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Research Article

SUBCLINICAL MASTITIS AT AN ORGANISED FARM: PREVALENCE, ETIOLOGY AND ANTIBIOGRAM

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ABSTRACT

A total of 262 quarters of 66 apparently healthy dairy buffaloes at an organised farm were screened for subclinical mastitis. Following IDF criteria the quarter-wise prevalence of subclinical, latent and non-specific mastitis was 4.58% (SCC> 500,000/ml and culturally positive), 13.74% (SCC <500,000/ml and culturally positive) and 2.62% (culturally negative and SCC> 500,000/ml), respectively. Out of 48 culturally positive quarters, 50 isolates were recovered and two quarters had mixed infection. *Staphylococcus* spp. (n=32) was observed to be the predominant pathogen followed by *Streptococcus* spp. (n=18). All the isolates were subjected to antimicrobial sensitivity test by disc diffusion method. Staphylococci showed 100% susceptibility to ampicillin, cloxacillin, amoxycillin, ceftriaxone and cefoperazone followed by 95% susceptibility to penicillin, chloramphenicol, tylosin and enrofloxacin. Streptococci revealed 100% sensitivity towards chloramphenicol, ampicillin, enrofloxacin, amoxycillin, ceftriaxone and cefoperazone.

Key words: Subclinical mastitis, prevalence, staphylococci, streptococci, antimicrobial sensitivity

Mastitis is an inflammatory reaction of the udder parenchyma which is caused by the bacterial infection and is characterized by physical, chemical and bacteriological changes in milk and glandular tissue (Radostits *et al*., 2000). Mastitis occurs in two forms viz. clinical and sub-clinical. Clinical mastitis is easily detected by abnormalities in milk or udder whereas clinical signs are not apparent in subclinical mastitis (SCM) thus posing difficulty in early diagnosis. Subclinical form of the disease is important because it is 15 to 40 times more prevalent than its clinical counterpart and usually precedes the clinical form.The selection of antibiotic for treatment of SCM depends upon the type of pathogens involved. Therefore, milk culture and antimicrobial sensitivity testing must be undertaken before developing a treatment protocol. Losses due to mastitis include reduced milk yield, increased culling rate, discarded milk and antibiotic treatment costs. In India, Dua (2001) has reported annual losses due to mastitis to the tune of Rs 60.5321 billion of which, Rs. 43.6532 billion has been attributed to subclinical mastitis. The present investigation was undertaken to diagnose SCM and to determine its aetiology and antimicrobial sensitivity pattern.

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MATERIALS AND METHODS

Source of Milk Samples: A total of 262 quarter buffalo milk samples were collected aseptically from 66 apparently healthy buffaloes of Murrah breed located at an organized farm, Hisar, Haryana.

Bacteriological Examination of Milk: The prevalence of SCM was determined following International Dairy Federation Criteria based on bacteriological examination of milk and somatic cell count (SCC). For bacteriological examination, milk samples were inoculated on blood agar and MacConkey's lactose agar plates and incubated at 37^0C for 18-24 hours. Bacterial identification was carried out by studying morphology, culture characteristics and Gram's staining (Quinn *et al*., 2004).

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

Antimicrobial Sensitivity Testing: A standard disc diffusion method (Bauer *et al*.,1966) was employed for carrying out antimicrobial sensitivity test using 14 antimicrobials viz., ampicillin (25 mcg), ceftriaxone (30 mcg), amoxycillin (10 mcg), streptomycin (25 mcg), cloxacillin (30 mcg), chloramphenicol (30 mcg), neomycin (30 mcg), tylosin (15 mcg), enrofloxacin (10 mcg), cefoperazone (75 mcg), amikacin (30 mcg), penicillin (10

units), gentamicin (30 mcg), and oxytetracycline (30 mcg) (Hi media). Zones of inhibition were measured and the results were interpreted as sensitive (S), intermediate (I) and resistant (R) using the chart provided by manufacturer.

