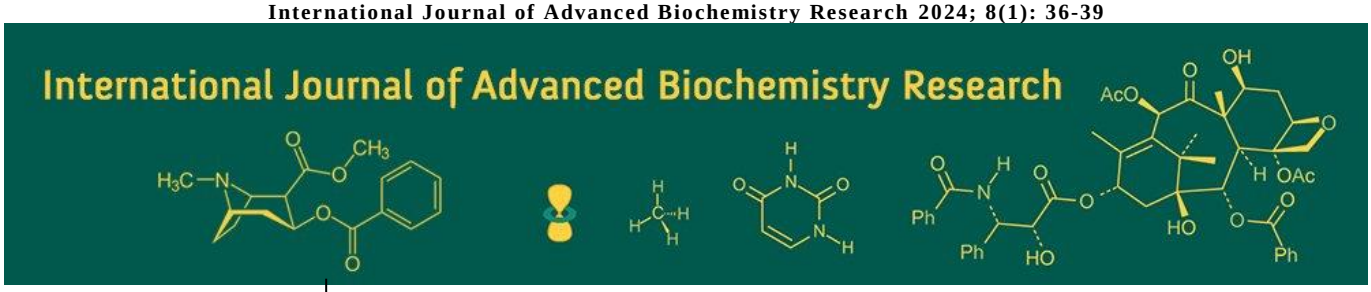

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Study of prevalence and isolation of pathogens in sub clinical mastitis

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Abstract
Two hundred quarter milk samples from fifty seemingly healthy cows with varying parities were tested using the California Mastitis test and culture analysis. On a quarterly basis, the prevalence determined by CMT was 36% (72/200) and determined by culture analysis, 30% (60/200). According to CMT and a culture examination, the prevalence on an animal basis was forty (20/50) and forty percent (23/50). The highest prevalence was seen in the IVth parity on a quarterly basis and in the Vth parity on a cow basis. 50.56% of the *Staphylococcus aureus* organism was determined to be predominant in this study based on culture testing. The major pathogen was identified as *Staphylococcus* spp. (42%), which was isolated by bacterial mPCR.

Keywords: Cow, sub clinical mastitis, California mastitis test

Introduction
One of the most common diseases known to affect cows is mastitis. Mastitis is categorized based on the severity of the condition and the level of inflammation. Awale *et al.* (2012) [1]. Since the subclinical mastitis has no symptoms, milk seems to be normal. In most dairy herds, this kind causes the largest loss and is 30-40 times more common than clinical mastitis. Can significantly affect the global dairy industry's financial standing (Bachaya *et al.*, 2012) [3]. The California Mastitis Test (CMT) is a quick, easy, and affordable screening tool. The gold standard technique for diagnosing subclinical mastitis and intra-mammary infections is bacteriological culture of milk samples. 1987 saw the International Dairy Federation.

Materials and Methods
A total of 200 quarters of milk from 50 cows that appeared to be in good health were aseptically collected and examined from several government veterinary hospitals, the College of Veterinary and Animal Science in Bikaner, and a few private dairies in the Bikaner District. The California Mastitis Test (CMT) and cultural analysis were performed on each sample.

Collection of milk samples: Samples of aseptic milk were taken after the udder and teats were thoroughly cleaned with water and allowed to dry naturally. Next, a spirit swab was used to clean each teat. The first two or three fore milk strippings were thrown away. Next, each teat's 30 milliliters of foremilk were collected in a test tube that had been disinfected. Left fore (LF), left hind (LH), right fore (RF), and right hind (RH) were marked on them.

California mastitis test: The California mastitis test was applied to every milk sample in accordance with the protocol provided by Schalm and Noorlander (1957). The tool used was a plastic paddle with four shallow cup chambers. Each quarter was stripped and 3 ml of milk sample was taken, labeled RF, RH, LF, and LH for the right fore, left fore, and left hind quarters, respectively, into the corresponding 4 cups. A test reagent in an equal quantity was added to the cup. By rotating the paddle in a horizontal plane for a short while, the contents were gradually combined. The gel formation shows the total number of cells. The color response with bromocresol purple indicates the pH change.

The sensitivity of this test for leukocytes in milk is excellent.

Culture Examination: Using a 4 mm platinum loop, a loopful of milk sample was streaked in primary, secondary, and tertiary form on nutritional agar plates, mannitol salt agar, and 5% sheep blood to produce isolated colonies of bacteria. Observations were made of colony features and hemolytic zones on blood agar plates after 24-48 hours of aerobic incubation at 37 °C in petri dishes. The plates were incubated for a further 24 hours if the colonies were tiny or nonexistent.

