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Lactating Cattle Subclinical Mastitis: Comparative Efficacy of Different Diagnostic Tests and Associated Risk Factors

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ABSTRACT

Subclinical mastitis (SCM) is a prevalent infection of the udder that causes significant economic losses in the dairy sector on a global scale. The primary objective was to assess and contrast the clinical efficacy of three screening tests that are currently available for identifying SCM. A total of 400 foremilk samples were obtained from 100 Lactating cattle in Menoufia governorate between May 2022 and April 2023. The diagnosis of mastitis was conducted by utilizing bacterial cultures of foremilk samples as a reference, which involved isolating the causal pathogens. For mastitis diagnosis in the field, the California Mastitis Test (CMT) was used. The BacSomatic® method was used to estimate the somatic cell count (SCC). Additionally, the pH of the milk was determined on-site using a pH meter. The performance of the tests was assessed by determining the test sensitivity, specificity, and accuracy at the appropriate cut-off point for each test. The BacSomatic® test demonstrated exceptional performance in identifying mastitis, with a sensitivity of 98.3%, a specificity of 96.8%, and an accuracy of 97.7% at a cut-off point of >200,000 cells/mL. When comparing several tests, it is found that CMT is the second most effective test when using an ideal cut-off point for a score that is not negative. This test has a sensitivity of 84.7%, a specificity of 85.3%, and an accuracy of 85%. The pH meter demonstrated satisfactory test performance, with a sensitivity of 80.9%, a specificity of 81.5%, and an accuracy of 81.3%. The BacSomatic® counter and CMT are deemed reliable diagnostic tests for mastitis. However, milk pH is not a therapeutically effective diagnostic tool for mastitis. Furthermore, the hind quarter was more affected than the other quarters, and cattle with higher parities were more susceptible to mastitis than others. Moreover, the early stage of lactation was more implicated in SCM than the mid-and late stages.

1. INTRODUCTION

Localized inflammation in CM causes discomfort, redness, oedema, heat, and irregular milk output, which might lead to clots or Mastitis is an endemic syndrome of dairy cows that is common in dairy herds, affecting production, animal health, welfare, and the economy of the industry worldwide (Ali et al., 2021; Du et al., 2022). Mastitis also decreases the likelihood of conception and has negative effects on animal welfare, leading to financial losses. Furthermore, the annual expenses associated with illness treatment impose a significant economic burden on dairy farmers (Gonçalves et al., 2018). The prevalence of SCM in Lactating Cattle in Egypt on a quarterly basis was 60.7% (Kandeel et al., 2023). The estimated yearly cost of clinical mastitis per cow in the United States varies from $71 to

$179, according to Bar et al. (2008). Bovine mastitis has two forms, subclinical mastitis (SCM) or clinical mastitis (CM), is the term used to describe inflammation of the udder in cows. discolouration. On the other hand, SCM is indicated by an increased SCC in the milk, despite its normal appearance (De Vliegher et al., 2012).

Veterinary services can promptly act on dairy cows with CM due to the observable signs of the condition. Consequently, the global occurrence of CM has significantly diminished due to the implementation of comprehensive control measures. Nevertheless, farm owners may fail to notice dairy cows with SCM due to the absence of observable symptoms in infected cows. Furthermore, the data that is now accessible indicates that the SCM prevalence is 15 to 40 times higher than that of (Pilla et al., 2013). Additionally, the classification is based on the cause of the condition, which might be either non-infectious or infectious. Infectious etiologies are predominantly observed, with bacterial infections being the prevailing manifestation in many instances among groups of animals. Bacterial pathogens are categorized into various classifications: The bacteria mentioned by Ndahetuye et al. (2019) are capable of spreading from person to person, thriving in various environments, and taking advantage of favourable conditions. Pathogens like E. coli. Staph. aureus, Strept. agalactiae, Strept. uberis and Klebsiella pneumoniae are most often linked to mastitis (Ashraf & Imran, 2020; Cadona et al., 2021; Kandeel et al., 2023; Morales-Ubaldo et al., 2023). Additionally, the species Corynebacterium spp., Pseudomonas spp., and Mycoplasma were identified by Yuan et al. (2011). Inflammation in the affected area, damage to glandular tissue, an increase in white blood cell counts, and the introduction of serum components into milk due to an increased permeability of the blood-milk barrier are some of the variations in milk's constituents caused by mastitis (Kitchen, 1981; Pyörälä, 2003).

