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Accuracy of Field Methods in Detection of Bovine Subclinical Mastitis

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ABSTRACT

Mastitis is a major challenge for the dairy industry. The current study aimed to evaluate the performance of Draminski® mastitis detector (DMD) and ultrasonography as cow-side tools for detecting bovine subclinical mastitis. To achieve this purpose, a total of 103 raw milk samples, including 48 samples from buffaloes and 55 from cows were tested using DMD, the California Mastitis Test (CMT), ultrasonography, and bacterial culture. Based on the results of CMT and bacterial culture, the study defined the quarter health status (QHS) as healthy, latent, or inflamed. The study evaluated the performance of the DMD and ultrasonography via Spearman's rank correlation and calculated the cut-point value for DMD, in addition to identifying the sensitivity, specificity, and predictive values for ultrasonography against QHS. Results showed that percentages of quarters defined as healthy or inflamed using QHS categorization were higher in buffaloes than cows, unlike the latent ones. The study found a significant inverse correlation between DMD and QHS (– 0.695 in buffalo and – 0.526 in cow samples). Using inflamed/non-inflamed QHS classification, the cut-point values for the DMD were 515 for buffaloes and 465 for cows. The diagnostic performance analysis of ultrasonography against inflamed/non-inflamed QHS revealed a sensitivity of 83% and specificity of 22%. The negative predictive and positive predictive values for ultrasonography against inflamed/non-inflamed QHS were 78% and 29%, respectively, and the overall accuracy was 39% as a screening method for subclinical infection. The study concluded that DMD is suitable for early detection of bovine subclinical mastitis. The cut-point value assigned by the DMD manufacturer is potentially low. Therefore, the study suggests a cut-point value of 515 for buffaloes and 465 for cows. Finally, ultrasonography is more appropriate for identifying structural intramammary changes rather than for screening subclinical infections.

Keywords: subclinical mastitis, mastitis screening, precise detection, Draminski, sustainable production

INTRODUCTION

Intramammary health is a major challenge for dairy profitability. The inflammatory process causes damage to mammary gland epithelial cells through different factors, including intracellular bacterial multiplication, bacterial toxins, inflammatory cell infiltration, and release of proteolytic enzymes from inflammatory cells (**1**). For instance, neutrophils and histone, a neutrophil's attachment protein, have cytotoxic, necrotic, pyroptotic, and apoptotic effects on mammary gland epithelial cells (**2**) Additionally, inflammatory reactions can compromise the blood-milk barrier integrity, which allows infiltration of blood constituents to enter the milk (**3**). These reactions decrease the number of healthy cells available for production, which reduces milk production and changes the milk composition, particularly the concentrations of protein, fat, and lactose (**4**).

Moreover, infiltration of the inflammatory cells increases the somatic cell count (SCC) in the milk. Altogether, they cause undesirable milk composition changes that can decrease milk quality, sustainability, and suitability for the dairy industry (**5**).

The manifestation of mastitis can be clinical with noticeable changes in milk and/or the gland, accompanied or not by systemic reactions, or subclinical, mainly manifested by increased SCC and detected through milk examination (**6**). The economic losses of subclinical infections are considered greater than those of clinical episodes and mainly impact milk yield and quality (**5**). Therefore, monitoring intramammary health can decrease the economic impact associated with subclinical infections (**7**). The California mastitis test (CMT) remains the most common cow-side test used to distinguish an intramammary infection based on predicting milk SCC from potentially affected quarters (**8**). Other screening methods include on-farm methods, such as testing the electrical conductivity via the Draminski® mastitis detector (DMD), and laboratory tests, such as SCC via the flow cytometry method (**9**). Udder ultrasonography has promising results as a practical and non-invasive on-farm method for investigating mammary gland health (**10**).

