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COMPARATIVE STUDY FOR EFFICACY OF DIFFERENT DIAGNOSTIC TESTS OF SUBCLINICAL MASTITIS IN GOATS IN TORO LOCAL GOVERNMENT AREA, BAUCHI STATE, NIGERIA

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ABSTRACT

This study was aimed to determine the comparative efficacy of various diagnostic tests for subclinical mastitis (SCM) in goats. The study was conducted on 210 (6 blind) quarter milk samples from 108 goats. Cultural examination was used as the gold standard to compare the accuracy of the following diagnostic tests: CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST and SCC. The incidence rates were highest with CMT (28,7%) and lowest with Mastidin (22.2%). Cultivation revealed the presence of Coagulase-positive *Staphylococcus*, Coagulase-negative *Staphylococcus*, *Streptococcus species*, *Bacillus species*, *Lactobacillus species*, *Escherichia coli*, *Salmonella species*, *Enterobacter species* and *Klebsiella species*. No growth was observed on Brucella Agar. The sensitivity, specificity and predictive value of various tests were recorded and it was found that CMT had the highest sensitivity (32.89%), specificity (96.55%) and predictive value (96.15%) than compare with the other diagnostic methods for diagnosis of sub clinical mastitis in goat. The accuracy of all the conducted diagnostic tests when compared cultural examination were considered to be statistically significant ($p<0.01$). It can be concluded thatCMT can be used as a first screening tool for detecting suspected samples for further investigation for SCM also as the decision criteria to treat or to cull the animals in herds with high prevalence of SCM.

Keywords: Comparative efficacy, Diagnose, Subclinical mastitis, Goat, Test, Bacteria

INTRODUCTION

Mastitis is a parenchymal inflammation of the mammary gland, characterized by physical, chemical, and usually bacteriological changes in milk and pathological changes in glandular tissues (Danmallam and Pimenov, 2017; Mishra *et al.* 2014). Mastitis is a major cause of substantial economic losses in dairy goat's flocks. Economic losses are due to decreased milk yield by about 21% (Bardhan, 2013), changes in milk quality and composition, increased cost of treatment, increased mortality of kids, potentially premature culling of small ruminants, and poses public health hazard due to persistence of antibiotic residues in the milk (Rakesh *et al.* 2018). The cost of mastitis in dairy ruminants exceeds € 230 per case (Bonestroo et al. 2023), while single case of subclinical mastitis may be around € 80. The cost for one farm usually exceeds € 53 on average per animal on the farm (Van Soest et al. 2016). In general, mastitis occurs in two forms which include clinical (overt) and subclinical (hidden) (Amare, 2016; Singh and Kumar, 2022). Clinical form of the disease is an individual animal problem and can be easily diagnosed during clinical examination whereas, subclinical mastitis (SCM) is a herd problem which may go unnoticed since no gross signs of inflammation and changes in the milk composition are evident and the milk and udder appear normal and remain a depot for spreading infection to the herd mates (Gere *et al*. 2006). Adoptability of tests as well as their sensitivity and accuracy are important factors to be taken into consideration for the correct, rapid and real-time diagnosis of the disease to determine the course of treatment (Otoo *et al.* 2022). The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the Modified White Side test (MWT), Somatic cell count (SCC), lactate dehydrogenase, N-acetyl-β-D-glucosaminidase (NAGase), Acute phase proteins, bromothymol blue (BTB) strip test, electrical conductivity (EC), pH, lactose, chlorine and catalase test (Rakesh *et al.* 2018; Sharma *et al.* 2010). The conventional methods (e.g., CMT) are rapid, relatively cheap, and have field applicability, but have the disadvantage of non-specific detection. Bacteriological culture of milk samples is the standard method for identifying mastitis. However, the logistic and financial considerations involved with sampling all fresh animals have precluded this technique from being widely adopted. Advanced diagnostic tools, viz. polymerase chain reactions, protein-based ELISA techniques, acute phase protein detection, quantitative PCR, MALDI-TOF, etc., are costly, requiring skilled technicians, a laboratory, and sophisticated infrastructure. The great advantages of these tools are the highly accurate and specific nature of the detection of mastitis-causing pathogens, even at the subspecies level, providing an efficient method of treatment. Considering this approach, this study aimed to assess the comparative efficacy of different diagnostic tests of subclinical mastitis in goats in Nigeria.

MATERIALS AND METHODS

This study was carried out on cases of subclinical mastitis in 34 herds selected (at random from Toro LGA located in Bauchi State, north-eastern part of Nigeria (Danmallam and Pimenov, 2018).

The udder and teats were cleaned with clean water and dried with clean towels. The teat orifice and the skin around the teat

were wiped with cotton soaked in 70% alcohol. About 10 ml of milk was collected from each individual quarter into different sterilized containers and labeled duly following aseptic precautions. A total of 210 milk samples from 108 goats were collected and remaining 6 quarters were blind.
The milk samples from different quarters were subjected to test with different mastitis detection reagents: California mastitis test (CMT), Masttest, Mastidin, Kerba test, Milk test, White side test (WST), Bromothymol blue strip test (BBST), Somatic cells count (SCC) and further subjected to bacteriological examination for isolation of etiological agents in order to detect SCM and the diagnostic efficiency, sensitivity, specificity and positive predictive value of each test taking cultural test as standard (Verma *et al.* 2024).

