The Research of Medical Science Review
ISSN: 3007-1208 & 3007-1216 Volume 3, Issue 7, 2025

STUDY ON PREVALENCE AND RISK FACTORS ASSOCIATED WITH SUB-CLINICAL MASTITIS IN KUNDHI BUFFALOES

Paio Khan[*1], Aijaz Ul Haq[2], Raza Ali Mangi[3], Reema Bughio[4], Faisal Khan[2], Mujeeb Ur Rehman[2], Riaz Ali Mangrio[2], Atiq Ur Rehman[2], Muhammad Ishaq[5], Habib Ur Rehman[2]

*[*1]Department of Epidemiology and Pubic Health, UVAS, Lahore*
[2]Livestock and Dairy Development Department, Quetta, Balochistan
[3]Department of Veterinary Anatomy and Histology, SBBUVAS, Sakrand
[4]Department of Animal Breeding and Genetics, SBBUVAS, Sakrand
[5]Department of Poultry Husbandry, SAU, Tandojam

[*1]payodummar@gmail.com

DOI: https://doi.org/10.5281/zenodo.16403632

Keywords
Buffalo, Sub-clinical, Mastitis, Prevalence, Risk factors

Article History
Received: 19 April, 2025
Accepted: 23 June, 2025
Published: 22 July, 2025

Corresponding Author: *
Paio Khan

Abstract

This study was conducted to investigate the prevalence, severity, and associated risk factors of sub-clinical mastitis (SCM) in Kundhi buffaloes across three districts of Sindh, Pakistan: Sanghar, Mirpurkhas, and Dadu. A total of 210 buffaloes and 840 udder quarters were examined using the California Mastitis Test (CMT). The overall prevalence of SCM was 54.71% at the animal level and 39.37% at the quarter level. District-wise, Sanghar showed the highest prevalence (61.62%), followed by Mirpurkhas (50.13%) and Dadu (49.75%). Quarter-wise analysis revealed that the front right (11.79%) and hind right (10.56%) quarters were more frequently affected than the front and hind left quarters. Age and parity were significant risk factors. Buffaloes aged 6–9 years showed the highest prevalence (57.15%), and those with 4–7 parities had a prevalence of 56.34%, indicating increased susceptibility with age and lactation cycles. The CMT-based grading of mastitis severity revealed a high proportion of moderate to strong positive reactions. Among all quarters, 63.61% tested strong positive, indicating chronic or advanced infections, particularly in Sanghar and Mirpurkhas. These findings highlight the endemic nature of sub-clinical mastitis in Kundhi buffaloes and underscore the need for routine diagnostic screening, improved farm hygiene, and targeted awareness programs. Identifying risk factors such as age, parity, and quarter susceptibility can aid in the development of effective mastitis control strategies, ultimately improving milk yield and animal welfare in the region.

INTRODUCTION

Mastitis is currently recognized as the foremost disease of dairy animals and posing unrestrained economical risk to the farmers of the world (Bhatt et al., 2012). The disease causes tenderness in the mammary glands and producing physical, chemical and bacteriological changes in milk and pathological changes in the glandular tissues (Shakoor, 2004). The ailment left unnoticed leads to deterioration of animal health till culling or death of the animals. Mastitis is fundamentally classified into clinical and

subclinical forms. The clinical mastitis causes udder inflammation with visible signs of redness, swelling, pain, reduction in milk production and can be perceived easily without the help of laboratory tests, whereas in case of subclinical mastitis, the udder appears normal and does not show symptoms of infection but requires laboratory tests for its diagnosis (Kumbhar et al., 2018 and Moroni *et al.*, 2006).

The subclinical form of mastitis has been reported by many workers which range from 60-70% of the total mastitic losses in advanced countries. The losses may even mount higher in Pakistan due to executing least virtuous management and preventive measures (Hoque *et al.*, 2015). Literature shows that quarter basis prevalence of intra-mammary infection (IMI) in buffaloes is about 66% and the incidence is getting higher during 30 days of parturition (Akhtar et al., 2012). The identification of mastitis has great consequence on account of growing public awareness about food safety, quality and as well as for animal welfare. Subclinical mastitis is commonly renowned after laboratory examination of the milk as there is no gross swelling of quarters or apparent deviance in the milk (Hameed *et al.*, 2012). Major bacterial agents responsible for subclinical mastitis in buffaloes and other animal species are *Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli and Streptococci* species. All such pathogens exist in animal environments including water, feed, bedding, manure and soil. Numerous other pathogens reported from infected mammary glands worldwide are *Actinomyces pyrogenes, Clostridium perfringenes, Pseudomonas aeroginosa, Klebsiella pneumonia and Pasteurella haemolytica* etc. (Javed *et al.*, 2013). Subclinical mastitis a potential risk to dairy health, the present investigation was undertaken to explore the current status of subclinical mastitis by observing into the prevalence of subclinical mastitis along with severity of the disease in Kundhi breed kept at different locations around Hyderabad of Sindh province. Further, it was aimed to demonstrate the risk factors associated with udder infections in buffaloes.

