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Effect of Diverse Factors on the Frequency of Clinical and Subclinical Mastitis in Kundhi Buffaloes of Sindh, Pakistan

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RR designed the study. HB carried out research and analysed the data. MRR wrote the manuscript. VK helped in correction of manuscript. NB and RKO helped in data collection and process.

Key words
Diverse factors, Affecting mastitis, Clinical mastitis, Subclinical mastitis, Kundhi buffaloes, Bacterial mastitis.

ABSTRACT

The present study was aimed at determining the effects of diverse factors associated with clinical and subclinical mastitis in buffaloes. Significantly higher prevalence (49. 07%) of clinical mastitis was observed in buffaloes with average age of 6 to 9 years whereas those of > 9 years of age showed higher (33.33%) but non-significant prevalence of subclinical mastitis. However, significant impact of the number of parity on the prevalence of clinical mastitis was observed in animals. Higher prevalence (48.20%) of clinical mastitis was observed in buffaloes with 4 to 7 calves. A similar trend of significant influence of the number of parity on the prevalence (39.72%) of subclinical mastitis was also noted in animals given more than 7 parity. Significant role of the humid hot summer month (August) on the occurrence of apparent and non-apparent mastitis in buffaloes was determined. The soil bedding material played significant role in the prevalence of both, clinical and subclinical mastitis. Significantly higher prevalence of clinical mastitis was observed in August (52, 10.74%), while the overall prevalence (26.65%) was determined during hot humid summer season. Similarly significantly higher frequency of non-apparent udder infection in animals was documented during August (36, 10.90%). The overall higher mean incidence (31.81%) of subclinical mastitis was detected in hot humid summer.

INTRODUCTION

The major sources of infection in the diseased udder are infected quarters, soiled udder, and contaminated milking machines, teat cups, hands of milkers, washing clothes, flies and surgical instruments. A significant role of stage (*P*<0.001) of lactation, lactation number, trauma to udders, dirty udders, leg hygiene, teat canal, teat end callosity, hyperkeratosis, body condition score, loose teat sphincters, lesions on teat skin, immunological status of each mammary gland, parity, live body weight, teat end to floor distance, udder depth, teat length, milk leakage, udder shape, pendulous udder, monthly milk yield and bulk of infection in the environment and management conditions are amongst the determinants that determine the level of mastitis prevalence (Radostits *et al*., 2007; Kavitha *et al*., 2009; Hussain *et al*., 2013; Shitu *et al*., 2012) in animals.

The occurrence, rigorousness, and monetary impact of udder infection depends upon the deterrent and management approaches. The frequency and the decorations of causal agents markedly differ from territory to territory, herd to herd, and period to period (Radostits *et al*., 2007). The higher prevalence of clinical mastitis has been recorded in buffaloes with 10 (86.66%) and 11 (15.38%) years of age, and in cows with 6 (57.44%) and 11 years (15.78%) of age (Sadashiv and Kaliwal, 2013). The role of diverse factors such as age, stage of lactation, parity, tick infestation, previous history of apparent mastitis in the incidence of mastitis in bovine has been observed (Reyher and Dohoo, 2011; Abera *et al*., 2012; Elbably *et al*., 2013).

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The high milk yielding animals are highly susceptible to mastitis than low milk yielding buffaloes (Kavitha *et al*., 2009), milking with folded thumb pressure as compared with those in which milk let down induced through suckling calves (Bilal *et al*., 2004), parity (Breen *et al*., 2009; Sharma *et al*., 2007; Rabbani and Samad, 2010; Belayneh *et al*., 2013; Elbably *et al*., 2013), tick infestation, previous history of clinical mastitis, farm hygiene and season are also considered major risk factors for clinical and subclinical mastitis (Osman *et al*., 2009).

