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Diagnostic Accuracy of the California Mastitis Test, White Side Test, and Chloride Test Against Fungal Culture and Host-Related Distribution of Mycotic Mastitis in Ewes in Al-Anbar Province, Iraq

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Abstract Mycotic mastitis in ewes frequently presents without overt clinical signs, while definitive fungal culture requires laboratory infrastructure and sufficient incubation time. Low-cost, animal-side screening tests can assist in flock monitoring, but their relative diagnostic accuracy against culture-confirmed fungal isolation in sheep remains incompletely characterized. This study aimed to estimate the diagnostic accuracy of the California Mastitis Test (CMT), White Side Test (WST) and chloride test for mycotic mastitis in ewes, using fungal culture as the reference standard, and to describe the distribution of infection by udder side, age and parity. Milk samples from both mammary halves of 100 lactating ewes (200 halves in total) from pastoral flocks around Fallujah, Al-Anbar Province, Iraq, were screened by CMT, WST and the silver nitrate chloride test, and cultured on Sabouraud dextrose and potato dextrose agar. Sensitivity, specificity, predictive values, likelihood ratios, Youden's index, and Cohen's kappa were calculated at the ewe level, with Wilson score 95% confidence intervals (CIs). Udder side, age, and parity were compared using the reported chi-square, McNemar, and Cochran–Armitage trend-test procedures, subject to the limitations of paired udder-half data. Fungal culture confirmed mycotic mastitis in 45 ewes (45.0%; 95% CI 35.6–54.8). At half level, CMT, WST and the chloride test were positive in 51.5%, 48.5% and 50.0% of halves, with no left–right difference for any test ($P \geq 0.32$). CMT had the highest sensitivity (88.9%; 76.5–95.2) and negative predictive value (88.1%), while the chloride test had the highest specificity (72.7%; 59.8–82.7). Overall accuracy (76.0–77.0%) and agreement with culture ($\kappa = 0.52$–0.55) were similar for the three tests, and only 40 ewes were positive on all three. Bilateral infection was found in 71.1% of positive ewes. Prevalence rose numerically from 35.0% at one year to 60.0% at five years of age and from 36.0% at parity 2 to 57.1% at parity ≥ 6, but neither trend was significant ($P = 0.12$ and $P = 0.18$). Among the three procedures, CMT provided the most favorable negative likelihood ratio for preliminary exclusion of culture positivity; nevertheless, none of these inflammation-based tests can reliably replace fungal culture. Because infection was frequently bilateral in this sample, both mammary halves should be evaluated, and individual management decisions should reflect culture results and clinical findings.

Index Terms ovine mastitis; mycotic mastitis; California Mastitis Test; White Side Test; chloride test; diagnostic accuracy; sensitivity and specificity; Iraq

I. Introduction

Mastitis is one of the most economically damaging diseases of dairy and dual-purpose sheep. It decreases both milk production and quality, impedes lamb growth, increases treatment costs and leads to premature culling [1]–[3]. A substantial proportion of intramammary infections in ewes are subclinical and may be identified only through systematic examination and appropriately selected laboratory testing of milk [1], [2], [4].

Fungal organisms are increasingly recognized as opportunistic agents associated with mastitis in domestic ruminants [5], [6]. Predisposing circumstances may include inadequate milking hygiene, contaminated bedding or feed, and indiscriminate intramammary antibiotic administration, which can disturb competing microbial populations in the mammary gland [6]–[8]. Because fungal infections do not respond to antibacterial agents and may become persistent or chronic, timely differentiation from bacterial disease is important for appropriate veterinary decision-making [8].

Previous investigations have documented clinical and subclinical mycotic mastitis in Iraqi ewes, including studies conducted in Al-Diwaniya and Fallujah [9], [10]. Additional research has recovered fungal organisms from ovine and other ruminant mastitis samples, highlighting the practical impor-

tance of establishing an appropriate mycological diagnosis [11]. Nevertheless, results obtained from different populations and screening procedures cannot establish the accuracy of all three indirect tests when they are applied concurrently to the same animals.