Result and Discussion

Prevalence of sub clinical mastitis: In the current investigation, the quarterly prevalence of subclinical mastitis was 36% (72/200) on a culture basis and 30% (60/200) on a CMT basis. In terms of animals, the prevalence in cows was 40% (20/50) based on CMT and 46% (23/50) based on Cultural testing. (No. 3 in the table)

Supriya and Leather (2010) [12] observed nearly identical findings, with a prevalence of 53.33 and 32.5%, respectively, for animals and quarters wise.

This study found that 50% of the cattle had involvement in the right hindquarters and 46% in the left, whereas 36% of the cattle in the right and 32% in the left forequarters were impacted. (Table 4). The maximum prevalence was found in the IVth parity quarter-wise and the Vth parity cow-wise (Tables Nos. 5 and 6).

Various Diagnostic Tests

California Mastitis Test (CMT): On a CMT basis, the quarterly prevalence of subclinical mastitis was 30% (60/200), while the prevalence in animals was 40% (20/50). Comparable findings were reported by Islam *et al.* (2011) [14] and Khelef *et al.* (2013) [6], who calculated the prevalence of subclinical mastitis based on CMT to be 29.20 percent and 29.50 percent, respectively. Additionally, 41.02% of animal cases of subclinical mastitis were reported by Ayano *et al.* (2013) [2].

Cultural Examination: Out of 200 quarter samples, 72 milk samples tested positive for cultural contamination. Upon conducting a cultural examination, it was discovered that out of the milk samples, 55 (76.38%) had a single bacterial infection and 17 (23.61%) had a mixed infection. Two genera combined were discovered in the mixed infection. In mixed infections, *Staphylococcus* species were isolated with Streptococcus species, *Klebsiella* species, and *E. coli*, respectively. (Table 7).

The relative frequency of 89 microorganism islotes from 72 healthy quarters is shown in (Table-8). Of these, 45 isolates (50.56%) of *Staphylococcus aureus* and 6 isolates (6.74%) of Klebsiella pneumoniae were the most commonly isolated. 7 (or 7.8%) *E. coli*, 2 (2.2%) *Staphylococcus epidermidis*, 12 (13.48%) *Staphylococcus agalactiae*, 5 (5.61%) Streptococcus *hyicus*, 2 (2.2%) *Streptococcus uberis*, 10 (11.23%) *Streptococcus dysgalactiae*,

Harini and Sumathi (2011) [4] and *Savita* (2016) [10] have observed similar results. According to Harini and Sumathi, the most common bacterial isolates were E. coli (23.5%) and *Staphylococcus aureus* (58%), followed by *Staphylococcus* epidermidis (8%), *Streptococcus* spp. (5.5%), and *Klebsiella* spp. (3%), in that order.

Table 1: CMT reaction was scored as followed

Negative	Mixture remained liquid with no precipitation
Trace (T)	Slight precipitation which tends to disappear after continued movements of the paddles.
+ (Weak positive)	Precipitate is distinct but no longer tendency towards gel formation.
++ (Distinct positive)	Mixture thicken immediately which give clear indication of gel formation. Swirl the mixture, it tends to move towards the centre and when the motion is stopped, the mixture is leaving the bottom of the outer edge of cup exposed, the mixture level out again covering the bottom of the cup.
+++ (Strong positive)	Mixture tends to adhere to the bottom of the cups

Table 2: Results of diagnostic tests used for detection of Sub-clinical mastitis (Quarter-wise)

Diagnostic Test	Subclinical mastitis affected quarters (n=200)	Percentage (%)
California mastitis test	60	30%
Bacterial culture	72	36%

Table 3: Results of diagnostic tests used for detection of Sub clinical mastitis (Animal-wise)

Diagnostic Test	Sub clinical mastitis affected animals (n=50)	Percentage (%)
California mastitis test	20	40%
Bacterial culture	23	46%

Table 4: Prevalence of sub clinical mastitis in cattle (Quarter wise)

S. No.	Quarter disposition	No. of quarters screened	No. quarters affected	Percentage
1	Left Fore	50	16	32%
2	Left hind	50	23	46%
3	Right fore	50	18	36%
4	Right hind	50	25	50%
		200	82	

Table 5: Parity wise Prevalence of Sub clinical Mastitis (Quarter-basis)

Parity	No of quarters examined	No. of quarters positive for subclinical mastitis	Percentage (%)
I	24	5	20.83
II	28	5	17.85
III	60	30	50
IV	40	28	70
V	32	11	34.37
VI	16	3	18.75

Table 6: Parity wise Prevalence of Sub clinical Mastitis (Animal-basis)

