Int.J.Curr.Microbiol.App.Sci (2021) *10*(02): 2165-2173

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 02 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1002.257

Study of Prevalence and Change in Milk Composition in Sub Clinical Mastitis Cattle

Kavita Jaidiya*, Anju Chahar, T. C. Nayak and Mahaveer Suresha

Department of Epidemiology and preventive Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner - 334001(Rajasthan), India

**Corresponding author*

Keywords

Cow, Sub clinical Mastits, California mastitis test

Article Info

Accepted:
17 January 2021
Available Online:
10 February 2021

A B S T R A C T

A total of 200 quarter milk samples from 50 apparently healthy cows of different parity were examined by California Mastitis test and cultural examination. Milk samples also subjected for Changes in composition Viz. fat, protein and lactose. The prevalence of subclinical mastitis based on culture examination was 30(60/200) and 36 per cent (72/200) based on CMT and Culture examination on quarter basis. The prevalence of subclinical mastitis on animal basis was 40 (20/50) and 46 per cent (23/50) based on CMT, and Culture examination. Highest prevalence was observed in IV^{th} parity on quarter basis and in V^{th} parity on cow basis. On culture examination, *Staphylococcus aureus* was the most prevalent organism (50.56%), followed by *Streptococcus dysaglactiae (*11.33%), *E.coli (*7.8 %), *Staphylococcus agalactiae* (13.48 %), *Staphylococcus epidermidis (*2.2 %), *Streptococcus hyicus* (6.94 %), *Streptococcus uberis* (5.16%), *Klebsiella pneumonia* (6.74 %). On isolation by bacterial mPCR, *Staphylococcus spp.* (42 %) was the major pathogen. Organisms isolated in mixed infections are *Streptococcus spp., Klebsiella pneumonia, E.coli* and *Psudomonas aerugenosa.* The average mean value of fat, protein and lactose content in subclinically affected milk samples were 3.40±0.101, 3.009±0.033 and 4.48±0.03 and the mean value of fat, protein and lactose content in normal milk were 4.13±0.035, 3.39±0.021 and 5.10±0.016.

Introduction

Mastitis is one of the most important disease of bovines and continues to have a major economic impact in the dairy industry throughout the world (Bachaya *et al.,* 2012). Mastitis is classified into clinical and sub clinical form, according to degree of inflammation and severity of disease (Awale *et al.,* 2012*).* The sub clinical mastitis is asymptomatic, therefore milk appears to be normal. This form of mastitis is+ 30-40 times more common than clinical mastitis and causes the great loss in most of the dairy

herds. Sub clinical mastitis is devasting disease in dairy industry through out the world and in India it is enzootic and alarming. The sub clinical mastitis usually goes unnoticed because the milk and udder appear normal.

The sub clinical mastitis is mainly caused by *Staphylococcus aureus, Streptococcus agalactiae* and *Streptococcus* species and *coliform* (Mpatswenumugabo *et al.,* 2017). The *S. aureus* is the major pathogen of sub clinical mastitis (Piepers *et al.,* 2007). Sub clinical mastitis lowers milk composition (fat, lactose, protein, solid not fat content) (Muhee *et al.,* 2017).

Bacteriological culture of milk samples is the gold standard test for identifying sub clinical mastitis and intra-mammary infection (International Dairy Federation, 1987). The California mastitis test is simple, inexpensive and rapid screening test.

Materials and Methods

A total of 200 quarter milk samples from 50 apparently healthy cows of different lacteal stage were collected aseptically from LRS, College of Veterinary and Animal Science, Bikaner and private dairies in surrounding areas of Bikaner.

All the milk samples were screened by California mastitis test (CMT) and cultural examination. Samples were also examined for changes in milk composition.

Collection of milk samples

Milk samples were collected aseptically. Udder and teats were washed with water and air dried. Then each teat was wipped off by spirit swab. First Two three strippings of fore milk were discarded. Approximately 30 ml of fore milk from each teat was collected in sterilized test tube with caps.

These were marked as right fore (RF), right hind (RH), left fore (LF), left hind (LH). Care was taken to avoid any type of contamination in the milk. The milk samples from a total of 200 quarters of 50 cattle collected in the present study.

All the samples of milk were brought to the laboratory and kept in refrigerator (4˚C) until analysed. Milk samples were subjected to California mastitis test and cultural examination and were also examined for fat, protein, lactose contents.

California mastitis test

The milk samples were subjected to California mastitis test as per the procedure given by Schalm and Noorlander (1957). A plastic paddle with four chambers of shallow cups was used to perform the test. 3 ml of milk was taken from each quarter stripped directly into the respective 4 cups labeled right fore, right hind, left fore, and left hind quarters as RF, RH, LF and LH.

