

Original Research Article

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## Prevalence of Sub Clinical Mastitis in an Organized Buffalo Dairy Farm along with Antibioqram

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### ABSTRACT

The present study was aimed to determine prevalence and etiology of sub clinical mastitis in an organized buffalo dairy farm of LUVAS, Hisar, India along with antibiogram against their respective causative organisms. A total of 401 quarter milk samples were collected from 102 apparently healthy buffaloes. According to the IDF criteria the percent animal wise prevalence of sub clinical mastitis on the basis of somatic cell count (SCC) (>500000/ml) was 2.94, whereas on the basis of cultural examination it was 29.41. However, the quarter wise percent prevalence was 18.20 and 0.74 based on cultural positivity and SCC, respectively. Out of 73 culturally positive quarters, a total of 96 organisms were recovered. Of these 63.54% were *Staphylococcus* species, 36.46% were *Streptococcus* species while 13.54% had mixed infection of both *Staphylococcus* and *Streptococcus* species. Based on the IDF criteria the quarter wise percent prevalence of sub clinical, latent and nonspecific mastitis was 0.74%, 17.45% and 0%, respectively. The antibiogram study revealed that the highest percent sensitivity of the *Staphylococcus* species isolates was 73.44% and 75% for moxifloxacin and enrofloxacin, respectively. Similarly the highest sensitivity of *Streptococcus* species isolates was 71.88% for moxifloxacin.

#### Keywords

Mastitis, Buffalo,  
Antibiogram,  
Somatic cell count,  
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### Introduction

Mastitis is defined as the infection of the udder tissue having inflammatory responses. It has been reported in different animal species but is major concern in cattle and buffalo which are common dairy animals.

Mastitis can be classified into two types namely clinical and subclinical. Clinical mastitis is characterized by acute onset, altered milk composition and appearance, decreased milk production and also the presence of the cardinal signs of inflammation in mammary glands. Whereas the subclinical

mastitis has no visible signs are seen on the udder or in the milk, but the milk production decreases and the somatic cell count increases (Abebe *et al.*, 2016). Subclinical mastitis has more economical impact due to constantly decreased milk production, higher prevalence and longer devastating effects (Mungube *et al.*, 2005).

Mastitis in buffalo herds have been reported to cause high antibiotic resistance due to which infection persists for longer duration and cause severe economic losses. The economic losses may be in terms of decreased quantity and quality of milk production, as well as increased costs of treatment and control. Although, buffalo has been traditionally considered less susceptible to mastitis (Wanasinghe, 1985) is still most difficult to cure due to high cost of treatment, antibiotic resistance and high rate of multiplying microorganism in nutritive media like milk. (Fagiolo and Lai, 2007). The total buffalo population in India is 108.7 million which is the largest in the world, among which 3.84% and 24.43% of buffaloes are affected with clinical and subclinical mastitis respectively every year (Singh and Singh, 1994). The annual economic losses due to mastitis are estimated to be Rs. 7165.51 crores in India, out of which 57.93% (Rs. 4151.16 crores losses) has been attributed to subclinical mastitis (Reshi *et al.*, 2015). Sharma *et al.*, 2018 have also reported that subclinical mastitis is more prevalent than clinical mastitis in buffalo farms accounting for 33.76% and 18.17% cases, respectively. Sub clinical mastitis drastically reduces milk yield, and usually precedes the clinical form which is the basis of herd problems when mastitis outbreaks occur.

The most common etiological agents of mastitis in high yielder riverine buffalo (murrh and nili ravi) are *Staphylococcus aureus* followed by *Streptococcus agalactiae*,

*Streptococcus dysgalactiae* and *Streptococcus uberis* (Fagiolo and Lai, 2007). Mastitis leads to reservoir of infection for human beings through increased amounts of drug residues in milk, increased antimicrobial resistance of the organisms in animals treated with antibiotics. Mastitis specifically sub clinical type is well known for development of multiple drug resistance strains and emergence of new pathogens due to indiscriminate use of antimicrobials over long period (Raberg *et al.*, 2007). Hence it becomes important to diagnose the etiological agent of subclinical mastitis and subsequently the effective antimicrobial to prevent the indiscriminate use of antibiotics.