The degree of physical damage to the udder tissue is indicated by the chemical changes. The rate at which milk is produced decreases when the blood-milk barrier is compromised because blood and components of the extracellular fluid escape into the alveolar lumen (Nguyen & Neville, 1998).

The combination of blood and extracellular fluid components with released milk in inflamed quarters results in changes to milk components. These changes include an increase in milk SCC and pH. The extent of the increase in these parameters is directly related to the severity of the inflammatory process. Elevation in SCC in the milk is also defined as mastitis. It occasionally may result in severe systemic clinical symptoms, such as sepsis, along with a fever (Shpigel et al., 2008).

Several factors can influence the SCC, including age, Lactating period, parity, season, stress, management, day-to-day variation, and most importantly, the presence of intramammary infection (IMI). An accurate interpretation of SCC relies on a comprehension of the variables that can influence the quantity of somatic cells (Minnat & Hammadi, 2015).

During breastfeeding, SCM is diagnosed using a variety of screening techniques based on changes in milk's physical and chemical composition (Sharma et al., 2010). One method for locating quarters with SCM is the CMT, a semi-quantitative, rapid, inexpensive, and popular cow-side test that has been in use for more than 60 years (Barnum & Newbould, 1961). According to Dingwell et al. (2003) and Sharma et al. (2010), the test measures the number of inflammatory (somatic) cells in the milk; higher scores are associated with a greater probability and severity of SCM.

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 the detection of the affected 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). So, the aim of this study was to compare the efficacy of different subclinical mastitis diagnostic tests and some associated risk factors in lactating cattle.

2. MATERIALS AND METHODS

2.1. Animals

One hundred native dairy cattle ranging in age from 2 to 7 years old and in different parties (1 to 5) provided milk samples. In moderate animal groups (10 to 50), cattle were the subjects of the investigation during the period from May 2022 to April 2023 in Menoufia governorate, Shibin El kom and Quesina city, Egypt. The animals under study were hand-milked twice daily. The majority of hand milkers do not regularly clean the udders before and lactation phase, Age, days in milk (DIM), parity, and milk production data have been collected.

Every animal received a physical examination (Duguma, 2016), to rule out any systemic illnesses. This included checking the body temperature, pulse, respiration rate, superficial lymph nodes, and rumen movement. The cardinal symptoms of inflammation and abnormal milk were among the anomalies that were palpated and examined to find any abnormalities.

Sample collection2.2 .

Four hundreds foremilk samples were retrieved from cattle (50 each), sequentially from each quarter. Using disposable gloves, according to (Hogan et al., 1999), the teat end of each quarter was washed by using water and then disinfected with 70% alcohol. After discarding the first streams of milk, 30 ml of sterile milk samples were manually stripped and collected in sterile screw-capped polypropylene falcon tubes. The CMT and measuring the milk pH using a pH meter (PHS-3C waterproof, pH-.meter, GZ) were performed cow-side

After being placed in an icebox, the milk samples were brought to the Animal Health Research Institute (AHRI) laboratory in Menoufia governorate. There, samples were kept in a refrigerator (4 °C) to conduct additional testing. Approximately 20 mL was utilized for additional measuring of the SCC using an automatic somatic cell count (FOSS-BacSomatic®) device Additionally, 10 mL was sent to the lab for additional bacteriological analysis without delay (two hours of collection).

2.3. Milk Analysis for SCC

2.3.1. California Mastitis Test (CMT)

The CMT was performed in the field, according to Schalm and Noorlander (1957). In a plastic plate, 5 ml of milk sample from each quarter was mixed with an equal amount of Schalm reagent (KERBL, Germany), followed by a gentle movement of the paddle in a circle. The CMT result was visually scored using four scale points [negative (< 200,000), + positive (200000 - 400,000), ++ positive (400,000- 750,000), +++ positive (> 750,000). The non-negative score (≥ +ve) was used as a cut-point for identifying infected quarters, and we considered the trace score as 1 positive.

2.3.2. FOSS-BacSomatic® automatic counter

The FOSS-BacSomatic® automatic cell counter (Rev. 4/18, FOSS, Denmark) was performed as described in the manual and instructions of the manufacturer (www.fossanalytics.com). An optimal cut-point of >200,000 cells/mL was used to identify SCM.