MATERIAL AND METHODS

A study was conducted on 210 buffaloes of Kundhi breed during year 2010-2012. The animals were raised at Sanghar (n=68), Mirpurkhas (n=71) and Dadu (n=71) locations of Sindh Province (Pakistan). Buffaloes of three locations were further segregated based on age (3-5, 6-9 and >10 years), parity (1-3, 4-7 and >7 calves) and number of lactations (1-9). Milk was collected from lactating buffaloes having no inflammatory signs in their glandular tissue. About 10ml milk from each quarter of the animal was collected aseptically into a sterile container after rejecting few initial milk drops. Each sample was labelled with locality, buffalo number and quarter side in addition to other information like age, parity and lactation stage.

Milk samples taken from 840 quarters were subjected to CMT for diagnosis of subclinical mastitis and severity level. Changes in the viscosity of the milk indicated subclinical mastitis positive, and the samples given partly, distinct, thick or strong gel were regarded as ' Moderate: 1+', 'Severe': 2+, 'More Severe': 3+ or 'Most Severe': 4+ level (Muhammad *et al.*, 2015). Identification of the isolates was carried out based on colony, morphology, Gram-stained and biochemical properties as described by Waage et al. (1999, 2001).

STATISTICAL ANALYSIS

Data were analysed with SPSS for windows 17.0. The prevalence of subclinical mastitis was expressed as percentage (%) on site, animal and quarter levels. The means (±SD) were compared by t-test and P

RESULT

Prevalence and Risk Factors Associated with Sub-Clinical Mastitis in Kundhi Buffaloes

The prevalence of sub-clinical mastitis (SCM) in Kundhi buffaloes was assessed across three districts of Sindh: Sanghar, Mirpurkhas, and Dadu (Table 1). A total of 210 buffaloes and 840 udder quarters were examined during the study. In Sanghar, out of 72 buffaloes examined, 45 were found positive for SCM, yielding an infection rate of **61.62 ± 0.892%,** which was the highest among the three districts. A total of 286 quarters were screened in Sanghar, of which 136 were positive, corresponding to a **48.65 ± 0.359%** quarter-level prevalence. In Mirpurkhas, 70 buffaloes were examined and 34 (50.13 ± 0.31%) were found to be affected. Out of 286 quarters examined in this district, 104 were

infected, giving a quarter-level infection rate of **35.21 ± 0.4113**%. Dadu district showed a buffalo-level prevalence **of 49.75 ± 1.73**%, with 35 out of 68 animals testing positive for SCM. From 268 udder quarters examined, 90 were positive, resulting in a **34.97 ± 0.534**% infection rate. Overall, the total prevalence of sub-clinical mastitis in Kundhi buffaloes across the studied districts was **54.71**% at the animal level and **39.37**% at the quarter level. The results indicate a high burden of sub-clinical mastitis, with Sanghar showing the highest rates at both the buffalo and quarter levels, suggesting potential district-wise variation in risk factors such as hygiene practices, milking procedures, and awareness among farmers.

Table-1 Prevalence and Risk Factors Associated with Sub-Clinical Mastitisin Kundhi Buffaloes

Location	Buffaloes			Quarters		
	Examined	Positive	Infected%	Examined	Positive	Infected%
Sanghar	72	45	61.62±0.892*	286	136	48.65±0.359
Mirpurkhas	70	34	50.13±0.31	286	104	35.21±0.4113
Dadu	68	35	49.75±1.73	268	90	34.97±0.534
Total	210	114	54.71	840	330	39.37

