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Frequency of clinical and subclinical mastitis in Kundhi buffalo breed of Sindh, Pakistan

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Abstract
To illuminate the magnitude of clinical and subclinical mastitis in buffaloes of Sindh province of Pakistan, a total of 423 buffaloes with clinical and subclinical mastitis and examined by cultural and CMT methods for bacterial infection. The overall frequency of clinical and subclinical mastitis was detected in 66.90% (n=283) buffaloes. Of the 423 buffaloes tested, 169 (39.95%) and 114 (26.95%) were detected positive for clinical and subclinical mastitis respectively. Statically higher prevalence of clinical mastitis was observed as compared to subclinical mastitis.
The frequency of one, two, three and four quarters in clinical and subclinical mastitis was determined as 14.79, 26.03, 19.52 and 39.64% and 14.91, 21.05, 24.56 and 39.47% respectively. Animals with four quarters affected by clinical and subclinical mastitis were determined as 39.64% and 39.47% respectively. From 484 clinically positive mastitis milk samples, 317 (65.49%) and 167 (34.50%) whereas from 330 subclinically positive samples, 197 (59.69%) and 133 (40.30%) were found with pure and mixed bacterial infections respectively.

Keywords: Kundhi buffalo, clinical and sub- clinical mastitis, CMT, frequency

Introduction
Mastitis is nowadays acknowledged as major udder disease of milch beasts and posing a significant economic threat to the holders/farmers world-wide [1]. The disease causes inflammation in the mammary glands fetching physical, chemical and pathological deviances in milk and glandular tissues [2, 3]. The problem left disregarded leads toward worsening of physical aptitude of animals until culling of the affected animals, or even to death. The infections of udder are generally categorized into apparent and unapparent forms of mastitis. The clinical mastitis causes udder tenderness through obvious signs of redness, hotness, inflammation, soreness and reduced milk production and therefore could be detected simply without assistance of laboratory examinations, however in imperceptible mastitis, the udder looks ordinary and not shows the signs of disease but obliges laboratory examinations for its diagnosis. The clinical and subclinical forms of mastitis have been reported to cause as high as 60 to 70% of the entire udder infection losses in the developed country [4]. Previous records show that quarter level occurrence of intra-mammary infection (IMI) in bubaline is around 66%, and frequency is attaining higher up to 30 days of parturition [5]. The diagnosis of mastitis overwhelms prodigious consequence on justification of rising public awareness about food safety and value as well for animal welfare. Sub-clinical disease of udder is generally recognized successively research laboratory analysis of the milk as there is no gross distension of quarters or obvious vicissitudes in the udder fluid. The diagnosis for such purpose largely needs screening tests like White Side Test (WST), Surf Field Mastitis Test (SFMT), California Mastitis Test (CMT), and Somatic Cell Count (SCC) [6]. The major pathogenic organisms accountable for clinical and subclinical mastitis in buffaloes and other animal species are *Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae* and *Streptococci* species. Many other bacterial pathogens reported from infected mammary glands universal are *Actinomyces pyrogenes, Clostridium perfringenes, Pseudomonas aeroginosa, Klebsiella pneumonia* and *Pasteurella haemolytic* [7, 8]. A little research work had been succeeded out on the occurrence of clinical and subclinical in buffaloes of Sindh province (Pakistan), and Kundhi breed in particular. Some fragmented information gathered from clinical and subclinical mastitis cases presented to veterinary clinic for treatment or drawn together over a limited area of the country other than Sindh [9-11, 3, 12]. Considering clinical and subclinical mastitis, a potential threat to animal status, the current study was designed to

explore the present situation of clinical and subclinical mastitis in Kundhi breed kept at various areas round Hyderabad of the jurisdiction of Sindh. Moreover, that probably this is first ever study of this kind conducted over this area which remained neglected so far. The current revision is considered to be helpful in updating the health of animals and future strategies in controlling of the disease which can be regarded as an important aspect in productivity enhancement.