On the basis of bacterial organisms, the highest incidence of clinical mastitis was recorded in the month of August caused by *Streptococcus uberis* when animals were on pasture while *Escherichia coli* in summer as compared to winter where animals were confined in houses (Hashemi *et al*., 2011). Higher incidence of mastitis in buffaloes was noted in August (50.0%) and March (65.0%) months, this variation could be due to monsoon during August where the climatic conditions remain moist and humid that might help in propagation of bacterial organisms (Baloch *et al*., 2013).

The cows housed on concrete-floored houses had lower prevalence (19.0%) of mastitis as compared to cows kept on soil-floored houses (47.6%). A lower occurrence of mastitis was detected in the cows of the farms did not use bedding (23.5%) as compared to farms used hay/straw(37.4%) bedding (Abera *et al*., 2012). Less incidence of clinical mastitis in animals provided sand flooring as compared to concrete. Higher incidence of clinical mastitis was demonstrated in animals had provided soil bedding material (Kavitha *et al*., 2009). Bilal *et al*. (2004) reported that concrete and brick grounds contribute more towards bovine mastitis as compared to kacha floors.

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, TandoAllahyar and Tandojam of Sindh province of Pakistan. The average rainfall in the area is 415 mm/year and average temperature is about 46^{o}C during months of May to August while in December and January, it is about 10^{o}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 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. No tick infestation was noted though the sheds and open yards where animals were kept were not properly managed and cleaned. The udders of the animals were soiled with manure, mud and urine. In majority of the sheds and open yards, clean water was not 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 (213 animals) and 840 milk samples (210 animals) were collected from lactating Kundhi breed buffaloes having inflammatory signs of clinical and subclinical mastitis, respectively 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 towel. Individual milk sample (100ml) was collected in sterile specimen container (completely wrapped/covered with aluminum foil) after discarding few initial milking streams. Each sample was labeled with locality, buffalo number, quarter site, number of sample, and other information related to the animals. Whereas the milk samples from apparently mastitis free buffaloes were collected aseptically and initial examination was done at field level by testing through California Mastitis Test (CMT) to confirm the positivity of milk samples. The positive results were interpreted according to the technique adopted by Muhammad *et al*. (1995) and David *et al*. (2005). 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 seconds 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 disease from lower to higher intensity as + = moderate/traces, + + = severe, + + + = more severe, + ++ + = very severe. The California positive subclinical mastitis milk samples collected were placed in icebox and transferred to the laboratory for bacteriological investigation. Before sampling, the whole udder of the buffaloes were washed thoroughly and then dried by 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 obtained in disinfected caped bottles, numbered and transported to the Laboratory of the section of Veterinary Microbiology, 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 bacteria. Similarly the California Mastitis Test positive samples were also subjected to bacteriological investigation to isolate pathogens. The samples of both, clinical and sub-clinical mastitis 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 colony, morphology, Gram-stained and biochemical properties as described by Waage *et al*. (1999, 2000 and 2001).

The significance of the data about different parameters was obtained by Chi-squire Test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The pathogenic species isolated and identified from clinical and subclinical mastitis samples were: *Staphylococcus aureus, Escherichia coli*, *Streptococcus dysgalactiae*, *Micrococcus luteus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, *Bacillus subtilis* and *Streptococcus pyogenes*. Sadashiv and Kaliwal (2013), Sharma *et al*. (2012), Supre *et al*. (2011), Zaki *et al*. (2010), Aiza *et al*., (2017) and Arshad *et al*. (2006) also isolated and identified the same bacterial pathogens from clinical and subclinical mastitis of buffaloes.