2.4. pH Analysis

pH-meter

In this study, the milk pH was assessed using (PHS-3C waterproof, pH-meter, GZ). pH-meter range is from 2 to 16 with a resolution of 0.1 to 0.01 according to the technical data of the manufacturer. The milk sample was dipped into the pH electrode without going above the maximum immersion level. Once the reading steadied, the pH was measured. An optimal cut-point of 6.6 was used to identify SCM (Kandeel et al., 2019)

2.5. Culturing method

Following the National Mastitis Council's guidelines (Hogan et al., 1999), the blood agar base media (HIMEDIA, M073), MacConkey plates (HIMEDIA, MM081), Edward's agar medium (HIMEDIA, M748), and mannitol salt agar (HIMEDIA, M118) were streaked with each milk sample. The samples were then incubated separately and aerobically at 37 °C for a duration of 24 to 48 hours. After that, all plates were incubated for 24 to 48 hours at 37°C in an inverted orientation. The isolated pathogens were identified by colony morphological appearance, haemolysis pattern; biochemical testing, including catalase and coagulase tests, Gram staining reaction, Cell morphology after gram staining was performed according to NMC recommendations (Hogan et al., 1999).

2.6. Statistical Analysis

The study's data were analyzed using ANOVA and Turkey-Kramer HSD post hoc test using

SPSS statistical software. P ≤ 0.001 was considered significant according to (Feldman et al., 2003).

Table (1) The prevalence of microorganisms isolated from 400-quarter milk samples collected from 100 dairy cattle.

Prevalence	No	Percent (%)
Negative growth	155	38,7
Positive growth	245	61,3
Mixed infection	90	36,7
Staph. aureus	44	17,9
E. coli	40	16,3
Klebsiella pp.	**25**	10,2
Streptococcus spp.	19	7,7
Staphylococcus vitulins	12	4,9
Proteus spp.	6	2,4
Corynebacterium spp.	5	2
Pasteurella multocida.	4	1,6

3.RESULTS

3.1. SCM prevalence

The prevalence of SCM based on the milk cultural method was 61.3% (n=245) while 155 (38.7%) of the examined samples were negative. The frequency of isolated pathogens in relation to positive samples is presented in Table (1). Most of the samples have more than one type of isolated microorganisms (mixed infection) (36.7%). Of the 400 quarter samples, contagious pathogens primarily Staph. aureus was identified in 44 samples (17.9%) while environmental pathogens such as E. coli were the most isolated environmental pathogens in 40 (16.3%). Also, Klebsiella spp., Streptococcus spp., Staph. vitulins, Proteus Spp., Corynebacterium spp and Pasteurella multocida. Were presented at the incidence of 25(10.2%), 19 (7.7%), 12 (4.9%), 6 (2.4%), 5 (2%), and 4 (1.6%), respectively.

3.2. Comparison between Bacsomatic® and CMT scores and pH values, sensitivity, specificity, and accuracy

Comparison between different tests, sensitivity, specificity, and accuracy were summarized in Table (2). Bacsomatic® automatic counter showed the highest sensitivity (98.3%) and specificity (96.8%) with a test accuracy of 97.7% while the sensitivity of CMT was 84.7% and specificity was 85.3% with an accuracy of 85%. On the other hand, the pH meter showed the lowest sensitivity (80.9%) and specificity (81.5%) with an accuracy of 81.3%.

3.3. The correlation between SCC measured by Bacsomatic® and CMT scores and pH values

The correlation between SCC measured by Bacsomatic® and CMT scores and pH values was presented in Figures. 1, 2 respectively, where there was a positive correlation between SCC and increased CMT scores (r = 0.97) and pH value (r = 0.35), where increased the probability of SCM was positively associated with an increased CMT score using a cut-point of score > negative and increased pH value. The high correlation between CMT and SCC (near 1) was observed while, a low correlation appeared between pH and SCC. The t-test showed significance at p ≤ 0.001.

3.4. The quarter-wise SCM prevalence

The quarter prevalence of SCM in the examined milk samples using CMT was recorded in Table (3). 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 higher test sensitivity than specificity is required. The prevalence of SCM using the CMT test was 57.5% (n=230). The (hind right) HR quarter showed the highest prevalence of mastitis (41.3%) (n=95), and the most frequent CMT score reported was two positives "++ve" (42.6%; n= 98). There were significant differences between the prevalence of SCM in different quarters (P ≤ 0.001).

Table (4) stated the quarter prevalence of SCM in the examined quarter milk samples using Bacsomatic®. The incidence of SCM using the Bacsomatic® was 60% (n=240) distributed among different quarters as the HR quarter showing the highest prevalence (36.3%; n=87). The SCC ranging from 400,000 – 750,000 cell/ml milk was the most frequently reported (42.5%; n=102).

