Pakistan J. Zool., vol. 56(3), pp 1379-1387, 2024 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20221111051117

Influence of Bacterial Species on Physical Characteristics and Somatic Cell Counts in Clinical and Subclinical Mastitis Milk of Kundhi Buffaloes

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Article Information
Received 11 November 2022
Revised 26 November 2022
Accepted 17 December 2022
Available online 01 March 2023 (early access)
Published 02 May 2024

Authors' Contribution
HB, RR and SHA conceived and designed the experiments. HB performed the experiments. DHK, HAB, SK, MI, HA and MM analyzed the data. HB wrote the paper.

Key words
Buffaloes, Mastitis, Milk characteristics, Somatic cell count, Total bacterial count

ABSTRACT

A total of 852 (213 animals) and 840 (210 animals) milk samples were collected from lactating buffaloes with signs of clinical and subclinical mastitis respectively. The California Mastitis Test was performed to confirm mastitis. The sub-clinical cases were categorized by severity of infection with moderate severe, severe, more severe and most severe. All the 646 positive samples analysed, 41.95% were found with flacks, 7.73% with watery consistency, 15.01% with pus, 10.06% with blood tinge, 21.67% had odour while 3.56% were noted with curd like consistency. *Staphylococcus aureus* was logged as the dominant species caused intensive gel formation in subclinical mastitis. However, 840 quarters were found with infection at diverse degree. Only 102 Most severe (4+) samples noted strong gel as with mean somatic cell count (SCC) of $8{,}1x10^5$ while 60 more severe (3+) samples showed distinct gel with mean SCC of $2{,}7x10^5$. Among the 102 strong positive samples, 57 (17.27%) contained pathogens. The correlation between SCCml^{-1} and colony forming unit (CFU) ml^{-1} of bacterial population was analysed. The mean SCC was counted as $82x10^5$ cells ml^{-1}in clinical and $50x10^5$ cells ml^{-1} in subclinical mastitis. *Staphylococcus aureus* was recorded as the most dominant species. The mean values of $93x10^5$ CFU ml^{-1} were counted for clinical mastitis while $51x10^5$ CFU ml^{-1} was measured for subclinical mastitis. A clear difference between Somatic Cell Count (SCC) and Total Bacterial Count (TBC) was observed in two groups of buffaloes. The significance of the study is considered useful in apprising health of the animals recommending future strategies to control the infections, an important factor in productivity enhancement.

INTRODUCTION

Pakistan has been blessed with two major breeds of buffaloes, Nili-Ravi and Kundhi which are located mostly in the irrigated areas of Punjab and Sindh provinces, respectively (Aujla, 2014). Both breeds are famous for high milk yield. Bovine mastitis is one of the greatest challenging maladies and have an endless fundamental commercial impact on the dairy cattle causing economic losses due to reduced yield and poor milk quality (Cheng and Han, 2020). Mastitis is the most common inflammatory disease of the mammary gland of cattle, detected by an increase in the number of somatic cell count (SCC) or visible abnormalities in milk (Metzger *et al*., 2018; Thabiso *et al*., 2017). The disease is characterized by escalation in the number of somatic cells, exclusively white blood cells in the udder secretions through irrational alterations in the mammary tissues. Somatic cell count is influenced by the cow's productivity, health, parity, lactation stage, and breed of the animal (Alhussien and Dang, 2018). Mastitis is classified into clinical and subclinical mastitis. Clinical mastitis is manifested by redness, heat