Impact of age

Table I show effect of age, parity, number of lactations, season of the year and floor types on the occurrence of clinical and subclinical mastitis in buffaloes. Significantly, high prevalence (106, 49%) of clinical mastitis was recorded in buffaloes of age group from 6 to 9 years. Sadashiv and Kaliwal (2013) observed higher prevalence of clinical mastitis in buffaloes with 6 (11.11%) and 11 (15.38%) years of age. Elbably *et al*. (2013) stated the strong relationship of the prevalence of clinical mastitis in animals with the age that varied from 6-8 years. They detected significantly higher prevalence (12.50%) of clinical mastitis in adult cows as related to young lactating (9.45%) animals with age that varied from 3-5 years. Similar attempt was made by Sharma *et al*. (2007) who found higher prevalence of clinical mastitis in buffaloes at age varied from 5 to 9 years. Sadashiv and Kaliwal (2013) also detected similar pattern of prevalence of clinical mastitis in buffaloes. However, the results about the effect of age on the prevalence of subclinical mastitis determined in buffaloes in the current survey are in accordance with the results of Salvador *et al*. (2012) who demonstrated the influence of age on the prevalence of subclinical mastitis in Bulgarian buffaloes. The younger buffaloes were found more susceptible as compared to those of 6 years old. The younger dams of 3 years showed 76% probability while those of above 3 years showed 82% susceptibility to subclinical mastitis. Shittu *et al*. (2012) recorded significantly lower risk of SCM among young lactating cows while Sadashiv and Kaliwal (2013) also conducted a study to observe the influence of age on the prevalence of subclinical mastitis in buffaloes, they reported that varied from 25-86% prevalence in buffaloes with age ranged from 5 to 11 years. Adane *et al*. (2012) studied age as a risk factor for the prevalence of subclinical mastitis in bovine and recorded 76.3% prevalence in cows with an average age above 10 years. The above authors support the results of the current investigation regarding the effect of age on the prevalence of subclinical mastitis noted in buffaloes.

Effect of parity

The influence of the number of parity on clinical and subclinical mastitis in buffaloes was also studied and the results are elucidated in the same Table I. A significant impact of the number of parity on prevalence of clinical mastitis in buffaloes was observed. The highest prevalence (94, 48%) of clinical mastitis was observed in buffaloes given out 4 to 7 calves. In contrast to clinical mastitis, a significant influence of the parity on the prevalence (29, 40%) of subclinical mastitis at χ^2 values 7.73, P-values 0.021 were noted in animals given out more than 7 parity. Dimitar and Metodija (2012) who stated that higher presence of udder infection in cows was noted with parity that varied from 1 to 3. Sharma *et al*. (2007) found higher prevalence of clinical mastitis in buffaloes at 3rd and 4th parity. The tendency of the influence of number of parity on clinical mastitis in buffaloes observed in this occasion is similar to the tendency of the above workers who observed that as figure of parity increased the prevalence of clinical mastitis also increased in animals. Aarestrup and Jensen (1997) described a clear correlation between parity and clinical mastitis as the parity increased there was also increase in the prevalence of mastitis n buffaloes. Kavitha *et al*. (2009) observed the association of parity with incidence of clinical mastitis in buffaloes and cows. As the parity increased the increase in the occurrence of clinical udder infection was recorded. The results regarding the association of the number of parity with the prevalence of clinical mastitis are in close agreement to the above workers. All the above authors support the observations noted and described in this study.

A significant effect of the number of parity >7 (40%) on prevalence of subclinical mastitis was observed in the current investigation (Table I). Hussain *et al*. (2013) observed a substantial ($P< 0.01$) positive association of subclinical mastitis with numbers of parity. Rabbani and Samad (2010) noted the number of parity as a risk factor against subclinical mastitis in buffaloes and recorded as 18.42%, 55.0%, 66.67%, 75.0% and 75.0% at 1st, 2nd, 3rd, 4th and 5th parity, respectively. Elbably *et al*. (2013) identified significantly ($P< 0.003$) higher prevalence of subclinical mastitis in cows with parity numbers that ranged from 2-4. Aarestrup and Jensen (1997) observed a marked influence of number of parity on the progress of mastitis. A clear correlation was noted between parity and subclinical udder infection as the parity increased however there was also increase in the prevalence of mastitis in animals. Adane *et al*. (2012) noted a significant ($P<0.00001$) influence of the parity on the prevalence of subclinical mastitis in bovine and observed 87.8 % in cows with more than 7 parity. However, similar conclusions were made in this study as drawn by the above workers as the parity increased; the opportunities of the prevalence of subclinical mastitis also increase in animals.