Definitive diagnosis of mycotic mastitis requires culture, which takes several days and a mycology laboratory. Flock-level control therefore relies on indirect tests that detect inflammation rather than the organism. The CMT and WST estimate the somatic cell content of milk, and the chloride test detects the leakage of chloride ions into milk when the blood–milk barrier is damaged [12]–[15]. These tests have been validated in ewes mainly against bacterial culture, with sensitivities of roughly 69–100% and specificities of 71–88% depending on the cut-off and pathogen [10], [13]; CMT scores also rise with the probability of a positive culture [16]. Earlier Iraqi work evaluated the California Mastitis Test in ewes with clinical and subclinical mastitis and reported mycotic infections [10]. However, the available literature does not establish a directly comparable, ewe-level assessment of all three specified screening tests against fungal culture in the same sample. Any claim of absolute novelty should therefore be interpreted cautiously.

This study therefore aimed (i) to estimate the sensitivity, specificity and predictive values of the CMT, WST and chloride test for mycotic mastitis in ewes, using fungal culture as the reference standard, and (ii) to describe the distribution of culture-confirmed infection by mammary side, age and parity.

II. Materials and Methods

This investigation used a cross-sectional diagnostic accuracy design, described with reference to the reporting principles of STARD 2015 [17]. It was carried out on lactating ewes kept in grazing flocks around Fallujah City, Al-Anbar Province, Iraq. One hundred ewes aged 1–5 years (age estimated from dental eruption) and with 2 to ≥ 6 previous parturitions were examined. Age, parity and date of last lambing were taken from farm records and owner history. The current analysis addresses the screening tests and culture status of these samples. A claimed companion publication describing the specific fungal isolates could not be verified from the supplied bibliographic information; therefore, no other published dataset is assumed to derive from these same ewes.

Each ewe underwent a general clinical examination (rectal temperature, pulse and respiratory rate) and inspection and palpation of both udder halves. Clinical mastitis was defined as heat, swelling, pain or induration of a half, or visible changes in the milk (clots, flakes, discoloration). Subclinical mastitis was defined as a half with normal udder and milk on inspection, but a positive screening test and a positive fungal culture.

Milk samples were collected separately from both mammary halves of each ewe (200 half-level samples in total), following the aseptic sampling procedures outlined by Markey et al. [18]. The udder and teats were washed and dried, teats were dipped in iodine and the orifice was scrubbed with 70% ethanol. The first three streams were discarded, and about 10 mL of mid-stream milk was collected into a sterile labelled tube. Samples were carried on ice in an insulated box and processed on the day of collection.

The results of all three screening tests were interpreted before fungal culture results became available, thereby reducing the possibility that knowledge of culture status influenced the initial interpretation of these index tests.

California Mastitis Test. Two millilitres of milk were mixed with 2 mL of CMT reagent (ImmuCell, USA) in a paddle and swirled for 10–15 s. Reactions were scored as negative, trace, 1+ (distinct precipitate), 2+ (gel formation) or 3+ (thick gel adhering to the paddle) [12], [13].

White Side Test. A few drops of milk were mixed with two drops of 4% sodium hydroxide on a dark glass plate and stirred for 20–30 s. Reactions were scored as negative (no change), trace (slight thickening), 1+ (small clumps), 2+ (large clumps with increased viscosity) or 3+ (solid gel) [2], [12].

Chloride Test. For the chloride test, 1 mL of milk was added to 5 mL of 0.1341% silver nitrate solution, followed by two drops of 10% potassium chromate indicator. A persistent brick-red coloration, associated with silver chromate formation, was interpreted as normal; yellow coloration was considered indicative of elevated chloride ($> 0.14\%$), while an intermediate coloration was recorded as borderline [15], [19].