The quarter prevalence of SCM in the examined quarter milk samples using pH-meter is presented in table

(5). The incidence of SCM using pH-meter was 52.5% (n=210), and the HR quarter showed the highest prevalence (33.3%; n=70). There was a significant difference and the highest reported pH range was 6.8 – 6.9 (42.4%; n=89).

3.5. Prevalence of subclinical mastitis based on parity

Table 6 shows the prevalence of SCM according to parity. This study found that the prevalence of SCM rose as the number of parities increased. The highest prevalence of SCM was found at the fourth and fifth parity by all three tests, which were 78.9% and 80% by CMT, 84.2 and 86.7 by BacSomatic®, and 68.4% and 73.3% by pH meter, respectively (animal-wise), and 78.9% and 83.3% by CMT, 82.9 and 85% by BacSomatic®, and 72.4% and 78.3% by pH meter, respectively (quarter-wise). The prevalence of SCM was substantially higher ($p<0.001$) in the fourth and fifth parities compared to other parities (quarter-wise). The lowest incidence of SCM was detected at first parity by all three tests, which were 45%, 50%, and 40% respectively.

3.6. Prevalence of subclinical mastitis based on lactation stage

Table 7 showed the occurrence of SCM relay by lactation stage, with 73.3%, 53.3%, and 40% (animal-wise) and 69.4%, 52.5%, and 42% (quarter-wise). BacSomatic® detected SCM at the early, mid, and late stages of lactation in 77.7%, 56.6%, and 48% of the animals, and 72.2%, 54.2%, and 45% of the quarters, respectively.

Table (2) The ability of the California Mastitis Test (CMT), BacSomatic, and pH-meter to diagnose mastitis with reference to cultural method .

Test	No. of Positive samples	TP	FP	Negative samples	TN	FN	Sensitivity %	Specificity %	Accuracy %
CMT	230 (57.5%)	195 (84.7%)	(15.2%)	170 (42.5%)	145 (85.2%)	25 (17.2%)	84.7	85.3	85
SCC	240 (60%)	236 (98.3%)	4 (1.6%)	160 (40%)	155 (96.8%)	5 (3.1%)	98.3	96.8	97.7
pH meter	210 (52.5%)	(80.9%)	40 (19.1%)	190 (47.5%)	(81.5%)	(18.4%)	80.9	81.5	81.3

TP=True Positive, FP=False Positive, TN=True Negative, FN=False Negative.

$$Sensitivity = \frac{\mathrm{TP}}{\mathrm{TP}+\mathrm{FN}} \times 100$$

$$Specificity = \frac{\mathrm{TN}}{\mathrm{FP}+\mathrm{TN}} \times 100$$

$$Accuracy = \frac{\mathrm{TP}+\mathrm{TN}}{\mathrm{TP}+\mathrm{FP}+\mathrm{FN}+\mathrm{TN}} \times 100$$

Table (3) Quarter-wise prevalence of mastitis of 400 quarter milk samples collected from 100 dairy cattle using California mastitis test

Test	Quarter side	Negative (%)	Positive (%)

			Total	+	++	+++
CMT	HR	5 (2.9%) a	95 (41.3%) a	95 (41.3%) a	37 (38.9%)	28 (29.5%)
	HL	45 (26.5%) a	55 (23.9%) b	17 (30.9%)	26 (47.3%)	12 (21.8%)
	FR	54 (31.8%) b	46 (20%) c	15 (32.6%)	21 (45.7%)	10 (21.7%)
	FL	66 (38.8%) c	34 (14.8%) c	10 (29.4%)	21 (45.7%)	10 (29.4%)
	Total	170 (42.5%)	230 (57.5%)	72 (31.3%)	98 (42.6%)	60 (26.1%)

LF=Left front quarter, LH=Left hind quarter, RF =Right front quarter, RH=Right hind quarter

Different letters in the same column are significantly different (P ≤ 0.001)

Table (4) Quarter-wise prevalence of mastitis of 400 quarter milk samples that collected from 100 dairy cattle using Bacsomatic®.

