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RESEARCH ARTICLE

STUDY ON QUARTER-WISE COMPARATIVE PREVALENCE, ETIOLOGY AND ANTIBIOGRAM OF BOVINE SUBCLINICAL MASTITIS

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ABSTRACT

The present study was undertaken to ascertain the quarter-wise comparative prevalence, distribution of etiological agents and drug sensitivity pattern of subclinical mastitis (SCM) in crossbred cows and buffaloes. Milk samples collected from 48 dairy animals (30 Jersey crossbred cows and 18 Graded Murrah buffaloes) were confirmed as subclinical mastitis by using six different indirect screening tests: pH, Electrical Conductivity, Chloride Test, Somatic Cell Count, Modified White Side Test and Modified California Mastitis Test and causative organisms were isolated and subjected to *in vitro* antibiotic sensitivity test. The overall quarter prevalence of subclinical mastitis in crossbred cows and buffaloes were (49.16%) and (45.83%) respectively. Among quarters, prevalence was higher in right hind quarter, followed by left hind quarter, right fore quarter and left fore quarter in crossbred cows and buffaloes. Among the isolates, *Staphylococcus spp.* showed the highest (40.68%) frequency, followed by *Streptococcus spp.* (37.29%), *E. coli* (13.56%) and *Bacillus spp.* (8.47%) in crossbred cows. In case of buffaloes *Staphylococcus spp., Streptococcus spp., E. coli* and *Bacillus spp.* were isolated from 39.39, 30.31, 18.18 and 12.13% milk samples respectively. Ceftriaxone showed the highest values of sensitivity for subclinical mastitis milk sample cultures of crossbred cows and buffaloes (89.83 and 87.87% respectively). It is concluded that right side quarters were found to be more susceptible for SCM than left side quarters because of farmers hand milking nature in crossbred cows and buffaloes. *Staphylococcus spp*. was the major pathogen responsible for SCM. Ceftriaxone and enrofloxacin were found to be the most effective drug under *in vitro* condition against the isolated pathogens in SCM affected crossbred cows and buffaloes.

INTRODUCTION

India continues to be the largest producer of milk in the world with 190 millions cattle and 108 million buffaloes as 19th Livestock Census, 2012. Mastitis is one of the important production diseases of dairy animals as it causes great financial losses worldwide due to lower milk yield, reduced milk quality, treatment cost, labour cost and discarded milk during infection and treatment. Bovine mastitis is the inflammation of mammary gland, usually due to microbial infection (Watts, 1988). Major organisms causing infection are *Staphylococcus spp*., *Streptococcus spp*. and gram negative bacteria (Mubarack *et al.,* 2012). Mastitis can be defined as clinical and subclinical (Duguma *et al.,* 2014). Subclinical mastitis is 3-40 times more common and causes great economic loss than clinical mastitis in most of dairy herds (Bachaya *et al.,* 2011). The prevalence of subclinical mastitis has increased enormously in India in the recent years than bovine clinical mastitis (NAAS, 2013) that varying from 10 to 50% in cows and 5 to 20% in buffaloes than clinical mastitis (1 to 10%). In India an economic loss due to mastitis was about 526 million dollars of which 70 per cent are due to subclinical mastitis (Varshney and Naresh, 2004). To avoid severe economic loss, it is highly important to identify the disease in early stage itself in subclinical form. Clinical detection of SCM is extremely difficult as there is no evidence / change in milk and udder, but milk production decreases, bacteria are present in the secretion and composition is altered (Eriskin, 2001). Therefore, diagnosis and treatment of mastitis in subclinical stage itself is more important in large-scale mastitis control programmes to avoid losses, which are apt to occur even after treatment of clinical form of mastitis. Mastitis is considered to be the most common cause of indiscriminate antibiotic use in dairy animals. Even though the treatment of mastitis was undertaken, some cases will end up in failure. This may be due to the late commencement of the treatment, improper antibiotic selection and resistance of the pathogen to the antibiotic.

In recent years, attempts to control the infection by antibiotic treatment are many times affected by differences in the antibiotic sensitivity and emergence of drug resistant bacteria.

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The major problem for the field veterinarian is identifying the etiological agent, selection of efficacious drugs commonly available, performing reliable diagnostic tests and initiation of proper control measures. Considering the above facts, the present study was undertaken to determine the quarter-wise comparative prevalence, etiological agents distribution and antibiotic sensitivity pattern of bacterial isolates responsible for subclinical mastitis in crossbred cows and buffaloes.