RESULTS AND DISCUSSION

The prevalence of SCM was found to be higher on the basis of cultural examination (28.78%) as compared to SCC (>5x10^5/ml; 13.63%) alone (Table 1). Quarter-wise prevalence by SCC and bacteriological examination was 7.25% and 18.30%, respectively. Pankaj *et al.* (2013) also reported higher prevalence by bacteriological examination which might be due to the presence of resident microorganisms contaminating the milk. By IDF criteria, 4.58% (SCC above 500,000/ml of milk and culturally positive), 13.74% (SCC below 500,000/ml of milk but culturally positive) and 2.62% (culturally negative and SCC above 500,000/ml) of the quarters were found to suffer from subclinical, latent and non-specific mastitis, respectively (Table 1). In contrast to our study, Sindhu *et al.* (2009) reported very low prevalence of SCM at the same farm. However, Sharma and Sindhu (2007), Chavan *et al*. (2007), Kurjogi and Kaliwal (2011) observed higher prevalence of SCM. Since bacteriological examination requires aseptic collection and SCC is influenced by several physiological factors (climatic condition, parity, stage of lactation), it is therefore, suggested that prevalence should be determined by both the tests following IDF criteria.

Of the 48 culturally positive quarters, a total of 50 isolates were recovered and two quarters showed mixed infection. *Staphylococcus* spp. was found to be predominant pathogen (64%) followed by streptococci (36%). Several reports (Chavan *et al*., 2007; Sharma and Sindhu, 2007; Roychoudhury and Dutta, 2009; Pankaj *et al*., 2013) from India have indicated staphylococci and streptococci to be the main etiological agents of mastitis.

The antibiotic susceptibility test revealed that *Staphylococcus* spp. were 100% susceptible to ampicillin, cloxacillin, amoxycillin, ceftriaxone and cefoperazone followed by 95% susceptibility to penicillin, chloramphenicol, tylosin and enrofloxacin (Fig. 1). *Staphylococcus* spp. isolates were found least susceptible (85%) to amikacin, neomycin and oxytetracycline. The results of this study with respect to cloxacillin, cefoperazone and amoxycillin are in close agreement with that of Malinowski *et al*. (2008). However, Ranjan *et al.* (2010) reported higher resistance of mastitis pathogens to cloxacillin, ampicillin and amoxycillin. While results with regard to enrofloxacin and amikacin were similar to sensitivity pattern observed in this study.

Chloramphenicol, ampicillin, enrofloxacin, amoxycillin, ceftriaxone and cefoperazone showed 100% sensitivity to streptococci spp. (Fig. 1). Higher sensitivity (90.90%) was also observed for amikacin, cloxacillin and tylosin and least sensitivity to neomycin (63.63%). Results of the sensitivity pattern to enrofloxacin, cefaperazone and amikacin are in close agreement to those reported by Ranjan *et al.* (2010), whereas these were different in case of ampicillin, amoxicillin and ceftriaxone. Studies conducted by several workers (Sharma *et al*., 2007; Chavan *et al*., 2007; Roychoudhury and Dutta, 2009; Sharma *et al*., 2010) have showed increased resistance of mastitis causing pathogens to different traditional and newly introduced antibiotics. Indiscriminate use of antibiotics, irregular doses of antibiotics or under dosing of antibiotics may lead to resistant mutants. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in a particular area; therefore, antimicrobial sensitivity is suggested before institution of treatment. In contrast to these studies, the antibiogram obtained in the current study indicated higher sensitivity to newer and older antibiotics, showing rational use of these antibiotics at farms under study.

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Table 1

Prevalence of sub-clinical mastitis based on IDF criteria in buffaloes at an organized farm, Hisar

No of buffaloes/ quarters	Animals culturally positive	Animals showing SCC>5 lac/ml	Quarters culturally positive	Quarters showing			
				SCC>5 lac/ml and culturally positive	SCC>5 lac/ml and culturally positive	SCC<5 lac/ml and culturally negative	SCC>5 lac/ml
66/262	19 (28.78%)	9 (13.63%)	48 (18.32%)	19 (7.25%)	12 (4.58%)	36 (13.74%)	7 (2.67%)

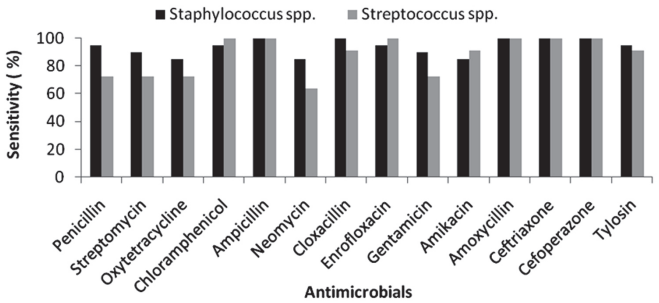

Fig 1. Sensitivity (%) of *Staphylococcus* and *Streptococcus* spp. to various antimicrobials.

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