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PREVALENCE OF SUB CLINICAL MASTITIS IN DAIRY BUFFALOES OF PUNJAB, PAKISTAN

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ABSTRACT

Six hundred lactating dairy buffaloes from four districts (Lahore, Sialkot, Narowal and Okara) of Punjab, Pakistan were screened for subclinical mastitis using White Side Test and subsequent bacterial isolation. Overall prevalence of subclinical mastitis was 44% (264/600). It was the highest (58%) in animals kept as individual holding at backyards followed by small holdings in periurban area (42%) and the lowest at organized farms with reasonable good managemental conditions (32%). Two hundred thirty four bacterial isolates of nine genera i.e. *Staphylococci, Escherichia, Streptococci, Pseudomonas, Salmonellae, Bacillus, Klebsiella, Enterococci* and *Corynebacterium* species were identified. The highest prevalence was of *Staphylococci* (28.32%) followed by *Escherichia coli* (16.18%), *Pseudomonas* (13.29%), *Bacillus* (12.42%), *Streptococci* (7.51%), *Salmonellae* (7.22%), *Corynebacterium* (6.64%), *Klebsiella (*5.20%) and *Enterococci* (3.17%).

Key words: Buffaloes, Subclinical Mastitis, White Side Test, Prevalence, Pakistan.

INTRODUCTION

Mastitis is one of the devastating maladies of milch animals causing huge production losses to livestock industry in Pakistan. Mastitis has been recognized as one of the most economically important diseases affecting dairy animals worldwide (Lightner *et al.,* 1988; Kanenne and Hurd, 1990; Miller *et al.*, 1993; Kossaibati *et al.,* 1998; Chishty *et al*., 2007). It causes production losses in the form of condemned milk, loss in milk yield, earlier culling of animals and replacements (Khan and Khan, 2006). Clinical mastitis can cause 10% or more milk loss on the quarter level (Dijkhuizen and Renkema, 1978) and 10% decrease in the remaining lactation after acute mastitis (Blowey, 1986). Symptoms are though not evident in subclinical mastitis (Blosser, 1979) however, milk yield can drop as much as 20% per infected quarter (Schepers and Dijkhuizen, 1991). Subclinical mastitis is hence a major problem in dairy buffaloes of Punjab, kept under various farming systems and deserves attention due to its potential impact on milk production and food security. Subclinical mastitis causes two third losses of the total milk production due to affected quarters of animal (Radostits *et al.,* 2007).

Most of the common pathogens isolated from mastitic milk are contagious. Bilal *et al.* (2004) reported 16.72 and 21.08% clinical mastitis among cattle and buffaloes, respectively. According to a report 70-80 % of all the clinical and subclinical mastitis cases were either infected with *Staphylococcus aureus* or *Streptococcus agalactiae* in cows and buffaloes (Memon *et al.*, 1999). Subclinical mastitis can be diagnosed by somatic cell counts, California Mastitis Test, White side test (WST) or Surf field mastitis test (Muhammad *et al.,* 1995; Muhammad *et al.,* 2010). In the present study, incidence of subclinical mastitis was assessed in dairy buffaloes kept under different farm managemental conditions in four districts of Punjab as well as the prevalence of pathogenic bacteria in infected animals was determined.

MATERIALS AND METHODS

Six hundred lactating buffaloes from organized, small holding and individual holding private dairy buffalo farms in four districts of Punjab (Narowal, Lahore, Okara and Sahiwal) were screened for subclinical mastitis. A total of 2400 milk samples were collected in sterilized screw caped test tubes. An on-farm screening was performed and milk samples declared positive for sub-clinical mastitis were transported to the laboratory. White Side Test (WST**)** was performed for screening of subclinical mastitis as described by Barnumt and Newbouldt (1960). A drop of 1N Sodium Hydro Oxide solution (NaOH) was mixed with 5 drops of milk on a glass plate and stirred with an applicator for 10 seconds. The gel formation immediately after mixing was considered as positive and no gel formation as negative for subclinical mastitis.

Collected milk samples were primarily cultured on blood agar using 'spread out technique' as described by Lafi and Hailat (1998). The plates were incubated for 24 hrs at 37°C. Bacterial growth was purified by streaking and Gram's staining was performed to study the morphology of bacteria. Pure bacterial cultures were

identified on the basis of cultural, morphological and biochemical characteristics (John, 2000).

RESULTS AND DISCUSSION

Subclinical mastitis is one of the major infections of cattle and buffalo playing havoc for economic loss in dairy farming and the incidence is increasing day by day. Amongst multiple reasons of mastitis, sustained level of wide variety of microbes, particularly different bacterial species is the most vital one. Bacteria which are encountered in most of the mastitis cases in cattle and buffaloes are *Staphylococcus aureus*, *Streptococcus agalactiae* and *E. coli* (Giraudo *et al*., 1997; Razzaq, 1998). The results of White Side Test are presented in Table 1.