California mastitis test (CMT), Masttest, Mastidin, Kerba test and Milk test
The principle of these tests is that the detergent causes rupture of somatic cells when added to a milk sample due to which DNA and other cell contents are released. Released DNA from ruptured cells unites to form a gel, the consistency of which depends upon the number of somatic cells.

Procedure
California mastitis test (CMT), Masttest (manufactured by Agrofarm, Russia), Mastidin (manufactured by Reagent, Ukraine), Kerba test (manufactured by KERBL, Russia) and milk test (farma, Ukraine) were done according to manufacturer's instruction. Briefly, 1 ml of milk was taken in the respective cup of paddle. An equal amount of reagent (separately for each 1ml of milk) was added to milk. The paddle was then rotated in circular motion to mix the contents. Scoring was done within 10-15 seconds keeping the paddle rotating. Interpretation and scoring were done as follows: negative (0) – Mixture of the milk with diagnosticum remains in the form of a homogeneous liquid, doubtful/Trace (1+) – there is precipitate but no gel formation, positive (2+) – precipitate thickens and forms gel towards the centre of the paddle and strongly positive (3+) – forms distinct gel that adheres to the bottom of the paddle (Kumar *et al.* 2023).

White Side Test (WST)
The white side test was performed on the milk samples as per the procedure of Chauhan (Chauhan, 2003). The principle of this test is that sodium hydroxide dissolves cell wall of the somatic cells, causing the release of DNA which unite to form viscid masses that break down to form flakes and shreds.

Procedure
In this test, 4-5 drops of the test milk sample was placed on a clean dry glass slide. To this a drop of 4% sodium hydroxide was added and mixed with a glass rod. Milk thickened and flakes appeared when the sample was positive for mastitis. Based on the degree of thickening and appearance of flakes, the results were graded as negative – (no clot or gel formation), 1+ (background is less opaque, with larger particles of coagulated materials thickly scattered and slight degree of clumping), 2+ (background is more watery with large clumps of coagulated materials. If the stirring is been rapid, fine threads or strings may be present) and 3+ (background is very watery and whey-like, with large masses of coagulated material forming into strings and shreds) (Chauhan, 2003).

Bromothymol blue strip test (BBST)
Bromothymol blue strips are cellulose based strips stabilized with ion sensitive indicator for detection of SCM. These strips are manufactured by Dabur Ayurvet Ltd, 22, Site IV, Sahibabad, Ghaziabad-201020, U.P; under trade name "Mastrip" (Chanawanno *et al.* 2023).

Procedure
A drop of milk sample was put over the strip and change in color, if any, was observed within 30 seconds and compared with the standard color chart provided with the packing and interpreted as: yellow- normal or negative (-), greenish yellow-mild to moderate SCM or positive (+), green-advanced (severe) SCM or strongly positive (++) and blue-Clinical mastitis

Somatic cell count (SCC)
The SCC of milk was performed by the method as described by Schalm *et al*. (1971). For staining of milk films pyronin Y-methyl green (PYMG) stain 1.2 g, Ethanol (95%) 54 ml, Tetrachloroethane 40 ml and Glacial acetic acid 6 ml) was used.

Preparation of milk films
The samples were mixed thoroughly so as to obtain uniform distribution of the cells. The sample was allowed to stand for two to five minutes to permit air bubbles and foam to settle down. A clean grease free slide was placed on a level area over template to outline 1.0 cm^2 area. With help of a 4.00 mm diameter platinum loop, 0.01 ml of milk was spread evenly over the first template on the left side of slide. This procedure was repeated with sample from each quarter. Slides were air dried and subjected to staining.

Staining
Slides were immersed for 30 seconds in Methylene green pyronin Y stain solution. Excess stain was drained off and the slides were air dried. Then slides were rinsed thrice under tap water, drained and rapidly air dried after gently blotting with filter paper. Somatic cells stained clearly with pale green background.

Calculation of working factor (WF) of the microscope
A binocular microscope was used with 10× ocular and 1.8 mm oil immersion objective. The diameter of field was measured with the help of a stage micrometer.
Diameter of microscopic field= 0.18 mm = 0.018 cm.
Area of field= $\pi r2 = 3.14 \times (0.009)\ 2 = 0.00025$ sq. cm
Since 0.01 ml of milk was spread in 1.0 sq. cm area, the possible number of fields which could be counted in 1.0 sq. cm is 4000.
Milk volume represented by each field=1/4000 × 1/100= 1/4000 ml
Hence, microscopic factor = 4,00,000.
Working factor = Microscopic factor/ No of fields counted
4,00,000/ 25 = 16,000.

Counting of cells
The stained cells were examined under oil immersion objective.
The cells in the required 25 fields were counted. Total numbers of cells counted were multiplied by working factor of the microscope to obtain the number of cells per ml of milk. Milk samples containing more than 2000×10^3 cells per ml was considered positive (Bogdanovicova *et al.,* 2016).