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0030-9923/2024/0003-1379 $ 9.00/0

and inflammation of udder, discomfort and loss of milk production with systemic signs such as fever, anorexia and depression. Consistently, symptoms with milk abnormalities are milk lumps, scales, watery secretions and blood (Cheng and Han, 2020; Souto *et al*., 2010). Clinical mastitis is established by direct reflection of solid emblems through the farmers (Hokmabad *et al*., 2011). Subclinical mastitis is the presence of infection without local inflammation resulting in an absence of visual signs, but there is an increase in milk SCC, which reduces the value of the milk (Sumon *et al*., 2020; De Vliegher *et al*., 2012). Subclinical mastitis is 30-40 times more common than the clinical mastitis and causes the greatest losses in most dairy herds (Romero *et al*., 2018). It is responsible for 70% of economic losses (Heleili *et al*., 2012; Dua, 2001) reported annual losses due to mastitis in the terms of Rs. 60.5321 billion from which Rs. 43.6532 billion has been attributed to subclinical mastitis. Subclinical mastitis is recognized by indirect detection of the somatic cell count in milk (Hokmabad *et al*., 2011) by animal-side milk tests (Bachaya *et al*., 2011). It can be diagnosed through somatic cell counts (SCC) by California mastitis test (CMT), white side test (WST) or surf field mastitis test (SFMT) (Ranjeet *et al*., 2018). Somatic cell count is widely used as an inflammatory indicator in diagnosis of subclinical mastitis in the bovine cases and buffaloes as well (Alhussien and Dang, 2018; Dhakal *et al*., 1992; Moroni *et al*., 2006). The European Union Directives set a limit of 400,000 cells ml^{-1} for SCC in buffalo milk. It has also been observed that the incidence and the patterns of causative agents markedly differ from place to place, herd to herd and time to time (Costa *et al*., 2020).

According to the reports published in literature suggested that about 70-80% of all the clinical and subclinical mastitis cases are caused by *Staphylococcus aureus; Streptococcus agalactiae; Escherichia coli; Corynebacterium pyogenes; Streptococcus dysgalactiae* and *Streptococcus uberis* in buffaloes (Babji *et al*., 2020; Baloch *et al*., 2013; Mork *et al*., 2005). The frequency of clinical and subclinical mastitis instigated by *Staphylococcus aureus* is noted in 53.85% buffaloes (Hameed *et al*., 2008). The quarter-wise and animal-wise occurrence of mastitis in animals at various locations of Attock had been investigated while at quarter level the prevalence has been demonstrated to be 44.75% whereas at buffalo level, the prevalence has been noted in 44% (Bachaya *et al*., 2005) animals. In spite of vulnerability to udder infection, a little has been observed in bubaline species as equated to cows (Khaled *et al*., 2010). To avoid udder infections and following mastitis, it may be important to find out ways to stimulate the animal's immune defence more efficiently to help against elimination of infection (Zecconi and Smith, 2000; Hase *et al*., 2013). Considering clinical and subclinical mastitis as potential threat to dairy and human health, the present study was therefore proposed to explore the status of the influence of individual and mixed bacterial species in physical characteristic changes and somatic cell counts in milk of buffaloes that would be helpful in concluding the clinical mastitis from subclinical ones in buffaloes.

MATERIALS AND METHODS

Study area

A total of 423 buffaloes, 213 with clinical and 210 with subclinical mastitis of Kundhi breed were examined from Hyderabad, Tando Allahyar and Tandojam of Sindh province of Pakistan. The average rainfall in the area is 415 mm/year and average temperature is about 46°C during months of May to August while in December and January, months; it is about 10°C (MSP, 2011). The animals were kept largely under natural climatic conditions and were raised mainly for milk purpose. Hand milking was practiced at each holding site using normal management practices.

Physiological status of animals

Generally, the physiological conditions of buffaloes such as health, age, lactation, parity and breed were recorded. In clinical mastitis, the nature of the whole udder or part of the udder, the number of quarters, wounds, injuries and damages involved were also recorded. While in subclinical mastitis, the animals were found healthy, alert and active. There was no tick infestation noted but their sheds and open yards were not properly managed and cleaned. The udders of the animals were soiled with manure, mud and urine. At majority of sheds and open yards, no clean water was being provided to the animals.