Test	Quarter side	Negative (%)	Positive (%)			
SCC			Total	200,000 – 400,000 cell/ml	400,000 – 750,000 cell/ml	> 750,000 cell/ml
	HR	13 (8.1%) a	87 (36.3%) a	29 (33.3%)	38 (43.7%)	20 (22.9%)
	HL	35 (21.8%) b	65 (27.1%) b	24 (36.9%)	28 (43.1%)	13 (20%)
	FR	45 (28.1%) b	55 (22.9%) b	19 (34.5%)	23 (41.8%)	13 (23.7%)
	FL	67 (41.8%) c	33 (13.8%) c	14 (42.4%)	23 (41.8%)	6 (18.2%)
	Total	160 (40%)	240 (60%)	86 (35.8%)	102 (42.5%)	52 (21.6%)

LF=Left front quarter, LH=Left hind quarter, RF =Right front quarter, RH=Right hind quarter Different letters in the same column are significantly different (P ≤ 0.001)

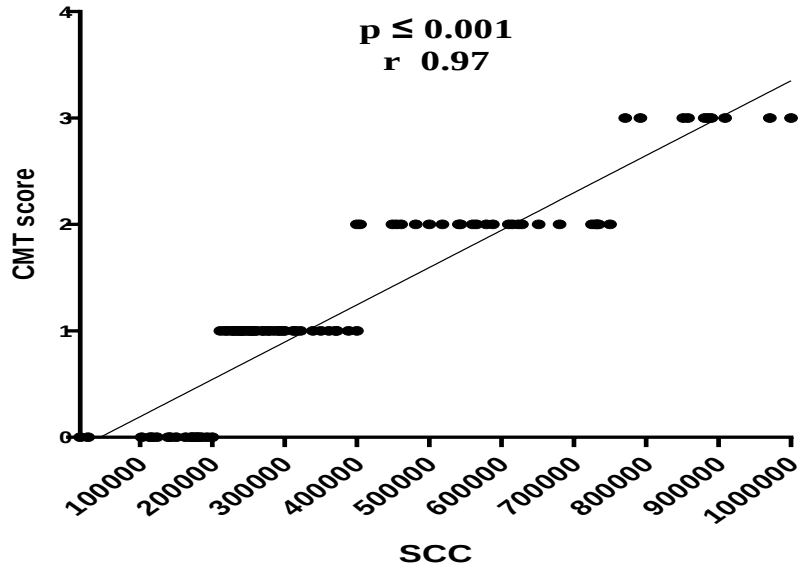

Fig (1) Scatterplot of the correlation between SCC measured by Bacsomatic® and CMT score for 400 quarter milk samples from 100 dairy cattle. The black line represents the diagonal solid line of identity.

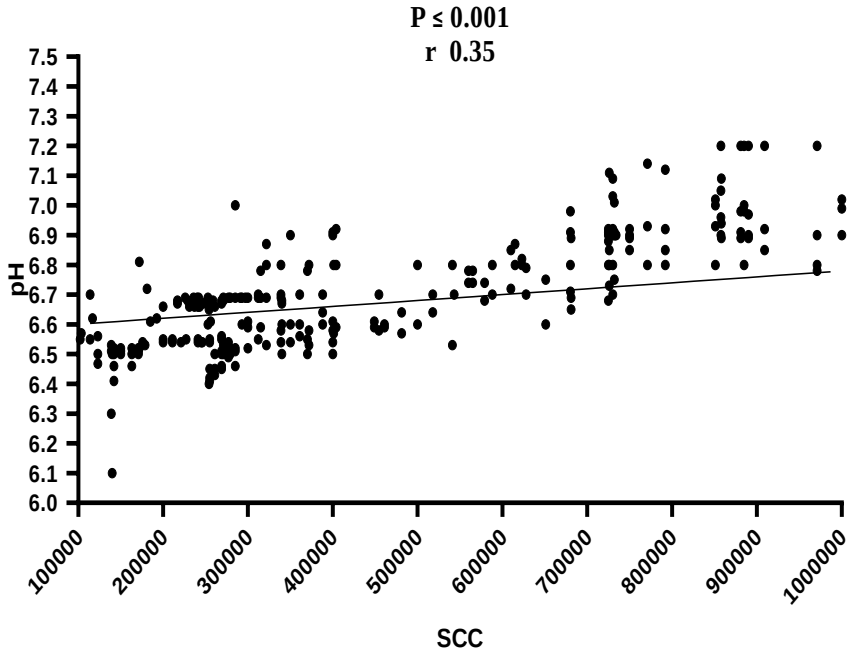

Fig 2: Scatterplot of the correlation between SCC measured by Bacsomatic® and pH value measured by pH-meter for 400-quarter milk samples from 100 dairy cattle. The black line represents the di

Table (5) Quarter-wise prevalence of SCM of 400 quarter milk samples collected from 100 dairy cattle using pH meter

