 92:13–17. 10.1016/j.rvsc.2010.10.006

BREEN, J.E., BRADLEY, A.J., and GREEN, M.J., 2009. Quarter and cow risk factors associated with a somatic cell count greater than 199,000 cells per milliliter in United Kingdom dairy cows. Journal of Dairy Science. 92:3106–3115. 10.3168/jds.2008-1562

BRITO, J.R.F., CALDEIRA, G.V., VERNEQUE, R.S., and BRITO, M.A.V., 1997. Sensitivity and specificity of the California Mastitis Test as a diagnostic tool for subclinical mastitis in quarter somatic cell count estimation. Brazilian Journal of Veterinary Research. 17:49. https://eurekamag.com/research/009/396/009396021.php

BRUCKMAIER, M., ONTSOUKA, E., and BLUM, W., 2004. Fractionized milk composition in dairy cows with subclinical mastitis. Veterinarni Medicina. 49:283–290. http://dx.doi.org/10.17221/5706-VETMED

CHERRINGTON, V. A., HANSEN, H. C., and HALVERSEN. W.V., 1933. The leucocyte content of milk as correlated with bacterial count andhydrogen ion concentration for the detection of mastitis. Journal of Dairy Science. 16:59–67. https://doi.org/10.3168/JDS.S0022-0302%2833%2993316-0

CONSTABLE, P. D. 1997. A simplified strong ion model for acid-base equilibria: Application to horse plasma. Journal of Applied Physiology. 83:297–311. https://doi.org/10.1152/jappl.1997.83.1.297

CONSTABLE, P. D., GELFERT, C. C., FURLL, M., STAUFENBIEL, R., and STAMPFLI, H. R., 2009. Application of strong ion difference theory to urine and the relationship between urine pH and net acid excretion in cattle. American Journal of Veterinary Research.70:915–925. https://doi.org/10.2460/ajvr.70.7.915.

CONSTABLE, P.D., HINCHCLIFF, K.W., DONE, S.H., and GRÜNBERG, W., 2017. Veterinary medicine. a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences. 11th ed. Philadelphia, Pennsylvania. WB Saunders 2017.1913. PMCID: PMC5603921.

DEGO, O.K., DIJK, J.E.V., and NEDERBRAGT, H., 2002. Factors involved in the early pathogenesis of bovine Staphylococcus aureus mastitis with emphasis on bacterial adhesion and invasion. a review. Veterinary Quarterly, 24(4): 181-198. https://doi.org/10.1080/01652176.2002.9695135

DINGWELL, R.T., KELTON, D.F., and LESLIE, K.E., 2003. Management of the dry cow in control of peripartum disease and mastitis. Veterinary Clinics of North America: Food Animal Practice. 19:235–265. https://doi.org/10.1016/s0749-0720(02)00072-5

DINGWELL, R.T., LESLIE, K. E., SCHUKKEN, Y. H., SARGEANT, J.M., and LEO. L.T., 2003a. evaluation of the california mastitis test to detect an intramammary infection with a major mastitis pathogen in early lactation dairy cows. Can. Vet. J. 44:413–415. http://www.ncbi.nlm.nih.gov/pmc/articles/pmc340150/

DOHOO, I.R., SMITH, J., ANDERSEN, S., KELTON, D.F., and GODDEN.S., 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. Journal of Dairy Science. 94:250–261. https://doi.org/10.3168/jds.2010-3559

DONAGH, P.B., and WILLIAM, J.M., 2005. Interdependence and distribution of subclinical

mastitis and intra-mammary infection among udder quarters in dairy cattle. Preventive Veterinary Medicine. (75): 81-91. 10.1016/j.prevetmed.2006.02.001

DUGUMA, A., TOLOSA, T., and YOHANNES, A., **2014.** Prevalence of clinical and sub-clinical mastitis on cross bred dairy cows at Holleta Agricultural Research Center, Central Ethiopia. Journal of Veterinary Medicine and Animal Health. (1): 13-17. http://dx.doi.org/10.5897/JVMAH2013.0259

EL-KHOLY, A.M. HASSAN, G.M. ZEINHOM, M.M.A., and EL-KLAWY, M.A., 2018. Detection of subclinical mastitis in a dairy farm in Beni Suef city, Egypt. Assiut Veterinary Medical Journal. 64 (157):18-24.
https://doi.org/10.21608/avmj.2018.168897

