Prevalence And Bacteriology Of Sub Clinical Mastitis In Buffaloes In And Around Peshawar District, Khyber Pakhtunkhwa Pakistan

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Abstract:

In the present study, eight hundred fore milk samples were collected from 800 mastitis quarters each 400 from Nili Ravi and Kundi respectively from peri urban and rural areas of Peshawar district, KPK Pakistan from November, 2012 to April, 2013 using Surf Field Mastitis Test for detection of sub clinical mastitis and subsequent bacterial isolation. Ninety, fungal, algae and bacterial isolates of six genera i.e. Streptococcus, Escherichia, Bacillus, Salmonella, Staphylococcus, Yeast (fungus) and Prototheca (algae). The highest prevalence was of Staphylococcus aureus which was the most frequently recovered bacterial species in both Nili Ravi and Kundi accounting 40.42% and 34.88% respectively of all isolates, followed by streptococcus agalactiae 36.17% and 32.55%, Bacillus cerueus 4.25% and 9.30%, Escherichia coli 4.25% and 6.97%, Streptococcus dysgalactiae 6.38% and 2.32%, Prototheca 2.12% and 6.97%, Salmonella spp 4.25% and 2.32% and Yeast 2.12% and 4.65% respectively. The overall quarter wise prevalence was accounted 47% and 43% in Nili Ravi and Kundi buffaloe respectively. The maximum one positive quarter wise prevalence was found in Nili Ravi and Kundi to be 60.08% and 77.32% respectively. The two positive quarter-wise comparative prevalence of mastitis in buffaloes (Nili Ravi and Kundi) was 31.91% and 22.67% respectively.

Key words: Sub clinical mastitis, buffaloes, Surf Field Mastitis Test, Peri urban, Rural, Nile Ravi, Kundi

Introduction:

Mastitis is renowned across the globe as serious and the most expensive disease of mulching animals (Lightner et al., 1988). It is an inflammatory condition of the udder irrespective of the cause and results in huge economic loses (Ahmad, 2001). An estimated loss of 35 billion US dollars worldwide caused by mastitis alone. Ratafia (1987) stated that losses might be much more in Pakistan due to mal practice of teat dipping and dry period antibiotic therapy as a preventive measure (Arshad, 1995). Field surveys of various diseases throughout Pakistan depicted that sub clinical mastitis is an important health hazard (Ajmal, 1990). Besides its drastic effects on economy it is important from zoonotic and milk processing point of view because the mastitic milk harbors different organism which are potentially pathogenic for humans and processing of affected milk results in substandard byproducts (Muhammad et al., 1995). Sub clinical mastitis is the most dreadful disease confronting the dairy industry throughout the world but the situation in Pakistan is particularly very alarming and demands great attention for its control because of high economic losses to this disease. Mastitis is one of the limiting factors in the development of dairy industry in Pakistan. In addition to causing colossal economic losses to the farmers, the disease is important from consumer's and milk processor's point of view. This is because the milk from affected animal may harbor the organisms potentially pathogenic for humans (Zoonosis) and processing of such milk results in sub optimal output of substandard finished fermented products like yogurt, cheese, etc. (Muhammad et al., 1995).

Mastitis has also been implicated in decreasing reproductive performance (Cullor, 1991; Moore et al., 1991; Moore & O'Connor, 1993). According to Ratafia (1987) annual losses caused by this disease were nearly $35 billion at world level. In Pakistan, statistics of current losses due to this disease are not available although it was estimated two decades ago that in Punjab province alone, the total losses caused by clinical mastitis amounted to Rs. 240 million per annum (Schltz et al., 1978). It is pertinent to mention that this survey did not take into account the losses caused by sub-clinical mastitis.
Mastitis is the outcome of interaction of interaction of different factors associated with the host, pathogen (s) and the environment. Infectious agents, in particular various species of bacteria, are the most important etiologic agents of mastitis,.

