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Research Article

PREVALENCE OF BUBALINE SUBCLINICAL MASTITIS ALONG WITH MICROBIAL PROFILE AND SENSITIVITY PATTERN

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ABSTRACT

A total of 280 quarter milk samples collected from 71 Murrah buffaloes in 2014, were screened for prevalence of subclinical mastitis (SCM) by cultural examination, somatic cell count (SCC), California mastitis test (CMT) and electrical conductivity (EC) test. Overall animal-wise prevalence of SCM on the basis of cultural examination and SCC was found to be 15.49 and 8.21%, respectively whereas quarter-wise it was 7.04 and 2.86%, respectively. On the basis of CMT and EC, quarter-wise prevalence was 2.85 and 17.14%, respectively and animal-wise prevalence was 7.04 and 25.35%, respectively. Following International Dairy Federation (IDF) criteria, prevalence of specific SCM, latent mastitis and nonspecific mastitis was found to be 1.43, 6.79 and 1.43%, respectively. Staphylococci (56.52%) and Streptococci (43.47%) were the organisms isolated on bacteriological examination. Staphylococci and Streptococci showed 100% sensitivity towards chloramphenicol, enrofloxacin, gentamicin, cefoperazone and ceftriaxone. In conclusion, IDF criteria based on cultural examination and SCC depicts reliable, comprehensive and true picture of prevalence of subclinical mastitis in buffaloes at the farm.

Key words: California mastitis test, cultural examination, electrical conductivity, somatic cell count, subclinical mastitis, .

Mastitis is an important production disease associated with reduction in milk yield, alterations in chemical composition of milk, loss of genetic potential and considerable economic losses to dairy farmers. In India, economic losses due to mastitis have been estimated to be Rs. 72 billion per year (Bansal and Gupta, 2009); posing a serious potential constraint for dairy sector development. Subclinical form of the disease is more significant due to its tricky diagnosis and more prevalence (15-40 times) than its clinical counterpart (Prabhu *et al*., 2015; Charaya *et al*., 2015). Subclinical mastitis (SCM) usually persists longer in the herd and serves as a continuous source of infection to other animals. The understanding of the pathogens involved is relevant for establishment of risk factors and formulating strategies for prevention and control of SCM. Therefore, the aim of present study was to investigate the prevalence of SCM at an organized buffalo farm along with microbial profile and antimicrobial sensitivity pattern.

MATERIALS AND METHODS

Source of Milk Samples: Apparently healthy, lactating buffaloes (71) reared at LUVAS farm, Hisar were included in the present study. A total of 280 quarter milk samples were collected aseptically during morning and immediately transported to laboratory on ice.

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Bacteriological Examination: The milk samples were shaken thoroughly and 0.01 ml of the milk was streaked on 5% sheep blood agar and MacConkey's lactose agar plates. The plates were incubated aerobically at 37^0C for 24 to 48 h. Subcultures of the resulting growth were made on blood agar for purification of isolates. Preliminary identification was done on the basis of Grams reaction, morphology, colony characteristics, catalase test and hemolysis patterns.

Somatic Cell Count: The somatic cell count (SCC) on milk samples was performed as described by Schalm *et al*. (1971) and the milk smears were stained with modified Newman-Lampert stain (HiMedia, Mumbai).

California Mastitis Test: The California mastitis test (CMT) was performed as per method Doxy (1985). The positive result was shown by the development of a viscous gel that tended to swirl towards the centre. The milk samples showing viscous gel in the range of '++' and above grade were taken as positive for SCM.

Electrical Conductivity Test: The change in the electrical conductivity (EC) of milk due to change in Na^+ and K^+ ions during mastitis was recorded with the help of a hand held milk checker (Eisai Co. Ltd., Tokyo, Japan).

***In-vitro* Drug Sensitivity Pattern:** Different strains of organisms isolated from udder infections were subjected to *in-vitro* drug sensitivity testing using 13

antimicrobials by disc-diffusion method (Bauer *et al.*, 1966). The sensitivity was observed on the basis of zone size interpretation chart provided by the manufacturer.

RESULTS AND DISCUSSION

Overall animal-wise prevalence of SCM on the basis of cultural examination and SCC on this farm was 15.49% and 8.21% whereas quarter-wise it was 7.04% and 2.86%, respectively. Almost similar results have been reported earlier (Charaya *et al*., 2013; Al-Zainy and Al-Jeburii, 2015). In contrast to our study, Kaliwal *et al*. (2011), Mir *et al*. (2014) and Prabhu *et al*. (2015) reported very high animal-wise and quarter-wise prevalence of SCM in buffaloes. Variations in prevalence rates might be due to adoption of different management and hygienic practices in dairy herds. Increased risk factor for mastitis are age, parity, breed, milk yield, udder morphology, immunity, genetic resistance and stage of lactation. The housing conditions, temperature, stress and climatic conditions also affect the prevalence of mastitis.