Effect of lactation

During present investigation significant effect of the number of lactations on the prevalence of clinical and subclinical mastitis was recorded in buffaloes. A higher occurrence (46%) of clinical mastitis was found in buffaloes with number of lactations that varied from 1-4, while the lower prevalence (31%) in buffaloes with number of lactations ranged from 5-8. Furthermore, in comparison to clinical, higher prevalence (39%) of

Table I.- Diverse factors related with occurrence of clinical and subclinical mastitis at animal level.

Diverse factors	Groups	Buffaloes examined	No. of positive (%)	Clinical mastitis		Subclinical mastitis	
				No. of positive (%)	χ2 and P-value Significance	No. of positive (%)	χ2 and P-value Significance
Age (years)	3-5	123	71 (58)	39 (32)	15.51 0.0004 **	32 (26)	2.21 0.33 NS
	6-9	216	160 (74)	106 (49)		54 (25)	
	>9	84	52 (62)	24 (29)		28 (33)	
Parity	1-3	155	93 (60)	58 (37)	14.40 0.0007 **	35 (23)	7.73 0.021 *
	4-7	195	144 (74)	94 (48)		50 (26)	
	>7	73	46 (63)	17 (23)		29 (40)	
No. of lactation	1-4	218	140 (64)	100 (46)	6.45 0.03 *	48 (22)	7.23 0.027 *
	5-8	151	100 (66)	47 (31)		45 (30)	
	>8	54	43 (80)	22 (41)		21 (39)	
Seasons/year	Autumn	72	47 (65)	27 (38)	1.62 0.65 NS	20 (28)	0.12 0.98 NS
	Winter	104	69 (66)	40 (38)		26 (25)	
	Spring	69	44 (64)	26 (38)		18 (26)	
	Summer	178	123 (69)	76 (43)		50 (28)	
Floor types	Cemented	106	49 (46)	29 (28)	12.43 0.002 *	20 (19)	5.72 0.05 *
	Sand	121	78 (64)	46 (38)		32 (26)	
	Soiled	196	156 (80)	94 (48)		62 (32)	

*Chi- square difference was significant ($p < 0.05$). NS, Chi-square P-value non-significant.

subclinical mastitis was determined in animals with number of lactations more than 8 (Table I). Bilal *et al*. (2004) detected extreme number of cases of udder infection during third lactation in both, peri-urban (19.00%) and rural (22.98%) locations. Sadashiv and Kaliwal (2013) detected higher prevalence of clinical mastitis in buffaloes at 7th month (25%) stage of lactation. Reyher and Dohoo (2011) observed the influence of the number and stage of lactations on the severity of clinical mastitis. Elbably *et al*. (2013) stated a strong relationship between prevalence of clinical mastitis and number of lactations in animals. Abd-Elrahman (2013) noted the higher incidence of clinical mastitis at 1st and 2nd months of post calving as 51.1% and 17.7%, respectively. However, the results and observations made in this survey are very close to those reported by the above workers irrespective of managemental conditions provided to animals, the findings of the study do agree with results mentioned earlier expressed by the researchers.