Collection of clinical and subclinical mastitis milk samples

The present study was based on cross-sectional design investigation. A total of 852 (from 213 animals with clinical mastitis) and 840 (from 210 animals with subclinical mastitis) milk samples were collected from lactating Kundhi breed buffaloes from the surroundings of Hyderabad, Tandojam and Tando Allahyar areas. Prior to collection of milk samples, the udders of both, clinical and subclinical buffaloes were washed thoroughly with lukewarm water and the tips of the quarters were cleansed with antiseptic agent and finally dried with a towel. Individual milk sample (100ml) was collected and placed in chilled in sterile specimen container (completely wrapped/covered with aluminium foil) after discarding few initial milking streams. Each sample was labelled with

locality, buffalo number, quarter site, number of samples and other information related to the animals. Whereas the milk samples from apparently mastitis free buffaloes were collected aseptically and initial examination was conducted at the field level by testing through CMT to confirm the positivity of milk samples. The positive results were interpreted according to the technique adopted by Ikram (1997). For the purpose, the quarter milk samples (ml^{-1}) and CMT reagent were mixed in equal quantities in paddle cups separately for each quarter. The paddle cups contained milk and reagent was rotated for 10 sec and results were recorded. The change in milk consistency indicated the sub-clinical mastitis while no change in milk consistency categorized as healthy samples. The sub-clinical mastitis was graded into four categories based on the severity of the disease from lower to higher intensity as + = moderate/ traces (Mean SCC $1.5x10^4$-$5x10^5$), + + = severe (Mean SCC $4x10^5$–$15x10^5$), + + + = more severe (Mean SCC $8x10^5$–$50x10^5$) and + ++ + = very severe (Mean SCC > $50x10^5$). The CMT subclinical mastitis milk samples collected were placed in the icebox and transferred to the laboratory for bacteriological investigation. Before sampling, the whole udder of the buffaloes was washed thoroughly and then dried with a clean towel. The teats of the buffaloes were disinfected with swabs soaked in 70% alcohol. After discarding first few drops of milk, 8-10 ml milk samples were collected in sterile caped bottles and numbered and then transported to the Laboratory of the Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Laboratory examination of samples

All the samples collected from buffaloes showing clinical signs of mastitis were directly processed for isolation and identification of bacterial isolates. Similarly, the CMT positive samples were also subjected to bacteriological investigation to isolate pathogens. The samples of both natures, clinical and subclinical were streaked on nutrient, blood and MacConkeys agars. The plates were incubated under aerobic conditions at 37°C for 24 h. The isolated bacterial organisms were further processed and biochemical tests were carried out to confirm the species. For the purpose biochemical tests have been developed to identify at genus and species level. However, different types of biochemical tests were performed according to the nature of the target isolate. The biochemical tests executed to identify the bacterial isolates were catalase, oxidase, Simmons's citrate, urease, coagulase, aesculin, methyl red, vogues-proaskeur, triple sugar iron agar and gelatine liquefaction. The other tests those needed to be very important were also carried- out on the basis of the results of tests and nature of target organisms. The identification of the isolates was carried out on the bases of the colony, morphology, Gram-stained and biochemical properties as described by Waage *et al.* (1999, 2000, 2001).

Statistical analysis

All the data were analyzed using SPSS version 15 statistical package software. The statistical analysis used included comparison of proportions and chi-square test that was applied to test significant association existed between predisposing risk factors such as season, dry period, location at buffalo quarter levels with clinical and subclinical mastitis positivity. For all the analysis done, $P<0.05$ was taken as statistically significant regarding different parameters obtained by chi-square test (Gomez and Gomez, 1984). Furthermore, the mean percentage was also adopted during analysis of data.

RESULTS AND DISCUSSION

Physical characteristic changes caused by bacterial species

Of the 646 positive milk samples analysed for physical characteristic changes caused by bacterial species in clinical mastitis, 271(41.95%) were demonstrated with large and small flacks, 50 (7.73%) with watery consistency, 97 (15.01%) with pus, 65 (10.06%) with blood tinge, 140 (21.67%) had white colour but with bad odour while 23 (3.56%) samples were found with curd like consistency. Among the physical characteristic changes produced by bacterial species, a significant higher physical change (χ^2 values = 13.14, P-values 0.0221*) in milk samples were observed with flakes, however, a majority of the samples contained flakes of different sizes and some other changes were also noted in the mastitis milk samples (Table I).