Quarter-wise Prevalence of Sub-Clinical Mastitis in Kundhi Buffaloes

The distribution of sub-clinical mastitis (SCM) in individual udder quarters of Kundhi buffaloes was evaluated across a total of 840 quarters (Table 2). Out of these, 330 quarters were found to be positive for SCM, resulting in an overall quarter-level prevalence of 39.37%. Among the individual quarters, the highest prevalence was recorded in the front quarters, with 97 (11.79 ± 0.07611%) being positive. The hind right quarters followed, with 89 (10.56 ± 0.095%) showing signs of infection. The front left quarters had a prevalence of 8.23 ± 0.075%, with 74 quarters affected, while the hind left quarters showed the lowest prevalence, with 71 (8.31 ± 0.085%) positive cases. The findings suggest that the front quarters, particularly the right side, are more susceptible to sub-clinical infections compared to the hind quarters. These differences may be attributed to anatomical exposure, milking practices, or variations in teat sphincter strength and hygiene. Statistically significant differences ($p<0.05$) were observed in the prevalence among some quarters, notably the front and hind left quarters. These results emphasize the importance of quarter-level monitoring in SCM control strategies and suggest that targeted intervention in specific udder quarters could help reduce the disease burden in affected herds.

Table 2: Prevalence of Sub-Clinical Mastitis in Buffaloes (Quarters=840) in Terms of Individual Quarters

Quarters	Positive	Prevalence (%)
Front	97	11.79±0.07611*
Front left	74	8.23±0.075
Hind right	89	10.56±0.095
Hind left	71	8.31±0.085*
Total	330	39.37%

Risk Factors Associated with Sub-Clinical Mastitis in Relation to Age and Parity in Kundhi Buffaloes

The influence of age and parity on the prevalence of sub-clinical mastitis (SCM) in Kundhi buffaloes was assessed and is presented in Table 3. A total of 210 buffaloes were categorized based on age and number of parities to evaluate these as potential risk factors.

Age-wise Prevalence

The age group 6–9 years exhibited the highest prevalence of SCM, with 48 out of 90 buffaloes

testing positive (57.15 ± 0.691%, $p<0.05$). Similarly, buffaloes aged more than 10 years showed a prevalence of 55.3 ± 1.21% (30/53), also statistically significant. The 3–5 years age group had the lowest prevalence, with 36 of 67 animals affected (49.00 ± 1.045%), suggesting that the risk of sub-clinical mastitis increases with age.

Parity-wise Prevalence

A similar trend was observed with parity. Buffaloes in their 4th to 7th parity had the highest infection rate, with 49 of 91 animals affected (56.34 ± 0.969%, $p<0.05$). Buffaloes with more than 8 parities showed a prevalence of 54.20 ± 1.343% (27/49), whereas those in their first to third parity had a lower prevalence of 51.11 ± 1.075% (38/70). These findings indicate that age and parity are significant risk factors associated with sub-clinical mastitis in Kundhi buffaloes. Increased age and repeated lactations may lead to a decline in udder immunity and tissue integrity, making the animals more susceptible to intramammary infections.

Table 3: The Risk Factors Associated with Subclinical Mastitis in Different Age Groups and Number of Parity in Buffaloes

Parameters	Total No. of buffaloes	Positive	
		No.	%
Age years			
3-5	67	36	49.00±1.045
6-9	90	48	57.15±0.691*
>10	53	30	55.3±1.21*
Parity			
1-3	70	38	51.11±1.075
4-7	91	49	56.34±0.969*
>8	49	27	54.20±1.343

Degree of Sub-Clinical Mastitis Based on California Mastitis Test (CMT) Severity in Buffaloes from Different Locations

The California Mastitis Test (CMT) was used to assess the severity of sub-clinical mastitis (SCM) in buffaloes across three districts: Sanghar, Mirpurkhas, and Dadu. A total of 840 udder quarters were tested and categorized based on CMT reaction intensity, ranging from Negative, Trace, Weak Positive (+1), Moderate Positive (+2), to Strong Positive (+3) (Table 4). In Sanghar, no quarters tested negative, while 5 (1.74 ± 0.1%) were trace positive. A total of 15 quarters (5.13 ± 0.7%) showed weak positive reactions, 72 (25.1 ± 0.3%) were moderately positive, and a substantial number of 181 quarters (65.75 ± 0.3%) showed strong positive reactions. The high proportion of strong positives in Sanghar was statistically significant ($p<0.001$), indicating more severe infections in this district. In Mirpurkhas, 2 quarters (0.71 ± 0.5%) were negative, and 6 (2.17 ± 0.1%*) were trace positive. Weak positive reactions were found in 19 quarters (6.79 ± 0.5%), while 78 quarters (26.17 ± 0.9%*) were moderate positive and 179 (61.03 ± 1.1%) showed strong positive reactions. The distribution in Mirpurkhas also indicated a predominance of moderate to severe cases, with several severity levels being statistically significant ($p<0.05$ to $p<0.001$). In Dadu, 3 quarters (1.03 ± 0.1%) were negative and 8 (2.91 ± 0.5%) were trace positive. Weak positive cases totaled 30 (10.76 ± 0.7%), moderate positives were 94 (37.1 ± 0.3%), and 149 quarters (51.57 ± 0.1%) were strong positive. Compared to the other districts, Dadu showed relatively higher rates of weak and moderate positives and a slightly lower proportion of strong positives. Overall, of the 840 quarters tested, 510 (63.61%) were strongly positive for SCM, 243 (27.97%) were moderate, 63 (7.6%) weak, 19 (2.31%) trace, and only 5 (0.63%) were negative. These results reflect a high burden and severity of sub-clinical mastitis, particularly in Sanghar and Mirpurkhas, underlining the need for urgent intervention and improved udder health management in the affected herds.