Alike investigation against subclinical mastitis was launched during current research work; a significant effect on the higher prevalence (39%) of subclinical mastitis was recorded in buffaloes with more than 8 numbers of lactations (Table I). Salvador *et al*. (2012) demonstrated the effect of the number of lactation on the prevalence of subclinical mastitis (SCM) in Bulgarian buffaloes. They noted 42.76% prevalence of subclinical mastitis in buffaloes, whereas the recurrence was recorded as 75.03%. They further reported that lactation length also influenced the occurrence of SCM. Kavitha *et al*. (2009) stated that the first phase of lactation (from 1 to 4 months and the last part of dry period (10 to 12 months) in buffaloes were also noted more vulnerable to in-apparent mastitis. Hussain *et al*. (2013) evaluated to ascertain the association of various risk factors of mastitis in water buffaloes. The frequency analysis showed a significant ($P<0.001$) positive difference among various groups including lactation stage on the prevalence of subclinical mastitis in buffaloes. Joshi and Gokhale (2006) reported the highest incidence of subclinical mastitis in purebred Holsteins and Jerseys while lowest in local cows and buffaloes. The higher frequency of subclinical udder infection was observed in animals with higher lactation numbers. Rabbani and Samad (2010) detected the highest prevalence (71.43%) of subclinical mastitis at late lactation stage as compared to mid and early lactation stages. Belayneh *et al*. (2013) and Elbably *et al*. (2013) reported the stages of lactation have substantial ($P<0.05$) effect on frequency of subclinical mastitis in animals. The results about the influence of the number of lactations on presence of subclinical mastitis observed during current investigation are closely coincided to the findings of the majority authors who also recorded similar conclusions as noted in this investigation.

Influence of season

During present study, all four seasons were analyzed and non-significant influence on the occurrence of clinical mastitis (76, 43%) in buffaloes was determined in summer season as compared to autumn, spring and winter seasons of the year. Similarly, non-significant influence of the seasons on the trend of prevalence (50, 28%) in subclinical mastitis was also recoded in buffaloes during summer season as observed in clinical mastitis (Table I). Osman *et al*. (2009) reported highest proportions of incidences were found during spring (10.71%) and winter (7.07%). Hashemi *et al*. (2011) conducted similar investigation to a certain the effect of season on the incidence of clinical mastitis in dairy cows in Iran. They did find the effect of season on the incidence of clinical mastitis but non-significant difference in the incidence of clinical mastitis among different seasons. The highest incidence of clinical mastitis was recorded during December and January months. A seasonal effect on the incidence of clinical mastitis was also studied by Shpigel *et al*. (1998) who observed the lower incidence of clinical mastitis in cows during dry summer months, the ratio of higher to lower incidence (1:62) was observed by January in Israel. Ranjan *et al*. (2011) determined highest incidence of clinical mastitis in winter followed by summer and least in rainy seasons. Riekerink *et al*. (2007) demonstrated the highest incidence of clinical mastitis in December to January. While, Sentitula *et al*. (2012) stated significantly higher incidence of mastitis in winter season as compared to summer. Baloch *et al*. (2013) determined the higher incidence of mastitis in buffaloes during August (50.0%) and March (65.0%) in their previous study. The results of this investigation with reference to the effect of seasons on the incidence of clinical mastitis determined in the survey are in partial agreement to the majority of the workers reported earlier; however, the findings of the current survey are in agreement to the observations made by Ranjan *et al*. (2011) in Indian buffaloes who recorded in winter season which was also noted in this study during winter climate in Pakistan. Baloch *et al*. (2013) recorded the higher incidence of mastitis in buffaloes during August (50.0%) and March (65.0%) in their previous study. They further defined that this variation might be due to monsoon in August where the climatic conditions remain moist and humid that could help in propagation of bacterial organisms.

At the same time the influence of the season on the prevalence of subclinical mastitis in buffaloes was also studied. A non-significant effect of the season on the trend of prevalence (29, 27.88%) was also recoded in buffaloes throughout winter season as observed in clinical mastitis in this survey (Table I). Joshi and Gokhale (2006) conducted investigation on subclinical mastitis in Indian buffaloes and recorded the incidence that did vary from 5-20%. Further informed that the monsoon season played a major role in subclinical mastitis than summer or winter. Elbably *et al*. (2013) stated significantly higher prevalence of subclinical mastitis (P<0.003) during summer months. Hashemi *et al*. (2011) and Riekerink *et al*. (2007) demonstrated the higher incidence of subclinical mastitis in bovine occurred during December and January. However, the results obtained for the influence of season on the percentage incidence of in-apparent mastitis in bubaline are in line to findings noted by Joshi and Gokhale (2006), they demonstrated 5-20% influence of summer and winter seasons on the prevalence of subclinical mastitis in buffaloes. Moreover, the influence of season on the prevalence of mastitis in buffaloes found during different seasons in the present study is also in accordance to the results acquired by the above workers because we are living in the same ecosystem therefore similar conclusions could be achieved.