Test	Quarter side	Negative (%)	Positive (%)			
pH meter			Total	pH 6.6 – 6.7	pH 6.8 – 6.9	pH > 6.9
	HR	30 (15.8%) a	70 (33.3%) a	21 (30%)	30 (42.9%)	19 (27.1%)
	HL	35 (18.4%) b	65 (31%) b	22 (33.8%)	25 (38.5%)	18 (27.7%)
	FR	48 (26.8%) c	52 (24.7%) c	15 (28.8%)	23 (44.2%)	14 (26.9%)
	FL	77 (25.3%) c	23 (11%) c	9 (39.1%)	11 (47.8%)	3 (13.1%)
	Total	190 (40.5%)	23 (11%) c	67 (31.9%)	89 (42.4%)	54 (25.7%)

LF=Left front quarter, LH=Left hind quarter, RF =Right front quarter, RH=Right hind quarter

Different small superscripted letters in the same column are significantly different ($P \leq 0.001$)

Table (6): Effect of number of parities on the prevalence of mastitis in 100 lactating cattle and 400 quarter samples

Test	**parity**	**Total animals (100)**	**Animal- Wise**		**Total quarters (400)**	**Quarter-wise**	
			Positive %	**Negative %**		**Positive %**	**Negative %**
CMT	**1st**	**20**	**9 (45%) a**	**11 (55%) a**	**80**	**28 (35%) a**	**52 (65%) a**
	2nd	**24**	**10 (41.6%) a**	**14 (58.3%) a**	**96**	**40 (41.7%) b**	**56 (58.3%) b**
	3rd	**22**	**13 (59.1%) b**	**9 (40.9%) b**	**88**	**52 (59.1%) c**	**36 (40.9%) c**
	4th	**19**	**15 (78.9%) c**	**4 (21.05%) c**	**76**	**60 (78.9%) d**	**16 (21.1%) d**
	5th	**15**	**12(80%) c**	**3 (20%) c**	**60**	**50 (83.3%) e**	**12 (16.7%) e**
SCC	**1st**	**20**	**10 (50%) a**	**10 (50%) a**	**80**	**29 (36.3%) a**	**51 (65%) a**
	2nd	**24**	**12 (50%) a**	**10 (50%) a**	**96**	**45 (46.9%) b**	**51 (53.1%) b**
	3rd	**22**	**13 (59.1%) b**	**9 (40.9%) b**	**88**	**52 (59.1%) c**	**36 (40.9%) c**
	4th	**19**	**16 (84.2%) c**	**3 (15.8%) c**	**76**	**63 (82.9%) d**	**13 (17.1%) d**
	5th	**15**	**13 (86.7%) c**	**2 (13.3%) c**	**60**	**51 (85%) d**	**9 (15%) d**
	1st	**20**	**8 (40%) a**	**12 (60%) a**	**80**	**21 (26.3%) a**	**59 (73.7%) a**
pH	**2nd**	**24**	**11 (45.8%) b**	**13 (54.2%) b**	**96**	**39 (40.6%) b**	**57 (59.4%) b**
	3rd	**22**	**10 (45.5%) b**	**12 (54.5%) b**	**88**	**48 (54.5%) c**	**40 (45.5%) c**

	4th	**19**	**13 (68.4%) c**	**6 (31.6%) c**	**76**	**55 (72.4%) d**	**21 (27.6%) d**
	5th	**15**	**11 (73.3%) d**	**4 (26.7%) d**	**60**	**47 (78.3%) e**	**13 (21.7%) e**

different small superscripted letters in the same column for each test are considered significantly different (P ≤ 0.001)

Table (7) Effect of lactation stage on the incidence of mastitis in 100 lactating cattle and 400 quarter samples

Test	**Lactation stage**	**Total animals (100)**	**Animal- Wise**		**Animal - Wise**	**Quarter-wise**	
			Positive %	**Negative (%)**		**Positive %**	**Negative %**
CMT	**Early (6-90d)**	**45**	**33 (73.3%) a**	**12 (26.7%) a**	**180**	**125 (69.4%) a**	**55 (30.6%) a**
	Mid (91-180d)	**30**	**16 (53.3%) b**	**14 (46.7%) b**	**120**	**63 (52.5%) b**	**57 (47.5%) b**
	Late (>180d)	**25**	**10 (40%) c**	**15 (60%) c**	**100**	**42 (42%) c**	**58 (58%) c**
SCC	**Early (6-90d)**	**45**	**35 (77.7%) a**	**10 (22.3%) a**	**180**	**130 (72.2%) a**	**50 (27.8%) a**
	Mid (91-180d)	**30**	**17 (56.6%) b**	**13 (43.4%) b**	**120**	**65 (54.2%) b**	**55 (45.8%) b**
	Late (>180d)	**25**	**12 (48%) c**	**13 (52%) c**	**100**	**45 (45%) c**	**55 (55%) c**
	Early (6-90d)	**45**	**30 (66.7%) a**	**15 (33.3%) a**	**180**	**120 (66.7%) a**	**60 (33.3%) a**
	Mid (91-180d)	**30**	**14 (46.7%) b**	**16 (53.3%) b**	**120**	**51 (42.5%) b**	**69 (57.5%) b**
	Late (>180d)	**25**	**9 (36%) c**	**16 (53.3%) b**	**100**	**51 (42.5%) b**	**61(61%) c**