More over increased somatic cell count and reduces protein, lactose, fat levels were observed in mastitis affected milk which results in reduced shelf life of processed milk and milk products (Said and Abd-el-Malik, 1968).
Susceptibility of cattle and Buffaloes to mastitis differs between both species and farmer mentioned are more susceptible (Thapa and Kaphle, 2002). Sub clinical mastitis causes a tremendous increase in the number of white blood cells in the milk. In the context of milk, these cells are called milk somatic cells
Somatic cells are part of the natural defense mechanism and include lymphocytes, macrophages, polymorpho-nuclear cells and some epithelial cells (Pillai et al., 2001). Somatic cell count (SCC) can be measured quantitatively by California mastitis test (CMT). It is a simple, easy and low cost screening test for subclinical mastitis at dairy farms (Dingwell et al., 2003). Validity of CMT in diagnosis of infected quarters was established in various Milking stages (Dingwell et al., 2003; Gharagozloo et al., 2003).

Commonly isolated pathogens from mastitis milk are pathogenic and 70-80 % of all mastitis cases were infected with either staphylococcus aureus or streptococcus agalactiae in cows and buffaloes (Memon et al., 1999 and Ali et al, 2008). No obvious clinical signs like swelling of quarters as well abnormality of milk observed in sub-clinical mastitis. Only can be diagnosed by laboratory test and a common farmer is not aware with sophisticated test and techniques. So the present study was designed to investigate the prevalence of sub-clinical mastitis in Buffaloes in Rural and Peri Urban area of District Peshawar. The objective of this study was the isolation of different types of microorganisms associated with sub clinical mastitis in buffaloes kept under field conditions in District Peshawar, Pakistan.

Materials and Methods:

Study was conducted in and around Peshawar District. For this purpose eight hundred milk samples from 800 quarters were collected from apparently healthy animals from November, 2012 to April, 2013. 100 samples from each buffaloe breed i.e. Kundi and Nili Raviwere were collected. In field, milk samples were tested for mastitis through Surf Field Mastitis Test and positive samples were transported to Veterinary Research Institute, Peshawar (Mastitis Section) and Dairy Technology Laboratory Livestock Research and Development Station Surezai, Peshawar. The principal of SFMT is that when milk samples mixed with detergent, rupture of somatic cells occurs and DNA along with cellular contents come out. Detergent and DNA forms gel, gel consistency depends upon the number of somatic cells. For the purpose of Study, 03 % surf solution was made by adding three grams of common detergent powder (Ariel by Procter & Gamble) in 100 ml of water. Equal volume of milk samples and surf solution were mixed in a petri-dishes, change in consistency of milk depicted mastitis while healthy samples resulted no change in consistency. On the basis of severity, the mastitis is graded in to four categories from higher to lower intensity as, more severe P3 (+++), severe P2 (++), moderate P1 (+) and doubtful T (±) respectively (Muhammad et al., 1995). The 3% solution of a house-hold detergent viz. Surf (Unilever, Pakistan) was used for an early farmer's level detection and enumerated elevated somatic cell count under the microscope which was an indicator of hidden mastitis. This test has been named as Surf Field Mastitis Test. Desirable sensitivity was detected using SFMT because all other tests were too much expensive. Availability of the required reagent, i.e. Surf Excel Powder (Lever Brothers Pak Ltd.) was almost available in every village. Milk samples were cultured on Nutrient Agar by "Spread out technique" Lafi and Hailat (1998). Incubation of Plates at 37 ^{0}C for 24 hours done. Pure colonies were obtained by streaking and organisms were identified by Colony Morphology, Gram's staining and biochemical tests (John, 2000).

Results and Discussion:

Table 1: Prevalence of Mastitis in buffaloes in peri-urban and rural areas of Peshawar according to position of quarters.

Area	Animal Observed	Mastitic Animals		Hind Quarters		Fore Quarter	
		No.	%	No.	%	No.	%
Peri-Urban	120	61	50.83	41	67.21	20	32.78
Rural	80	29	36.25	20	68.96	9	31,03

The occurrence and prevalence of mastitis in buffaloes in per-urban and rural areas of district Peshawar is depicted in table (1). Prevalence of mastitis was assessed in population of dairy buffaloes raised under organized, small holdings and individual holdings farming conditions in peri- urban and rural areas shown in the table 1. Incidence of mastitis was found to be higher in peri-urban areas (50.83%) as compared to rural areas (36.25%). The herd size in per- urban areas is generally greater than that in rural areas. Mastitis is one of the major infections of buffalo playing havoc for economic loss in dairy farming and generally of contagious nature (Allore, 1993). As the herd size increases, the prevalence of mastitis increases (Fazal-ur-Rehaman, 1995). Another probable reason might be that in peri-urban areas, majority of the animals were milked by laborers rather than by their owners. The laborers could not be as careful as the stock owners. The laborer could not be as careful as the stock owners themselves. Therefore, due to their careless attitude in milking, incidence of mastitis increases. In addition brick floor/ hard bedding and dirty floor could also be a predisposing factors leading to higher incidence of mastitis in peri- urban areas (Joseph, 1996). These findings are in line with those of Kalara and Duanda (1964) who reported that prevalence of mastitis was more in peri- urban than rural areas. Variation in prevalence of mastitis might be due to the different regions, therapeutic practices, manage mental conditions and presence of microorganisms in environment.