Each sample was inoculated onto Sabouraud dextrose agar and potato dextrose agar (HiMedia, India), both supplemented with chloramphenicol (0.05 g/L). Plates were incubated aerobically at 25 °C and 37 °C for up to 7 days and examined daily. Isolates were identified by colony morphology and lactophenol cotton blue mounts of slide cultures; yeasts were further examined by Gram stain and germ-tube test [18]. Species identification is reported separately [11].

The unit of the accuracy analysis was the ewe. A ewe was culture-positive if fungi grew from at least one half. A ewe was considered index-test positive if at least one mammary half scored trace or higher on the CMT or WST, or if either half was classified as borderline or elevated on the chloride test. These criteria were applied consistently for the reported ewe-level diagnostic calculations. Half-level results were used to compare the left and right sides.

Sensitivity, specificity, positive and negative predictive values (PPV, NPV), overall accuracy, positive and negative likelihood ratios (LR+, LR−), Youden's index (J = sensitivity + specificity − 1) and Cohen's kappa (κ) against culture were calculated from 2×2 tables. Proportions are given with Wilson score 95% CIs [20]; κ was interpreted after Landis and Koch [21].

The original analysis compared the proportions of positive left and right halves using Pearson's chi-square test for the screening tests and an exact McNemar test for fungal culture. However, because both halves originated from the same ewe, independent-sample chi-square comparisons do not account for within-ewe pairing. Paired-test reanalysis of each screening method would require the unavailable individual-

animal cross-tabulations; the original values are retained and identified as unadjusted comparisons. Prevalence across age and parity groups was compared with the chi-square test for heterogeneity and the Cochran–Armitage test for linear trend [22]. A two-sided significance threshold of $P < 0.05$ was specified, and the reported calculations were performed in Python 3 using SciPy. The original individual-ewe records and exact analysis code were not provided with the manuscript, limiting independent reconstruction of paired comparisons and joint-test results.

III. Results

Fungal culture confirmed mycotic mastitis in 45 of 100 ewes (45.0%; 95% CI 35.6–54.8). Of these, 33 (73.3%) were subclinical and 12 (26.7%) clinical; species-level identification of the present isolates is not reproduced in this article. Ewes with acute clinical mastitis had a rectal temperature of about 41.3 °C, pulse of 109–115 beats/min and respiration of 55–60 breaths/min, against 38.3–39.9 °C, 70–90 beats/min and 12–20 breaths/min in clinically normal ewes.

Table 1: California Mastitis Test, White Side Test, and Chloride Test Results by Mammary Half

Test and score	Left, n (%)	Right, n (%)	Total, n (%)
California Mastitis Test (CMT)			
Negative	52 (52.0)	45 (45.0)	97 (48.5)
Trace	10 (10.0)	8 (8.0)	18 (9.0)
1+	18 (18.0)	16 (16.0)	34 (17.0)
2+	12 (12.0)	19 (19.0)	31 (15.5)
3+	8 (8.0)	12 (12.0)	20 (10.0)
Positive ($\geq$ trace)	48 (48.0)	55 (55.0)	103 (51.5)
White Side Test (WST)			
Negative	55 (55.0)	48 (48.0)	103 (51.5)
Trace	10 (10.0)	9 (9.0)	19 (9.5)
1+	16 (16.0)	18 (18.0)	34 (17.0)
2+	11 (11.0)	14 (14.0)	25 (12.5)
3+	8 (8.0)	11 (11.0)	19 (9.5)
Positive ($\geq$ trace)	45 (45.0)	52 (52.0)	97 (48.5)
Chloride Test			
Normal	53 (53.0)	47 (47.0)	100 (50.0)
Borderline	12 (12.0)	14 (14.0)	26 (13.0)
Elevated ($> 0.14\%$)	35 (35.0)	39 (39.0)	74 (37.0)
Positive (borderline and elevated)	47 (47.0)	53 (53.0)	100 (50.0)

From 100 left and 100 right mammary halves. The source reports unpaired left–right chi-square comparisons: CMT $\chi^2 = 0.98$, $P = 0.322$; WST $\chi^2 = 0.98$, $P = 0.322$; chloride $\chi^2 = 0.72$, $P = 0.396$ (df = 1). Full score-distribution comparisons were reported as CMT $P = 0.52$, WST $P = 0.83$, and chloride $P = 0.69$. Because halves are paired within ewes, these comparisons require paired-data reanalysis.