MATERIALS AND METHODS

Source and collection of milk samples

Milk sample of each quarter was aseptically collected in sterilized vials from 48 dairy animals (30 Jersey crossbred cows and 18 Graded Murrah buffaloes) brought to the Large Animal Clinic Medical Outpatient Unit, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, Chennai with the history of reduction in milk yield were subjected to determine subclinical mastitis by using six different indirect screening tests: pH (Rosenberger, 1979), Electrical Conductivity (Hillerton and Walton, 1991), Chloride Test (Yadav *et al*., 1993), Somatic Cell Count (Booth *et al.,* 1984), Modified White Side Test (Doxey, 1983) and Modified California Mastitis Test (Sharma and Rajani, 1969).

Isolation and identification of causative organism

The isolation and identification of causative organisms from milk samples were done as per standard microbiological procedures suggested by Quin *et al.* (2002).

In vitro antibiotic sensitivity testing

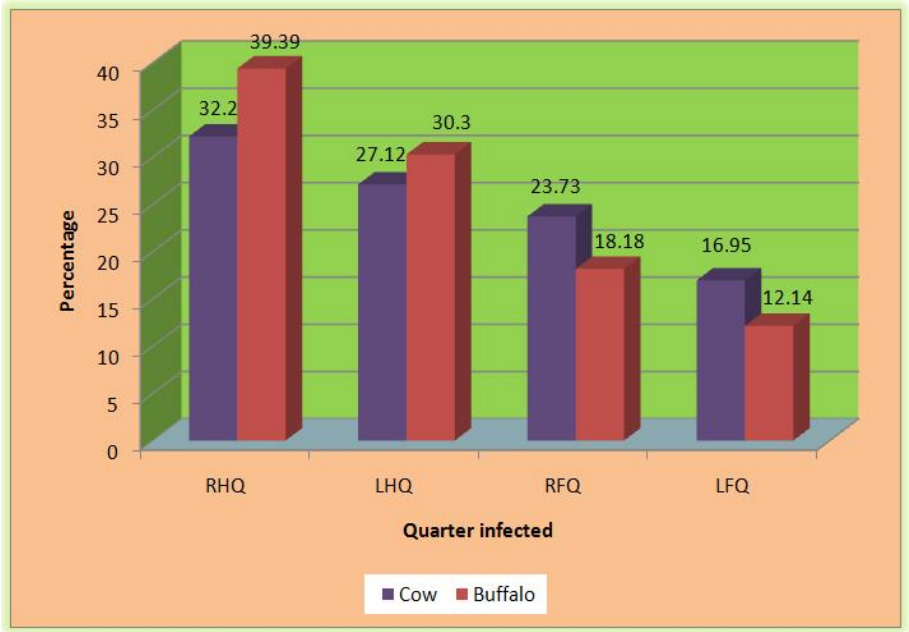
The organisms isolated from quarter foremilk samples were tested for sensitivity to 7 different antimicrobials agents as per the method suggested by Bauer *et al.* (1966).

RESULTS AND DISCUSSION

The present study shows, an overall quarter-wise prevalence of subclinical mastitis in crossbred cows and buffaloes were 49.16% and 45.83% respectively. The lower prevalence in buffaloes in comparison to crossbred cows can be that the former is generally more resistant than the later. This was in agreement to the earlier report of Thapa and Kaphle (2002) who reported that buffaloes to be less susceptible to mastitis than cattle. It has also been ascribed to more perfect sphincter mechanism, which prevents entry of infection into teat canal (Uppal *et al.,* 1994).

Quarter-wise prevalence of subclinical mastitis in crossbred cows and buffaloes has been shown in Fig. 1. As regard to distribution among infected quarters higher prevalence of SCM in crossbred cows was recorded in right hind (32.2%), followed by left hind (27.12%) while it was (23.73%) in right fore and (16.95%) in left fore quarters respectively. In case of buffaloes, higher prevalence of SCM was recorded in right hind (39.39%), followed by left hind (30.3%) while it was (18.18%) in right fore and (12.13%) in left fore quarters respectively. This similar pattern of affection of quarters i.e. right hind followed by left hind, right fore and left fore were observed by Zeryehun *et al.* (2013) and Hase *et al.* (2013). The result shows that the hind quarters are affected more than the front quarters. This could be attributed to the high production capacity of the hind quarters (Duguma *et al.,* 2014; Radostits *et al.,* 1994) and the high chance of getting fecal and environmental contamination (Hase *et al.,* 2013).

Though, there was higher prevalence of SCM in hindquarter in buffaloes than crossbred cows and among hindquarters, right hind quarters were found to be more susceptible than the left hind quarters. In case of forequarters, there was higher prevalence in crossbred cows than buffaloes and among forequarters, right forequarters were found to be more susceptible to SCM in our study.