Table 2: Frequency distribution of various bacterial, fungal and algae genera isolated from milk samples positive for sub clinical mastitis.

Name of the bacteria	Total cases		Total Positive Cases		No. of Isolates		Frequency	
	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi	Kundi	Nili Ravi %	Kundi%
	100	100	47	43				
Streptococcus agalactia					17	14	36.17	32.55
Escherichia coli					2	3	4.25	6.97
Bacillus cereus					2	4	4.25	9.30
Streptococcus dysgalactiae					3	1	6.38	2.32
Salmonella spp					2	1	4.25	2.32
Yeast					1	2	2.12	4.65
Protothecad					1	3	2.12	6.97
Staphylococcus aureus					19	15	40.42	34.88
Total	100	100	47	43	47	43	47%	43%

A total of 90 isolates 47 from Nili Ravi buffaloes and 43 from Kundi buffaloes were found positive in which 8 different microbial species were recovered (Table 2). Staphylococcus aureus was the most frequently recovered bacterial species in both Nili Ravi and Kundi accounting 40.42% and 34.88% respectively of all isolates, followed by streptococcus agalactiae 36.17% and 32.55%, Bacillus cerueus 4.25% and 9.30%, Escherichia coli 4.25% and 6.97%, Streptococcus dysgalactiae 6.38% and 2.32%, Protothecad 2.12 and 6.97%, Salmonella spp 4.25% and 2.32% and Yeast 2.12% and 4.65% respectively. Mastitis is the inflammation of mammary glands. It is the outcome of various factors associated with the host, environment and the pathogens. Among pathogens, bacteria are by far the most frequently associated etiological agent of the disease Ali et al., (2008). Different bacterial genera as Stphylococcus, Streptococcus and Escherichia cause clinical bovine mastitis (Allore, 1993; Ahmad, 2001). These findings are in agreement with our findings because animal surroundings such as bedding and manure are source of common contagious pathogens, these pathogens may be present in soil and air as environmental microorganisms and are considered the basic cause of mastitis. Milker's hands, cloth, towels and flies spread these pathogenic bacteria to clean udders during the milking process and responsible for most of the sub clinical cases (Allore, 1993).

Pitkala et al., (2004) reported microbial growth in 21-33% of milk samples, whereas, Iqbal et al., (2004) reported only 15.16% in dairy buffaloes, whereas our findings resulted 47% and 43% mastitis in Nili Ravi and Kundi buffaloes. This variation may be due to season, manage mental conditions at the farm, area tranportation conditions, difference in sample handling in the laboratory and use of antibiotics. In the present study, Staph. aureus 40.42% and 34.88% in Nili Ravi and Kundi buffaloes was isolated as top ranking pathogen from cases positive for mastitis. In previous studies, it was also reported as major pathogen (Kapur et al., 1992; Allore, 1993; Rabello et al., 2005; Arshad et al., 2006; Ebrahimi et al., 2007; Ali et al., 2008 Botrel et al.,2009). Ebrahimi et al., (2007) reported 8.33% Streptococcus agalactiae and 9.44% E.coli isolates from subclinical bovine mastitis milk samples while Ali et al ., (2008) reported 30% growth of Strep. agalactiae, Staphylococcus aureus and Strep. dysgalactiae these findings are in line with our results in which the same pathogens were obtained from the 82.23% of the positive cases and similar findings have been reported by Ahmad et al.,(2001) and Allore (1993). The previous studies conducted in India (Joshi and Gokhale, 2006), Indonesia (Estuningsih et al., 2002) and Italy (Moroni et al., 2006) endowed that clinical as well as sub-clinical mastitis in dairy animals is predominantly contagious in nature.

Table 3: Quarter wise prevalence of sub clinical mastitis in Nili Ravi and Kundi buffaloes in and around District Peshawar.