Cultural isolation
The isolation and identification of bacterial pathogens were performed according to the procedure described by Mbindyo *et al.* (2014). In Brief, 0.1 ml milk sample was cultured on

blood agar, nutrient agar, MacConkey agar, and Brucella agar base. The inoculated plates were incubated at 37°C aerobically for 24-48 h, while those on Brucella agar were incubated at 37°C anaerobically for 7 days. The bacterial pathogens were identified by morphology, hemolysis, gram staining, and biochemical tests such as catalase, oxidase, coagulase, reaction on sulfite, indole, and motile medium, and fermentation of sugars.

Statistical analysis

The data was entered and managed in Microsoft Excel spread sheet. The percentage accuracy of the tests and sensitivity, specificity, and the predictive values of the CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST and SCC results, compared to culture results, were calculated using standard two-by-two contingency tables. Data were also analyzed by Chi-square test to observe the significant between the dependent variables.

RESULTS AND DISCUSSION

A total of 210 (6 blind) quarter milk samples from 108 goats were subjected to different diagnostic tests i.e. CMT, masttest, mastidin, kerba test, milk test, WST, BBST and SCC taking cultural examination as standard for detection of SCM. The animal wise and quarter wise incidence of subclinical mastitis by various diagnostic tests are shown in the table 1.

According to CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST, SCC and cultural examination, the animal wise incidence was 28.7%, 25.9%, 22.2%, 24.1%, 25.9%, 22.2%, 26.8%, 26.8% and 80.6%, respectively, whereas quarter wise incidence was 24.8%, 23.3%, 17.6%, 22.4%, 22.9%, 17.1%, 23.8%, 23.8% and 72.38%, respectively.

Table 1: Comparative study on the detection of subclinical mastitis in goats using different diagnostic tests

S. No	Name of Test	Number of animals			Number of quarter		
		Tested	Positive	Incidence (%)	Tested	Positive	Incidence (%)
1.	CMT	108	31	28.70	210	52	24.76
2.	Masttest	108	28	25.93	210	49	23.33
3.	Mastidin	108	24	22.22	210	37	17.62
4.	Kerba test	108	26	24.07	210	47	22.38
5.	Milk test	108	28	25.93	210	48	22.86
6.	WST	108	24	22.22	210	36	17.14
7.	BBST	108	29	26.85	210	50	23.81
8.	SCC	108	29	26.85	210	50	23.81
9.	Cultural examination	108	87	80.56	210	152	72.38

California Mastitis Test (CMT)

Various grades of CMT reaction obtained on screening of 108 goats with 210 quarter milk samples by CMT and the infectious status are presented in the table 2. Of the 77 goats were negative for CMT reaction, 58 (75.32%) were culturally positive, further 16, 10 and 5 goats were showing 1+, 2+ and 3+ CMT reactions out of which 14/16 (87.50), 10/10 (100.00%) and 5/5 (100.00%) were culturally positive, respectively. On the other hand, 158 quarter milk samples were negative for CMT reaction, 102 (64.56%) were culturally positive, while 24, 19 and 9 quarters were showing 1+, 2+ and 3+ CMT reactions out of which 22/24 (91.67%), 19/19 (100.00%) and 9/9 (100.00%) were culturally positive, respectively.

Table 2: Grades of CMT reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	CMT reaction grade	Number of animals showing CMT reaction	Number of animals		Number of quarters showing CMT reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	77	58 (75.32)	19 (24.68)	158	102 (64.56)	56 (35.44)
2.	+	16	14 (87.50)	2 (12.50)	24	22 (91.67)	2 (8.33)
3.	++	10	10 (100.00)	0 (0.00)	19	19 (100.00)	0 (0.00)
4.	+++	5	5 (100.00)	0 (0.00)	9	9 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Masttest

Various grades of Masttest reaction obtained on screening of 108 goats with 210 quarter milk samples by Masttest solution and the infectious status are presented in the table 3. Of the 80 goats were negative for masttest reaction, 61 (76.25%) were culturally positive, further 13, 10 and 5 goats were showing 1+, 2+ and 3+ Masttest reactions, respectively and of which 11/13 (84.61%), 10/10 (100.00%) and 5/5 (100.00%) samples were culturally positive. On the quarter level, 161 milk samples were negative for Masttest reaction, 107 (66.46%) were culturally positive, while 24, 18 and 7 quarters were showing 1+, 2+ and 3+ Masttest reactions, respectively out of which 20/24 (83.33%), 18/18 (100.00%) and 7/7 (100.00%) were culturally positive.