Thus the present study was designed to determine the prevalence of subclinical mastitis in organized buffalo dairy farm. The study also included the determination of organisms responsible to its causation and antimicrobial sensitivity towards common and newer antimicrobials.

## **Materials and Methods**

### **Source of milk samples and collection method**

A total of 401 quarters of buffalo milk samples from 102 apparently healthy buffaloes of organized dairy farm, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, India were screened for sub clinical mastitis.

Milk was collected under aseptic conditions. For this the udders of buffaloes were cleaned thoroughly with dilute potassium permanganate solution. After washing hands properly with soap and water the teats were disinfected with 70% alcohol. There after first three to four milk stripping were discarded and 15-20 ml milk sample of each quarter was collected separately in a sterilized test tube.

These test tubes were marked as right fore (RF), right hind (RH), left fore (LF) and left hind (LH). Each test tube was given the number possessed by the animal.

### **Bacterial examination**

According to International Dairy Federation Criteria the prevalence of sub-clinical mastitis should be determined on the basis of on bacteriological examination of milk and somatic cell count (SCC).

For bacteriological examination, the milk samples were shaken thoroughly to remove the fat and 10 µl of milk was streaked on both 5% sheep blood agar and MacConkey's lactose agar plates. The plates were incubated aerobically at 37°C for 24 to 48 hours. The resulting growth was sub-cultured on blood agar for purification of isolates and bacterial isolates were further subjected for identification and confirmation on the basis of gram's staining, morphology and colony characteristics. All the isolates were identified following standard bacteriological procedures as per Cruickshank *et al.*, (1975).

### **Somatic cell counts**

The SCC was performed as per the original method introduced by Schalm *et al.*, (1971). It involved the spreading of 0.01 ml milk over an area of 1 cm<sup>2</sup> on a microscope slide, followed by fixing, defatting and staining with the Newman-Lampert stain (HiMedia Laboratories Ltd., Mumbai).

### **Antimicrobial sensitivity testing**

The antimicrobial sensitivity testing was performed as per disc-diffusion method suggested by Bauer *et al.*, (1966). The interpretation was made based on the zone size interpretation chart provided by the manufacturer of discs. The fifteen different antimicrobials (Hi Media Laboratories

Ltd., Mumbai) namely Penicillin (10 I.U), Streptomycin (10 µg), Moxifloxacin (5 µg), Oxytetracycline (30 µg), Chloramphenicol (30µg), Ampicillin(10 µg), Neomycin (10 µg), Cloxacillin, Enrofloxacin (10 µg), Gentamicin (50 µg), Amikacin (30 µg), Amoxicillin (30 µg), Ceftriaxone (30 µg), Cefoperazone (75 µg) and Levofloxacin (5 µg) were used. The results were recorded as sensitive, intermediate and resistant.

### **Results and Discussion**

Bacterial culture examination on 401 quarters of 102 buffaloes showed that 73 quarters were culturally positive and total of 96 organisms were isolated from these quarters. Out of these 61 (63.54%) were positive for *Staphylococcus* species, 35 (36.46%) were positive for *Streptococcus* species while 13 (13.54%) quarters had mixed infection of *Staphylococcus* and *Streptococcus* species combined.

The result of somatic cell count (SCC) in the organized buffalo farm is presented in table 1. The animal wise percent prevalence of sub-clinical mastitis was found to be 2.94% on the basis of SCC (>500000/ml) while on the basis of cultural examination was 29.41%. Whereas the quarter-wise percent prevalence on the basis of SCC was 18.02 % while on the basis of bacteriological examination was 0.74%.

According to IDF criteria based on SCC and bacterial examination, the mastitis can be classified as sub clinical when SCC is above 500,000/ml of milk and culturally positive. The latent mastitis occurs when SCC below 500,000/ml of milk but culturally positive. Mastitis is said to be nonspecific when culturally negative and SCC above 500,000/ml. In the present study the 0.74%, 17.45% and 0% of the quarters were found to be suffering with sub clinical, latent and nonspecific mastitis respectively.