RHQ- Right Hind Quarter LHQ- Left Hind Quarter RFQ- Right Fore Quarter LFQ- Left Fore Quarter

Fig. 1. Quarter wise prevalence of subclinical mastitis in cows and buffaloes

Similar results have been reported by Shahid *et al.* (2011). The higher prevalence of right side quarters were ascribed due to the common practice of milkmen milking the animals, while sitting on the left side of the animals; while they exert pressure on the right side of quarters.

The major pathogens isolated from milk samples were *Staphylococcus spp.* 24(40.68%), followed by *Streptococcus spp.* 22(37.29%), *E coli* 8(13.56%) and *Bacillus spp.* 5(8.47%) in crossbred cows. In case of buffaloes *Staphylococcus spp.* 13(39.39%), followed by *Streptococcus spp.* 10(30.31%), E. *coli* 6(18.18%) and *Bacillus spp.* 4(12.13%) were isolated (Fig. 2). Similar results were observed by Khan and Muhammad (2005) and Akram *et al.* (2013) who reported similar pattern of isolates in crossbred cows and buffaloes affected with SCM. The higher prevalence of *Staphylococcus spp.* followed by *Streptococcus spp.* in SCM has been highlighted in the study of many workers in cows (Duguma *et al.,* 2014; Khan and Muhammad, 2005; Elango *et al.,* 2010) and buffaloes (Khan and Muhammad, 2005; Bhalerao *et al*., 2000; Pankaj *et al*., 2013). Similar to our findings, other workers from India have also reported *Staphylococci* and *Streptococci* to be the main etiological agents of SCM (Pankaj *et al.,* 2013; Sharma and Sindhu, 2007). The higher incidence of *Staphylococci* indicates unhygienic milking practices as these pathogens are mainly spread during milking via milker's hands (Bradley, 2002).

In the present study, *Streptococcus spp.* was the second prevalent bacterial species isolated which agrees to the Radostits *et al.* (1994) who stated that *Streptococcus spp.* is the most prevalent along with *Staphylococcus spp.* However, the lower prevalence as compared to *Staphylococcus spp.* is because *Streptococcus spp.* survives poorly outside the udder, and established infections are eliminated by frequently use of penicillin and other antibiotics. In our study, prevalence of *E. coli* and *Bacillus spp.* were quite low. The incidence of *E coli* mastitis may have been due to poor hygienic conditions as *E coli* originates from the cows environment and infect the udder via the teat canal as reported by Bradley (2002). Mir *et al.* (2014) who isolated *E coli* and *Bacillus spp.* from cows affected with SCM. A high prevalence of *E coli* in SCM affected buffaloes was reported by Naiknaware *et al.* (1998). Palanivel *et al.* (2005) who isolated *Bacillus spp*. from SCM affected buffaloes. Our study is in agreement with the above authors. All the bacteria isolated were tested *in vitro* for their sensitivity to 7 different antibiotics that are commonly used in veterinary practices. The descending order of antibiotic sensitivity in crossbred cows and buffaloes affected with SCM were ceftriaxone, enrofloxacin, gentamicin, chloramphenicol, cloxacillin, ampicillin and amoxicillin (Table 1 and 2).

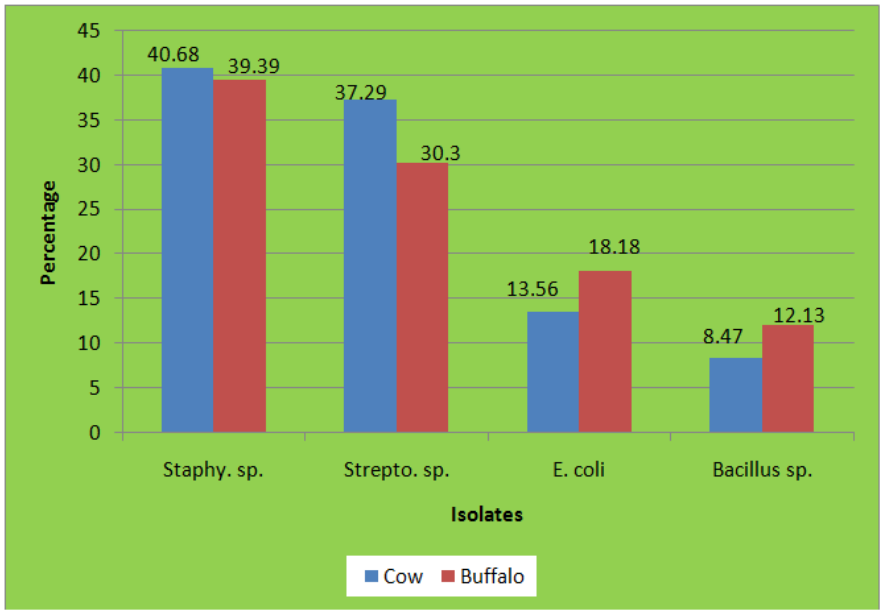


Fig. 2. Bacterial isolates in subclinical mastitis milk of cows and buffaloes

Table 1. *In vitro* antibiogram of bacterial isolates (n=59) from subclinical mastitis in Cows

