PREVALENCE, ETIOLOGY AND ANTIBIOGRAM OF BACTERIAL ISOLATES RECOVERED FROM MASTITIS OF BUFFALOES

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ABSTRACT

The study was carried out in 1299 lactating buffaloes to determine prevalence and antimicrobial sensitivity of causative organisms of mastitis in Haryana State, India. Out of 4452 quarter milk samples tested, 1503 (33.76%) and 809 (18.17%) cases were found positive for subclinical mastitis and clinical mastitis, respectively. Majority of clinical cases of mastitis were of chronic nature, occurring during first two parities and first five months of lactation. *Staphylococci* (51.16%) were the most prevalent organism followed by *Streptococci* (37.94%), *Escherichia coli* (8.41%) and *Corynebacterium pyogenes* (1.62%). All the organisms except *E. coli* revealed high sensitivity against Ceftriaxone and Cefaperazone. *E. coli* isolates showed multiple resistance patterns. Regular monitoring of udder health and determination of antimicrobial sensitivity of causative agents prior to initiation of mastitis treatment is suggested.

Keywords: *Bubalus bubalis*, buffalo, mastitis, prevalence, etiology, antibiogram

INTRODUCTION

Buffalo farming holds the greatest promise for food security and sustainable growth in the 21st century. Mastitis continues to be a foremost problem of dairy buffaloes. Mastitis, the inflammation of the mammary gland, is a multi-etiological disease with varying degrees of clinical intensity and variations in duration. The disease is characterized by physical and chemical changes, usually associated with bacteriological changes in milk and pathological alterations of glandular tissue (Radostits *et al*., 2007). Mastitis is responsible for massive economic losses, quantitatively as well as qualitatively including shortened lactation period, premature culling of animals and treatment losses (Degraves and Fetrow, 1993). In India, about 3.84% and 24.43% of buffaloes, respectively, are affected with clinical and subclinical mastitis every year (Singh and Singh, 1994). The annual losses due to clinical and subclinical mastitis were estimated to be Rs. 6053.21 crores in India (Dua, 2001). Development of multiple drug resistance strains and emergence of new pathogens due to indiscriminate use of antimicrobials over long periods is a constant threat of increasing amounts of drug residues in milk, a potential biohazard

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(Restif and Koella, 2004; Raberg *et al*., 2007). Therefore, the present study was focussed on isolation of the microorganisms associated with mastitis in buffaloes and antimicrobial sensitivity of the isolates.

MATERIALS AND METHODS

Source of milk samples

Milk samples brought to College Central Laboratory, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana from July 2012 to June 2013 were analysed for bacterial culture isolation and their antibiogram. A total of 4452 quarter milk samples from 1299 buffaloes of different parts of Haryana were screened for mastitis pathogens.

Bacterial culture isolation and antibiogram

Bacterial isolation was performed from milk samples of different quarters on 5% defibrinated sheep blood agar and McConkey's lactose agar following method of Carter *et al.* (1995). The plates were incubated at 37°C overnight in an incubator. Bacterial isolates were identified by their colonial morphology and Gram's staining. Antimicrobial sensitivity was performed as per standard procedure of Bauer *et al.* (1966).

RESULTS AND DISCUSSION

Mastitis is the top ranking infectious disease of dairy industry (Sordillo and Streitcher, 2002; Zecconi, 2003) which causes tremendous losses to dairy farmers. Mastitis is a multi-etiological disease and a total of 137 bacterial species have been isolated from bovine intramammary infections (Watts, 1988; Hawari and Al-Dabbas, 2008), of which the most common being *Streptococcus* and *Staphylococcus* spp.