Table 1: Animal wise prevalence of subclinical mastitis in Buffaloes screened by White Side Test

Type of Farm	Total Number of Animals	Animals positive for Mastitis	Prevalence Percent
Organized	200	64	32
Small holdings	200	84	42
Individual holding	200	116	58
Total	600	264	44

Prevalence of subclinical mastitis was assessed in population of dairy buffaloes raised under organized, small holdings and individual holdings farming conditions in Narowal, Lahore, Okara and Sahiwal using White Side Test (WST). The prevalence was higher at individual holding buffalo farms (52%) as compared with small holdings (48%) and organized (32%) farms. Difference may be due to poor hygienic and managemental conditions. Cumulative percentage of mastitis in buffaloes observed by WST was 44% which is much lower than that of 92% recorded by Lafi and Hailat (1998). Similarly, an incidence of 54.7, 32.85 and 23.18% has been reported by Getahun *et al*., (2007), Pitkala *et al*., (2004) and Iqbal *et al*., (2004), respectively. Variation in prevalence of mastitis might be due to the different regions, therapeutic practices, management conditions and presence of microorganisms in environment. The data of district wise prevalence for subclinical mastitis is presented in Table 2. Prevalence recorded was the highest (48%) for district Narowal followed by 40.67% for Lahore, 42% for Okara and 45.33% for Sahiwal, respectively. This variation may be due to difference in qualified veterinary cover, availability of quality medicine and managemental trends.

Table 2: District wise prevalence of subclinical mastitis screened by White Side Test

Districts	Total Number of Animals	Animals positive for Mastitis	Percent Prevalence
Narowal	150	72	48
Lahore	150	61	40.67
Okara	150	63	42
Sahiwal	150	68	45.33
Total	600	264	44

The percentage of bacterial growth obtained from mastitis positive milk samples of different districts is recorded in Table 3.

Table 3: Numerical bacterial growth isolated from milk samples positive for mastitis in different Districts

Area	No. of Samples Positive	No. of Samples Yielded Bacterial Growth	Percen-tage	No. of Isolated Bacteria
Narowal	156	41	26.28	90
Lahore	130	37	28.46	89
Okara	134	35	26.11	79
Sahiwal	146	40	27.39	88
Total	566	153	27.03	346

Different bacterial genera as Staphylococcus, Streptococcus and Escherichia cause clinical bovine mastitis (Allore, 1993; Ahmad, 2001). 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. Milker's hands, cloth towels and flies spread these pathogenic bacteria to clean udders during the milking process and are responsible for most of the clinical cases (Allore, 1993). The data of bacterial isolates is presented in Table 4. In the recent study, bacterial growth was observed only in 27.03% of milk samples. Bacteria identified were of nine different genera as *Staphylococcus* (28.32%), *E. coli* (16.18%), *Streptococcus* (7.51%), *Pseudomonas* (13.29%), *Salmonella* (7.22%), *Bacillus* (12.42%), *Klebsiella* (5.20%), *Enterococcus* (3.17%) and *Corynebacterium* (6.64%).

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. This variation may be due to season, managemental conditions at the farm, area, transportation conditions, difference in sample handling in the laboratory and use of antibiotics.

In present study, *Staph. aureus* (26.92%) 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) obtained 30% growth of *Strep. agalactiae* and *Strep. dysgalactiae* and 42.6% *Staph. aureus*. Contaminated environment of farm is a main source of coliforms and mostly cause clinical infections. Ebrahimi *et al*. (2007) obtained 3.88% coagulase-negative staphylococci (CNS), 8.33% *Streptococci* other than agalactiae and 9.44% *E. coli.* Botrel *et al.* 2009 isolated 30.2% CNS, 13.7% coagulase-negative staphylococci and 9.3% *Strep. dysgalactiae* from subclinical mastitis milk samples.

Mastitis control programs reported in recent years may need to be evaluated under our farming systems and local conditions.

Table 4: Various Bacterial Genera isolated from milk samples positive for mastitis

Name of Bacteria	District				Total No of Isolates	Percentage
	Narowal	Lahore	Okara	Sahiwal		
Staph. aureus	30	21	18	29	98	26.92
E. coli	15	14	15	12	56	15.38
Strep. aglactiae	05	07	08	06	26	7.14
Strep. Dysaglactiae	03	05	06	04	18	4.94
Pseudomonas	15	14	05	12	46	12.63
Salmonellae	05	07	07	06	25	6.86
Bacillus	10	12	10	11	43	11.81
Klebsiella	00	07	05	06	18	4.94
Enterococcus	05	00	06	00	11	3.02
Corynebacteria	05	07	05	06	23	6.31
Total	93	94	85	92	364	100

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