ENANY, M.E., EL-ATTAR A.A., KHAFAGY, A.A.R., and EL-GAMMAL, A.M., 2007. Bacterial and mycotic causes of subclinical bovine mastitis. Suez Canal Veterinary Medical Journal, XII (2): 107–114. https://www.researchgate.net/publication/328695905

FAHIM, K.M., ISMAEL, E., KHALEFA, H.S., FARAG, H.S., and HAMZA DA., 2019. Isolation and characterization of E. coli strains causing intra-mammary infections from dairy animals and wild birds. International journal of veterinary science and medicine, 7(1): 61-70. https://doi.org/10.1080/23144599.2019.1691378

GODDEN, S. M., ROYSTER, E., TIMMERMAN, J., RAPNICKI, P., and GREEN, H., 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early-and late-lactation quarters and cows. Journal of Dairy Science. 100:6527–6544. https://doi.org/10.3168/jds.2017-12548

GONZALO, C., MARTÍNEZ, J.R., CARRIEDO, J.A., and SAN PRIMITIVO, F., 2003. Fossomatic cell-counting on ewe milk: comparison with direct microscopy and study of variation factors. Journal of Dairy Science. 86:138-145. https://doi.org/10.3168/jds.S0022-0302(03)73593-0

GREEN, M.J., GREEN, L.E., SCHUKKEN, Y.H., BRADLEY, A.J., PEELER, E.J, BARKEMA, H.W., DE HAAS, Y., COLLIS, V.J., and MEDLEY, G.F., 2004. Somatic cell count distributions during lactation predict clinical mastitis. Journal of Dairy Science. 87:1256–1264. 10.3168/jds.S0022-0302(04)73276-2

HALTIA, L., HONKANEN-BUZALSKI, T., SPIRIDONOVA, I., OLKONEN, A., and MYLLYS, V., 2006. A study of bovine mastitis, milking procedures and management practices on 25 Estonian dairy herds. Acta Veterinaria Scandinavica, 48(1), 1-6. 10.1186/1751-0147-48-22

HELEILI, N., AYACHI, A., MELIZI, M., KASSAH, A.L., and MAMACHE, B., 2012. Prevalence of subclinical bovine mastitis and the in vitro sensitivity of bacterial isolates in Batna Governorate, East of Algeria. Journal of animal science advances. 2: 576-582.https://www.researchgate.net/publication/281526588

HUSSAIN, R., KHAN, A., JAVED, M.T., and ALI, F., 2013. Morphometric and pathological studies on mammary gland of slaughtered Nili-Ravi buffaloes. Pakistan Journal of Agricultural Sciences. 50(1): 123-130. https://pakjas.com.pk/papers/2130.pdf

HUSSEIN, O.H., ABDEL HAMEED, K.G., and EL-MALT, L.M., 2022. Prevalence and public health hazards of subclinical mastitis in dairy cows. SVU-International Journal of Veterinary Sciences, 5(3), 52-64. 10.21608/SVU.2022.131652.1189

HUSSEIN, S.A. 2012. Prevalence and bacterial etiology of subclinical mastitis in dairy cows in Al Sulaimaniyah District. Kufa Journal for Veterinary Medical Sciences. (3): 190-203. https://www.iasj.net/iasj/article/62298

JENSEN, A.L., and KJELGAARD-HANSEN, M., 2006. Method comparison in theclinical laboratory. Veterinary Clinical Pathology. 35(3) : 276-286. 10.1111/j.1939-165x.2006.tb00131.x

KADER, M.A., SAMAD, M.A., and SAHA, S., 2003. Influence of host level factors on prevalence and economics of subclinical mastitis in dairy cows in Bangladesh. Indian Journal of Dairy Science, 56(4): 235-240.
https://api.semanticscholar.org/CorpusID:77585292?utm_source=wikipedia

KAMAL, R.M., BAYOUMI, M.A., and ABD EL AAL, S.F.A., 2014. Correlation between some direct and indirect tests for screen detection of subclinical mastitis. International Food Research Journal, 21(3): 1249-1254.
https://www.researchgate.net/publication/263377486

KANDEEL, S.A., MEGAHED, A.A., ARNAOUT, F.K., and CONSTABLE, P.D., 2018. Evaluation and comparison of two on-farm tests for estimating somatic cell count in quarter milk samples from lactating dairy cattle. Journal of veterinary internal medicine. 32:506–515. https://doi.org/10.1111/jvim.14888