Materials and Methods

Study area:

A total of 423 Kundhi buffaloes breed from which 213 and 210 with clinical and subclinical mastitis respectively were studied from the areas of Hyderabad, TandoAllahyar and Tandojam of Sindh province of Pakistan. The average rainfall in the area was 415 mm/year and temperature was about 46ºC during months of May to August while 10ºC from December to January months [13]. 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 during 2016 and 2017.

Physical status of animals

Physiological conditions of animals such as health, age, lactation, parity and breed were documented. In clinical mastitis, the nature of whole udder or part of the udder, the number of quarters, wounds, injuries and damages involved were also noted. While in unapparent mastitis, the animals were found healthy, alert and active. No any parasitic infestation was detected but their sheds and open backyards were not properly accomplished 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 mastitis milk samples

The current study design was based on cross-sectional investigation. A total of 852 (213 animals) and 840 milk samples (210 animals) were collected from lactating Kundhi buffaloes with clinical and subclinical mastitis respectively from the areas of Hyderabad, TandoAllahyar and Tandojam of the province of Sindh, Pakistan. Before 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 preliminary milking streams. Individual specimen was labeled by area, animal 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 [14, 15]. 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 CMT 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 collected in sterile caped bottles, numbered and then transported to the Central Veterinary Diagnostic Laboratory, Tandojam.

Examination of milk samples

Milk samples collected from buffaloes showing clinical signs of mastitis were directly processed for isolation and identification of bacterial isolates. Likewise the California Mastitis Test positive samples were also subjected to bacteriological investigation to isolate pathogens. The samples of both natures, clinical and sub-clinical were streaked on nutrient, blood and MacConkeys agars. The plates were incubated under aerobic conditions at 37 °C for 24 hours. 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 documentation of the bacterial species was done through colony charactertics, morphology of the bacterial organisms, Gram-reaction and chemical properties as prescribed by [16- 18].

The significance of the data about different parameters was obtained by Chi-squire Test [19].

Results and Discussion

An epidemiological survey on clinical and subclinical mastitis was carried out to demonstrate the frequency of bacterial mastitis in Kundhi buffaloes of the province of Sindh, Pakistan. For the purpose, 423 buffaloes were examined through their milk samples by cultural and CMT, the frequency of clinical and subclinical mastitis was noted in 283 (66.90%) buffaloes. From 283 buffaloes, 39.95% (n=169) was detected positive for clinical mastitis while 26.95% (n=114) buffaloes was found positive for subclinical mastitis. Significantly higher frequency of clinical mastitis was observed in buffaloes as compared to subclinical mastitis (Table 1) [20]. Examined 80 mastitis milk samples and recorded 70% mastitis in buffaloes [21-23]. reported the overall prevalence of clinical mastitis in 21.08, 53.85 and 40.35% buffaloes respectively [24]. Detected clinical mastitis in buffaloes from different areas that varied from 19.74 – 25.12% in Pakistan. [25] Investigated 840 milk samples and noted 25.55 and 29.23% clinical mastitis in buffaloes at Sharkia and Dakahlia Governorates in Egypt respectively [26]. Studied 10 farms in the Latina province of Italy and detected 50% prevalence of clinical mastitis in buffaloes. The findings of the present study do agree with the findings of [20, 22, 23], they recorded 70, 53.85 and 40.35% respectively in Pakistani

buffaloes, but not with rest of workers who found somewhat lower prevalence in different countries.

On the other hand, the overall prevalence (26.95%) of subclinical mastitis in buffaloes was also determined [27, 28]. detected the prevalence that varied from 5-20% and 64.96% mastitis in buffaloes of North Karnataka Region of India [29]. Observed animal-wise prevalence of subclinical mastitis in dairy buffaloes in and around Faisalabad, Pakistan and reported 51% prevalence in buffaloes by CMT. While [10] examined milk samples from 300 buffaloes apparently mastitis free by Surf Field Mastitis Test (SFMT), observed the overall animal-wise prevalence as 53%. Whereas [30, 31, 25] tested milk samples, 56.8, 44 and 40% buffaloes were detected positive with subclinical mastitis. In general, that the ecological and managemental circumstances are similar for the prevalence of subclinical as demonstrated by the above authors in their countries. Hence, the current values about the overall frequency of clinical and subclinical mastitis in animals are in agreement to the findings of the earlier mentioned scientists who also recorded similar figures as recorded in this survey.