An equal quantity of the above test reagent was added into cup. The content was gently mixed by circular movement of the paddle in horizontal plane for few seconds.

Total cell count is reflected by the degree of precipitation or gel formation that occurs. The pH change associated with abnormal milk is indicated by a colour reaction with bromocresol purple. This test has specificity for leukocytes in the milk.

Culture examination

The Milk samples collected aseptically were shaken thoroughly. A loopful of milk sample was streaked on 5% sheep blood agar, nutrient agar plate, mannitol salt agar and Mac-Conkey agar plate with the help of 4mm diameter platinum loop in primary secondary and

tertiary fashion in order to obtain isolated colonies of bacteria. Petri dishes were incubated aerobically at 37˚C for 24-48 hours. The plates were observed for colonial characteristics and haemolytic zones on blood agar plates. In case the colonies were small or absent, then the plates were incubated for further 24 hours.

Changes in constituents of milk

Measurement of changes in composition of mastitic milk was done by passing milk through the milk analyzer because mastitis leads to changes in composition of milk viz. protein, fat and lactose. This was determined by milkoScan.

Results and Discussion

Prevalence of sub clinical mastitis

In present study, the quarter wise prevalence of Sub clinical mastitis in cows was 30 per cent (60/200) and 36 per cent (72/200) based on CMT and Cultural examination, respectively. Animal wise prevalence of sub clinical mastitis in cows was 40 (20/50), and 46 per cent (23/50) based on CMT and Culutral examination (Table no. 1–3)

Almost similar results have been reported by Supriya and Leather (2010) they recorded 53.33 and 32.5 per cent prevalence on animal wise and quarters wise. In this study 50 per cent of cattle showed involvement of right hind quarters and 46 per cent showed in left hind quarters whereas right fore and right fore quarters were affected as 36 and 32 per cent, respectively (Table no.4). Highest prevalence was observed in IV^{th} parity on quarter basis and in V^{th} parity on cow basis. This could be due to lowered resistance of the animals within increase in parity due to improper functioning of the teat sphincter as reported by Singh and Baxi (1980) (Table 5 and 6).

Status of various diagnostic tests

California Mastitis Test (CMT)

The prevalence of sub clinical mastitis based on CMT was 30 per cent (60/200) on quarter basis and 40 per cent (20/50) on animal basis. Similar findings were observed by Islam *et al.*, (2011) and Khelef *et al.*, (2013) who reported the prevalence of sub clinical mastitis on the basis of CMT as 29.50 and 29.20 per cent, respectively. Ayano *et al.*, (2013) also reported 41.02% prevalence of sub clinical mastitis on animal basis.

Cultural examination

Out of 200 quarter milk samples, 72 samples were found positive on cultural examination. Out of these, 55 (76.38 %) milk samples were having single bacterial infection whereas 17 (23.61%) milk samples were having mixed infection respectively. In mixed infections combination of two genera were found. Oragnisms isolated in mixed infections were *Staphylococcus* spp. in combination with *Streptococcus* spp, *Klebsiella* Spp. and *E.coli*, respectively (Table-7; Fig. 1–4).

The relative frequency of different microorganisms in 89 islotes from 72 apparently healthy quarter is presented in (Table-8). *Staphylococcus aureus* was the most frequent isolate, accounting for 45 isolates (50.56%), followed by *Streptococcus dysaglactiae* 10 (11.23 %), *E. coli* 7 (7.8 per cent), *Staphylococcus agalactiae* 12 (13.48 %), *Staphylococcus epidermidis* 2 (2.2%), *Streptococcus hyicus* 5 (5.61%), *Streptococcus uberis* 2 (2.2 %), *Klebsiella pneumoniae* 6 (6.74 %). Amongst different bacteria isolated in present study *Staphylococcus aureus* was found to be the most prevalent organism based on isolation on specific media and microscopic identification of organism.

Table.1 CMT reaction was scored as followed

(-) ve	**Mixture remained liquid with no evidence of formation of precipitate**
Trace (T)	A slight precipitate which tends to disappear with continued movements of the paddles.
+(Weak positive)	A distinct precipitate but no tendency towards gel formation.
++(Distinct positive)	The mixture thick immediately with indication of gel formation. As the mixture is caused to swirl, it tends to move towards the centre, leaving the bottom of the outer edge of cup exposed, when the motion is stopped, the mixture level out again covering the bottom of the cup.
+++ (Strong positive)	A distinct gel forms that tends to adhere to the bottom of the cups and during swirling a distinct central peak is formed

Table.2 Results of various diagnostic tests used for detection of sub-clinical mastitis (Quarter-wise)

