

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

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Abstract: The present study aimed to elucidate the magnitude and distribution of subclinical mastitis and associated risk factors in 'Kundhi' buffaloes of Hyderabad, Tandojam and Tando Allahyar locations of the province of Sindh, Pakistan. Milk samples (n=840) collected from 210 buffaloes were evaluated for the presence of subclinical mastitis in buffaloes using California Mastitis Test (CMT) and severity; moderate, severe, more severe, most severe. The positive samples were further processed for isolation and identification of bacterial pathogens. The overall intensity of subclinical mastitis at animal level was recorded as 54.29% while significantly higher prevalence ($P < 0.05$) of subclinical mastitis was observed in buffaloes of Tandojam ($60.56 \pm 0.996\%$) location. The prevalence of the subclinical at quarter-wise noted as 39.29% given maximum frequency ($11.31 \pm 0.085\%$) with hind left quarters. The age and parity were found potential risk factors in Kundhi buffaloes, and susceptibility to subclinical mastitis was higher beyond the age of 6-9 years and 4-7 calving. The higher prevalence as well as the intensity of the disease was determined in buffaloes located at Tandojam than Hyderabad and Tando Allahyar. The ailment was higher in animals at 4th lactation likewise crossing 6 years of age and 4-7 parity. The old age animals were more prone to subclinical mastitis than the younger animals. Overall, the prevalence of the subclinical mastitis at animal and quarter levels was observed in buffaloes.

Keywords: Kundhi buffalo, sub-clinical, mastitis, prevalence, risk factors.

INTRODUCTION

Mastitis is currently recognized as the foremost disease of dairy animals and posing unrestrained economical risk to the farmers of the world [1]. The disease causes tenderness in the mammary glands and producing physical, chemical and bacteriological changes in milk and pathological changes in the glandular tissues [2, 3]. 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.

The subclinical form of mastitis has been reported by many workers which range from 60-70% of the total mastitic losses in advanced countries [4]. The losses may even mount higher in Pakistan due to executing least virtuous management and preventive measures [5]. 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 [6]. 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 [7].

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

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worldwide are *Actinomyces pyrogenes*, *Clostridium perfringenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pasteurella haemolytica* etc. [8, 9].

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.

MATERIALS AND METHODS

A study was conducted on 210 buffaloes of Kundhi breed during year 2010-2012. The animals were raised at Hyderabad (n=68), Tando Allahyar (n=71) and Tandojam (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 [10]. Identification of the isolates was carried out based on colony, morphology, Gram-stained and biochemical properties as described by Waage *et al.* (1999, 2001) [11, 12].

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 (\pm SD) were compared by t-test and $P < 0.05$ was considered as significance level.

RESULTS AND DISCUSSION

Prevalence of Subclinical Mastitis in Buffaloes

During present study, at buffalo and quarter level prevalence of subclinical mastitis in Kundhi breed of buffaloes was investigated and the results are summarized in Table 1. Out of the 210 animals examined from all the three localities through California Mastitis Test, 114 animals were found positive with subclinical mastitis and the overall prevalence was recorded as 54.29%. Of the 840 apparently healthy quarters examined, 330 quarters were found positive and the overall prevalence was recorded as 39.29%. The prevalence of subclinical mastitis at buffalo level determined in the present investigation are within the range (51.6 to 62%) demonstrated by previous studies [13-16]. At animal level, the highest prevalence of subclinical mastitis ($60.56 \pm 0.996\%$, 43/71) was recorded in buffaloes located at Tandojam (Table 1). Significantly lower prevalence of subclinical mastitis was noted in the animals raised at Hyderabad ($51.47 \pm 1.04\%$, 35/68) and Tando Allahyar ($50.70 \pm 0.996\%$, 36/71) sites. The frequency of the infection at the quarter-level was found again highest ($47.54 \pm 0.257\%$, 135/284) at Tandojam locality and intensity of the infection was much lower in animals located at Hyderabad ($33.09 \pm 0.26\%$, 90/272) and Tando Allahyar ($36.97 \pm 0.247\%$, 105/284) locations respectively. A previous study reported 77.98 and 58.75% prevalence of subclinical mastitis at animal and quarter-levels in buffaloes respectively from Attock district of the Punjab, Pakistan [17]. A relatively lower

Table 1: The Prevalence of Subclinical Mastitis in Buffaloes at Different Locations