Effect of type of floor bedding

Considering the type of bedding as a risk factor worldwide, A significant increase in the prevalence (94, 48%) of clinical mastitis was encountered in animals kept on soil floored houses while lower prevalence (29, 27%) was recorded in animals provided cemented and sand bedding materials (Table I). Furthermore, significant effect of soil bedding on the prevalence of subclinical mastitis was also found in animals as recorded in clinical mastitis. Abera *et al*. (2012) observed a clear association of the floor typing and bedding material (P< 0.05) on the prevalence of clinical mastitis. The animals kept on concrete-floored houses had lower prevalence (19.0%) of mastitis as compared to animals kept on soil-floored houses (47.6%). Kavitha *et al*. (2009) recorded less incidence of clinical mastitis in animals provided sand flooring (14.2%) as compared to concrete (18.5%). Bilal *et al*. (2004) reported that concrete and brick floors added more towards clinical infection of udder in bovine as compared to kacha grounds. Fadlelmoula *et al*. (2007) encountered a higher probability (P<0.001) of mastitis in tie-stall housed cows. The influence of type of housing and bedding material on the prevalence were also demonstrated by various workers in terms of favorable conditions for bacterial growth and ultimately the occurrence of mastitis would be inevitable in animals. They further noted that management of bedding had a big influence on exposure of teats to mastitis causing bacteria (Zdanowicz *et al*., 2004; Ruegg, 2012). Also cleared that sand bedding usually has the least bacterial populations but using of recycled sand had increased the moisture content and resulted in increased growth of mastitis pathogens.

A significant effect of soil bedding on the prevalence (32%) of subclinical mastitis was also observed in animals during present study (Table I). Kavitha *et al*. (2009) detected higher prevalence (21.2%) of subclinical mastitis in buffaloes kept on soil floored bedding as compared to sand (14.2%) and concrete (14.8%). Alemu *et al*. (2013) indicated in their studies that along with other risk factors, housing and bedding had significant effect on the prevalence of subclinical mastitis in cows. They further signified that the animals were kept at very good condition houses showed 88.9% mastitis while animals kept at poor houses with poor bedding showed 33.1% mastitis in Ethiopian cows. Furthermore, Fadlelmoula *et al*. (2007) also demonstrated higher incidence of mastitis in tie-stall housed cows in Germany. The observations subtracted for the effect of bedding on the prevalence of subclinical mastitis made in the current examination are in accordance to the outcomes of Kavitha *et al*. (2009). Ruegg (2012) defined that sand bedding usually has the least bacterial populations but using of recycling sand had increased the moisture content and resulted in increased growth of mastitis as compared to inorganic bedding. He also mentioned that organic bedding materials had supported the bacterial.

Different types of floors such as cemented, sand and soiled were studied from study areas. At Hyderabad, TandoAllahyar and Tandojam areas, the higher prevalence of clinical mastitis was observed in 29 (48%), 31 (48%) and 34 (47%) animals, respectively, while in subclinical mastitis, it was detected in 17 (28%), 19 (30%) and 26 (36%) animals, respectively. When the prevalence of clinical and subclinical mastitis was equated among three locations and types of floors provided, significantly higher influence of the soiled floor on the prevalence of clinical and subclinical mastitis was observed in animals at Hyderabad. However, similar significant effect of the soiled floor on the frequency of apparent and un-apparent clinical udder infections in bubaline of TandoAllahyar was also demonstrated (Table II).