different small superscripted letters in the same column for each test are considered significantly different (P ≤ 0.001)

3. DISCUSSION

Subclinical mastitis in cows affects their health, wellbeing, longevity, and performance, leading to reduced productivity and profit. Early prediction of subclinical mastitis can enable dairy farmers to perform interventions to mitigate its effect (Pakrashi et al., 2023). The dairy business experienced significant growth and advancement during the past two decades. Nevertheless, SCM remains a prevalent and expensive ailment that impacts dairy cows, even with the adoption of rigorous preventative measures (Bi et al., 2016; Yang et al., 2019).

According to the bacteriological investigation, the most common bacteria are Staph. aureus, E. coli, and Klebsiella species. During the milking process, infectious germs are transferred from infected to uninfected teats and stored in the udder. According to Ashraf and Imran (2020) and Cobirka et al. (2020), Streptococcus agalactiae and Staph. aureus are the most common types of bacteria discovered. The results were higher than that reported previously by El-Fattah et al. (2023) who isolated E. coli from 14% of the examined milk samples. A previous study reported that E. coli caused over 80% of cases of coliform mastitis (Fahim et al., 2019). The prevalence 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 and using unclean washing towels (Ayano et al., 2013).

Staph. aureus, the most major contagious mastitis-causing bacterium with a high degree of penetration that develops deep-seated foci in the infected glands,

was the predominant isolated contagious pathogen (Ranjan et al., 2011). This may result is serious issues and have an expense effect on dairy animals (Dego et al., 2002). A number of factors, such as inadequate hand hygiene before and during milking, a lack of teat dipping after milking, the failure to cull animals with chronic infections, and the lack of dry cow therapy in many dairy herds, may be responsible for the high prevalence of Staph. aureus in our study (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. Furthermore, Staph. vitulinus was detected by Nobrega et al. (2018) and Alkhouly et al. (2023) from mastitic milk samples.

The sensitivity, specificity, and accuracy of the testes used for the diagnosis of subclinical mastitis are listed in Table 2. The Bacsomatic® sensitivity and specificity were higher than those of CMT and pH. The sensitivity and specificity of pH were lower than those of others. 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 also 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 strongest correlation (r=0.97) was observed between SCC and CMT, but a low correlation (r=0.35) was found between SCC and pH. The present investigation clearly demonstrated that Bacsomatic® was the most dependable test and exhibited the closest correlation with the bacteriological data. The current results are consistent with the findings of Neelesh et al. (2008). According to their findings, Bacsomatic® was deemed the most precise diagnostic test for SCM, with the California mastitis test (CMT) following closely behind. In their study, Reddy et al. (1998) conducted a comparison between the specificity and sensitivity of CMT and SCC using a conventional cultural test. They found that somatic cell counter had a specificity of 84.84% while CMT had a specificity of 73.30%. Tanwar et al. (2001) conducted a comparison of different diagnostic tests for detecting mastitis. They found that the somatic cell counter had a sensitivity of 100% and the CMT reaction had a sensitivity of 96%. According to a study conducted by Sargeant et al. (2001), it was shown that CMT (California Mastitis Test) can be utilized as a screening test in a dairy herd monitoring program to identify newly lactating cows with intramammary infection caused by significant pathogens. According to Barbosa et al. (2002), there is a strong correlation between the SCC and the California Mastitis Test (CMT) when it comes to diagnosing mastitis.

For almost 60 years, the California mastitis test has been a semiquantitative screening test conducted at the cow's side and offers results within one minute, despite the significant variation in SCC within each CMT score. Our study showed good sensitivity (84.7%) and specificity (85.3%) of CMT using a threshold reaction ≥ +ve (i.e., any non-negative CMT score). Interpreting the CMT as negative or positive can maximize its clinical utility in the diagnosis of SCM while maintaining the best sensitivity and appropriate specificity. 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).