Table 4: California Mastitis Test (CMT) Based Degree of Severity of Subclinical Mastitis in Buffaloes of various locations

Sanghar	272	Nil	-	5	1.74±0.1	15	5.13±0.7	72	25.1±0.3	181	65.75±0.3***
Mirpurkhas	282	2	0.71±0.5	6	2.17±0.1*	`9	6.79±0.5***	78	26.17±0.9***	179	61.03±1.1**
Dadu	284	3	1.03±0.1**	8	2.91±0.5**	30	10.76±0.7***	94	37.1±0.3***	149	51.57±0.1
Overall	840	5	0.63	19	2.31	63	7.6	243	27.97	510	63.61

Discussion

The present study investigated the prevalence and associated risk factors of sub-clinical mastitis (SCM) in Kundhi buffaloes across three major districts of Sindh Sanghar, Mirpurkhas, and Dadu using clinical examination and the California Mastitis Test (CMT). The findings revealed a notably high overall prevalence of SCM (54.71%) at the animal level and 39.37% at the quarter level, indicating a widespread occurrence of intramammary infections among apparently healthy buffaloes. District-wise analysis showed that Sanghar had the highest prevalence (61.62%), followed by Mirpurkhas (50.13%) and Dadu (49.75%), suggesting possible geographical or management-related variations. These findings are consistent with previous studies (Getahun *et al.*, 2008; Sharma *et al.*, 2007) reporting high SCM prevalence in buffaloes under poor hygienic conditions and traditional farming practices in rural areas of Pakistan. The quarter-wise analysis revealed that front quarters, especially the front right (11.79%) and hind right (10.56%), were more frequently affected. This pattern may be associated with anatomical exposure or preferential milking practices, which increase susceptibility to infections in specific quarters. Similar observations were reported by Patel *et al.* (2015), who emphasized the uneven distribution of mastitis across quarters in dairy buffaloes. Age and parity were also identified as significant risk factors. Buffaloes aged 6–9 years (57.15%) and those with 4–7 parities (56.34%) had the highest prevalence. Increased susceptibility in older and multiparous animals may be due to repeated exposure to pathogens, reduced teat sphincter strength, and compromised immune responses (Zeryehun & Abera, 2017). Younger animals and those in early parities showed lower infection rates, supporting the hypothesis that risk accumulates with age and lactation cycles. The CMT-based assessment of mastitis severity revealed a concerning trend toward higher-grade infections, with 63.61% of the affected quarters testing strong positive, and only 0.63% showing negative results. Sanghar district had the highest proportion of strong positive cases (65.75%), indicating not just high prevalence but also more severe and chronic forms of infection. This may reflect prolonged neglect of udder health, delayed treatment, or ineffective mastitis control programs. The predominance of moderate to strong positive reactions aligns with findings by Iqbal *et al.* (2016), highlighting the chronicity of sub-clinical mastitis in indigenous breeds. Overall, these findings stress the need for routine screening using CMT, targeted awareness campaigns, and improved husbandry and hygiene practices to mitigate the impact of sub-clinical mastitis in Kundhi buffaloes. Strategic control measures tailored to age, parity, and quarter-specific susceptibility could significantly reduce the disease burden and improve dairy productivity in Sindh.

CONCLUSION

Subclinical mastitis was found to be more prevalent in buffaloes at the Sanghar location, with higher rates in animals at the 4th lactation and 4-7 parity. Older buffaloes (above 6 years) were more susceptible to the disease compared to younger ones (3-5 years).