Table 1: The overall number and percentage prevalence of clinical and subclinical mastitis in buffaloes.

Animal species	No. of buffaloes Examined	No. of positive buffaloes	% of positive buffaloes	Clinical mastitis		Subclinical mastitis	
				No. of positive buffaloes	% of positive buffaloes	No. of positive buffaloes	% of positive buffaloes
Buffalo	423	283	66.90	169	39.95	114	26.95
χ^2 values = 16.06, *df* = 1, P-values = 0.0001*							

*Chi- square difference was significant (P < 0.05).

From 213 buffaloes, 169 (79.34%) were found positive with clinical mastitis. Likewise, 210 buffaloes tested by CMT and confirmed through cultural growth, 114 (54.28%) animals were identified and noted with subclinical mastitis. When clinical and subclinical mastitis were analyzed on quarter basis, all 1692 quarters examined, 814 (48.10%) were found affected by both, clinical and subclinical mastitis. The results of the present study indicate that in all respect the prevalence of clinical mastitis was higher in buffaloes as compared to subclinical mastitis (Fig. 1).

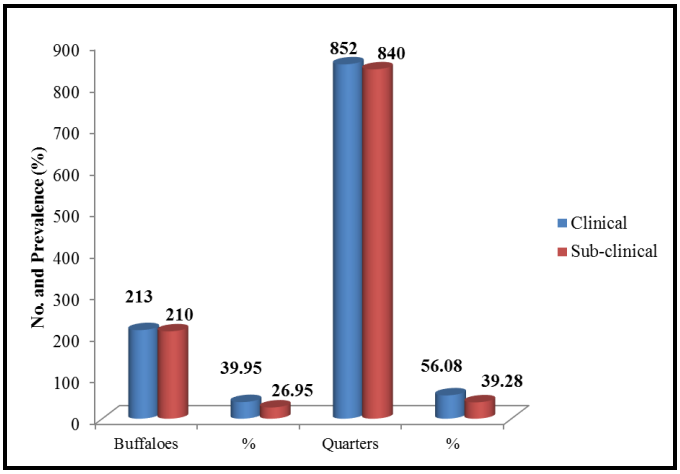

Fig 1: Number and percentage prevalence of clinical and subclinical mastitis at buffalo and quarter levels

Similar investigation with regards to the prevalence of clinical mastitis at buffalo level was conducted by [28] who reported 64.86% prevalence of clinical mastitis in buffaloes of North Karnataka Region India [8]. Observed clinical mastitis in mixed breed and recorded 70% prevalence of clinical mastitis in Pakistani buffaloes [21-23]. documented clinical mastitis at buffalo level as 21.08, 53.85 and 4053% respectively, [24, 25, 32] reported at buffalo level prevalence of clinical mastitis that varied from 19.74-50% from different countries and areas is lower than the present study about prevalence of clinical mastitis at animal level. However, the prevalence of clinical mastitis at buffalo level demonstrated by [28, 8] are in line to the findings regarding the prevalence of clinical mastitis observed in this survey. Though, the overall prevalence of clinical mastitis at quarter level (48.10%) detected in the present survey is some extent far to the overall prevalence of clinical mastitis observed by [33], documented 29. 08% prevalence of clinical mastitis in the quarters of cows suffering with mastitis while lower prevalence was noted by [34], they recorded 15.06% in buffaloes per lactation.

A like-wise study on the status of subclinical mastitis at buffalo level was also undertaken. Of the 210 buffaloes tested by CMT and cultural growth, 114 (54.28%) were found positive for subclinical mastitis are in close agreement to the results of [29, 10, 9, 30, 31], they recorded the frequency of subclinical mastitis at buffalo level in different countries and Pakistan as well that did vary from 44-53% by various techniques. Moreover, on overall at quarter level, the frequency of unapparent mastitis was observed in this survey is also in complete agreement to the prevalence of subclinical mastitis at quarter level recorded by [10], who stated 45.67% overall occurrence of subclinical mastitis at quarter level in Pakistani bubaline. The findings about subclinical mastitis at buffalo level demonstrated in this survey are in accordance to the results observed by the above scientists in their studies carried out in different countries.