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 bad management systems and intramammary infection that are in agreement with other previous studies (Minnat & Hammadi, 2015). These variations might be assigned to the complex nature of mastitis, which involves the interactions of various factors such as management practices, environmental factors, animal-related factors, and the causative pathogen (Constable et al., 2016).

Our results showed that, due to the declining sensitivity and specificity of milk pH measurement as an on-farm diagnostic test for SCM, milk pH does not offer a feasible and helpful screening tool for identifying SCM in on-farm settings. Our investigation indicates that the primary reason for this is the reduced sensitivity of milk pH in identifying mastitis. The poorer sensitivity of the

test is attributed to the wide range of milk pH values even in uninfected quarters and the comparatively small pH increase in infected quarters (Cherrington et al., 1933). This could be explained by the fact that, in contrast to blood pH, milk pH is not strictly controlled; as a result, even in healthy environments, a larger range or fluctuation in milk pH is predicted. However, milk pH may be more accurate and useful in the clinical setting for identifying subclinical infected quarters when used in conjunction with other diagnostic techniques like SCC. Although the precise physicochemical mechanism responsible for the rise in milk pH in infected quarters has not yet been established, the strong ion difference theory applied to milk, along with the physicochemical models developed for urine (Constable et al., 2009) and plasma (Constable, 1997), it may be assigned to an increase in the concentration differential between sodium (primary strong cation) and the sum of chloride and casein (main strong anions) in milk, which consequently increases the strong ion difference (Ogola et al., 2007).

It is commonly stated that the pH of milk, excluding mastitic and colostrum milk, ranges from 6.4 to 6.8 (Schalm & 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 mastitis. However, another recent study revealed an optimal pH cut point of ≥6.67 for cows at dry-off and 6.52 for freshening cows for diagnosing mastitis using a pH meter with adjusted milk temperature (Kandeel et al., 2019).

The incidence of SCM rose as parity increased. This observation is reinforced by Rasool et al. (1985) and Devi et al. (1997), who found that the prevalence of SCM increased with parity. The risk tends to be greatest in older parity cows (Rahularaj et al., 2019) and early in lactation (Fox, 2009). The strength of the associations between risk factors and subclinical mastitis can also differ by parity, as reflected by 2-way interactions between parity and risk factors for clinical mastitis previously reported in dairy cows (Pakrashi et al., 2023).

According to Batra and McAllister (1984), Somatic Cel1 Count and CMT score grew from first to fourth parity, while conductivity generally increased from second to fourth parity. Lee JeongChi and Lee ChaiYong (2007) found that higher parity was associated with increased SCC. Badiuzzaman et al. (2015) found that cows with third and fourth parity had considerably greater ($p \leq 0.001$) SCM compared to other early phases.

The data indicated that SCM impacted all three stages of Lactating in cows. The prevalence of SCM was highest during the early stage of lactation, according to all measuring tests, California Mastitis Test (CMT), SCC, and pH. The mid-stage of lactation had a slightly lower incidence, while the late-lactation stage had the lowest incidence of SCM. The prevalence of SCM during the early stage of lactation was substantially greater ($p \leq 0.001$) compared to other stages of lactation (both at the animal and quarter level). In their study, Lalrintluanga et al. (2003) found that the occurrence of mastitis was more frequent in the initial phase of the third lactation, with a rate of 36.60%. Nevertheless, SCC has been observed to rise during the initial days of Lactating and may remain elevated for up to one month (Koç, 2008). Conversely, an increase in SCC towards the end of lactation is regarded as a normal physiological response. Also, Badiuzzaman et al. (2015) discovered that the highest occurrence of SCM was observed during the early breastfeeding stage as the prevalence rates, based on CMT, and SCC tests were 78.43% and 76.47% at the animal level, while, they were 70.58% and 64.22% at the quarter level. The occurrence of SCM was notably greater ($p < 0.001$) during the initial phase of Lactating compared to other stages of lactation (on a per-quarter basis). Sederevičius et al. (2006) recorded a short-lived rise in SCC right after calving. This was because the udder was changing from not lactating to lactating. However, during the latter stages of lactation, SCC typically remained within the normal range.

4. CONCLUSIONS

The obtained results showed a high incidence of SCM among lactating cattle in Menoufia governorate. The BacSomatic® 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 test sensitivity and specificity of the CMT were higher than those of milk pH when it was applied cow-side with a cut-point larger than negative. The incidence of SCM varied depending on a variety of parameters, including the fact that the back quarters were more impacted, that the

prevalence rose with the number of parties involved, and that the early stages of lactation were more likely to be associated with SCM than the mid- and late-stages.

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