Buffaloes Species	Area	Total Quarter tested	Affected quarters				One Positive Quarter		Two Positive quarters	
			Front right	Front left	Rear right	Rear left	No.	%age	No.	%age
Nili Ravi	Peshawar	400	21(11.17%)	19(10.1%)	80(42.5%)	68(36.17%)	128	68.08	60	31.91
			Total Nili Ravi Affected quarters 188 (47%)							
Kundi	Peshawar	400	17(9.88%)	13(7.55%)	61(35.46%)	81(47.09%)	133	77.32	39	22.67
			Total Kundi Affected quarters 172 (43%)							

Prevalence of quarter wise and animal wise mastitis was assess in population of dairy buffaloes raised under organized, small holdings and individual holdings farming condition in Peshawar District using Surf Field Mastitis Test. In this present study the quarter wise prevalence was found to be 47% and 43% respectively. Contrary to our findings, Butt, (2005) reported higher prevalence of mastitis (96.36%). The causes of this difference might be manage mental condition, method of detection, breeds of animals, immune response and climatic condition, animal health, stress, hence immunity decreases and manage mental conditions are probably risk factors of mastitis and may

be explain the difference in prevalence of Subclinical mastitis observed in the present and previous studies. The value of incidence of mastitis reported in this study 47% and 43% was similar to that obtained in France. Longe et al. (1994) reported a prevalence of 25% in the basis of California Mastitis Test. In Spain, (Ares et al., 1995) used cultural examination and reported a figure of 33.5% for cow. In venezuela, (Ferraro et al., 1999) reported a prevalence of 30.18% of subclinical mastitis in cows, on the basis of direct, indirect and cultural examination. Helelll (2012) observed the prevalence of 50% in cows (quoted by Bouaziz, 2005), Bachaya et al., (2011) used Surf Field Mastitis (SFMT) and found that 45% buffaloes suffered from subclinical mastitis these results are in line with our findings. Hussain et al., (1984) documented a prevalence of 33% in cows and 8 percent in buffaloes on the basis or results of Whiteside test. Shah (1987) used Ciba-Geigy Mastitis test and found that 34.48% buffaloes suffered from sub- clinical mastitis. Anwar and Chaudry (1978) reported a prevalence of 47.5 % in buffaloes after using Strip Cup test, pH test and Whiteside test. The difference in prevalence of sub-clinical mastitis observed in the present and the previous studies may be due to differences in immune response of animals and climatic conditions, Hygienic condition and applications of early mastitis detection methods. The milk samples from udder quarters affected with sub-clinical mastitis showed floccules or gel formation when subjected to Surf Field Mastitis Test. In a previous study, quarter-wise milk samples were examined by Surf Field Mastitis Test to determine the prevalence of mastitis (Muhammad et al., 1995). Fresh milk was collected from buffaloes considered apparently normal. Present study focused on determining the percentage of sub clinically affected animals. Gel formation was divided into four categories i.e. += moderate, ++=Severe, +++= more severe, ++++=very severe. Only first two conditions were present, while other two were absent.

In the study area, there are extreme weather conditions and also deficiency of fodder is a major problem. These factors may favor mastitis and create stress to the body; hence immunity decreases, leading to increased sub- clinical incidence associated with contagious and environmental mastitogens. In the sub-clinical mastitis the most important factor affection somatic cell count in milk is mammary gland infection (Eberhart et al., 1979; Reneau, 1986). Inflammation of mammary glands increases the number of somatic cells in milk. When milk from sub- clinically mastitis quarters is mixed with anionic detergent solutions such as California Mastitis Test or Surf Field Mastitis Test reagent, a Chemical reaction causes the gel formation (Schalm et al., 1971). Ideally milk from all four quarters should be tested separately. Mastitis milk is unwholesome for human consumption due to the presence of bacteria and their toxins, as well as high number of white blood cells. Non-infected animals should be milked ahead of infected ones. Using a simple test like Surf Field Mastitis Test, farmers should test their dairy animals before purchasing, if positive; avoid buying such animals.

Conclusion and Recommendations:

The cases of mastitis can be reduced to an appreciable extent and the production can be increased by adopting following manage mental measures on priority basis, 1. Preventing the calf to cause any injury on teat/udder, 2. Full hand milking should be practiced, 3. Keeping the animals on wet and dirty floors should be discouraged, IV. Pacca floor must be even and properly bedded.

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