Locations	Buffaloes			Quarters		
	Examined	Positive	Infected (%)	Examined	Positive	Infected (%)
Hyderabad	68	35	51.47 \pm 1.04	272	90	33.09 \pm 0.26
Tando Allahyar	71	36	50.70 \pm 0.996	284	105	36.97 \pm 0.247
Tandojam	71	43	60.56 \pm 0.996*	284	135	47.54 \pm 0.257*
Total	210	114	54.29	840	330	39.29

*Values differ significantly using t-test ($p < 0.05$).

prevalence as 64 and 30.5% at animal and quarter levels respectively had also been reported by Muhammad *et al.* (1995) [10]. The difference in prevalence of subclinical mastitis observed in the present and reported by the above authors may be due to differences in management practices, methods of detection, breeds of the animals, immune response of animals and climatic conditions.

During present study, at quarter level, the prevalence of subclinical mastitis in Kundhi buffaloes was investigated and the results are summarized in Table 2. Of the 840 apparently healthy quarters examined, 330 quarters were found positive. The quarters of the hind sides were found relatively more affected (10.36 and 11.31%) than those of the front sides (8.69 and 8.93%). A significant difference in the prevalence of the mastitis between front and hind quarters ($p < 0.05$) was noted. Similar findings were also reported by Khan and Muhammad (2005) [18], who recorded 27% (54/200) prevalence of subclinical mastitis at quarter level in buffaloes, and that was comprised of 8 (14.8%) right fore, 16 (29.6%) right hind, 10 (18.5%) left fore and 20 (37%) left hind quarters. The higher prevalence of subclinical mastitis in hind quarters of buffaloes than cross bred and local cows. They further noted that between hind quarters, the right hind quarters were found more susceptible than the left hind ones. On the other hand, Javaid *et al.* (2013) investigated at quarter level prevalence of subclinical mastitis in cows; a higher prevalence was recorded in the left rear quarters (26.7%) [15]. Joshi and Gokhle (2006) recorded a higher prevalence of subclinical mastitis in hind quarters (56.52%) as compared to four quarters (43.47%). They also suggested that clean and dry farm premises ensure the risk free environment for animal health [19].

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

Quarters	Positive	Prevalence (%)
Front right	73	8.69±0.085
Front left	75	8.93±0.085
Hind right	87	10.36±0.085*
Hind left	95	11.31±0.085*
Total	330	39.29%

*Values differ significantly using t-test ($p < 0.05$).

Lactation

The prevalence of subclinical mastitis in buffaloes at different number of lactations was analysed based on

three stages of lactations i.e. early (1-4), mid (5-8) and late (>8). During 1st two lactations, 17 out of 43 buffaloes (39.54%) were found infected by subclinical mastitis. The mastitis level, soon after 2nd lactation started increasing rapidly approaching to a maximum of 70.83% (17/24) while on 4th lactation, significant rise in the prevalence (31.29%) was demonstrated (Figure 1). However, the intensity of the disease showed a gradual decline in further increment in the number of lactation, the trend continued till end of the lactation (9/24, 37.5%). The buffaloes gradually received udder bulging with high anxiety after 2nd to 4th lactation and then started recovering up. It was reported that buffaloes in the first stage of lactation (1-4 months) and the last part of dry period (7-9 months) were found more prone to mastitis [20].

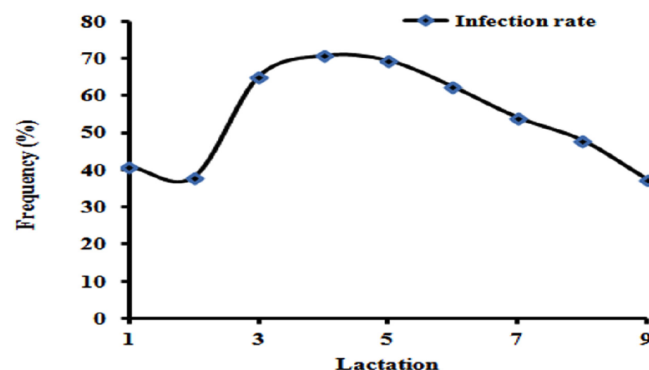


Figure 1: Lactation-wise prevalence of subclinical mastitis in buffaloes.