Bilal *et al*. (2004) documented the association of the floor as a risk factor that influenced the prevalence of mastitis in animals. They reported that concrete and block grounds promoted further towards clinical mastitis in bovine as accorded to mud made floors. Kavitha *et al*. (2009) stated that the occurrence of udder infection was lower on silt flooring than cemented. A mud used bedding material provided higher frequency of clinical and subclinical infection of udder (21.2%) in buffaloes was observed as compared to sand and concrete floor bedding. The conclusions drawn with regards to the floor management as a risk factor for contribution of prevalence of both mastitis, clinical and subclinical in buffaloes in the current survey are also concluded by Bilal *et al*. (2004)

and Kavitha *et al*. (2009), they noted the higher prevalence of clinical and subclinical mastitis in animals which were provided soil floor bedding as revealed in this study. Therefore, the present findings are in agreement to the observations made by the above workers.

Significantly higher incidence of clinical mastitis was observed in the month of August (52, 11%), while the overall prevalence (27%) was noted during hot humid summer season. Similarly, significantly higher incidence of subclinical mastitis in buffaloes was recorded during August (36, 11%) month of the year. Overall higher mean incidence (32%) of subclinical mastitis was detected in hot humid summer season of the year (Table III). Elbably *et al*. (2013) studied significantly (*P*<0.000) higher occurrence of subclinical mastitis in summer months and recorded 2.94, 5.63, 5.63 and 8.75% prevalence during autumn, winter, and spring and summer seasons of the year in dairy cows, respectively, in Egypt. Baloch *et al*. (2013) in their previous study observed higher prevalence of clinical mastitis in buffaloes in the months of August (50.0%) and March (65.0%). They described August month as a humid hot summer month with

Table II.- Divers factors associated with floor management in the prevalence of clinical and subclinical mastitis in buffaloes at Hyderabad, Tando Allahyar and Tandojam locations.

Locations	Floor types	Total buffaloes examined	Clinical mastitis			Subclinical mastitis		
			Total No.	Positive No. (%)	χ2 and P-value significance	Total No.	Positive No. (%)	χ2 and P-value significance
Hyderabad	Cemented/bricked	51	26	10 (20)	17.77 0.0001 *	25	08 (19)	6.14 0.046*
	Sand	43	28	16 (37)		15	10 (20)	
	Soiled	60	32	29 (48)		28	17 (28)	
Tando Allahyar	Cemented/bricked	33	15	11 (33)	5.68 0.058 *	18	07 (21)	2.05 0.35 NS
	Sand	39	18	15 (39)		21	10 (26)	
	Soiled	64	32	31 (48)		32	19 (30)	
Tandojam	Cemented/bricked	22	10	08 (36)	3.52 0.17 NS	12	05 (23)	3.59 0.166 NS
	Sand	39	17	15 (38)		22	12 (31)	
	Soiled	72	35	34 (47)		37	26 (36)	
Total		423	213	169 (40)		210	114 (27)	

*Chi-square difference was significant (P<0.05). NS, Chi-square P-value was non-significant.

Table III.- The mean number and percentage incidence of clinical and subclinical mastitis in buffaloes during different months of the year.