Considering the lack of a definitive gold standard method for the diagnosis of subclinical mastitis in animals and the fact that the most methods used for this purpose are indirect (**11,12**), such as CMT, SCC, and DMD, selecting an appropriate method would rely on the cost-effectiveness and non-invasiveness of the test. A previous study compared the performance of CMT and DMD based on microscopic SCC in detecting subclinical mastitis in lactating buffaloes (**13**). In that study, the area under the curve was 80.1% and 81.4% for CMT and DMD, respectively. The current study hypothesizes that DMD and ultrasonography have reliable performance in detecting bovine subclinical mastitis as a portable cow-side method that does not require reagent addition, as is the case for CMT. Therefore, the objective of this study was to evaluate the performance of DMD and ultrasonography in detecting bovine subclinical mastitis.

Materials And Methods

Ethical Approval

The scientific Institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul approved conducting this study (Approval Ref. is UM.VET.2023.158 on 15/12/2023).

Study Animals and Samples

Study animals included 21 buffaloes and 18 cows. A total of 103 milk samples were aseptically collected (48 from buffaloes and 55 from cows), which represented quarter-level milk samples, except for 11 composite milk samples (6 from buffaloes and 5 from cows), summarized in **Table 1**. Because study objective was to evaluate the performance of field screening tests, the sample type (composite or quarter-level milk samples) was not considered critical.

Table 1. Summary of animals and samples used in the study

Animals		Composite samples	Quarter-based samples	Total samples
Type	Number			
Buffalo	21	6	42	48
Cow	18	5	50	55

Detection of Mastitis in Study Animals

Draminski® Mastitis Detector (DMD)

The first foremilk (about 20 mL milk) was collected into the measuring cup of the Draminski® Mastitis Detector 1Q (Dramiński S. A., Gietrzwałd, Poland) directly from the teat, turned on the power, and recorded the value of the electrical resistance displayed on the screen. According to the manufacturer's manual, a reading of less than 300 indicated evidence of infection. Additionally, the cut-point of 515 for buffalo milk was considered as previously assigned by Ali and Dahl (**13**).

California Mastitis Test (CMT)

Equal amounts of milk samples were mixed with previously prepared CMT reagent (ImmuCell Co., Portland, ME, USA) inside the cups of the CMT paddle, gently rotated the paddle for 30 seconds, and recorded the result as previously described by Fitts et al. (**14**).

Ultrasonography

The udder skin was thoroughly cleaned and disinfected using 70% alcohol and applied a gel to the examination area. For scanning purposes, a 7.5 MHz ultrasound linear transducer was longitudinally placed on the udder surface, starting from the right hind quarter along the entire udder (**15**), and displayed mammary gland structure images using a portable ultrasound scanner KX5100 (KAIXIN-BIO-VET, China). The ultrasonography images were interpreted taking into consideration the structure of the normal quarter is homogenous and echogenic (**15**).

Bacterial Culture

Milk samples were cultured on four different media: (i) Nutrient Agar (NA) as a non-selective medium capturing a wide range of bacteria, (ii) Mannitol Salt Agar (MSA) as a selective medium for *Staphylococcus* spp., (iii) Eosin Methylene Blue (EMB) as a selective medium for *Escherichia coli*, and (iv) Edward's Medium Base, Modified (ED) as a selective medium for *Streptococcus agalactiae* and other streptococci. All media were provided by HiMedia Laboratories Pvt Ltd., India, and prepared according to the standard techniques for this purpose (**16**). Teats were first disinfected with 70% alcohol, the first foremilk was discarded, approximately 10 mL of milk was collected in a sterile container, and directly the samples were transported to the laboratory. In a sterile environment, the plates were streaked according to the standard methods (**17**). After overnight incubation at 37°C, the morphology and color of the colonies were visually inspected to identify the bacterial growth. The following characteristics were considered positive on bacterial culture: any growth of colonies on NA, yellow colonies on MSA, shiny colonies (metallic sheen) on EMB, and transparent colorless pinheads on Edward's Medium Base, Modified (**16**).