The antibiogram of different organisms isolated from the 73 culturally positive quarters is in terms of percent sensitivity is presented in table 2. About 73.44% and 75% of the *Staphylococcus* species isolated revealed high sensitivity towards moxifloxacin and enrofloxacin, respectively. However, only 1.56% and 7.81 % of the *Staphylococcus* isolates were sensitive to oxytetracycline and streptomycin, respectively. While 71.88% of the *Streptococcus* species isolated revealed higher sensitivity towards moxifloxacin. Whereas around 68.75% *Streptococcus* species had

higher sensitivity towards enrofloxacin. In the *Streptococcus* species the least effective antimicrobials were oxytetracycline and Streptomycin with only 1.56% as percent sensitivity. Both *Staphylococcus* and *Streptococcus* species showed lowered sensitivity towards amikacin with percent sensitivity of 18.75 and 12.50%, respectively. Similarly sensitivity towards other antibiotics was found little. It was interesting to note that none of the antimicrobials had 100 percent sensitivity towards either *Staphylococcus* or *Streptococcus* species.

**Table.1** Prevalence of sub clinical mastitis based on IDF criteria in buffalo dairy farm, LUVAS

Animals culturally positive	Animals showing SCC > 5 lac/ml	Quarters culturally positive	Quarters showing			
			SCC > 5 lac/ml	SCC > 5 lac/ml and culturally positive	SCC < 5 lac/ml and culturally positive	SCC > 5 lac/ml and culturally negative
30 (29.41%)	03 (2.94%)	73 (18.20%)	03 (0.74%)	03 (0.74%)	70 (17.45%)	0 (0%)

**Table.2** Percent antimicrobial sensitivity of bacterial isolates

Antimicrobial Drug	Percent sensitivity	
	Staphylococci (n= 61)	Streptococci (n= 35)
Penicillin	23.44	21.88
Streptomycin	7.81	1.56
Moxifloxacin	73.44	71.88
Oxytetracycline	1.56	1.56
Chloramphenicol	45.31	40.63
Ampicillin	23.44	21.88
Neomycin	34.38	34.38
Cloxacillin	31.25	25.00
Enrofloxacin	75.00	68.75
Gentamicin	32.81	25.00
Amikacin	18.75	12.50
Amoxycillin	31.25	25.00
Ceftriaxone	28.13	26.56
Cefoperazone	39.06	31.25
Levofloxacin	46.88	37.50

India is known for the largest buffalo population of the world that includes high milk yielder 'Black Gold' Murrah buffalo. Therefore, disease like mastitis becomes economically devastating for the buffalo dairy industry. The cost of treatment is also high and even leads to antimicrobial resistances. Hence, mastitis in dairy industry is said to be the top ranking infectious disease (Zecconi, 2003) leading to heavy economic losses. Mastitis is known to be caused by over 150 different contagious or environmental microorganisms (Kuang *et al.*, 2009), but the most common pathogens of economic and epidemiological importance are species of staphylococci, streptococci and coliforms (Shome *et al.*, 2011).

In the present study a total of 96 microorganisms were isolated from 73 quarters chiefly *Staphylococcus* and *Streptococcus* species and their mixed infection, which are contagious bacteria causing mastitis. These infections are usually spread from animal to animal at the time of milking. These findings were similar to that of Bansal *et al.*, (1995), Petzer *et al.*, (2009) and Pankaj *et al.*, (2013). Among the 96 microorganisms, 61 were *Staphylococcus* species isolates which showed high prevalence of *Staphylococcus* species. The high prevalence of staphylococci has also been reported by several other workers in India (Kalorey *et al.*, 1983; Javed and Siddique, 1999; Kaya *et al.*, 2000; Sharma *et al.*, 2007) and abroad (Tenhagen *et al.*, 2009 and Nickerson and Stephen, 2009). The prevalence of mastitis varies with breed, age, lactation and season (Rani *et al.*, 2008) therefore distribution of pathogens changes over time hence, the study suggests that bacteriological examination at the herd level must be taken regularly to monitor udder health status. Sumathi *et al.*, (2008) reported that coliform mastitis indicate poor hygienic conditions as *E. coli* originates from the