Of the 283 affected animals were further analyzed on location-wise for clinical and subclinical mastitis, a higher number (100, 23.64%) of the prevalence of clinical and subclinical mastitis were detected in animals at Tandojam while lower at Hyderabad (90, 21.27%) and Tando Allahyar (93, 21.98%) locations. Moreover, when further comparison was made between overall clinical (169, 39.95%) and subclinical mastitis (114, 26.95%) in animals of the study areas, a significantly higher number (χ^2 values 9.24, P-values 0.009) of the prevalence of the affected animals were observed for clinical mastitis (Table 2) [23]. Examined 272 buffaloes in and round the district of Lahore, found 20.98% clinical mastitis in animals while the overall prevalence of clinical mastitis in buffaloes was observed as 40.35% [24]. Detected higher prevalence of clinical mastitis in buffaloes from peri-urban (25.12%) than rural (19.74%) of Faisalabad areas of Pakistan [25]. Collected 840 milk samples from 220 lactating buffaloes with history of clinical mastitis and noted 25.55 and 29.23% mastitis in buffaloes of Sharkia and Dakahlia Governorates respectively.

On the other hand [10], collected milk samples from apparently mastitis free 1200 quarters of 300 buffaloes and examined by Surf Field Mastitis Test (SFMT). The highest animal wise

prevalence of subclinical mastitis was observed to be 50, 53 and 47% in buffaloes of Tehsils of Prowa, Dera Ismail Khan and Paharpur respectively [9]. Surveyed 300 Pakistani buffaloes by Surf Field Mastitis Test. The maximum prevalence was recorded as 82.61% in Tehsil Pinighaib followed by 73.33, 80.0 and 76.0% in Tehsils Attock, Jand and Fateh Jang respectively [31]. Screened out 600 lactating dairy buffaloes from four districts of Lahore, Sialkot, Narowal and Okara of Punjab province of Pakistan for subclinical mastitis using White Side Test and subsequent bacterial isolation. The overall prevalence of subclinical mastitis in these districts was observed as 44%. The variation in results of the dominance of clinical mastitis at various locations observed in buffaloes during present study are in close agreement in all respects to the findings of [23, 24, 31, 25]. They all recorded more or less similar findings regarding the prevalence of clinical mastitis from different areas are also observed for clinical mastitis from different areas during present study. The causes for the similarity in the values are that above workers carried out investigations in circumstances wherever managemental and topographical atmospheres are prevailing with similar configuration, even the same species of the buffaloes since no perfect descriptions about the animals are given by the investigators. Therefore the findings regarding the prevalence of clinical mastitis observed from different locations during current investigation are in accordance with the values of the above workers who also recorded from different places and got similar results as recorded in this investigation for clinical mastitis in buffaloes. While [31] screened-out 600 lactating dairy buffaloes from four districts of Lahore, Sialkot, Narowal and Okara of Punjab province of Pakistan for subclinical mastitis and demonstrated similar results as recorded in the present study for different areas/ localities but they determined for tehsils. However, the Tehsils and Talukas or even localities are same but terms are being used locally by the people in various areas of the country. Therefore, the results of the existing investigation regarding the prevalence of subclinical mastitis and the findings observed by [9] for Pakistani buffaloes at Tehsil level are could not be compared because they recorded higher prevalence of subclinical mastitis at Tehsils Pinighaib, Attock, Jand and Fateh Jang of the Punjab province of Pakistan. This difference may be due to change in the pattern of managemental and environmental systems governing there.

Table 2: Percentage prevalence of clinical and subclinical mastitis at buffalo level from Hyderabad, TandoAllahyar and Tandojam areas.

Study areas	Prevalence of mastitis			Clinical mastitis		Subclinical mastitis	
	No. of buffaloes tested	No. of affected Buffaloes	% of affected buffaloes	No. of positive Buffaloes	No. of positive buffaloes	No. of positive Buffaloes	% of positive buffaloes
Hyderabad	154	90	21.27	55	13.00	35	8.27
Tando Allahyar	136	93	21.98	57	13.47	36	8.51
Tandojam	133	100	23.64	57	13.47	43	10.16
Total	423	283	66.90	169	39.95	114	26.95
χ^2 values = 9.24, *df* = 2, P-value = 0.009 *							

*Chi- square difference was significant ($P < 0.05$).