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

Table.3 Results of various diagnostic tests used for detection of sub clinical mastitis (Animal-wise)

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

Table.4 Quarter wise prevalence of sub clinical mastitis in cattle

S. No.	Quarter disposition	No. of quarters screened	No. quarters affected	Percentage
1	**Right Fore**	50	18	36%
2	**Right hind**	50	25	50%
3	**Left fore**	50	16	32%
4	**Left hind**	50	23	46%
		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.	*Streptococcus agalactiae*	7	9.72
3.	*Staphylococcus hyicus*	5	6.94
4.	*Streptococcus uberis*	2	2.77
5.	*Klebsiella pneumoniae*	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 dysaglactiae*	5	6.94
10	*Staphylococcus aureus+ Escherichia coli*	3	4.16
11	*Staphylococcus aureus +Streptococccus agalactiae*	5	6.94
12	*Staphylococcus aureus + K. pneumoniae*	4	5.55
	Total	72	

Table.8 Isolation of organism by cultural method

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

Fig.1 Photograph showing *Staphylococcus aureus* (mannitol fermentor) colony on MSA agar

Fig.2 Photograph showing *Klebsiella* on Macconkey agar

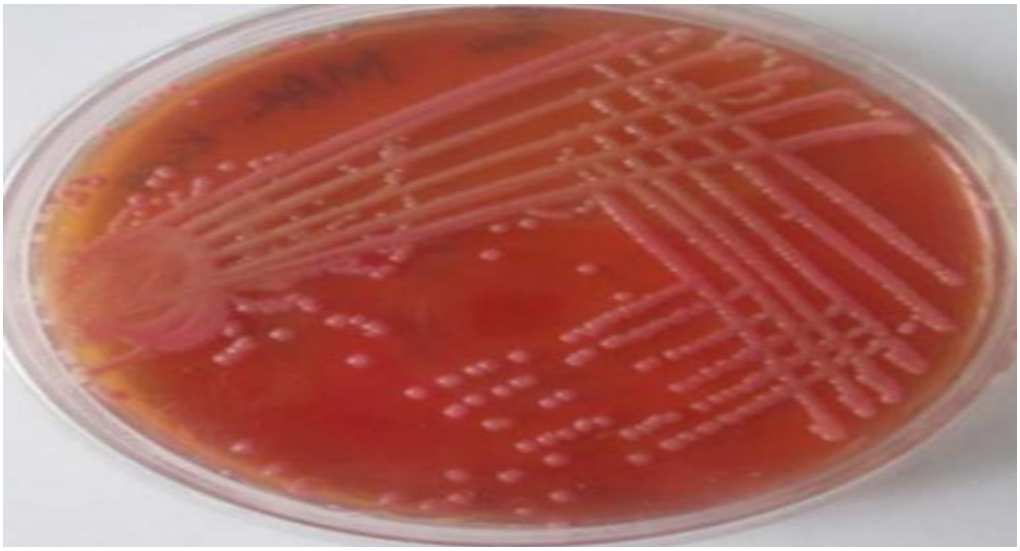
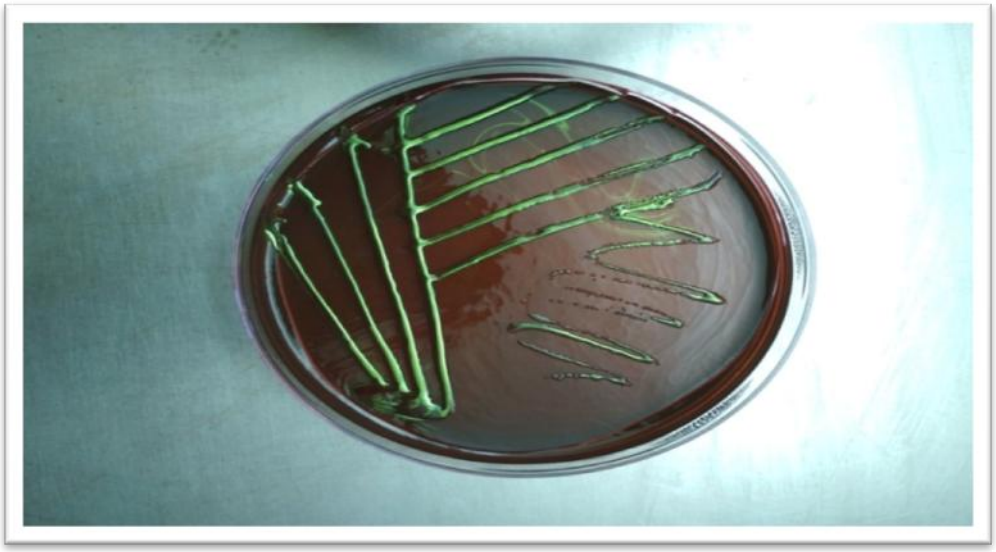