Diagnostic Performance: DMD and Ultrasonography

In this analysis, the quarter health status (QHS) was defined as healthy, latent, or inflamed based on the results of CMT and bacterial culture (**Table 2**). This approach is a modification of that used by Tamburini et al. (**18**) for quarter health status classification. The performance of DMD and ultrasonography as a cow-side procedure for identification of mastitis was evaluated compared to QHS as the reference standard. Spearman's rank correlation was applied to assess the relationship between QHS (healthy, latent, or inflamed) and DMD readings. The receiver operating characteristic (ROC) curve was used to calculate the cut-point value for DMD based on treating QHS as a binary variable: non-inflamed (healthy/latent) = 0 and inflamed = 1 (**13,19**). In this analysis, the cut-point value represented the minimum value for the absolute difference between ROC curve sensitivity and specificity (19). The DMD data in cattle and buffalo was examined separately because milk fat influences DMD results (13). On the other hand, the sensitivity, the specificity, the predictive values, and the accuracy were calculated as measures of the diagnostic performance of ultrasonography for identifying subclinical mastitis against inflamed/non-inflamed QHS. Finally, the analysis was performed by STATA 13.0, Stata Corp.; IBM SPSS Statistics, V 21, IBM Corp.; and Microsoft 365 Spreadsheet, Microsoft Corp.

Table 2. Classification of QHS based on CMT and bacterial culture

CMT	Bacterial culture	QHS
0 or T	Negative	Healthy
0 or T	Positive	Latent
1+, 2+, or 3+	Negative or Positive	Inflamed

0: Negative, T: Trace, 1+: Weak positive, 2+: Distinct positive, 3+ Strong positive

Results

Descriptive Analysis

Among samples from buffaloes, 8% and 37% gave a reading of less than 300 and 515 with DMD, respectively, whereas all samples from cows were above the cut-point value (**Table 3**). On the other hand, 56% and 80% of samples from buffaloes and cows, respectively, gave negative readings with CMT, whereas other readings were between trace and strong positive (**Table 3**). However, samples from buffaloes gave a lower percentage of bacterial culture than those of cows (**Table 3**). Finally, the percentages of quarters defined as healthy or inflamed using QHS categorization were higher in buffaloes than cows, unlike the latent ones (**Table 3**).

Table 3. Details of the Draminski® mastitis detector (DMD), California mastitis test (CMT), bacterial culture, and quarter health status (QHS) results using 103 milk samples, 48 buffalo-milk samples, and 55 cow-milk samples

Tests Readings	Buffalo (n=48)	Cow(n=55)	Total(N=103)
Draminski mastitis detector (DMD)			
Cut-point value 300			
≥ 300	44 (92%)	55 (100%)	99 (96%)
< 300	4 (8%)	0	4 (4%)
Cut-point value 515			
≥ 515	30 (63%)	*	
< 515	18 (37%)	*	
California Mastitis Test (CMT)			
0: Negative	27 (56%)	44 (80%)	71 (69%)
T: Trace	2 (4%)	4 (7%)	6 (6%)
1+: Weak positive	8 (17%)	3 (6%)	11 (11%)
2+: Distinct positive	10 (21%)	4 (7%)	13 (14%)
3+ Strong positive	1 (2%)	0	1 (1%)
Bacterial Culture (positive samples)			
Nutrient Agar (NA)	16 (33%)	33 (60%)	49 (48%)
Mannitol Salt Agar (MSA)	1 (2%)	7 (13%)	8 (8%)
Eosin Methylene Blue (EMB)	3 (6%)	3 (5%)	6 (6%)
Edward's Medium Base, Modified (ED)	1 (2%)	9 (16%)	10 (10%)
NA only	12 (25%)	16 (29%)	28 (27%)
NA and at least another media	4 (8%)	17 (31%)	21 (20%)
Quarter Health Status (QHS)			
Healthy	25 (52%)	22 (40%)	47 (46%)
Latent	4 (8%)	26 (47%)	30 (29%)
Inflamed	19 (40%)	7 (13%)	26 (25%)

* Cut-point value 515 for Draminski mastitis detector (DMD) was not used in cows.

Ultrasonographic images

The ultrasonographic examination revealed that the normal mammary gland has a uniform homogenous echogenic structure with clear anatomical structure representing visible hypoechoic vessels and clear distal spectrum of the examined quarter (**Figure 1**). In contrast, marked alterations in the echogenic structure were observed in the inflamed quarters (**Figure 2**). In cows, a heterogenous pattern was observed characterized by hyperechoic areas with echogenic dots representing inflammatory cell aggregation and branching area fluid-filled known as a ramified hypoechoic structure, in addition to disappearance of the distal spectrum of the examined quarter (**Figure 2A**). On the other hand, affected buffalo-quarter also exhibited hyperechoic structure with appearance of distal spectrum of the examined quarter as an echogenic line (**Figure 2B**).