Of the 852 quarters examined from clinical mastitis, 484 (56.80%) were detected to be positive for bacterial organisms while similar investigation was conducted against 840 milk samples obtained from quarters of subclinical cases, 330 (39.28%) quarters were found positive. Significantly (χ^2-values 52, P-values 0.000) higher prevalence was recorded in quarters of animals suffering from clinical mastitis as compared to the quarters of buffaloes affected by subclinical mastitis (Table 3).

Table 3: The number and percentage incidence of clinical and subclinical bacterial mastitis in milk samples obtained from quarters of buffaloes

Total No. of quarters examined	Clinical mastitis			Subclinical mastitis		
	No. of quarters Examined	No. of positive quarters	% of positive quarters	No. of quarters examined	No. of positive quarters	% of positive Quarters
1692	852	484	56.80	840	330	39.28
χ^2 values = 52.02, *df* = 1, P-values = 0.0000 *						

*Chi- square difference was significant (P < 0.05)

[35-37] recorded the overall prevalence of clinical mastitis at quarter level in 9.64%, 4% and 39.2% quarters of buffaloes and cows respectively. However, the present prevalence of clinical mastitis determined at quarter levels are somehow closer to [37], they recorded the overall prevalence at quarter basis as 39.2%. But the results regarding existence of udder infection at quarter levels determined in bubaline in this study are higher than the findings of [36] they detected only 4% in quarters of buffaloes. This variation in the occurrence of clinical mastitis at quarter levels noted in this survey and those of the above workers may be explained by different managemental factors such as specific dry period management strategies.

In current survey, the overall presence subclinical mastitis at quarter level was also investigated and presented in Table-3. [10] Examined milk samples from 1200 quarters by Surf Field Mastitis Test (SFMT). The quarter-wise prevalence was recorded as 45.67%. [38] Analyzed milk samples of 300 buffaloes of Iran by CMT, the prevalence of subclinical mastitis was determined in 13.87% quarters [39, 9]. studied the prevalence of subclinical mastitis in buffaloes, found 8% and 51-67% at quarter level while lowest prevalence (30%) was reported by [40, 41] Reported the CMT quarters related prevalence in 58% farm buffaloes suffering from subclinical mastitis. The results obtained at quarter basis for presence of un-apparent mastitis in this study are in close agreement with majority of the authors reported earlier [10]. noted the overall prevalence at quarter level as 45.67% is also in comparable to the prevalence of clinical mastitis at quarter level recorded in this survey; we also recorded 39.67% which is not so far from the findings of [10].

A total of 213 each, from right front and left front quarters were examined, 106 right and 110 left front quarters were found affected with clinical mastitis, the frequency was recorded as 49.76% and 51.64% respectively. A similar number 213 each, from right and left hind quarters were tested, 128 right and 140 left rear quarters were also affected by clinical mastitis, the prevalence of clinical mastitis in right and left hind quarters was detected as 60.09 and 65.72% respectively. Likewise 210 each, from right front and left front quarters were tested from subclinical mastitis, right front (n=73) and left front (n=79) quarters were affected by non-clinical infection, frequency was observed as 34.76 and 37.61% respectively while right hind (n=83) and left hind (n=95) quarters were determined with subclinical mastitis, the prevalence was observed as 39.52 and 45.23% respectively (Fig. 2).