Fig.3 Photograph showing *E. coli* (lactose fermentor) colony on Macconkey agar

Fig.4 Photograph showing metallic sheen of *E. coli* on Eosine methylene blue agar



Similar results have been reported by Harini and Sumathi (2011) and Savita (2016). Harini and Sumathi reported that predominant bacterial isolates were *Staphylococcus aureus* (58 %) and *E. coli* (23.5%) followed by *Staphylococcus epidermidis* (8 %), *Streptococcus* spp. (5.5 %), *Klebsiella* spp. (3 %), respectively.

Changes in the milk constituents

The average mean value of fat, protein and lactose content in sub clinically affected milk samples were 3.40 ± 0.0101, 3.009 ± 0.033 and 4.48 ± 0.036 (ranged between 2-4, 2.7-3.7 and 4-5.1) and the mean value of fat, protein and lactose content in normal milk were 4.13±0.035, 3.39±0.021 and 5.10±0.016 (ranged between 3.2-4.9, 3-3.9 and 4.8-5.5).

References

Awale, M.M., Dandhatra, G.B., Avinash, K. Chauhan, B.N., Modi, C.M., and O' Kennedy, R. (2012) Bovine mastitis: a threat to economy. *Open Access Scientific Reports*, 1(15): 295.

Ayano, *A.*, Hiriko, F., Simyalew, A and Yohannes, A. (2013). Prevalence of Sub clinical mastitis in lactating cows in selected commercial dairy farms of Holeta. *J. Vet. Med.*, 5(3): 67-72.

Bachaya, A., Raza, M.A. and Akbar, R. (2011). Sub clinical bovine mastitis in Muzaffar Garth district of Punjab (Pakistan). *Journal of Animal Sciences*, 21(1): 16-19.

Harini, H. and Sumathi, B.R. (2011) Screening of bovine milk samples for sub clinical mastitis and antibiogram of bacterial isolates. *Veterinary World*, 4(8): 358-359.

International Dairy Federation (1987). Bovine mastitis: Defination and guidelines for diagnosis. *Bulletin International Dairy Federation, No 211, Brussels, Belgium.*

Khelef, D., Saidi, R. and Kaidi, R. (2013). Bovine mastitis: Prevalence of bacterial pathogens and evaluation of early screening test. *African Journal of Microbiology Research.*, 7(9): 777-782.

Mpatswenumugabo, J.P., Bebora, L.C., Gitao, G.C., Moblegi, V.A., Iraguha, B,. Kamana, O., Gitao, G.C., Mobegi,

V.A., Shumbusho, B. (2017). " Prevalence of subclinical Mastitis and Distribution of pathogens in Dairy farms of Rubare and Nyabihu Districts, Rawanda. *Journal of Veterinary Medicine*, pp 8.

Muhee, A.., Malik, H.U., Asharf, Shah, O,S., Jian, A; Rather, W. and Muzzamil, S (2017). Biochemical and mineral alteration of milk chemistry in mastitis.

Piepers, S., Meulemeester, L. De., Kruit, A. De, Opsomer, G., Barkema. H.W., and Vliegher S.De., 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium, Journal of Dairy Research., 74: 478-483.

Savita (2016). Studies on some aspect of sub clinical mastitis in cattle. M.V.Sc. Thesis submitted to Rajasthan University of Veterinary and Animal Sciences, Bikaner Rajasthan, India.

Singh, K.B. and Baxi, K.K. (1980) Studies on the incidence and diagnosis of sub clinical mastitis in Dairy Cows. *Indian Veterinary Journal*, 57: 723-729.

Supriya, N.A and Sharma, N. (2010). Prevalence of sub-clinical mastitis in an organized cow herd. *Haryana Veterinary, 49: 64-65.*

Yang, F. Li., Li, X.S., He, B.X., Yang, X.L., Li, Liu, P., Huang, Q.H., Pan, X.M. and Li, J. (2011) Malondialdehyde level and some enzymatic activities in subclinical mastitis milk. *African Journal of Biotechnology*, 10(28): 5534-5538.

How to cite this article:

Kavita Jaidiya, Anju Chahar, T. C. Nayak and Mahaveer Suresha. 2021. Study of Prevalence and Change in Milk Composition in Sub Clinical Mastitis Cattle. *Int.J.Curr.Microbiol.App.Sci.* 10(02): 2165-2173. doi: https://doi.org/10.20546/ijcmas.2021.1002.257