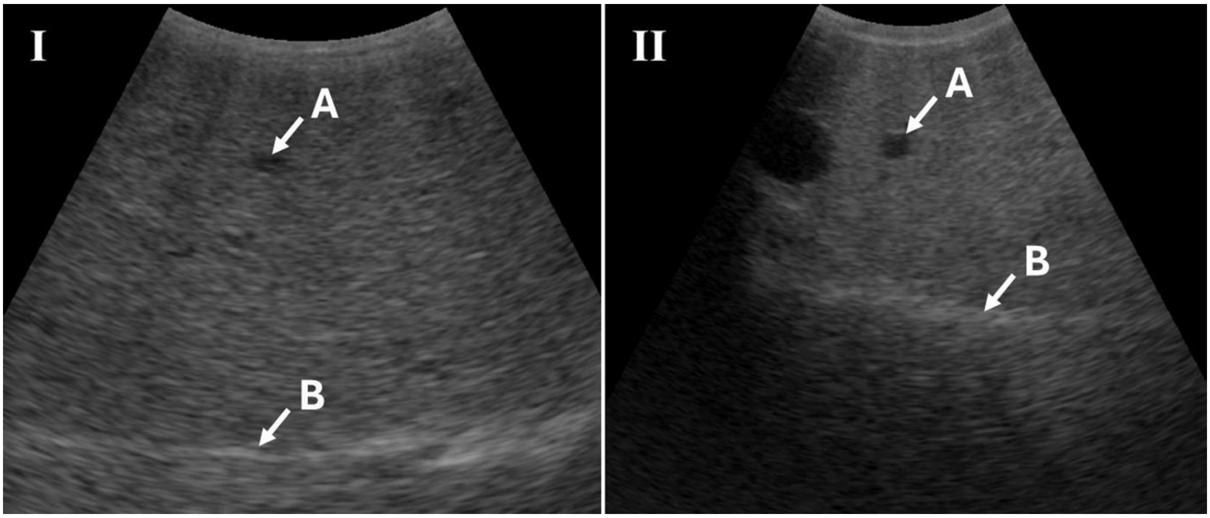

Figure 1. Longitudinal sonogram for normal mammary gland of cow **(I)** and buffalo **(II)** using a 7.5 MHz transducer. For each image, the left line represents the dorsal part of the animal, while the right line is the ventral part. The images show a uniform homogenous echogenic pattern with clear anatomical structures, such as visible hypoechoic vessel **(A)** and distinct echogenic distal spectrum **(B)** of the examined quarter