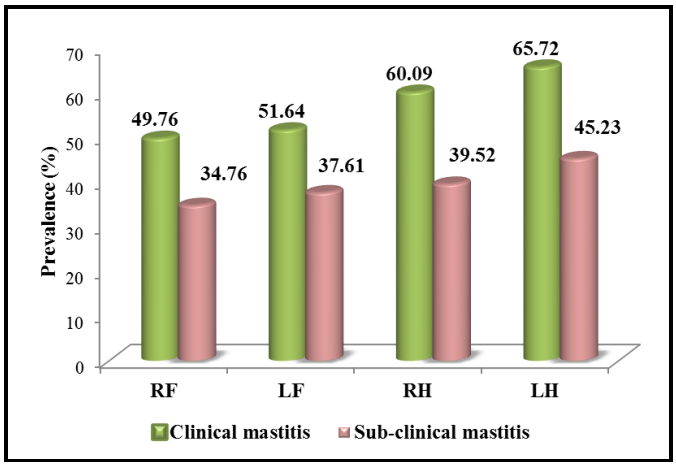

Fig 2: Quarter-wise prevalence of clinical and subclinical mastitis in buffaloes

Similar pattern of survey at the quarter level of infected udder was conducted by [23], who examined 272 milk samples from buffaloes, the prevalence of mastitis was observed to be 0, 68.96, and 11. 49 and 19.54% in the left front left rear, right front and right rear quarters independently [24]. Detected higher prevalence of clinical mastitis in rear-quarters and demonstrated as 73.3 and 63.1% than in forequarters as 26.6 and 36.8% in peri-urban and rural areas respectively [34]. Recorded higher prevalence of clinical mastitis in rear quarters (49.39%) in comparison to the front one (33.04%) while 17.55% prevalence was observed in both, either front one and rear quarters. Determined similar results as noted in the present investigation in all respects. However [34], recorded the prevalence of clinical mastitis in rear quarters of buffaloes in their study is in close agreement to the values of the present study. But the variation in the prevalence of clinical mastitis in buffaloes of the current and of those authors could be based on managemental factors and breed of the buffaloes under examination. The quarter-wise incidence of non-clinical mastitis in bovine was also studied (Fig. 2). However, the results of the present study are in accordance to the observations of [36, 23, 42, 36] recorded 14.8, 29.6, 18.5 and 37.5% prevalence in right forefront, right rear, left fore and left hind separately. Furthermore, the present results do agree with the findings of the above authors with regards that they noted increased occurrence of mastitis in back quarters as recorded in this study in buffaloes.

The distribution of mastitis in animals with one, two, three and four quarters affected by clinical mastitis recorded were 25, 44, 33 and 67, the prevalence was recorded as 14.79, 26.03, 19.52 and 39.64% respectively. On the other hand, the animals with one, two, three and four quarters affected by subclinical mastitis were 17, 24, 28 and 45, the distribution of subclinical mastitis in quarters was recorded as 14.91, 21.05, 24.56 and 39.47% respectively. A higher number of animals with four quarters affected by clinical and subclinical mastitis were determined in 67 (31.45%) and 45 (21.42%) buffaloes respectively (Fig. 3) [28]. recorded the prevalence of clinical mastitis in one, two and three quarters in buffaloes was noted as 9.37, 2.70 and 6.25% respectively. The results of the present study regarding the one, two, three and four quarters infected by clinical mastitis of individual animal are in similar pattern as recorded by the above authors but not exactly the same.

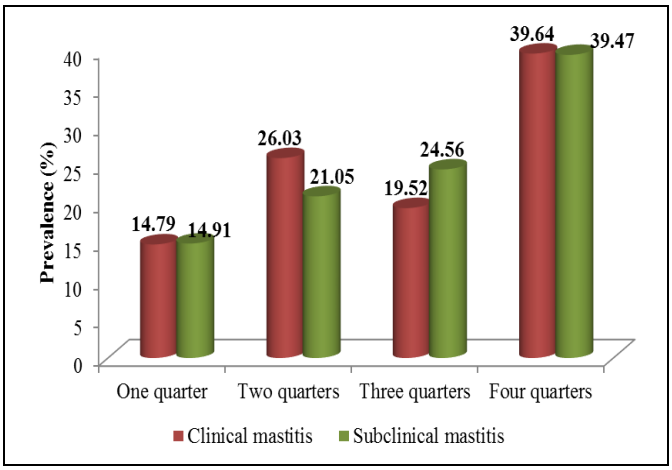

Fig 3: The number and percentage prevalence of clinical and subclinical mastitis in different quarters of buffaloes.