REFERENCES

Arshad, M., Mahmood, F., Akhtar, M., & Hussain, R. (2018). Epidemiological studies on subclinical mastitis in buffaloes and antimicrobial resistance of the pathogens in Southern Punjab, Pakistan. *Pakistan Veterinary Journal*, **38**(3), 303–308. https://doi.org/10.29261/pakvetj/2018.048.

Akhtar A, Habibullah S, Ameer M, Hidayatullah S, Arshad M. 2012. Prevalence of subclinical mastitis in buffaloes in

district D.I Khan. Pak J Sci; 64: 132-8.

Bhatt VD, Ahir, VB, Koringa PG. 2012. Milk microbiome signatures of subclinical mastitis-affected cattle analyzed by shot gun sequencing. J Appl Microbiol. 112:4 639-50.

Getahun KB, Kelay M, Bekana, Lobago F. 2008. Bovine mastitis and antibiotic resistance patterns in Selalle small holder dairy farms, central Ethiopia. Trop An Heal Prod; 40: 12-17.

Hoque, MN, Das ZC, Talukder AK, Alam MS, Rahman AN. 2015 Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh. Trop Anim Health Pro; 47: 79-86. http://dx.doi.org/10.1007/s11250-014-0688-0

Iqbal, M., Khan, M. Z., Avais, M., Khan, A., & Khan, J. A. (2016). Evaluation of severity of subclinical mastitis and its association with milk quality and yield in Nili-Ravi buffaloes. *Journal of Animal and Plant Sciences*, **26**(5), 1231–1236.

Hameed S, Arshad M, Ashraf M, Avais M, Shahid MA. 2012. Cross sectional epidemiological studies on mastitis in cattle and buffaloes of Tehsil Burewala, Pakistan. J Anim Plant Sci; 22: 375-380.

Javed M, Kashif J, Yaqoob M, Liping W, Yang Y, Hongjie F. 2013. Molecular characterization and antimicrobial sensitivity of pathogens from sub-clinical and clinical mastitis in eastern China. Pak Vet J 2013; 33: 170-4.

Khan, M. S., Khan, M. A., & Riaz, M. (2019). Prevalence and associated risk factors of sub-clinical mastitis in dairy buffaloes. *Buffalo Bulletin*, **38**(3), 451–457.

Kumbhar, M., Kandhro, S., Rind, R., Kaleri, R.R., Chandio, I.S., Farooq, T., Rehman, A., Junejo, A., Qureshi, F.N. and Kumar, M., 2018. Prevalence and Incidence of Staphylococcus aureus from Wound of Different Animal Species. *Journal of Basic & Applied Sciences*, *14*, pp.12-16.

Muhammad G, Athar M, Shakoor A, Khan MZ, Fazal-ur Rehman, Ahmed MI. Surf field mastitis test: An expensive new tool for evaluation of wholesomeness of fresh milk. Pak J Food Sci 1995; 5: 91-3.

Moroni P, Rossi CS, Pisoni G, Bronzo V, Castiglioni B, Boettcher PJ. 2006. Relationship between somatic cell count and intramammary infection in buffaloes. J Dairy Sci 2006; 89: 998-1003.

Patel, Y. G., and M. M. Trivedi. "Quarter-wise prevalence of subclinical mastitis in crossbred cows." *Trends Biosci* 8.17 (2015): 4727-4729.

Shakoor A. 2004. Preparation and evaluation of staphylococcus aureus vaccines for the control of mastitis in dairy buffaloes (*Bubalis bubalis*). 2004; PhD Thesis, Univ. Agri. Faisalabad, Pakistan.

Sharma N, Maiti, SK, Sharmaand K, Kant K. Prevalence, aetiology and antibiogram of microorganisms associated with sub-clinical mastitis in buffaloes in Chhattisgarh state of India. Int J Dairy Sci 2007; 2: 145-51. 261-8.

Sharma, N., Singh, N. K., & Bhadwal, M. S. (2020). Relationship of somatic cell count and mastitis: An overview. *Asian-Australasian Journal of Animal Sciences*, **33**(4), 634–646. https://doi.org/10.5713/ajas.19.0493

Zeryehun, T., & Abera, G. (2017). Prevalence of subclinical mastitis in dairy cows and associated risk factors in and around Sebeta Town, Central Ethiopia. *International Journal of Livestock Research*, **7**(2), 1–10. https://doi.org/10.5455/ijlr.20170221032613