In the present study, out of 4452 quarter milk samples tested, subclinical mastitis was found more prevalent than clinical mastitis accounting for 1503 (33.76%) and 809 (18.17%) cases respectively. Clinical form of mastitis can easily be diagnosed as there is gross abnormality in milk whereas in subclinical mastitis apparently milk is normal hence most of times it goes unnoticed and contributes as a reservoir of microorganisms leading to the spread of infection to the other animals within herd. Sharif *et al.* (2007) also reported that sub clinical form is 15 to 40 times more prevalent than clinical form and may account for 70% to 80% of the total losses. The occurrence of clinical mastitis would be proportional to the prevalence of subclinical mastitis, because an existing sub-clinical phase of intramammary infection predisposes the former causing more economic losses due to decreased milk production, expenditure on treatment and culling (Sindhu *et al*., 2009). Therefore, timely detection of subclinical mastitis is very important to initiate proper treatment, control and preventive measures.

Parity wise prevalence of clinical mastitis is shown in Figure 1. Dhakal *et al.* (2007) also reported more incidence of mastitis in first and second calving which get decreased with increasing number of calving and is in line as reported by our study. High milk production in early lactation predisposes dairy animals to intramammary infections. Also, the higher prevalence of disease during first and second parity may be due to persistence of intramammary infections of preceding lactation or new intra-mammary infections developing during the dry period which flare-up due to sudden change in the udder environment (Dingwell *et al*., 2003).

Incidence of mastitis was found in declining trend from 5th parity onwards could be due to reduction in milk yield. Prevalence of clinical mastitis was also determined according to stage of lactation which is shown in Figure 2. Similar to our study, Corbett (2009); Fadlelmula *et al*. (2009) reported higher number of mastitis cases in first two months of lactation. Sharma and Sindhu (2007) reported majority of mastitis cases in first five months of lactation. It may be due to physiological and oxidative stress, low antioxidant defence and alterations in homeostasis (Sharma *et al.*, 2011).

On the basis of severity of symptoms, clinical mastitis is categorised as peracute, acute, subacute and chronic. Out of 809 clinical mastitis cases, 2.80%, 12.52%, 41.46% and 43.19% of buffaloes were reported suffering from peracute, acute, subacute and chronic clinical mastitis, respectively. Higher number of chronic mastitis cases can be explained in terms of delayed treatment, indiscriminate use of antimicrobials, and inadequate antibiotic concentration in udder tissue due to biofilm formation by microbes. However, Tufani *et al.* (2012) observed higher cases of acute mastitis (55.56%) followed by sub-acute (25.40%) and chronic (19.05%) mastitis. This may turn out due to different geographical locations, knowledge of farmers and diverse management practices.

Pattern of isolation of organisms from subclinically and clinically infected quarters was similar. A total of 2580 bacterial isolates were obtained from infected quarters, including 51.16% *Staphylococcus* spp., 37.94% *Streptococcus* spp., 8.41% *E. coli*, 1.62% *Corynebacterium* spp., 0.19% *Klebsiella*, 0.46% *Pseudomonas aeruginosa* and 10.38% mixed infections encapsulated in Table 1. *Staphylococci* were found to be a major etiological agent of disease and its high prevalence had been reported by several workers in India (Bhalerao *et al*., 2000; Sharma and Prasad, 2002; Sharma *et al*., 2007) and internationally (Hawari and Dabas., 2008; Nickerson, 2009). *Streptococci* accounted for the second most prevalent causative agent of mastitis. This finding is in close agreement with that of Khan *et al*. (2004); Sharma and Sindhu (2007). On contrary, Kumar *et al*. (2009) reported *Streptococci* to be more prevalent than *Staphylococci*. In present study, 8.41% mastitis cases were due to *E. coli* infection. Sharma and Sindhu (2007) also recorded 11.80% occurrence of coliform mastitis in infected buffaloes. On contrary, Awandkar *et al*. (2009) reported higher incidence of *E. coli* infections (40%) in bovine mastitis. Coliform mastitis may indicate poor hygienic conditions as *E. coli* originates from the environment and infects the udder via teat canal (Sumathi *et al.*, 2008). Higher incidence of clinical mastitis via environmental pathogens may be due to poor animal housing and bedding practices (Radostits *et al*., 2007).