Ali *et al.* (2021) observed and recorded in their investigations that bacterial species in clinical mastitis caused large and small flacks with watery consistency, pus, blood tinge, white colour with bad odour and curd like consistency. Significantly higher physical changes in milk samples are found with flakes, however, a majority of the samples contained flakes of various sizes and some other changes were also noted in the mastitis milk samples. Further physical changes in the milk samples were bad taste, odour, watery, mucus mixed with blood tinge, flakes, pus, white colour with odour acquired from buffaloes were suffering from clinical mastitis (Imran *et al.*, 2021). The findings observed about the physical characteristic variations produced through different bacterial isolates in udder secretions of animals are in agreement with conclusions made by the former scientists.

They recognized the same pathogenic species as noted in the current study. A number of deviations in the milk samples were likewise demonstrated with flakes, watery, pus, blood tinge and white colour with odour obtained from buffaloes affected by clinical mastitis (Mustafa *et al*., 2012; Baloch *et al*., 2011).

The incidence of bacterial species isolated from clinical mastitis milk samples of buffaloes

All bacterial species produced some physical changes in mastitis milk samples of buffaloes. *Staphylococcus aureus* produced smaller and some larger flacks in 112 (7.33%) milk samples. Moreover, it also changed milk into watery (17, 2.63%), milk with pus (18, 2.78%), milk with blood tinge (22, 3.40%), milk with unpleasant odour (44, 6.81%) and curd like milk (04, 0.61%). Whereas *Escherichia coli* and all other bacterial isolates produced similar changes as produced by *Staphylococcus aureus* in the mastitis milk samples of buffaloes (Table II).

The physical characteristic variations made by various bacterial species in the mastitis milk samples of buffaloes noted in this study. The workers observed that *Staphylococcus aureus* produced smaller and some larger flacks in milk samples. Moreover, it also changed milk into watery, milk with pus, milk with blood tinge, milk with unpleasant odour and curd like consistency. *Bacillus cereus* produced changes in milk samples with flakes and watery whereas *Escherichia coli* changed the physical properties of milk into flakes, watery, pus and white colour with odour (Ali *et al*., 2021). Likewise, *Micrococcus luteus* yielded physical characteristics deviation in mastitis milk samples were: flakes and white colour with odour. Similarly, *Streptococcus dysgalactiae* and *Streptococcus uberis* formed flakes and white colour with odour in mastitis milk samples. This kind of familiarity in findings might be due to that the hygienic and managemental facilities are being provided to the animals at farms or at backyard heights (Ali *et al*., 2021; Mustafa *et al*., 2012; Zaki *et al*., 2010).

Table I. The physical characteristic changes caused by bacterial species in mastitis milk samples of buffaloes.

Milk samples examined	Physical characteristics of milk with					
	Flacks	Watery consistency	Pus	Blood tinge	Odour	Curd like consistency
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
No. of samples examined 852	297 (34.85)	90 (10.56)	148 (17.37)	110 (12.91)	169 (19.83)	38 (4.46)
No. of positive samples 646	271 (41.95)	50 (07.73)	97 (15.01)	65 (10.06)	140 (21.67)	23 (3.56)

χ^2 values= 13.14; df = 5; P-values= 0.0221; **Chi- square difference was significant ($p < 0.05$).

Table II. The bacterial species isolated from different physical conditions of clinical mastitis milk samples obtained from buffaloes.