Parity	No of cows examined	No. of cows positive for subclinical mastitis	Percentage
I	7	2	28.57
II	6	2	33.33
III	10	6	60
IV	15	9	60
V	8	5	62.5
VI	4	2	50

Table 7: Relative frequency of bacterial isolates in sub clinical mastitis infected quarters by culture isolation

S. No.	Bacterial isolate	No. of infected quarters 72 (55 single + 17 mixed bacterial infection)	Percentage (%)
1.	*Staphylococcus aureus*	28	38.88
2.	*Staphylococcus hyicus*	5	6.94
3.	*Streptococcus agalactiae*	7	9.72
4.	*Klebsiella pneumoniae*	2	2.77
5.	*Streptococcus uberis*	2	2.77
6.	*E.coli*	4	5.55
7.	*Streptococcus dysgalactiae*	5	6.94
8.	*Staphylococcus epidermidis*	2	2.77
9	*Staphylococcus aureus+ Streptococcus dysgalactiae*	5	6.94
10	*Staphylococcus aureus+ Escherichia coli*	3	4.16
11	*Staphylococcus aureus + K. pneumonia*	4	5.556
12	*Staphylococcus aureus + Streptococcus agalactiae*	5	.94
	Total	72	

Table 8: Isolation of organism by cultural method

S. No.	Bacterial Isolates	No. of isolates	Percentage (%)
1	*E. coli*	7	7.8
2	*Streptococcus dysgalactiae*	10	11.23
3	*Staphylococcus hyicus*	5	5.61
4	*Klebsiella pneumonia*	6	6.74
5	*Staphylococcus aureus*	45	50.56
6	*Streptococcus uberis*	2	2.2
7	*Streptococcus epidermidis*	2	2.2
8	*Streptococcus agalactiae*	12	13.48
	Total	89	

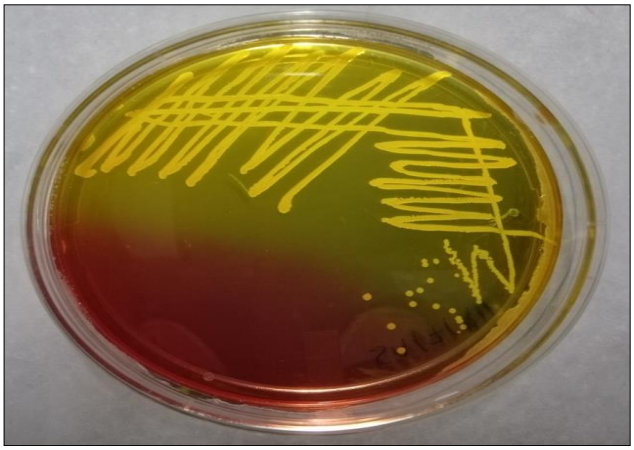

Fig 1: Photograph showing *Staphylococcus aureus* mannitol salt (MSA) agar

Fig 2: *Klebsiella* on Macconkey agar

Fig 3: *E. coli* (lactose fermenter) colony on MacConkey agar

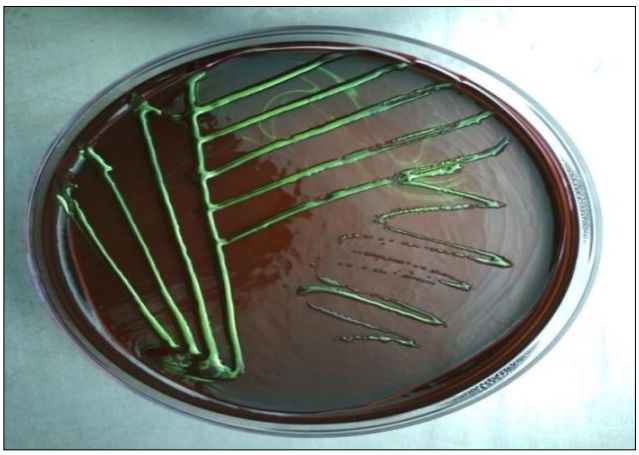

Fig 4: Metallic sheen of *E. coli* on Eosin Methylene Blue agar

Conclusions

Subclinical mastitis prevalence, determined through California Mastitis Test and culture analysis, was noteworthy at 36% and 30%, respectively, on a quarterly basis. Staphylococcus aureus emerged as the predominant pathogen (50.56%), with Staphylococcus spp. identified as the major contributor (42%). The 4th parity exhibited the highest quarterly prevalence, while the 5th parity had the highest prevalence on a cow basis. These findings underscore the significance of regular monitoring and early detection to address subclinical mastitis in dairy herds, crucial for minimizing economic losses and sustaining the industry's health.

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