KANDEEL, S.A., MORIN, D. E., CALLOWAY, C.D., and CONSTABLE, P. D., 2018a. Association of California Mastitis Test Scores with intramammary infection status in lactating dairy cows admitted to a veterinary teaching hospital. Journal of veterinary internal medicine. 32:497–505. 10.1111/jvim.14876

KANDEEL, S.A., MEGAHED, A.A., EBEID, M.H. and CONSTABLE, P.D., 2019. Ability of milk pH to predict subclinical mastitis and intramammary infection in quarters from lactating dairy cattle. Journal of dairy science, 102(2), 1417-1427. 10.3168/jds.2018-14993

KITCHEN, B. J. 1981. Review of the progress of dairy science: Bovine mastitis: Milk compositional changes and related diagnostic tests. Journal of Dairy Research. 48:167–188. 10.1017/s0022029900021580

LANCELOT, R., FAYE, B., and LESCO-URRET, F., 1997. Factors affecting the distribution of clinical mastitis among udder quarters in French dairy cows. Veterinary Research. 28: 45–53. PMID: 9172840

MARSCHKE, R. J., and KITCHEN, B. J., 1985. Detection of bovine mastitis by bromothymol blue pH indicator test. Journal of Dairy Science. 68:1263–1269. 10.3168/jds.S0022-0302(85)80955-3

MIDDLETON, J. R., HARDIN, D., STEEVENS, B., RANDLE, R., and TYLER, J. W., 2004. Use of somatic cell counts and California Mastitis Test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. Journal of the American Veterinary Medical Association. 224:419–423. 10.2460/javma.2004.224.419

MINNAT, T.R., and HAMMADI, K.M., 2015. Detection of clinical and subclinical mastitis in dairy cows of Diyala Province, Iraq. Journal of Wassit For Science and Medicine, *8*(3), 71-81. https://www.iasj.net/iasj/article/162381

MOHAMMED, S., BAKR, N., and SAYED, M., 2019. Detection of subclinical mastitis in milk of dairy cows in Sohag city, Egypt. Assiut Veterinary Medical Journal. *65*(160), 51-58. 10.21608/avmj.2019.167298

MOURYA, A., SHUKLA, P.C., GUPTA, D.K., SHARMA, R.K., NAYAK, A., SINGH, B., JAIN, A., and PRADHAN, S., 2020. Prevalence of subclinical mastitis in Cows in and around Jabalpur, Madhya Pradesh. Journal of Entomology and Zoology Studies, 8(4): 40-44. : https://www.researchgate.net/publication/343826574

MUREITHI, D.K., and NJUGUNA, M.N., 2016. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. *Livestock* Research for Rural Development. 28(2): 13. https://www.lrrd.cipav.org.co/lrrd28/2/mure28013.html

NMC (NATIONAL MASTITIS COUNCIL), 1999. Laboratory Handbook on Bovine Mastitis. Natl. Mastitis Counc. Inc., Madison, WI.

NGUYEN, D. A. D., and NEVILLE, M. C., 1998. Tight junction regulation in the mammary gland. Journal of Mammary Gland Biology and Neo*plasia*. 3:233–246. 10.1023/a:1018707309361

OGOLA, H., A. SHITANDI, and NANUA, J., 2007. Effect of mastitis on raw milk compositional quality. Journal of Veterinary Science. 8:237–242. 10.4142/jvs.2007.8.3.237

PROUTY, C.C. 1934. A comparison of the leucocyte count, the brom thymol blue reaction and the catalase content of freshly drawn milk. Journal of Dairy Science. 17:75–81. https://doi.org/10.3168/jds.S0022-0302(34)93222-7

PYORALA, S. 2003. Indicators of inflammation in the diagnosis of mastitis. Veterinary Research. 34:565–578. 10.1051/vetres:2003026

QAYYUM, A., KHAN, J. A., HUSSAIN, R., AVAIS, M., AHMAD, N., and KHAN, M. S., 2016. Investigation of milk and blood serum biochemical profile as an indicator of sub-clinical mastitis in Cholistani cattle. The Pakistan Veterinary Journal. 36:275–279. http://www.pvj.com.pk/pdf-files/36_3/275-279.pdf

RANJAN, R., GUPTA, M.K., and SINGH, K.K., 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Veterinary World, 4(5): 205-208. 10.5455/VETWORLD.2011.205-208