Table 3: Grades of Masttest reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	Masttest reaction grade	Number of animals showing Masttest reaction	Number of animals		Number of quarters showing Masttest reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	80	61 (76.25)	19 (23.75)	161	107 (66.46)	54 (33.54)
2.	+	13	11 (84.61)	2 (15.38)	24	20 (83.33)	4 (16.67)
3.	++	10	10 (100.00)	0 (0.00)	18	18 (100.00)	0 (0.00)
4.	+++	5	5 (100.00)	0 (0.00)	7	7 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Mastidin

Various grades of Mastidin reaction obtained on screening of 108 goats with 210 quarter milk samples by Mastidin solution and the infectious status are presented in the table 4. Of the 84 goats were negative for Mastidin reaction, 65 (77.38%) were culturally positive, further 12, 9 and 3 goats were showing 1+, 2+ and 3+ Mastidin reactions, respectively out of which 11/12 (91.67%), 8/9 (88.89%) and 3/3 (100.00%) were culturally positive. On the quarter level, 173 quarter milk samples were negative for Mastidin reaction, 117 (67.63%) were culturally positive, while 17, 15 and 5 quarters were showing 1+, 2+ and 3+ Mastidin reactions, respectively of which 15/17 (88.24%), 15/15 (100.00%) and 5/5 (100.00%) were culturally positive.

Table 4: Grades of Mastidin reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	Mastidin reaction grade	Number of animals showing Mastidin reaction	Number of animals		Number of quarters showing Mastidin reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	84	65 (77.38)	19 (22.61)	173	117 (67.63)	56 (47.87)
2.	+	12	11 (91.67)	1 (8.33)	17	15 (88.24)	2 (11.76)
3.	++	9	8 (88.89)	1 (11.11)	15	15 (100.00)	0 (0.00)
4.	+++	3	3 (100.00)	0 (0.00)	5	5 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Kerba test

Various grades of Kerba test reaction obtained on screening of 108 goats with 210 quarter milk samples by Kerba test solution and the infectious status are presented in the table 5. Of the 82 goats were negative for Kerba test reaction, 63 (76.83%) were culturally positive, further 14, 9 and 3 goats were showing 1+, 2+ and 3+ Kerba test reactions, respectively of which 13/14 (92.86%), 8/9 (88.89%) and 3/3 (100.00%) samples were culturally positive. On the quarter level, 163 milk samples were negative for Kerba test reaction, 109 (66.87%) were culturally positive, while 24, 16 and 7 quarters were showing 1+, 2+ and 3+ Kerba test reactions, respectively of which 21//24 (87.50%), 15/16 (93.75%) and 7/7 (100.00%) were culturally positive, respectively.

Table 5: Grades of Kerba test reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	Kerba test reaction grade	Number of animals showing Kerba test reaction	Number of animals		Number of quarters showing Kerba test reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	82	63 (76.83)	19 (23.19)	163	109 (66.87)	54 (33.13)
2.	+	14	13 (92.86)	1 (7.14)	24	21 (87.50)	3 (12.50)
3.	++	9	8 (88.89)	1 (11.11)	16	15 (93.75)	1 (6.25)
4.	+++	3	3 (100.00)	0 (0.00)	7	7 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Milk test

Various grades of Milk test reaction obtained on screening of 108 goats with 210 quarter milk samples by milk test solution and the infectious status are presented in the table 6. Of the 80 goats were negative for Milk test reaction, 62 (77.50%) were culturally positive, further 13, 10 and 5 goats were showing 1+, 2+ and 3+ Milk test reactions, respectively and of which 12/13 (92.31%), 8/10 (80.00%) and 5/5 (100.00%) samples were culturally positive. On the quarter level, 162 milk samples were negative for Milk test reaction, 107 (66.05%) were culturally positive, while 23, 18 and 7 quarters were showing 1+, 2+ and 3+ Milk test reactions, respectively out of which 22/23 (95.65%), 16/18 (88.89%) and 7/7 (100.00%) were culturally positive.

Table 6: Grades of Milk test reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	Milk test reaction grade	Number of animals showing Milk test reaction	Number of animals		Number of quarters showing Milk test reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	80	62 (77.50)	18 (22.50)	162	107 (66.05)	55 (33.05)
2.	+	13	12 (92.31)	1 (7.67)	23	22 (95.65)	1 (4.35)
3.	++	10	8 (80.00)	2 (20.00)	18	16 (88.89)	2 (11.11)
4.	+++	5	5 (100.00)	0 (0.00)	7	7 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

White Side Test (WST)

Various grades of WST reaction obtained on screening of 108 goats with 210 quarter milk samples by WST solution and the infectious status are presented in the table 7. Of the 84 goats were negative for WST reaction, 66 (78.57%) were culturally positive, further 12, 9 and 3 goats were showing 1+, 2+ and 3+ WST reactions, respectively of which 9/12 (75.00%), 9/9 (100.00%) and 3/3 (100.00%) were culturally positive. On the quarter level, 174 quarter milk samples were negative for WST reaction, 120 (68.97%) were culturally positive, while 17, 14 and 5 quarters were showing 1+, 2+ and 3+ WST reactions, respectively of which all 13/17 (76.47%), 14/14 (100.00%) and 5/5 (100.00%) were culturally positive.