In this survey, the higher prevalence was recorded for animals affected with four quarters while the above authors recorded higher number of animals affected with one quarter. But generally, a few observations of this study are very close to the findings of [34]. A lower prevalence of clinical mastitis in number of quarters of individual animal was observed by [as compared to this study, this variation in lower prevalence at quarter level might be due to different factors such as management, size and shape of quarters and breed etc. [28] demonstrated as 39.86, 18.75, 6.25 and 0% in one, two, three and four quarters affected by subclinical mastitis of individual animal respectively. Whereas one, two, three and quarters of individual animal affected with subclinical mastitis was also determined by [29] who recorded 13.72, 45.49, 11.76 and 49.01% respectively. Therefore, the observations made during present survey about the number of quarters affected by subclinical mastitis in individual buffalo are in line to the findings of the above workers, they also recorded more or less similar findings as noted in the present study.

The frequency of clinical and subclinical mastitis in individual buffalo at different locations with one, two, three and four quarters was studied (Table-4). The maximum number of animals was found with four quarters affected by mastitis. From Hyderabad, TandoAllahyar and Tandojam areas, the incidence of clinical and subclinical mastitis was recorded as 32.72, 37.14%; 40.35, 41.66%; 45.61 and 39.53% respectively during current survey [9]. observed single quarter incidence of subclinical mastitis in individual buffalo as 38.33, 50, 48.33 and 43.33% from tehsils of Attock, Jand, Pindighaib and Fatah Jang in Pakistan whereas two quarters

in individual buffalo was observed as 13.33, 16.66, 11.67 and 13.33% respectively. Furthermore, they did not observe three and four quarters incidence in individual buffalo during their investigation at all. In general, it could be concluded that the findings of the present survey in terms of one and two quarters affected by subclinical mastitis are in line to the results of [9], who recorded the influence of the area in the pattern of incidence of clinical and subclinical mastitis in the quarters of individual buffalo as recorded in this study for both, clinical and subclinical mastitis in buffaloes of Sindh province of Pakistan.

From 484 clinically positive mastitis milk samples, 317 (65.49%) and 167 (34.50%) were determined having pure and mixed bacterial species respectively. While utmost similar pattern of pure and mixed infections in milk samples of subclinical mastitis was also demonstrated during present study. From 330 positive samples, 197 (59.69%) and 133 (40.30%) were found with pure and mixed bacterial infections respectively (Fig. 4). [26] studied 10 farms in the Latina province of Italy to record the clinical mastitis in buffaloes. They detected 50% clinical mastitis was caused by one microbial strain while remaining was observed more than one microbial strain [8]. detected 70 positive mastitis milk samples, 55 (78.57%) and 15(21.43%) were determined having pure and mixed bacterial species respectively.

Table 4: The pattern of incidence of clinical and subclinical mastitis in number of quarters of individual buffalo detected from Hyderabad, Tando Allahyar and Tandojam locations.

Quarters involved	Locations						Total number. and % of CM and SCM quarters examined		Mean Number difference in quarters
	Hyderabad		Tando Allahyar		Tandojam				
	CM	SCM	CM	SCM	CM	SCM			
	No & % of positive quarters	No. & % of positive quarters	No. & % of positive quarters	No. & % of positive Quarters	No. & % of positive quarters	No. & % of positive quarters	Total No. & % of CM positive quarters	Total No. & % of SCM positive quarters	
One quarter	09 (16.36%)	5 (14.28%)	12 (21.05%)	5 (13.88%)	04 (07.01%)	07 (16.27%)	25 (14.79%)	17 (14.91%)	07.0
Two quarters	16 (29.09%)	6 (17.14%)	13 (22.80%)	9 (25.0%)	15 (26.31%)	09 (20.93%)	44 (26.03%)	24 (21.05%)	11.33
Three garters	12 (21.81%)	11 (31.42%)	09 (15.78%)	7 (19.44%)	12 (21.05%)	10 (23.25%)	33 (19.52%)	28 (24.56%)	10.16
Four quarters	18 (32.72	13 (37.14%)	23 (40.35%)	15(41.66%)	26 (45.61%)	17 (39.53%)	67 (39.64%)	45 (39.47%)	18.66