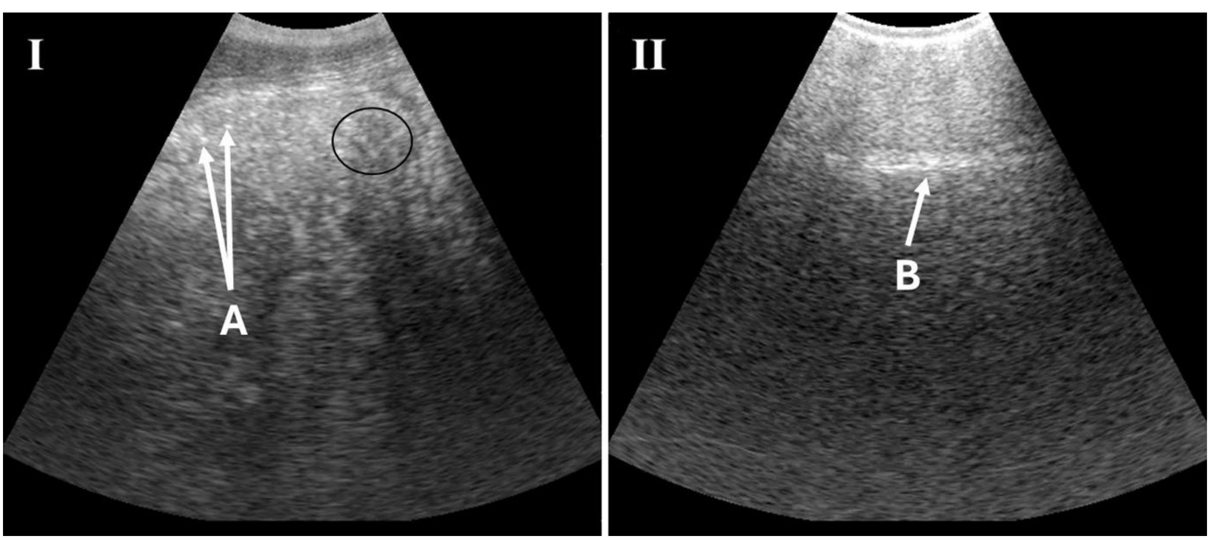

Figure 2. Longitudinal sonogram for inflamed mammary glands of cow **(I)** and buffalo **(II)** with a 7.5 MHz transducer. For each image, the left line represents the dorsal part of the animal, while the right line is the ventral part. In cow (I), inflamed quarter shows a heterogeneous structure with a hyperechoic area including echogenic dots reflecting aggregation of inflammatory cells (A) and a fluid-filled branching area known as a ramified hypoechoic structure (circle). The distal spectrum of the quarter was not visible. In buffalo (II), the quarter shows a hyperechoic and undefined structure, while the distal spectrum of the examined quarter remains visible as a clear echogenic line **(B)**

Diagnostic Performance: DMD and Ultrasonography

Spearman's analysis revealed a significant inverse correlation between DMD and QHS ($P < 0.01$). The correlation coefficient was – 0.695 in buffalo and – 0.526 in cow samples (**Figure 3**). Based on inflamed/non-inflamed QHS, the cut-point values for the DMD were 515 for buffaloes and 465 for cows. On the other hand, quarters identified as abnormal ultrasonographic images represented 83% of those identified as healthy QHS, 71% latent, and 83% inflamed (**Figure 4**). The diagnostic performance analysis of ultrasonography against inflamed/non-inflamed QHS revealed a sensitivity of 83% and specificity of 22%. The negative predictive value was 78%, while the positive predictive value was 29% (**Table 4**). The overall accuracy was 39%.