Table 7: Grades of WST reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	WST reaction grade	Number of animals showing WST reaction	Number of animals		Number of quarters showing WST reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	84	66 (78.57)	18 (21.43)	174	120 (68.97)	54 (31.03)
2.	+	12	9 (75.00)	3 (25.00)	17	13 (76.47)	4 (23.53)
3.	++	9	9 (100.00)	0 (0.00)	14	14 (100.00)	0 (0.00)
4.	+++	3	3 (100.00)	0 (0.00)	5	5 (100.00)	0 (0.00)
5.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Bromothymol blue strip test (BBST)

Various grades of BBST reaction obtained on screening of 108 goats with 210 quarter milk samples by BBST and the infectious status are presented in the table 8. Of the 79 goats were negative for BBST reaction, 60 (75.95%) were culturally positive, further 18 and 11 goats were showing 1+ and 2+ BBST reactions, respectively out of which 16/18 (88.89%) and 11/11 (100.00%) were culturally positive. On the quarter wise, 160 quarter milk samples were negative for BBST reaction, 106 (66.25%) were culturally positive, while 31 and 19 quarters were showing 1+ and 2+ BBST reactions, respectively, of which 27/31 (87.10%) and 19/19 (100.00%) were culturally positive.

Table 8: Grades of BBST reaction VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	BBST reaction grade	Number of animals showing BBST reaction	Number of animals		Number of quarters showing BBST reaction	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	79	60 (75.95)	19 (24.05)	160	106 (66.25)	54 (33.75)
2.	+	18	16 (88.89)	2 (11.11)	31	27 (87.10)	4 (12.90)
3.	++	11	11 (100.00)	0 (0.00)	19	19 (100.00)	0 (0.00)
4.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Somatic cell count (SCC)

Table 9 presents the SCC score and infectious status of mastitis on screening of 108 goats with 210 quarter milk samples. Of the 79 goats were negative for mastitis score, 60 (75.95%) were culturally positive, further, 29 goats show positive score for mastitis of which 27 (93.10%) of the samples were culturally positive. On the quarter wise, 160 milk samples showed negative SCC score for mastitis, of this, 105 (65.63%) were culturally positive. 50 quarter sample showed positive SCC score for mastitis of which 47 (94.00%) were positive on culture.

Table 9: Grades of SCC scores VS status of infection of affected animals (n=108) and quarters (n=210)

S. No	SCC reaction grade	Number of animals showing SCC score	Number of animals		Number of quarters showing SCC score	Number of quarters	
			Culturally positive (%)	Culturally negative (%)		Culturally positive (%)	Culturally negative (%)
1.	-	79	60 (75.95)	19 (24.05)	160	105 (65.63)	55 (36.25)
2.	+	29	27 (93.10)	2 (6.90)	50	47 (94.00)	3 (6.00)
3.	Total	108	87 (80.56)	21(19.44)	210	152 (72.38)	58 (27.62)

Cultural isolation results

Bacteriological test results of the mammary gland secretion of mastitic goats are shown in table 10 and 11. The cultural isolation results from a milk sample of a goat, analyzed for the diagnosis of mastitis. Highest growth of microorganism including Coagulase-positive *Staphylococcus*, Coagulase-negative *Staphylococcus*, *Streptococcus species* and *Bacillus species* were observed on Blood Agar 84 (49.70%) of the total isolates, followed by nutrient agar 50 (29.59%) isolates including Coagulase-positive *Staphylococcus*, Coagulase-negative *Staphylococcus*, *Bacillus species*, and *Lactobacillus species*, then Mac Conkey agar 35 (20.71 %) isolates consisting *Escherichia coli*, *Salmonella species*, *Enterobacter species*, and *Klebsiella species*. Brucella Agar showed no growth, suggesting the absence of *Brucella species*, which are typically less common in such infections. Overall, the presence of these pathogens in the milk sample supports the diagnosis of mastitis in the goat.

Table 10: Positive growth of microorganisms and their frequency and percentage on different agar media

Cultured media	Growth	Identified organisms	Frequency (%)	Percentage
Blood agar	Positive	*Coagulase positive Staphylococcus*	18 (10.65)	84 (49.70)
		Coagulase negative Staphylococcus	28 (16.57)	
		Streptococcus spp.	32 (18.93)	
		Bacillus spp.	6 (3.55)	
Nutrient agar	Positive	*Coagulase positive Staphylococcus*	12 (7.10)	50 (29.59)
		Coagulase negative Staphylococcus	25 (14.79)	
		Bacillus spp.	6 (3.55)	
		Lactobacillus spp.	7 (4.14)	
Mac Conkey agar	Positive	*E. coli*	12 (7.10)	35 (20.71)
		Salmonella spp.	3 (1.78)	
		Enterobacter spp.	12 (7.10)	
		Klebsiella spp.	8 (4.73)	
Brucella agar	Negative		0 (0.00)	0 (0.00)
Total			169 (100.0)	169 (100.0)

Table 11 shows the type, frequency and percentage of isolates from the milk samples of mastitic goats. A total of 169 bacterial isolates were recovered from 204 mammary gland secretion. The most predominant isolates were Coagulase-negative *Staphylococcus* 53 (31.36%), followed by *Streptococcus spp.* 32 (18.93%) and Coagulase-positive *Staphylococcus* 30 (17.75%), then *Enterobacter spp., E. coli* and *Bacillus spp.* with frequency of 12 (7.10%) occurrence each, *Klebsiella spp.* 8 (4.73%), *Lactobaccilus spp.* 7 (4.14%). *Salmonella spp.* was the isolate with less frequency of 3 (1.78%) occurrence.