Bacterial organisms	Milk with flacks	Watery milk	Milk with pus	Milk with blood tinge	White with odor	Curd like appearance
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Staphylococcus aureus	112 (7.33)	17 (2.63)	18 (2.78)	22 (3.40)	44 (6.81)	04 (0.61)
Escherichia coli	49 (7.85)	14 (2.16)	20 (3.09)	09 (1.39)	19 (2.94)	07 (1.08)
Streptococcus dysgalactiae	13 (2.01)	02 (0.30)	26 (4.02)	08 (1.23)	25 (3.86)	00 (00)
Micrococcus lutus	57 (8.82)	02 (0.30)	00 (00)	01 (0.15)	08 (1.23)	02 (0.30)
Streptococcus agalactiae	06 (0.92)	07 (1.08)	18 (2.78)	06 (0.92)	19 (2.94)	06 (0.92)
Bacillus cereus	18 (2.78)	02 (0.30)	06 (0.92)	04 (0.61)	10 (1.54)	01 (0.15)
Staphylococcus epidermidis	10 (1.54)	01 (0.15)	01 (0.15)	09 (1.39)	03 (0.46)	12 (1.85)
Pseudomonas aeruginosa	02 (0.30)	04 (0.61)	01 (0.15)	03 (0.46)	02 (0.30)	00 (00)
Streptococcus uberis	03 (0.46)	00 (00)	00 (00)	01 (0.15)	07 (1.08)	01 (0.15)
Bacillus subtilis	01 (0.15)	01 (0.15)	04 (0.61)	02 (0.30)	03 (0.46)	00 (00)
Streptococcus pyogenes	00 (00)	00 (00)	03 (0.46)	00 (00)	00 (00)	00 (00)

The CMT and the status of infection in subclinical mastitis

The data regarding positive CMT and status of infection in quarters of buffaloes are presented in Table III. The severity level of subclinical mastitis in 840 quarters from all the sites was found with infection of diverse degree. Only 102 samples formed strong gel with CMT and recorded as most severe (4+) [SCC, 8, $1x10^5$] while 60 samples formed distinct gel with CMT and thus detected as more severe (3+) [SCC, 2, $7x10^5$]. Similarly, 72 samples in overall formed weak gel with CMT and were diagnosed as severe (2+) [SCC, $4x10^5$] whereas 359 quarters received an infection of moderate severe (1+) [SCC, $3x10^5$] for producing gel trace amount. As many as 247 milk samples however, produced no change with CMT and hence considered to be normal. Furthermore, from 102 strong positive samples, only 57 (17.27%) contained bacterial organisms.

Table III. The relation between positive CMT and the status of infection in quarters of subclinical mastitis of buffaloes.

CMT scores	No. of quarters examined	No. of bacterial isolates	%	Mean of SCC ml^{-1}
Most severe (4+)	102	57	17.27	$8,1x10^5$
More severe (3+)	60	29	08.78	$2,7x10^5$
Severe (2+)	72	42	12.72	$4x10^5$
Moderate severe (1+)	359	202	61.21	$3x10^5$
Negative (-)	247	0	0	$15x10^4$
Total	840	330	39.28	-

The severity level of subclinical mastitis in quarters from all the sites was found to have an infection of diverse degree. The samples formed strong gel with CMT and recorded as most severe intensity while samples formed distinct gel with CMT and thus detected as more severe, severe, moderate severe for producing gel trace amount (Baloch *et al*., 2016). However, a large number of milk samples however, produced no change with CMT and hence considered to be normal. Furthermore, strong positive samples contained bacterial organisms. The results of the CMT and the presence of bacterial isolates in subclinical mastitis samples were observed (Islam *et al*., 2011; Suwito *et al*., 2021). Through surf field mastitis test (SFMT), the severity of subclinical mastitis in buffaloes on the basis of gel formation was recorded as moderate (+), severe (2+), more severe (3+) and very severe (4+) throughout the study. Similar results were also noted as moderate (1+), severe (2+), more severe (3+) and very severe (4+) in subclinical mastitis of dairy cows in selected areas of Bangladesh further categorized into light gel, light persistent gel with crumbly filaments, thick viscous cluster and thick gel with the consistency of egg white (Islam *et al*, 2011). Milk samples obtained from 35(2.5%) quarters showed 3+ degree of gel formation while 45 (3.22%) samples showed 2+ degree of gel formation whereas 120 (8.57%) samples exhibited 1+ degree of gel formation. The rest (85.71%) of samples were detected as negative (Abdel-Radi and Sayed, 2009). The subclinical mastitis milk samples collected from buffaloes and recorded the degree of gel formation that varied between 30 and 75% which presented 3+ and 2+ degrees of gel formation, respectively (Attia *et al*., 2003; Raghavendran *et al*., 2020). As well as the somatic cell count (SCC) in subclinical mastitis of buffaloes with different degrees of udder infections concerned counted SCC > 200,000 cells ml^{-1} and suggested as an indicator of udder infection (Dhakal *et al*., 1992; Singh and Ludri, 2001; Moroni *et al*., 2006). However, European Union Directives set a limit of 400,000 cells ml^{-1} for SCC in buffaloes. The findings regarding the relationship among the positive CMT, subclinical status of the udder and somatic cell count in the milk samples of buffaloes are demonstrated.