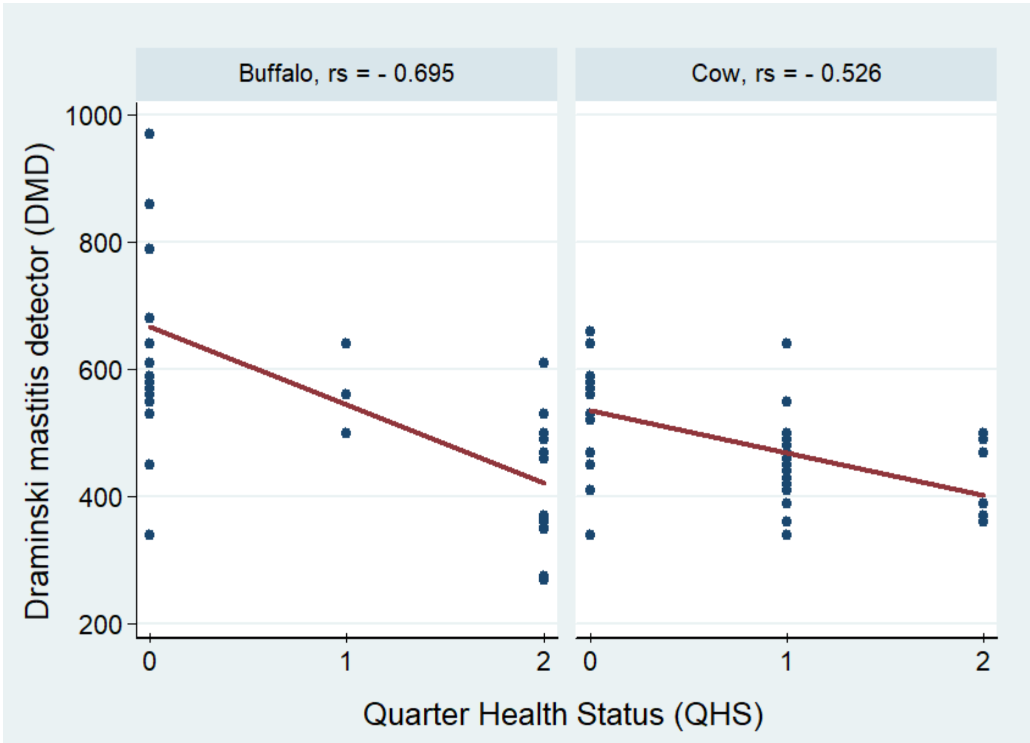

Figure 3. Scatter plot showing the inverse Spearman's rank correlation between quarter health status (QHS) and Draminski® mastitis detector (DMD) readings in milk samples from buffaloes (r_s = - 0.695) and cows (r_s = - 0.526). Blue dots represent individual DMD readings, whereas red lines indicate fitted values

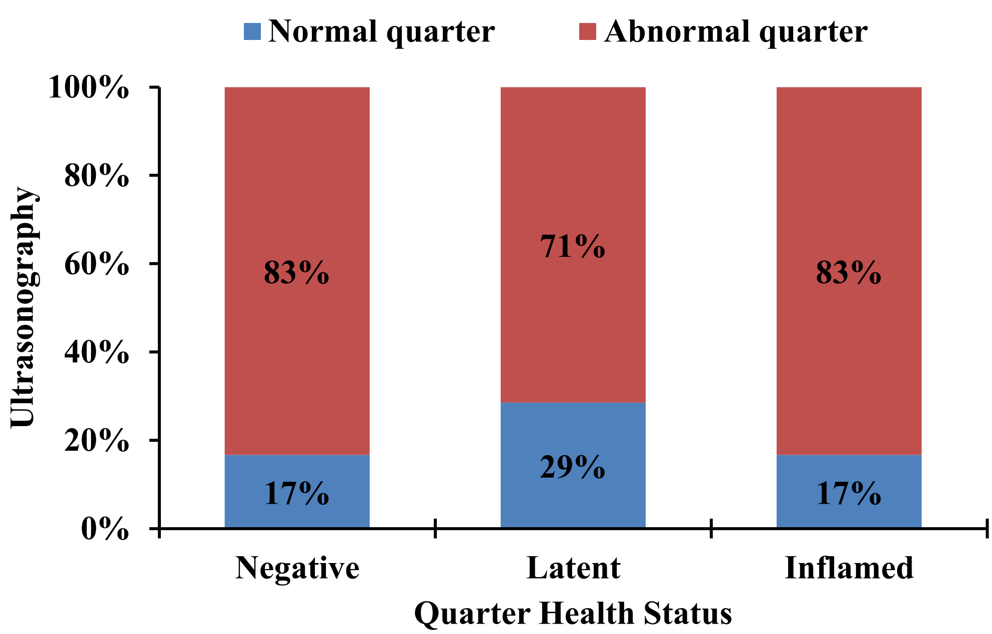

Figure 4. Stacked column graph comparing the distribution of ultrasonographic findings (normal/abnormal quarters) across quarter health status categories in buffalo and cows

Discussion

The study conducted here evaluated the diagnostic accuracy of some cow-side methods for the detection of subclinical mastitis in buffaloes and cows. In this study, DMD appears as a promising screening tool for bovine subclinical mastitis, with a cut-point value higher than that assigned by the manufacturer. On the other hand, while ultrasonography is a good diagnostic method for pathological changes of the mammary gland, it might not be applicable to be used for large numbers of animals and without trained personnel. This study has the strength of defining a healthy/inflamed quarter used for evaluating DMD readings and ultrasonography results. The current study used two parameters to define the quarter health status (QHS), a combination of the CMT readings and bacterial culture results.