Table 11: Bacteria isolated from the milk samples of mastitic goats

Isolates	Frequency and percentage of isolates, n (%)
Coagulase positive Staphylococcus	30 (17.75)
Coagulase negative *Staphylococcus*	53 (31.36)
Streptococcus spp.	32 (18.93)
Lactobacillus spp.	7 (4.14)
Enterobacter spp.	12 (7.10)
E. coli	12 (7.10)
Bacillus spp.	12 (7.10)
Klebsiella spp.	8 (4.73)
Salmonella spp.	3 (1.78)
Total	169 (100.00)

Sensitivity, Specificity and Accuracy

The per cent accuracy of various diagnostic tests for the detection of SCM, with cultural examination as standard are shown in table 12. The animal wise per cent accuracy of CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST and SCC was 44.44, 41.67, 37.96, 39.81, 39.81, 36.11, 42.59 and 42.59, respectively. The false positive reactions were more with WST (12.50), followed by Milk test (10.71), Mastidin (8.33), Kerba test (7.69), Masttest (7.14) and least with BBST, SCC (6.90) each and CMT (6.45), whereas, false negative reactions were more with WST (78.57), followed by Milk test (77.50), Mastidin (77.38), Kerba test (76.83), Masttest (76.25) and least with BBST, SCC (75.95) each and CMT (75.32). On the quarter level, the percent accuracy of CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST and SCC was 50.48, 47.14, 43.33, 47.62, 43.33, 46.19, 47.62 and 47.62, respectively. All tests were considered to be statistically significant ($p<0.01$).

Table 12: Percent, % accuracy of various diagnostic tests taking cultural examination as standard

S. No.	Name of the test, -ssample received from	Total samples examined	Number of positive samples	Number of test reaction as compared to cultural examination					Level of significance
				True positive, %	False positive, %	True negative, %	False negative, %	Percent accuracy	
1.	**CMT**								**
	- goat	108	31	29 (93.54)	2 (6.45)	19 (24.68)	58 (75.32)	48 (44.44)	
	- quarter	210	52	50 (96.15)	2 (3.85)	56 (35.44)	102 (64.56)	106 (50.48)	**
2.	**Masttest**								**
	- goat	108	28	26 (92.86)	2 (7.14)	19 (23.75)	61 (76.25)	45 (41.67)	
	- quarter	210	49	45 (91.84)	4 (8.16)	54 (33.54)	107 (66.46)	99 (47.14)	**
3.	**Mastidin**								**
	- goat	108	24	22 (91.67)	2 (8.33)	19 (22.61)	65 (77.38)	41 (37.96)	
	- quarter	210	37	35 (94.59)	2 (5.41)	56 (47.87)	117 (67.63)	91 (43.33)	**
4.	**Kerba test**								**
	- goat	108	26	24 (92.31)	2 (7.69)	19 (23.17)	63 (76.83)	43 (39.81)	
	- quarter	210	47	43 (91.49)	4 (8.51)	54 (33.13)	109 (66.87)	97 (46.19)	**
5.	**Milk test**								**
	- goat	108	28	25 (89.29)	3 (10.71)	18 (22.50)	62 (77.50)	43 (39.81)	
	- quarter	210	48	45 (93.75)	3 (6.25)	55 (33.05)	107 (66.05)	100 (47.62)	**
6.	**WST**								**
	- goat	108	24	21 (87.50)	3 (12.5)	18 (21.43)	66 (78.57)	39 (36.11)	
	- quarter	210	36	32 (88.89)	4 (11.11)	54 (31.03)	120 (68.97)	91 (43.33)	**
7.	**BBST**								**
	- goat	108	29	27 (93.10)	2 (6.90)	19 (24.05)	60 (75.95)	46 (42.59)	
	- quarter	210	50	46 (92.00)	4 (8.00)	54 (33.75)	106 (66.25)	100 (47.62)	**
8.	**SCC**								**
	- goat	108	29	27 (93.10)	2 (6.90)	19 (24.05)	60 (75.95)	46 (42.59)	
	- quarter	210	50	46 (92.00)	4 (8.00)	54 (33.75)	106 (66.25)	100 (47.62)	**

**Significant at 1% level ($p<0.01$)

$$\% \text{ Accuracy} = \frac{\text{Number of true positive animals (quaters)} + \text{ Number of true negative animals (quaters)}}{\text{Number of samples examined}} \times 100$$

$$\% \text{ False positive} = \frac{\text{Number of false positive samples}}{\text{Number of samples positive by test}} \times 100$$

$$\% \text{ False negative} = \frac{\text{Number of false negative samples}}{\text{Number of samples negative by test}} \times 100$$

Sensitivity, specificity and predictive value of different diagnostic test taking cultural examination as standard are shown in table 13. The animal wise decreasing order of sensitivity was for CMT (33.33%), same for BBST and SCC (31.03%), Masttest (29.89%), Milk test (28.74%), Kerba test (27.59%), Mastidin (25.29%) and WST (24.14%), while the quarter wise decreasing order of sensitivity was for CMT (32.89%), the same for BBST and SCC (30.26%), Masttest (29.61%), Milk test(29.60%), Kerba test (28.29%), Mastidin (23.03%) and WST (21.05%) whereas, the animal wise specificity were highest and same for CMT, Masttest, Mastidin, Kerba test, BBST and SCC (90.48%) and least for Milk test and WST (85.71%) while, the quarter wise specificity was highest and same for CMT and Mastidin (96.55%) followed by Milk test (94.83%) and least and same for Masttest, Kerba test, WST, BBST and SCC (93.10%), respectively.