environment and infects the udder via teat canal. Higher incidence of sub clinical mastitis via environmental pathogens may be due to poor animal housing and bedding practices. In the present study no isolation of environmental pathogens of mastitis such as *E. coli* etc. indicated good hygiene condition of animal house.

On the basis of IDF criteria the prevalence of quarter wise subclinical mastitis was 0.74% whereas the latent mastitis was 17.45% which was in contrast to high sub clinical mastitis prevalence reported by several other workers (Kalorey *et al.*, 1983; Chavan *et al.*, 2007 and Sharma *et al.*, 2018). These variations in the prevalence rates of sub clinical mastitis could be due to difference in management and hygienic practices adopted in different dairy herds. While other factors like breed, milk yield, stage of lactation (Sharma *et al.*, 2007) and udder morphology could also lead to difference in prevalence. The high latent infection can also denote possibility of teat canal infections serving as a potential source of infection to the milk secretory tissue (Nickerson *et al.*, 1986). None of the quarters were affected with nonspecific mastitis could be due to the failure to detect pathogens because of intermittent excretion of the organisms or their disappearance because of spontaneous recovery (Sindhu *et al.*, 2009).

The indiscriminate use of antibiotics in treatment of mastitis has led to antimicrobial resistance and even a reservoir of infection for humans through antimicrobial residue in the milk of the treated animals. In *Staphylococci* infections around 73.44% and 75% of the isolates were sensitive to moxifloxacin and enrofloxacin, respectively. This depicts an increase in antimicrobial resistance in *Staphylococci* infections as previous studies on buffalo subclinical mastitis reported higher sensitivity towards ceftriaxone, cefaperazone and ampicillin in the range from 89.56, 93.40

and 99.62 percent, respectively (Sharma *et al.*, 2018). In an another study, all the strains of staphylococci and streptococci were found sensitive to cloxacillin, ceftriaxone and cefoperazone (Pankaj *et al.*, 2013) which was not observed in our study. The present study suggests that *Staphylococcus* and *Streptococcus* species isolated from buffaloes milk sample of LUVAS farm showed high sensitivity towards moxifloxacin followed by enrofloxacin. Ranjan *et al.*, (2010) also found high sensitivity towards enrofloxacin (91.67%) similar to the present study. This indicates that fluroquinolone group of antimicrobials which inhibit the DNA gyrase enzyme and eventually DNA and RNA synthesis are still effective against majority of *Staphylococcus* and *Streptococcus* species isolates from buffalo mastitis cases.

Earlier studies have reported higher sensitivity towards chloramphenicol and oxytetracycline to be in the range from 81.59 to 82.46% (Sharma *et al.*, 2018) which was not seen in the present study. The sensitivity towards chloramphenicol and oxytetracycline was much reduced in the range 40.5 % to as low as 1.56 % which may indicate multiple drug resistance as the gene encoding for resistance to chloramphenicol and oxytetracycline are present on the same plasmid (Chopra and Roberts, 2001). Thus in contrast to the earlier studies the current study antibiogram indicated reduced sensitivity towards newer antibiotics like third and fourth generation cephalosporins and older antibiotics such as penicillin and streptomycin. This demand for rationalisation of use of antimicrobials in the treatment of mastitis especially in context to sub clinical mastitis cases in buffaloes. The evolution of drug resistant organisms leading to mastitis due to indiscriminate use of broad spectrum antibiotics is well established therefore, antimicrobial sensitivity is suggested before treatment. Above all distribution of pathogens

changes over time from different farms, regions, states and countries hence bacteriological examination at herd level must be performed regularly to monitor udder health status and sub clinical mastitis for early, prompt and specific treatment.

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