In this study, significant and strong inverse correlation was observed between DMD readings and QHS in both buffalo and cow samples. The inverse correlation reported here does not mean disagreement between these two methods. Instead, it reflects the opposite direction of the correlation as a function of the measurement scales of DMD and QHS. That is, DMD readings constitute the values of electrical resistance of milk, which decreases in milk from inflamed quarter as a function of Na^+, Cl^-, K^+ and lactose concentrations imbalance (**13**). In contracts, the value of QHS was higher in this study as a function of positive CMT readings. Indeed, this direction and magnitude of the correlation supports that DMD is a good tool for detection of mammary gland inflammation. However, the correlation was higher in buffaloes than in cows. The results of the current study are in line with those of a previous local study by Ali and Dahl (**13**), although the previous study compared DMD readings with microscopic SCC in buffalo samples only. On the other hand, these results contrast Galfi et al. (**20**) and Pisestyani et al. (**21**) findings, who reported poor performance of DMD in cow samples. Nevertheless, Galfi et al. (**20**) compared DMD with bacterial culture only, while Pisestyani et al. (**21**) compared it with an SCC-based device. The current study offers a more rigorous evaluation of DMD readings than the studies by Galfi et al. (**20**) and Pisestyani et al. (**21**), as it assessed DMD readings using the QHS, which combines CMT readings and bacterial culture results.

In the current study, the DMD cut-point values for both buffaloes and cows were higher than that assigned by the manufacturer's guidelines. The cut-point value of 515 for DMD buffalo milk samples in this study is in line with a previous study by Ali and Dahl (**13**), which could indicate that the value of 515 aligns more closely with buffalo milk samples than the value of 300 assigned by the manufacturer. This difference is biologically plausible because buffalo milk contains a higher fat than that of cow, which can elevate DMD readings above the average (**13**). The current study also found a higher cut-point value for cows than that assigned by Draminski® guidelines, which could indicate that the assigned cut-point DMD value by the manufacturer might be too low and thus limit the number of animals identified as having subclinical mastitis. A previous study by Pisestyani et al. (**21**) reported low sensitivity and high specificity of DMD, which could urge the need of re-evaluating the current Draminski® guidelines for the DMD cut-point value.

The ultrasonography successfully differentiated between healthy and unhealthy mammary gland tissues. Ultrasonography is an excellent tool for visualizing morphological features of the mammary gland (**22**). In this study, ultrasonography had high diagnostic sensitivity. It was able to successfully identify latent- and inflamed-QHS quarters as unhealthy quarters. However, its specificity is limited, as it poorly identified quarters without subclinical mastitis as healthy quarters (83% of them had some pathological changes). False-positive quarters identified by ultrasonography could be attributable to morphological changes resulting from previous infections or non-infectious conditions. For instance, necrosis and granulomatous lesions of the mammary gland parenchyma are associated with different bacterial infections (**23**). Therefore, the current study suggests that ultrasonographic examination is performed as a diagnostic method to identify mammary tissue pathological or structural changes rather than a tool for subclinical mastitis screening.

In the current study, buffaloes showed a greater percentage of subclinical mastitis identified by both CMT and DMD compared to cows. A similar finding was observed by Ali et al. (**24**) in Northwest Pakistan. In contrast, the current study found that the number of samples positive for bacterial culture was greater in cows than those for buffaloes. One reason is that buffaloes could have developed prolonged inflammatory responses, where the anti-inflammatory mechanisms are activated but they are not sufficient to counterbalance the proinflammatory response, resulting in persisting the inflammation instead of resolving it (**25**). This is supported by observations of previous investigations that revealed that the number of cows reported with clinical mastitis was higher compared to buffaloes (**24,26**). Additional local study indicated that mastitis in buffaloes is mostly chronic in nature (**27**), which supports the explanation of the prolonged inflammatory responses. On the other hand, the differences in the isolation percentages between buffaloes and cows using different media suggest that the prevalence of pathogens causing mastitis in buffaloes and cows is different. Further studies are required to confirm these differences in the prevalence of pathogens causing mastitis between both species, as it is important in designing control and prevention programs. Finally, the current study has a limitation of the unavailability of a flow cytometry technique for SCC, which could improve the identification of quarters with subclinical mastitis. However, the study defined the quarter health status based on a combination of the CMT readings and bacterial culture results.