The severity of subclinical mastitis caused by bacterial species determined by CMT

During present study, a total of 479 milk samples were found positive by CMT, however, 286, 50, 66 and 77 milk samples with different degree of severity of infection caused by bacterial species diagnosed as moderate-severe (1+); severe (2+), more-severe; (3+) and most-severe (4+), respectively (Table IV). Majority of the pathogens produced different intensity of gel formation in the milk. Among the bacterial species, *Staphylococcus aureus* was noted as the most dominant species that changed milk in moderate severe (78); severe (11); more-severe (13) and most-severe (32) gel formation in majority of the samples collected and tested by CMT while the second most pathogenic species observed in this survey was the *Escherichia coli*, it produced similar changes in small number of samples as compared to *Staphylococcus aureus* (Table IV).

Similar study was conducted by (Guha *et al*., 2013) they stated that milk samples were found positive by CMT, with different degree of severity of infection caused by bacterial species recognized as moderate severe (1+); severe (2+), more severe; (3+) and most severe (4+). Majority of the pathogens produced different intensity of gel formation in the milk. Among the bacterial species, *Staphylococcus aureus* was noted as the most dominant species that changed milk in moderated severe; severe; more severe and most severe gel formation in majority of

the samples collected and tested by CMT while the second most pathogenic species observed was the *Escherichia coli*, that produced similar changes in small number of samples as compared to *Staphylococcus aureus* to make comparison between CMT scores with bacterial cultural growth. *Staphylococcus* species produced trace, weak, distinct positive and strong gel formation; whereas *Streptococcus* and *Escherichia coli* made similar changes in milk samples obtained from subclinical mastitis in India (Guha *et al*., 2013). Further stated that physical changes in mastitis milk samples such as traces (1+), weak (2+) and distinct (3+) gel formation produced by *Bacillus* species while *Escherichia coli* did not show any kind of gel formations in milk samples (Ali *et al*., 2021).

Table IV. The distribution of bacterial pathogens in subclinical mastitis milk samples of buffaloes and CMT status of the quarters.

Bacterial species	CMT status					% of samples
	1+	2+	3+	4+	Total	
Staphylococcus aureus	78	11	13	32	134	27.97
Escherichia coli	59	09	10	08	86	17.95
Streptococcus agalactiae	29	10	12	15	66	10.43
Streptococcus dysgalactiae	28	05	09	08	50	07.93
Bacillus cereus	38	03	09	00	50	13.77
Streptococcus uberis	17	05	10	12	44	10.43
Micrococcus lutus	30	07	01	00	38	02.29
Pseudomonas aeruginosa	07	00	02	02	11	09.18
Total						100

Table V. The mean bacterial population (bacterial load) and somatic cell count in clinical and subclinical mastitis milk samples of buffaloes.

Forms of mastitis	No. of positive samples	Mean SCC ml^{-1}	Mean CFU ml^{-1}
Clinical	484	82 x 10^5	93 x 10^5
Subclinical	330	50 x 10^5	51 x 10^5

The relation between bacterial population and somatic cell count

The correlation between SCC/ml^{-1} and CFU ml^{-1} of bacterial population between clinical and subclinical mastitis in buffaloes was investigated. In this regard, milk samples were obtained from clinical mastitis buffaloes and the mean value of SCC was counted as 82x10^5 cells ml^{-1} whereas the buffalo with subclinical mastitis the mean values of SCC was determined as 50x10^5 cells ml^{-1} (Table V). Meanwhile the milk samples from the both groups, clinical and subclinical were cultured to determine correlation of TBC with value of SCC in terms of severity between clinical and subclinical mastitis groups of buffaloes. In this contest the milk samples of both cases, the clinical and subclinical mastitis were cultured on the medium. The mean values of 93x10^5cfu ml^{-1} were counted for clinical mastitis whereas 51x10^5 cfu ml^{-1} was counted for subclinical mastitis. However, a clear difference between SCC and TBC was observed between two groups of buffaloes.