Table 13: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural examination as standard

S. No.	Name of the test	Sensitivity		Specificity		Predictive value of positive test	
		goat	Quarter	goat	quarter	goat	quarter
1.	CMT	33.33	32.89	90.48	96.55	95.55	96.15
2.	Masttest	29.89	29.61	90.48	93.10	92.86	91.84
3.	Mastidin	25.29	23.03	90.48	96.55	91.67	94.59
4.	Kerba test	27.59	28.29	90.48	93.10	92.31	91.49
5.	Milk test	28.74	29.60	85.71	94.83	89.29	93.75
6.	WST	24.14	21.05	85.71	93.10	87.50	88.89
7.	BBST	31.03	30.26	90.48	93.10	93.10	92.00
8.	SCC	31.03	30.26	90.48	93.10	93.10	92.00

Table 14: Codes

D+ (Disease present)	D- (Disease negative)
A	b
c	d
a+c	b+d

Positive (T+)
Negative (T-)
a = Disease positive and test positive (true positive)
b = Disease negative but test positive (false positive)
c = Disease positive but test negative (false negative)
d = Disease negative and test negative (true negative)
Sensitivity = a/ (a+c) × 100
Specificity = d/ (b+d) × 100
Predictive value for +ve test = a / (a+b) × 100

Discussion

In subclinical mastitis, irrespective of the etiological agents involved, the first pathological change observed is the passage of leukocytes and erythrocytes into the milk as a result of increased permeability of the gland capillaries to inflammatory reaction. The intensity of the inflammation can be estimated qualitatively by indirect diagnosis method such as CMT and quantitatively by SCC, while the presence of the causative microorganism in the milk can be identified by cultural examination. The comparative incidence of different test for the present study in descending order was for CMT (28.70%), same for BBST and SCC (26.85%), Masttest and Milk test (25.93%), Kerba test (24.07%), least and same for Mastidin and WST (22.22%). These descending orders of incidence were in closely finding with the result of Aliev *et al.* (2014) who reported certain case in sheep with the result for Masttest (86%), Milk test (79.5%), Kerba test (76.4%) and Mastidin (73.1%).

SCC values in healthy sheep (4.86 Log10), which are lower than the SCC values observed in ewes with mastitis (5.9 Log10) (Ariznabarreta et al. 2002). the somatic cell count (SCC) of a healthy udder in milk-producing ewes has not yet been established. Studies shows that the SCC levels vary greatly, reaching a count up to 1.5x106.mL-1 in a healthy udder (Mavrogenis et al. 1995). However, a count limit above 250,000 cells.mL-1 (Hag, 2002) or below 500,000 cells.mL-1 (Bianchi et al. 2004) has been suggested for healthy udders. On the other hand, ewes with mammary infection will have a SCC above 1 million cells, in at least two consecutive samplings (Bianchi et al. 2004).

Other method to diagnose mastitis widely used in bovines is the CMT. This test is used worldwide to diagnose subclinical mastitis and, additionally, it can be carried out in the herd when the animals are being milked. The interpretation of the CMT is based on the visual inspection of the milk after the reagent is mixed in. The reaction takes place between the reagent and the genetic material from somatic cells found in the milk, forming a gel whose concentration is proportional to the number of somatic cells. However, in small ruminants, this test still generates controversies, because the amount of physiological somatic cells from these animals is very large, causing a false-positive result.

The animal and quarter wise per cent accuracy was CMT (44.44 and 50.48%), Masttest (41.67 and 47.14%), Mastidin (37.96 and 43.33%), Kerba test (39.81 and 46.19%), Milk test (39.81 and 47.62%), WST (36.11 and 43.33%), BBST (42.59 and 47.62%) and SCC (42.59 and 47.62%), respectively. The per cent accuracy of various diagnostic tests is usually analysed taking into consideration the cultural isolation of mammary pathogens as a standard procedure in the diagnosis of subclinical mastitis. Though, there is a lack of data for comparative test for diagnosing SCM in small ruminants but, these findings are in agreement with Anusha *et al.* (2018) who reported in cow the highest accuracy for CMT (76.52%), followed by SCC (73.39%) and least WST (71.30%). Hoque *et al.* (2015) who also reported for accuracy of CMT (70%), WST (64.8%) and SCC (85.2%).