CM = Clinical mastitis
SCM = Subclinical mastitis

Of the positive subclinical mastitis samples, 197 (59.69%) and 133 (40.30%) were determined with pure and mixed bacterial infections respectively [43]. examined 81 subclinical mastitis milk samples, significantly (P <0.05) higher mastitis was caused by pure (27, 17, 1%) bacterial species as compared to mixed (17, 10.8%) bacterial species in cows [44]. found 7 (31.8%) pure and 17 (68.2%) mixed bacterial infections from subclinical mastitis in cross-bred cows. The findings noted for pure and mixed bacterial infections in subclinical mastitis in buffaloes in the present survey are in agreement to the results observed by the above authors, whose studies revealed similar trend of pure and mixed bacterial infections in subclinical mastitis of cows as demonstrated in buffaloes during present study.

The number of bacterial species in a single sample was demonstrated in clinical and subclinical mastitis during present study (Table 5). The mixed infections with 2 species in 135 (27.89%) while 3 species were counted in 32 (6.61%) clinical mastitic milk samples. Similarly, the trend of mixed infections with 2 bacterial species were noted in 115 (34.84%) while 3 species in 18 (5.45%) subclinical samples [45]. recorded mixed bacterial species rather than single species in a single mastitis milk specimen of buffaloes [46]. counted 2-4 diverse bacterial species in an individual wound sample [47]. enumerated 2-4 bacterial isolates in a single sample [8]. also detected mixed (2-3) microbial agents in an individual milk specimen obtained from buffaloes. Further stated that a single bacterial species was recovered from 55% samples; more than 2 bacterial isolates were counted in 10.0% milk samples whereas 3 bacterial species were noted in 5.0% samples. Considering the results of the earlier mentioned workers and the findings of the current survey indicate that mixed species are common whether it would be wound or mastitis, in both the situations, the chances of the mixed bacterial infections are generally common since the wounds on skins/hides and the openings of the quarters are directly involved to the contaminated soil, hence there are a lot of probabilities for the bacterial organisms to invade any breach in skins or injuries in the quarters or udders.

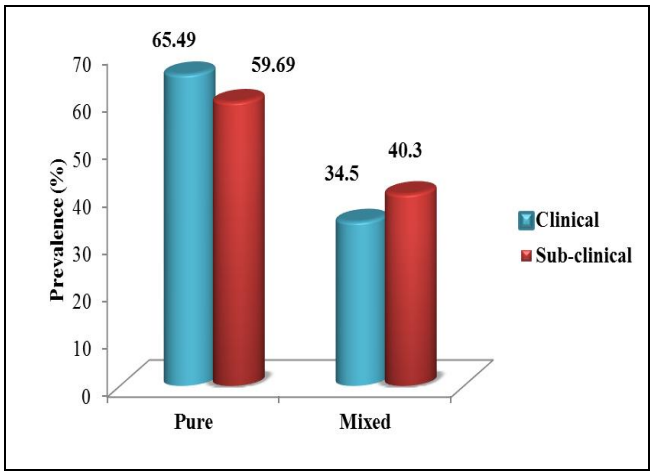

Fig 4: The number and percentage incidence of pure and mixed bacterial species in clinical and subclinical mastitis milk samples of buffaloes

Table 5: The number occurring of bacterial species in individual mastitis milk samples obtained from buffaloes

No. of species in samples	1		2		3	
	CM	SCM	CM	SCM	CM	SCM
No. of samples occurring in	317	197	135	115	32	18
Percentage of total samples	65.49	59.69	27.89	34.84	6.61	5.45

CM = Clinical mastitis SCM = Subclinical mastitis

Conclusions

From the present study, it is concluded that significantly higher (χ2-values 16.06, P-values 0.0001) prevalence of clinical mastitis was observed in buffaloes as compared to subclinical mastitis. A higher (23.64%) prevalence of clinical mastitis was detected in animals at Tandojam than other areas. It is also concluded that higher bacterial mastitis of clinical (65.72%) and subclinical (45.23%) was demonstrated in the left hind quarters. Further concluded that mixed infections was common in mastitis.

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