Seasons of the year	Months of year	Total No. of samples examined	Clinical mastitis				Sub-clinical mastitis			
			No. of samples examined	No. of positive samples (%)	Overall % of season	χ2 and P-value Significance	No. of samples examined	No. of positive samples (%)	Overall % of season	χ2 and P-value Significance
Winter	December	152	84	42 (9)	24	1.09 0.58 NS	68	32 (10)	25	5.89 0.05*
	January	124	60	32 (7)			64	19 (6)		
	February	140	72	42 (9)			68	33 (10)		
Spring	March	144	80	49 (10)	16	1.76 0.18 NS	64	25 (8)	16	0.05 0.85 NS
	April	132	60	30 (6)			72	27 (8)		
Dry Hot Summer	May	132	68	39 (8)	17	1.42 0.23 NS	64	17 (5)	9	0.98 0.32 NS
	June	160	88	42 (9)			72	14 (4)		
Humid hot summer	July	144	76	43 (9)	27	13.20 0.001*	68	25 (8)	32	7.00 0.03*
	August	124	64	52 (11)			60	36 (11)		
	September	152	64	34 (7)			88	44 (13)		
Autumn	October	152	72	40 (8)	16	0.40 0.52 NS	80	33 (10)	18	0.68 0.40 NS
	November	136	64	39 (8)			72	25 (8)		
Total		1692	852	484 (29)			840	330 (20)		

*Chi- square difference was significant (p < 0.05). NS, Chi-square P-value non-significant.

high rainfall of the year while March month is noted to be spring season that provides suitable environmental conditions in terms of appropriate temperature and moisture for the growth of bacterial organisms. In contrast to above workers, Hashemi *et al*. (2011) obtained the highest incidence of clinical mastitis in December and January months. Shpigel *et al*. (1998) who observed lower incidence of clinical mastitis in cows throughout dry summer months while the ratio of higher to lower incidence (1:62) was observed in January in Israel. Ranjan *et al*. (2011) calculated the highest incidence of clinical mastitis in winter followed by summer and least in rainy season. Riekerink *et al*. (2007) reported the highest incidence of clinical mastitis from December to January in cows whereas Shitu *et al.* (2012) explored significantly higher incidence of mastitis in winter season as compared to summer in buffaloes.

The results with regard the mean incidence of clinical mastitis in buffaloes in different months and seasons of the year observed in this study are in line to the incidence of clinical mastitis presented by Elbably *et al*. (2013) and Baloch *et al*. (2013) in their studies in cows and buffaloes, respectively.

Subclinical mastitis in buffaloes was also followed during present survey to compare the incidence in between two different cases. Significantly higher incidence of subclinical mastitis in buffaloes was recorded in month of August (36, 11%) of the id summer season of the year (Table III). Joshi and Gokhale (2006) stated the overall higher incidence (31.81%) of subclinical mastitis during hot-humid monsoon season that played a major role in the incidence of subclinical mastitis in animals than summer or winter seasons. Elbably *et al*. (2013) demonstrated significantly (P<0.000) higher occurrence of subclinical mastitis in summer months than the other months of the year and recorded 14.7, 25.91, 25.97 and 27.5% throughout autumn, winter, spring and summer months of the year in cows, respectively. Similar conclusions were presented by Abdel-Rady and Sayed (2009) who detected higher prevalence of subclinical mastitis in hot-summer (9.14%) and spring (4.86%) seasons and months than winter (2%) and autumn (3.14%) seasons and months of the year. The observations noted in this study about the mean incidence of clinical and subclinical mastitis in buffaloes during different seasons and months of the year are coincided to the figures presented by the above workers. However, present values are in complete agreement to the results obtained by Elbably *et al*. (2013) who recorded similar incidence of subclinical mastitis in Egyptian cows. Irrespective of animal species, the weather conditions of Egypt are similar as in Pakistan. In general, bacterial organisms can cause infection in all animal species because the intrinsic environment of the udder of animal species is alike and can favour for propagation of bacterial species.

CONCLUSIONS

It is concluded that the age had significant influence on the prevalence of clinical mastitis in buffaloes with the average age ranged from 6 to 9 years than the younger animals. Significantly influence of the number of parity on the frequency of apparent (48.20%) and in-apparent mastitis (39.72%) was observed in buffaloes given 4 to 7 and more than 7 parity, respectively. However, significant role of the humid hot summer months (August) on the occurrence of apparent and unapparent mastitis in buffaloes was determined. It was further concluded that soil bedding material played significant role in the prevalence of mastitis in both, clinical and subclinical mastitis in buffaloes.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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