Risk Factors

Buffaloes with 3 to 5 years of age had $50.00 \pm 1.043\%$ (34/68) subclinical mastitis while animals with 6 to 9 years of age or above had $56.18 \pm 0.792\%$ (50/89) to $56.6 \pm 1.33\%$ (30/53) subclinical mastitis (Table 3). It was observed that animals above 6 years of age were more prone to subclinical mastitis than the younger animals (3-5 years). The effect of the number of parity on the prevalence of subclinical mastitis in buffaloes was also studied. Buffaloes with 1-3 calves had $52.17 \pm 1.025\%$ (36/69) mastitis, whereas animals with higher number of calves (4-7) had $55.44 \pm 0.767\%$ (51/92) subclinical mastitis while $55.10 \pm 1.443\%$ (27/49) mastitis was noted in buffaloes given >8 calves. The udder infection appeared significantly higher ($p < 0.05$) in the animals given higher number of calves (>8 calves) and recorded as a risk factor associated with subclinical mastitis. The number of parity has positive correlation with subclinical mastitis [20]. As number of parity increased the prevalence of subclinical mastitis also

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

Parameter	Total No. of buffaloes	Positive	
		No.	%
Age (Years)			
3-5 years	68	34	50.00±1.043
6-9 years	89	50	56.18±0.792*
> 10 years	53	30	56.6±1.33*
Parity			
1-3	69	36	52.17±1.025
4-7	92	51	55.44±0.767*
> 8	49	27	55.10±1.443*

*Values differ significantly using t-test ($p < 0.05$).

increased in buffaloes. The present findings are in line with Sharma *et al.*, who reported higher prevalence of subclinical mastitis in buffaloes with age and number of parity that ranged from 5- 9 years and 3-4 parities respectively [3].

Severity Status

Only a very nominal number of the milk samples (5/840, 6%) produced a strong gel with CMT and found infected to most severe level of intensity (Table 4). The extreme severity (+4) was noted from Tandojam (3/284, 1.06±0.2%) and Tando Allahyar (2/284, 0.70±0.2%) locations. However, only 5 (1.84±0.3%), 6 (2.11±0.3%) and 8 (2.82±0.4%) out of 840 (2.26%) milk samples obtained from Hyderabad, Tando Allahyar and Tandojam farms formed a distinct gel by CMT and regarded as 'More Severe' (+3) mastitis respectively. Similarly 7.5% (n=63) out 840 milk samples formed weak gel and noted as 'Severe' (+2) level of mastitis, whereas 28.93% (243/840) of the quarters showed

'Moderate' (+1) level of mastitis caused gel in trace amount. As much as 60.71% (510/840) of the milk samples however produced no change with CMT and hence considered normal. Comparing severity levels existed at the three sites it appears that the milk samples from Tandojam location got significantly highest magnitude of infection at all the severity levels ($p < 0.01$) but significantly minimum in buffaloes from Hyderabad location. The present results are similar to the findings of several other studies reported from different parts of the country [17, 21].

CONCLUSIONS

The prevalence of the subclinical mastitis at animal and quarter levels was observed in buffaloes. Significantly higher prevalence as well as the intensity of the disease was determined in buffaloes located at Tandojam location. The ailment was higher in animals at 4th lactation likewise crossing 6 years of age and 4-7 parity. It was observed that animals above 6 years of

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

Taluka	Quarter	Quarters infected at various degree of severity									
		Most Severe (+4)		More Severe (+3)		Severe (+2)		Moderate (+1)		Negative (-)	
		No.	%	No.	%	No.	%	No.	%	No.	%
Hyderabad	272	Nil	-	5	1.84±0.3	14	5.15±0.5	71	26.1±0.6	182	66.91±0.2***
Tando Allahyar	284	2	0.70±0.2	6	2.11±0.3*	19	6.69±0.7***	78	27.47±0.8***	179	63.03±1.0**
Tandojam	284	3	1.06±0.2**	8	2.82±0.4**	30	10.56±0.4***	94	33.1±0.7***	149	52.47±0.3
Overall	840	5	0.60	19	2.26	63	7.5	243	28.93	510	60.71

*Values significantly differ using t-test ($p < 0.05$).

**Values significantly differ moderately at $p < 0.01$.

*** Values significantly high at $p < 0.001$.

age were more prone to subclinical mastitis than the younger animals (3-5 years).

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