The present study demonstrates that DMD has acceptable diagnostic performance for detecting bovine subclinical mastitis, supporting its use as a rapid, affordable, and portable cow-side device for screening and early detection. The current findings support the evidence that the manufacturer-recommended cut-point value may not be optimal for buffalo milk samples. The manufacturer's cut-point value is potentially low, and 515 is more appropriate for buffalo milk samples. These results highlight the necessity of species-specific calibration of such devices. Nevertheless, DMD remains a practical option for the routine screening of evidence of mastitis in lactating animals. In contrast, ultrasonography is considered a good method for diagnostic and structural evaluation of mammary gland disorders rather than for screening of subclinical infections.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this work.

ARTIFICIAL INTELLIGENT DECLARATION

The authors declare that they are responsible for the accuracy and integrity of all content of the manuscript, including part generated by AI, and it is not used as a co-author.

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دقة الطرائق الحقلية في الكشف عن التهاب الضرع البقري تحت السريري

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فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

يمثل التهاب الضرح تحدياً كبيراً لقطاع انتاج الحليب ومشتقاته. هدفت الدراسة الحالية تقييمَ أداء جهاز درامنيسكي الكاشف لالتهاب الضرع وتقنية التصوير بالموجات فوق الصوتية بوصفها اختبارات ميدانية بجانب الحيوان في الكشف عن التهاب الضرع تحت السريري. ولتحقيق هذا الهدف، تم تحليل 103 عينة حليب خام شملت 48 عينة من الجاموس و 55 عينة من الأبقار باستعمال جهاز درامنيسكي الكاشف لالتهاب الضرع، واختبار كاليفورنيا، وصور الموجات فوق الصوتية، والزرع الجرثومي. تم تصنيف حالة أرباع الضرع بناءً على نتائج اختبار كاليفورنيا والزرع الجرثومي إلى ثلاث فئات: سليم، وكامن، وملتهب. تم تقييم أداء جهاز درامنيسكي الكاشف لالتهاب الضرع وتقنية التصوير بالموجات فوق الصوتية من خلال إيجاد معامل ارتباط الرتب لسبيرمان، وتحديد القيمة الفاصلة لجهاز درامنيسكي، وحساب الحساسية والخصوصية والقيم التنبؤية لتقنية التصوير بالموجات فوق الصوتية مقارنة بحالة أرباع الضرع. أظهرت النتائج أن نسبة الأرباع السليمة أو الملتهبة كانت أعلى في الجاموس مقارنة بالأبقار، خلافاً لتلك التي شخّصت على أن الاتهاب فيها كامن. كما وجد ارتباط عكسي معنوي بين قيم جهاز درامنيسكي وحالة الأرباع بلغت – 0.695 في عينات الجاموس و – 0.526 في عينات الأبقار. واستناداً إلى تصنيف الأرباع إلى ملتهب وغير ملتهب، بلغت قيمة الحد الفاصل لجهاز درامنيسكي 515 للجاموس و 465 للأبقار. وقد أظهرت تقنية التصوير بالموجات فوق الصوتية مقارنة بحالة الأرباع حساسية 83% وخصوصية 22%، في حين بلغت القيمة التنبؤية السلبية 78% والإيجابية 29% والدقة العامة 39% بوصفه طريقة للتحري عن الالتهابات تحت السريرية. استنتجت الدراسة الحالية أن جهاز درامنيسكي مناسب للكشف المبكر عن التهاب الضرع البقري تحت السريري، وأن القيمة الفاصلة المعتمدة من الشركة المصنة قد تكون منخفضة، لذلك تقترح الدراسة قيمة فاصلة تبلغ 515 للجاموس و 465 للأبقار. وختاماً، تلائم تقنية التصوير بالموجات فوق الصوتية تحديد التغيرات في نسيج الضرع أكثر منها كطريقة للتحري عن الالتهابات تحت السريرية.

الكلمات المفاحية: التهاب الضرع تحت السريري، التحري عن التهاب الضرع، الكشف الدقيق، درامنيسكي، الانتاج المستدام