To avoid indiscriminate use of antimicrobials and to decide most appropriate drug, antimicrobial sensitivity test was carried out and results have been encapsulated in Table 2. In *Staphylococci* infections, 89.56, 93.40 and 99.62% of isolates were found sensitive to Ceftriaxone, Cefaperazone and Ampicillin respectively. Sensitivity towards Amoxycillin, Enrofloxacin, Chloramphenicol and Oxytetracycline was in range from 81.59 to 82.46%. Sensitivity towards other antibiotics was found little. Thaker *et al.* (2013) reported higher sensitivity (80%) towards Oxytetracycline which is parallel to our results, on contrary, lower sensitivity towards Ampicillin (60%) and higher sensitivity (90%) towards Gentamicin was observed.

As many as 94.28 and 95.23% *Streptococci* showed sensitivity to Ceftriaxone and Cefaperazone

Table 1. Prevalence and frequency of different microorganisms associated with mastitis in buffaloes.

Quarters/Isolates	Clinical cases (n=463)	Subclinical cases (n=836)	Total
Total quarters	1852	3344	5196
Quarters examined	1558	2894	4452
Quarters Culturally positive	809	1503	2312
Quarters Culturally negative	749	1391	2140
Total isolates	931	1649	2580
Staphylococci	467	853	1320
Streptococci	357	622	979
Escherichia coli	72	145	217
Corynebacterium pyogenes	32	10	42
Klebsiella spp.	0	5	5
Pseudomonas aeruginosa	3	9	12
Bacillus	0	5	5
Mixed infections	122*	146**	268

* *Staphylococci* + *Streptococci* (100), *Streptococci* + *C. pyogenes* (10), *Staphylococci* + *C. pyogenes* (1), *Staphylococci* + *E. coli* (4), *Streptococci* + *E. coli* (7)

** *Staphylococci* + *Streptococci* (129), *Staphylococci* + *E. coli* (12), *Staphylococci* + *C. pyogenes* (1), *Streptococci* + *E. coli* (1), *Streptococci* + *C. pyogenes* (2), *E. coli*+ *Pseudomonas aeruginosa* (1)

Table 2. *In vitro* percent drug sensitivity of various isolates from cases of mastitis in buffaloes.

Antimicrobial drug	*Staphylococci* (n=1320)	*Streptococci* (n=1019)	*E. coli* (n=217)	*C. pyogenes* (n=42)
Penicillin	64.83	61.90	0	91.66
Streptomycin	54.12	58.00	32.91	75.00
Oxytetracycline	82.41	83.80	60.75	66.66
Chloramphenicol	81.04	80.04	58.22	97.05
Ampicillin	99.62	78.09	26.58	91.66
Neomycin	68.13	64.76	62.02	50.00
Cloxacillin	79.67	78.09	0	83.33
Enrofloxacin	81.86	80.04	58.22	91.66
Gentamicin	75.82	77.14	77.21	83.33
Amikacin	70.05	64.76	72.15	83.33
Amoxycillin	81.59	78.09	62.02	97.05
Ceftriaxone	89.56	94.28	63.28	97.05
Cefoperazone	93.40	95.23	75.94	97.05