REDDY, B.S.S., KUMARI, K.N., REDDY, Y.R., REDDY, M.V.B., and REDDY, B.S., 2014. Comparison of different diagnostic tests in subclinical mastitis in dairy cattle. International Journal of Veterinary Science, *3*(4), 224-228. https://www.researchgate.net/publication/327679091

REYES, J., CHAFFER, M., SANCHEZ, J., TORRES, G., MACIAS, D., JARAMILLO, M., DUQUE, P.C., CEBALLOS, A., and KEEFE, G.P., 2015. Evaluation of the efficacy of intramuscular versus intramammary treatment of subclinical Streptococcus agalactiae mastitis in dairy cows in Colombia. Journal of Dairy Science, *98*(8), pp.5294-5303. 10.3168/jds.2014-9199

SARGEANT, J. M., LESLIE, K. E., SHIRLEY, J. E., PULKRABEK, B. J., and LIM, G. H., 2001. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. Journal of Dairy Science. 84:2018–2024. 10.3168/jds.S0022-0302(01)74645-0

SCHALM, O. W., and NOORLANDER, D. O., 1957. Experiments and observations leading to development of the California mastitis test. Journal of the American Veterinary Medical Association. 130: 199–204. https://digital.nls.uk/dcn6/7549/75495602.6.pdf

SCHEPERS, A.J., LAM, T.J., SCHUKKEN, Y.H., WILMINK, J.B., and HANEKAMP, W.J., 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. Journal of Dairy Science. 80:833–1840. 10.3168/jds.S0022-0302(97)76118-6

SCHUKKEN, Y.H., WILSON, D.J., WELCOME, F., GARRISON-TIKOFSKY, L., and GONZALEZ, R.N., 2003. Monitoring udder health and milking quality using somatic cell counts. Veterinary Research. 34:579–596. 10.1051/vetres:2003028

SHARMA, N., PANDEY, V., and SUDHAN, N.A., 2010. Comparison of some indirect screening tests for detection of subclinical mastitis in dairy cows. Bulgarian Journal of Veterinary Medicine. 13: 98. http://tru.uni-sz.bg/bjvm/BJVM%20June%202010%20p.98-103.pdf

SRI BALAJI, N., and SENTHILKUMAR, A., 2017. Prevalence of subclinical mastitis in dairy cows of theni district in tamilnadu. International Journal of Science, Environment and Technology. 6 (6*)*, 3427 – 3432. https://www.ijset.net/journal/1982.pdf

SUMON, S.M., EHSAN, M.A., and ISLAM, M., 2017. Subclinical mastitis in dairy cows. somatic cell counts and associated bacteria in Mymensingh, Bangladesh. Journal of the Bangladesh Agricultural University, 15(2): 266-271. 10.3329/jbau.v15i2.35073

TAWFIK, N. F., EFFAT, B.A., SHAFEI, K. E., DAIROUTY, R. E., and SHARAF, O. M., 2014. Mastitis and antibiotic residues in Egyptian raw milk with lactic acid bacteria population in dairy products retailed in Cairo and Giza area. Glob. Vet. 13:696–703. https://doi.org/10.5829/idosi.gv.2014.13.05.85190

URDEA, M., PENNY, L.A., OLMSTED, S.S., GIOVANNI, M.Y., KASPAR, P., SHEPHERED, A., WILSON, P., DAHL, C.A., BUCHSBAUM, S., MOELLER, G., and HAY BURGESS, D.C., 2006. Requirements for high impact diagnostics in the developing world. Nature. 1:73–79. 10.1038/nature05448

WAHBA, N., ALI, M.M., and ABDEL-HAFEEZ, M.M., 2005. Microbiological profile of subclinical mastitic cow milk and its correlation with field tests and somatic cell count. Assiut Veterinary Medical Journal. 51(104):62-75. 10.21608/avmj.2005.177366

WENZ, J.R., BARRINGTON, G.M., GARRY, F.B., ELLIS, R.P., and MAGNUSON, R.J., 2006. Escherichia coli isolates' serotypes, genotypes, and virulence genes and clinical coliform mastitis severity. Journal of dairy science, *89*(9), pp.3408-3412. 10.3168/jds.S0022-0302(06)72377-3

ZHAO, X., and LACASSE, P., 2008. Mammary tissue damage during bovine mastitis: Causes and control. Journal of Animal Science. 86(Suppl.):57–65. https://doi.org/10.2527/jas.2007-0302