Approximately half of all mammary halves showed a positive reaction on each screening test, as detailed in Table 1. CMT was positive (trace or higher) in 103 of 200 halves (51.5%), WST in 97 (48.5%) and the chloride test in 100 (50.0%, of which 74 elevated and 26 borderline). Most positive CMT and WST reactions were mild to moderate (trace to 2+); strong 3+ reactions made up 10.0% and 9.5% of halves. Right halves reacted slightly more often than left halves on all three tests, but none of the differences was significant (CMT and WST $\chi^2 = 0.98$, P = 0.32; chloride $\chi^2 = 0.72$, P = 0.40).

Table 2: Ewe-Level Diagnostic Accuracy of Three Screening Tests Against Fungal Culture

Index	CMT ($\geq$ trace)	WST ($\geq$ trace)	Chloride ($\geq$ borderline)
Test-positive ewes, n	58	55	52
True positives / false positives	40 / 18	38 / 17	37 / 15
False negatives / true negatives	5 / 37	7 / 38	8 / 40
Sensitivity, %	88.9 (76.5–95.2)	84.4 (71.2–92.3)	82.2 (68.7–90.7)
Specificity, %	67.3 (54.1–78.2)	69.1 (56.0–79.7)	72.7 (59.8–82.7)
PPV, %	69.0 (56.2–79.4)	69.1 (56.0–79.7)	71.2 (57.7–81.7)
NPV, %	88.1 (75.0–94.8)	84.4 (71.2–92.3)	83.3 (70.4–91.3)
Accuracy, %	77.0 (67.8–84.2)	76.0 (66.8–83.3)	77.0 (67.8–84.2)
LR+	2.72	2.73	3.01
LR−	0.17	0.23	0.24
Youden's index	0.56	0.54	0.55
Cohen's κ	0.55	0.52	0.54

The reference standard was fungal culture: $n = 100$ ewes, of whom 45 were culture-positive and 55 culture-negative. Parentheses give reported Wilson 95% confidence intervals. PPV and NPV are specific to the observed culture-positive proportion of 45%. Diagnostic indices computed from the printed 2×2 counts were checked independently and agree to rounding.

As shown in Table 2, the CMT yielded the highest sensitivity and negative predictive value, whereas the chloride test yielded the highest specificity and positive predictive value in the sampled ewes. The reported 95% confidence intervals were broadly overlapping, and overall accuracy (76.0–77.0%), Youden's index (0.54–0.56) and agreement with culture (κ = 0.52–0.55, moderate) were almost identical for the three tests. CMT missed 5 culture-positive ewes, WST 7 and the chloride test 8. Forty ewes (40.0%) were positive on all three tests, five fewer than the 45 confirmed by culture.

Table 3: Culture-Confirmed Mycotic Mastitis According to Ewe Age and Parity

Group	Examined n	Positive n	Subclinical n	Clinical n	Prevalence, % (95% CI)
Age (Years)					
1	20	7	5	2	35.0 (18.1–56.7)
2	25	10	7	3	40.0 (23.4–59.3)
3	30	14	10	4	46.7 (30.2–63.9)
4	15	8	6	2	53.3 (30.1–75.2)
5	10	6	5	1	60.0 (31.3–83.2)
Parity					
2	25	9	6	3	36.0 (20.2–55.5)
3	28	12	9	3	42.9 (26.5–60.9)
4	25	12	9	3	48.0 (30.0–66.5)
5	15	8	6	2	53.3 (30.1–75.2)
≥ 6	7	4	3	1	57.1 (25.0–84.2)
Total	100	45	33	12	45.0 (35.6–54.8)

The original values are retained. Age: $\chi^2 = 2.42$, df = 4, $P = 0.658$; age trend $\chi^2 = 2.41$, $P = 0.120$. Parity: $\chi^2 = 1.80$, df = 4, $P = 0.773$; parity trend $\chi^2 = 1.78$, $P = 0.182$. The chi-square tests for heterogeneity agree with the printed counts; trend-test procedures require confirmation against the authors' analysis code.