S. No.	Isolates	Ci	Ex	G	C	Clx	A	Am
1.	*Staphylococcus sp.*	19 (79.1)	19 (79.1)	20 (83.3)	15 (62.5)	12 (50)	10 (41.6)	9 (37.5
2.	*Streptococcus sp.*	22 (100)	21 (95.4)	16 (72.7)	10 (45.4)	4 (18.1)	5 (22.7)	4 (18.1)
3.	*E. coli*	8 (100)	8 (100)	8 (100)	5 (62.5)	1 (12.5)	2 (25)	2 (25)
4.	*Bacillus sp.*	4 (80)	4 (80)	3 (60)	3 (60)	2 (40)	1 (20)	2 (40)
	Total	53 (89.83)	52 (88.13)	47 (79.66)	33 (55.93)	19 (32.20)	18 (30.50)	17 (28.81)

Figures in parenthesis indicates percentage Ci- Ceftriaxone, Ex- Enrofloxacin, G- Gentamicin , C- Chloramphenicol Clox- Cloxacillin, A- Ampicillin, Am- Amoxycillin

Table 2. *In vitro* antibiogram of bacterial isolates (n=33) from subclinical mastitis in Buffaloes

S.No.	Isolates	Ci	Ex	G	C	Clx	A	Am
1.	*Staphylococcus sp.*	10 (79.9)	9 (69.2)	11 (84.6)	8 (61.5)	15 (38.4)	6 (46.15)	4 (30.76)
2.	*Streptococcus sp.*	10 (100)	9 (90)	7 (70)	4 (40)	3 (30)	2 (20)	2 (20)
3.	*E. coli*	6 (100)	6 (100)	6 (100)	3 (50)	3 (50)	1 (16.6)	2 (33.3)
4.	*Bacillus sp.*	3 (75)	3 (75)	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)
	Total	29 (87.87)	27 (81.81)	26 (78.78)	18 (54.54)	12 (36.36)	10 (30.30)	9 (27.27)

Figures in parenthesis indicates percentage, Ci- Ceftriaxone, Ex- Enrofloxacin, G- Gentamicin, C- Chloramphenicol Clox- Cloxacillin, A- Ampicillin, Am- Amoxycillin

The present observation is in agreement with the Lairintluanga *et al.* (2003) who reported *Staphylococcus spp.* was found highly sensitive to enrofloxacin, gentamicin and least sensitive to amphicillin. *Streptococcus spp.* was found highly sensitive to ceftriaxone. This observation is in agreement with the trial of Umakantan (1998) and Ramprabhu *et al.* (2004). *E. coli* was found highly sensitive for enrofloxacin, gentamicin which is in accordance with the report of Lairintluanga *et al*. (2003).

Bacillus spp. was found highly sensitive to enrofloxacin, gentamicin, chloramphenicol and least sensitive to amoxicillin and amphicillin. This agreed with reports of Sharma and Prasad (2004). *Staphylococcus spp*. and *Streptococcus spp.* were found least sensitive to amoxicillin. This observation is similar with the report of Ramprabhu and Rajeswar (2007) who reported that it is due to indiscriminate use of this antibiotic in the recent past. In our study, Ceftriaxone, enrofloxacin, gentamicin are less commonly used for treatment of mastitis in the area of studying resulting in higher efficacy of these drugs. On the other hand isolates showed least sensitivity or resistance to cloxacillin, ampicillin and cloxacillin. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Antibiotic resistance patterns vary among different farms, regions, states and countries depending upon the type of organisms and use of antibiotics in particular area; therefore, antibiotic sensitivity is suggested before institution of treatment.

Conclusion

Subclinical mastitis (SCM) is a common and serious problem of dairy animals. The prevalence of SCM increases in dairy animals with a history of reduction in milk yield. Various indirect diagnostic, cost effective tests are easily applicable for regular screening of SCM for early detection and treatment. The present study showed that contagious (*Staphylococcus spp*.), environmental (*Streptococcus spp.; E coli*) and opportunistic (*Bacillus spp.*) microorganisms are responsible for SCM in dairy animals. For controlling subclinical and clinical form of mastitis it is necessary to apply good sanitary and hygienic measures at every aspect of animals as well as milkmen. Isolation and identification of SCM causing bacteria, establishment of correct *in vitro* antibiogram are important prerequisite for implementation of effective control of mastitis. At the same time farmers should be aware about the mastitis management, udder health management, shed management and nutrition management for clean milk production.

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