In buffaloes following IDF criteria, prevalence of SCM (SCC above 5,00,000/ml of milk and culturally positive), latent mastitis (SCC below 5,00,000/ml of milk but were culturally positive) and nonspecific mastitis (SCC above 5,00,000/ml of milk and culturally negative) was found to be 1.43%, 6.79% and 1.43%, respectively. In contrast to present study, Charaya *et al*. (2013) and Singh *et al.* (2014) reported high prevalence of SCM at the same farm that may be due to seasonal difference. The previous studies on this farm were carried out in summer season whereas; the present work was carried out in winter season. Sindhu *et al*. (2009) and Pankaj *et al*. (2013) reported low occurrence of latent mastitis in 3.51% and 4.6% quarters, respectively. The significance of latent mastitis cannot be undermined, since some of these are likely to convert into sub-clinical form followed by clinical mastitis particularly under unfavourable environmental conditions. Charaya *et al*. (2013), Pankaj *et al.*(2013) and Singh *et al*. (2014) reported 2.62%, 3% and 3.95% quarters having non-specific mastitis, respectively. Failure to detect pathogens in such cases might be due to intermittent excretion of the organisms or their disappearance because of spontaneous recovery. Possibility of mastitis due to mycoplasma or mycobacteria may not be ruled out in such cases, since these organisms cannot be cultivated on common bacteriological media.

By CMT and EC, quarter-wise prevalence was found to be 2.85% and 17.14% and animal-wise prevalence was reported as 7.04 and 25.35%, respectively. CMT is considered as a rapid and characteristics indicator for the intramammary infections followed by SCC (Viguier *et al*., 2009). However, scoring of the CMT by farm worker depends on variation due to its subjective nature, false positive reactions occurring at early or late lactation (Kamal *et al*., 2014). EC of the milk increases due to an increased concentration of Na^+ and Cl^- and subsequent loss of predominantly lactose and K^+ (Kasikci *et al*., 2012). False positive rates have been associated with use of EC thereby limiting its potentials as a diagnostic tool for mastitis detection. Factors other than mastitis like breed, lactation stage, milking interval and milk composition may affect milk EC (Kamal *et al.,* 2014).

A total of 23 organisms were isolated from culturally positive quarters. Staphylococci (56.52%) and streptococci (43.47%) were the major organisms isolated. Charaya *et al.*(2013), Mir *et al*. (2014) and Ali *et al*. (2015) reported persistently highest prevalence of staphylococci in SCM cases. Streptococci were the second most prevalent organisms associated with SCM in present study which isin agreement to findings of Pankaj *et al.* (2013) while in contrast to our study Kurjogi and Kaliwal (2011) observed *E. coli* being the second most prevalent organism.

Staphylococci showed 100% sensitivity towards chloramphenicol, enrofloxacin, gentamicin, amikacin, cefoperazone and ceftriaxone whereas comparatively lesser sensitivity towards neomycin (92.3%), streptomycin (76.92%), penicillin (61.53%), ampicillin (69.23%), amoxicillin (76.92%), oxytetracycline (84.61%) and cloxacillin (69.23%). Streptococci showed 100% sensitivity towards neomycin, enrofloxacin, gentamicin, streptomycin, chloramphenicol, ceftriaxone and cefoperazone whereas 80 to 90% sensitivity towards oxytetracycline, penicillin, ampicillin, amoxicillin, cloxacillin and amikacin was found in present study. Pankaj *et al*. (2013) and Charaya *et al*. (2013) also reported high sensitivity of streptococci towards enrofloxacin, cefoperazone, ceftriaxone and gentamicin. Mir *et al*. (2014) reported enrofloxacin as the most effective drug followed by erythromycin and tetracycline.

Our study indicates that IDF criteria based on cultural examination and SCC depicts reliable, comprehensive and true picture of prevalence at farm in comparison to prevalence determined by cultural examination, SCC, CMT or EC alone which may provide false positive results. Surveillance of

antimicrobial resistance is important to ensure optimal results of antimicrobial use and also minimizes the risk for selection and spread of antimicrobial resistance.

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