Infection was usually bilateral. Of the 45 culture-positive ewes, 32 (71.1%; 95% CI 56.6–82.3) had fungi in both halves, 8 (17.8%) in the right half only and 5 (11.1%) in the left half only. The right half was infected in 40 ewes (88.9%) and the

left in 37 (82.2%); this difference was not significant (exact McNemar test on the 13 unilateral cases, P = 0.58).

Observed prevalence increased numerically across the ordered age and parity categories, but neither reported trend reached the prespecified significance threshold, as indicated in Table 3. It increased from 35.0% in one-year-old ewes to 60.0% in five-year-old ewes, and from 36.0% at the second parturition to 57.1% at six or more. Subclinical cases outnumbered clinical cases in every age and parity group.

IV. Discussion

This study provides an animal-level comparison of CMT, WST, and chloride-test findings against fungal culture within the same sampled ewes. Earlier work from Fallujah had already reported CMT diagnostic performance in an ovine mycotic-mastitis investigation [10]; therefore, the contribution is the concurrent, explicitly defined comparison rather than an unprecedented evaluation of CMT in fungal mastitis. Each of the three indirect procedures identified most culture-positive ewes but also produced positive results in some culture-negative animals. The CMT had the lowest reported negative likelihood ratio, yet a negative screening result could not exclude all culture-positive ewes. Fungal culture consequently remains important when clinical management requires evidence of fungal isolation.

The sensitivity observed for CMT in this sample (88.9%) exceeds the 69.3% sensitivity reported by Hueston and colleagues [13] for a broader category of ovine intramammary infections. However, differences in reference standards, pathogens, sample characteristics, and positivity thresholds limit direct comparisons. Its specificity of 67.3% was below the comparison values of approximately 76.5–87.2% discussed in earlier studies of ovine mastitis [10], [13]. These figures describe distinct diagnostic settings and should not be interpreted as evidence of a statistically established difference between studies. Arsenault et al. [16] investigated risk factors and consequences of clinical and subclinical mastitis in meat-producing sheep, providing useful epidemiological context for the present observations. However, differences in disease aetiology, sampling procedures, and diagnostic objectives prevent that study from serving as a direct validation of the present screening tests for fungal infection. Fthenakis [12] evaluated the CMT and WST in dairy ewes and reported their usefulness for detecting subclinical mastitis when appropriate score thresholds were applied. Those findings support using these tests as inflammatory screening instruments, not as specific tests for a fungal aetiology.

The practical value of CMT is better understood through its observed diagnostic likelihood ratios. Among the three tests, CMT had the lowest negative likelihood ratio (0.17), indicating that a negative result decreased the probability of culture-positive fungal infection in the sampled setting, although it did not exclude infection with certainty. It may therefore be useful as an initial screening procedure when follow-up culture remains available. The positive likelihood ratios were also modest, ranging from approximately 2.7 to 3.0. At the observed 45% culture-positive proportion, roughly three in ten index-test-positive ewes were culture-negative according to the reported positive predictive values. These findings favor confirmatory testing and individualized veterinary evaluation rather than treating a positive indirect test alone as sufficient evidence for antifungal therapy.

A plausible explanation for modest specificity is that CMT and WST measure milk changes associated with inflammation rather than directly identifying the responsible microorganism [14], [15], [23]. Some animals with positive screening tests but negative fungal cultures may have had bacterial intramammary infections that were not investigated by simultaneous bacteriological culture. Physiological variation in milk somatic cell counts is another possibility, particularly because baseline counts and lactation-related patterns differ between sheep and cattle [14], [24].