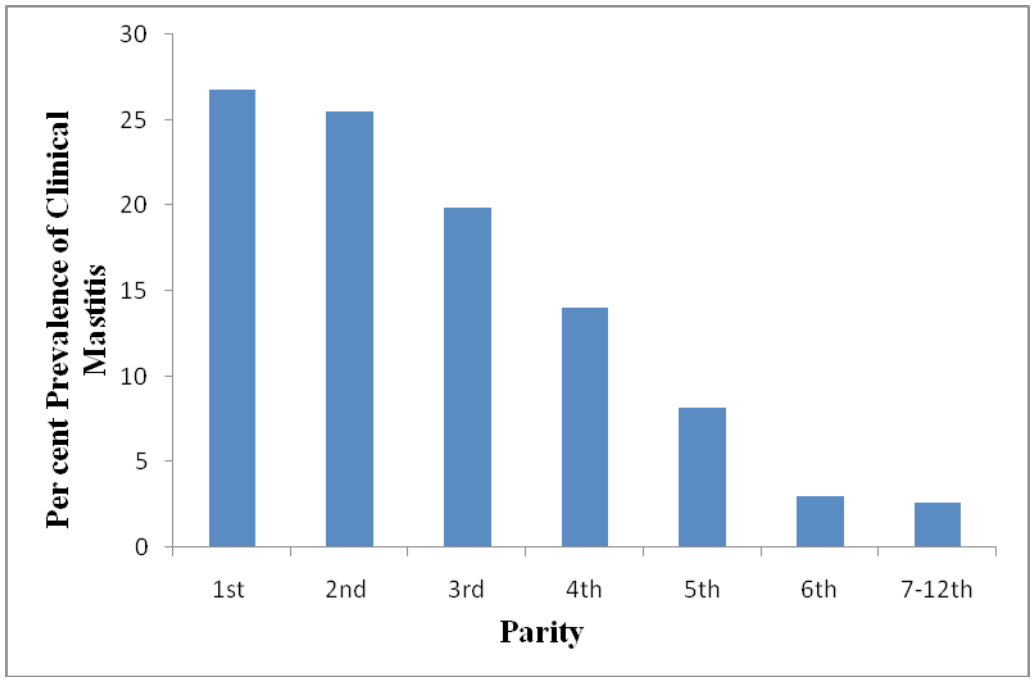

Figure1. Prevalence of clinical mastitis in buffaloes with respect to parity.

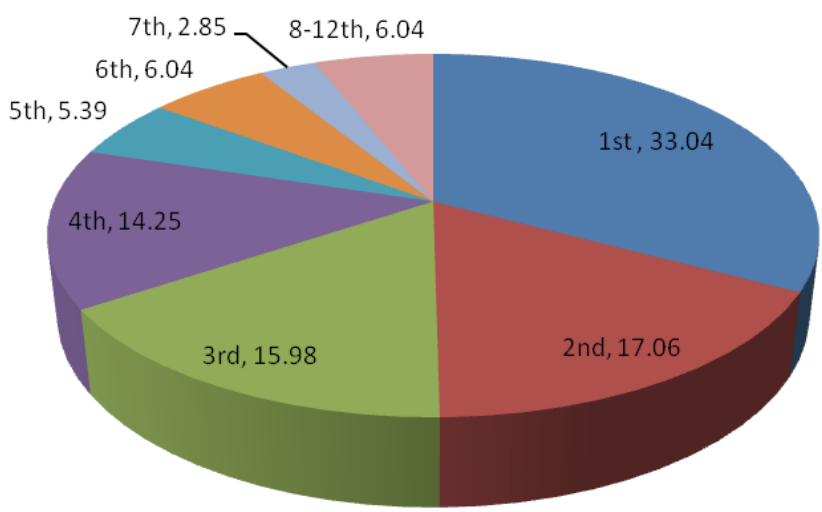

Figure 2. Prevalence of clinical mastitis in buffaloes with respect to stage of lactation.

respectively. Sensitivity towards Enrofloxacin, Chloramphenicol and Oxytetracycline ranged from 80.04 to 83.80% whereas 100% sensitivity was reported towards Chloramphenicol in Karnataka region of India by Kurjogi and Kaliwal (2011).

Corynebacterium isolates revealed high sensitivity (83.33 to 97.05%) towards majority of antimicrobials used except Neomycin, Streptomycin and Oxytetracycline. On the other hand, *E.coli* isolates revealed low sensitivity (26.58 to 77.2%) towards antimicrobials tested.

It can be concluded that the prevalence of clinical and subclinical mastitis is high in buffaloes and both contagious and environmental pathogens are involved. Distribution of pathogens changes over time, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health status. The emergence of drug resistant organisms causing mastitis due to indiscriminate use of antibiotics is well established. Therefore to avoid the drug residues in milk, emergence of antimicrobial resistant strains and to ascertain an effective treatment of mastitis, *In vitro* antimicrobial sensitivity testing of mastitogenic isolates is suggested.

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