Mwanza *et al*. (2013) determined the mean bacterial population in the transfer line milk samples collected from different locations and cultured on the medium, the mean was counted as 483.3 cfu ml^{-1} at 1st day of milk collection while on 5th day of the collection, the mean was recorded as 1150 cfu ml^{-1}. On the other hand, the same was conducted against the milk samples obtained from bulk tank unit; the bacterial contamination was counted as 483 cfu ml^{-1} on 1st day whereas on the 2nd day, it increased to 883.3 cfu ml^{-1} and then dropped to 316.7cfu ml^{-1} on the 5th day. A study was conducted to demonstrate the correlation between SCC ml^{-1} and CFU ml^{-1} of bacterial population among three groups of animals, the healthy, subclinical and clinical mastitis in cows. The mean value of SCC in healthy was counted as 130x10^3 cells ml^{-1} while cows with subclinical mastitis, the SCC was measured as 1,073x10^3 cells ml^{-1} whereas the cows with clinical mastitis, the SCC was determined as 4, 350x10^3 cells ml^{-1}. Further, that when the milk samples of three groups were cultured on the medium to obtain the correlation between TBC and SCC among three groups of cows, the mean values of 90x10^3cfu ml^{-1}, 1,077x10^3 cfu $ml^{-1}$and 5,783x10^3cfu ml^{-1} were counted for healthy, subclinical and clinical mastitis cows, respectively. However, a significant difference between SCC and TBC was observed among three groups of cows (Mwanza *et al*., 2013; Moura *et al*., 2017).

CONCLUSIONS

From the present study, it is concluded clearly that bacterial species are responsible for different physical characteristic changes in milk. However, during investigation, 271(41.95%) samples were detected with large and small flacks, 50 (7.73%) with watery consistency, 97 (15.01%) with pus, 65 (10.06%) with blood tinge, 140 (21.67%) had white colour but with bad smell while 23 (3.56%) samples were found with curd like consistency. Significant physical changes (χ^2 values 13.14, P-values 0.0221*) in milk was observed with flacks in clinical mastitis. The pathogenic bacterial species, *Staphylococcus aureus* was recorded as the most dominant species caused moderate-severe (78); severe (11); more-severe (13) and most-severe (32) degree of intensive gel formation in

subclinical mastitis in majority of the samples collected and tested by CMT, respectively. Milk samples from clinical mastitis analysed, the mean of SCC was counted as $82x10^5$cells ml^{-1} whereas the buffaloes with subclinical mastitis, the mean of SCC was determined as $50x10^5$ cells ml^{-1}. The mean values of $93x10^5$cfu ml^{-1} were counted for clinical mastitis while $51x10^{-5}$ cfu ml^{-1} was measured for subclinical mastitis. However, a clear difference between SCC and TBC was observed between two groups of buffalo.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks the help of Dr. Aslam Pervez Umerani, Director, Central Veterinary Diagnostic Laboratory Tandojam, Sindh, Pakistan for providing funds and assistance ship, which enabled us to complete this project.

Funding

The research was funded by the Central Veterinary Diagnostic Laboratory and Sindh Agriculture University, Tanojam.

IRB approval

The research project was approved by the Directorate of Advanced Studies, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

Ethical statement

The research project was approved by Research Ethics Committee of Department Veterinary Microbiology and Directorate of Advanced Studies, Sindh Agriculture university, Tandojam (Letter # No. DAS/2689/of 2013 31/10/2013).

Statement of conflict of interest

The authors have declared no conflict of interest.

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