The chloride test had the highest specificity and LR+ but the lowest sensitivity. Inflammation may impair mammary epithelial barrier integrity and increase the transfer of blood-derived electrolytes into milk, leading to higher measured chloride concentrations [15], [23]. Such barrier changes may develop differently from the cellular inflammatory response in early or mild disease, potentially contributing to false-negative results. Moreover, the test relies on a visually interpreted color reaction, and its borderline category introduces additional scope for observer-dependent classification.

Most infected ewes (71.1%) carried fungi in both halves. Mørk and colleagues [25] documented the bacteriological and clinical characteristics of mastitis in ewes, providing comparative context. However, their bacterial-mastitis observations should not be taken as direct confirmation of the bilateral distribution of fungal infection reported here. Several features of mycotic mastitis favour bilateral infection. The usual sources are environmental (soil, bedding, mouldy feed), so both teats are exposed at once [6], [8]. Intramammary antibiotics are usually given to both halves, and Las Heras et al. [7] linked *Aspergillus fumigatus* mastitis to antibiotic dry therapy. Fungal infections are also chronic, which leaves time for the second half to become infected. These observations support examining and sampling both halves when fungal mastitis is suspected. Whether treatment, further surveillance, or culling is appropriate should be determined by the clinical condition of the individual ewe and confirmatory laboratory findings; bilateral treatment or culling cannot be justified solely from the distribution observed here.

No statistically significant left–right difference was reported for fungal culture, and the original screening comparisons also failed to identify a difference. Nevertheless, those screening comparisons used independent-sample chi-square statistics despite paired sampling, so confirmation requires McNemar testing of each index test using the original paired observations.

The observed culture-positive proportion increased by approximately five percentage points across consecutive age and parity categories, but neither reported trend test was statistically significant. Older and multiparous ewes have more cu-

mulative exposure, more teat-end damage and more previous treatments, and age and parity are established risk factors for ovine mastitis [24]–[26]. The non-significant findings should be interpreted as inconclusive rather than as proof of no association. Several older or higher-parity strata included only 7–15 ewes, resulting in wide 95% confidence intervals and limited precision for subgroup comparisons.

V. Limitations

The study had several limitations. Ewes came from one district and were sampled by convenience, so prevalence and predictive values may not apply elsewhere. Bacteriological culture and somatic cell counts were not done, so the cause of test-positive, fungus-negative reactions could not be established. Fungal culture is itself an imperfect reference, and a single sample may miss intermittent shedding. Finally, with 45 positive ewes the study could estimate sensitivity to within about ± 10 percentage points [27], but was too small to show differences between tests of this size.

VI. Conclusion

The CMT, WST and chloride test each detected 82–89% of ewes with culture-confirmed mycotic mastitis, but their specificity was only 67–73%. CMT produced the highest sensitivity and lowest negative likelihood ratio among the three studied procedures, supporting its use as an initial screen in similar settings, subject to local validation and confirmatory testing. No indirect test, alone or in combination, can replace fungal culture for confirming fungal infection. Culture positivity involved both halves in approximately seven of every ten affected ewes and increased numerically, but not significantly, with age and parity. Screening of both halves, mycological confirmation where indicated, and additional multi-region studies incorporating bacterial culture, somatic cell counts, and appropriately paired diagnostic comparisons are recommended to clarify the clinical and epidemiological implications.

Ethical Approval

The study protocol was approved by the Animal Ethics Committee of the College of Veterinary Medicine, University of Fallujah.

Author Contributions

N.M.H. contributed to sampling, laboratory investigations, data curation, and preparation of the original manuscript draft. M.S.H. contributed to conceptualization, supervision, critical review, and manuscript editing. Both authors should confirm final approval of the submitted version.

Conflict of Interest

The authors declare that they have no known conflicts of interest related to this study.

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Data Availability

The animal-level screening results, culture results, and supporting data are available from the corresponding author upon reasonable request, subject to applicable institutional and research-data requirements.

Study Provenance

This article is derived from the first author's MSc thesis at the University of Fallujah.

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