Rakesh K. *et at.* (2018) compared the sensitivity of the BBST, CMT and SCC for the detection of SCM in dairy cow and reported that CMT and SCC (55.50%) were more than that of BBST. Buragohain and Dutta (1998), Reddy *et al.* (1998) and Sahay *et al.* (2002) also reported a higher sensitivity of CMT as compared to other tests. Bulla (2002) reported somewhat closely sensitivity of SCC (47.91%) as compared to that recorded in the present study, however Reddy *et al.* (1998) reported higher sensitivity of SCC (65.21%). In our study animal and quarter wise sensitivity of WST was 21.05 and 24.14%, respectively, which is lowered than the findings of Anusha *et al.*, (2018). Animal and quarter wise sensitivity of BBST for detection of SCM, was found to be 31.03 and 30.26%, respectively. However other workers Buragohain and Dutta (1998), Tiwari and Sisodia (2001) and Sahay *et al.* (2002) reported a higher sensitivity of BBST ranging between 69.38 to 83.15%.

In our study CMT was found to have highest specificity (96.55%) for quarter wise and predictive value of positive test for (96.15%), as compared to mastidin (96.55 and 94.59%), milk test (94.83 and 93.75%) same for masttest, kerba test, WST, BBST and SCC (93.10%) and predictive value of positive test (91.84, 91.49, 88.89, 92.00 and 92.00%), respectively. However other researchers Ghulam *et al.* (2010), Rakesh *et al.* (2018) and Anusha *et al.* (2018) also reported higher specificity and predictive value of CMT, WST, SCC and BBST for detection of SCM which were also similar to that in the present study.

The sensitivity and specificity of the above eight tests were determined by considering cultural examination as standard test. The reason for variation in the sensitivity and specificity and predictive value of different diagnostic tests in the present study might be due to the fact that they are designed to detect different types of changes in the subclinical infected milk.

In the present study, false positive reactions were more with WST (12.50%) followed by milk test (10.71%), Mastidin (8.33%), Kerba test (7.69%) Masttest (7.14%) and least and same with SCC and BBST (6.90%) and CMT (6.45%) and the false negative reactions were more with WST (78.57%) followed by Milk test (77.50%), Mastidin (77.38%), Kerba test (76.83%), Masttest (76.25%) and least and same with SCC and BBST (75.95%) and CMT (75.32%). Langer *et al.* (2014) reported 8.2% and 20.3% false positive reactions and 27.2% and 18.0% false negative reactions with SCC and CMT, respectively, taking culture as standard. Studies of

Anusha *et al.* (2018) showed 21.43%, 20.45% and 22.64% false positive and 25.42%, 33.80% and 29.03% false negative reactions with CMT, WST and SCC, respectively, and accuracy of 76.52% for CMT, 71.30% for WST and 73.39% for SCC. Sharma *et al.* (2010) showed 23.79% and 1.63%, false positive and 25.72% and 15.48% false negative reactions with CMT and SCC, respectively, and accuracy of 75.52% for CMT and 91.94% for SCC. Reddy *et al.* (2014) reported 73.33% and 71% accuracy for CMT and SCC, respectively. Siji and Vijayakumar, (2006) reported that the accuracy of CMT was 83.5%. All the results are in agreement with the present finding and indicate that CMT is a best field side diagnostic indicator taking cultural examination as a gold standard laboratory test.

The milk sample from a goat, analyzed for mastitis, revealed several pathogens across different culture media. Blood Agar showed growth of Coagulase positive Staphylococcus (*Staphylococcus aureus), Streptococcus species, Coagulase negative Staphylococcus, and Bacillus species*, key mastitis pathogens. Nutrient Agar identified *S. aureus, Bacillus species, and Lactobacillus species*, indicating a diverse microbial presence. MacConkey Agar detected *Escherichia coli, Salmonella species, Enterobacter species,* and *Klebsiella species*, highlighting potential contamination or infection. No growth was observed on Brucella Agar, indicating the absence of *Brucella species*. These findings support the diagnosis of mastitis in the goat which tally with the work of Podhorecká *et al.* 2021 that also isolate *Staphylococcus aureus, Staphylococcus chromogenes, and Bacillus species* in goat milk. Also correspond with the work of Kováčová *et al.,* 2021 that also isolate *Enterobacter species* and *S. aureus.*

However, in the present study per cent accuracy of various (CMT, Masttest, Mastidin, Kerba test, Milk test, WST, BBST and SCC) tests taking cultural test as standard is summarized that CMT test has high specificity (96.55%) and predictive value (96.15%) with sensitivity of (32.89%) than compare with other diagnostic tests. Though the sensitivity is low, a high specificity and predictive value of the positive test in the present study enables CMT to be used as the decision criteria to treat or to cull the animals in herds with high prevalence of sub clinical mastitis in dairy.

CONCLUSION

The diagnosis of mastitis is based on identification of the causative agent. CMT or SCC are the most common diagnostic tests used for the detection of SCM. From this study the sensitivity, specificity and predictive value of different diagnostic tests were studied and it was found that CMT had the highest sensitivity (32.89%), it also had the highest specificity (96.55%) among the tests evaluated. CMT can be used as a reliable diagnostic method in field conditions. However, since the utilization of only one diagnosis method in goat mastitis, without confirmation by bacteriologic test is not conclusive, CMT should be used cautiously in small ruminant mastitis diagnosis.

RECOMMENDATIONS

Further studies will be required: to determine the SCC of goat, to evaluate and improve the use of CMT on milk from individual quarters as an indicator of subclinical mastitis, also to explore additional diagnostic methods or combine multiple tests to enhance overall diagnostic accuracy.

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