

Original Research Article

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A Study on Quarter-wise Incidence of Mastitis in Dairy Animals and Antibigram of Associated Bacteria

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ABSTRACT

The present study was done on milk samples collected from the 469 milch animals (1765 quarters/teats), of which 296 were buffaloes (1120 quarters) and 173 were cows (645 quarters) at Disease Investigation Laboratory (DI lab), Bhiwani during the period from July, 2016 to June, 2017 to know quarter-wise association of mastitis in bovines and to understand current susceptibility pattern of the isolates to various antibacterial drugs. Overall prevalence was observed to be 42.86 % by WST. Species-wise higher percentage of cows (47.40%) were positive for mastitis compares to buffaloes (40.20%). Among the affected animals, the maximum were having only single quarter infection among both species i.e., 35.47% of cows and 41.18% of buffaloes. Incidence observed on front quarters and hind quarters basis lead to the inference that hind quarters (32.31%) are more prone to mastitis than front quarters (15.46%). At species level also, higher incidences were noted in hind quarters. The result leads to inference that the hind quarters are more susceptible to mastitis than the front quarters. Categorization of data according to position of four teats/quarters viz., left hind, right hind, left front and right front quarter, overall as level at species level basis right hind quarter was having the highest prevalence while left front quarter was found to be the least affected by mastitis. In bacteriological examination total 177 (88.06%) milk samples were found to be positive in cultural examination. Of the examined samples 37 (84.09%) of cows while 140 (89.17%) of buffaloes, respectively, were positive for microbial isolation. On Gram's staining it was observed that the majority of isolates were Gram-positive bacteria (59.89%). Based on colony and morphological characteristics among the Gram-positive bacteria *Staphylococcus sp.* bacteria were the major pathogens while *E. coli* was the major pathogen amongst the Gram-isolates. The antibiotic sensitivity pattern of isolates was studied. Ceftriaxone-sulbactam was found to be the most effective (92.09%) among the tested antibiotics followed by enrofloxacin (90.39%) while Penicillin-G was found to be least effective (11.30 %) among the battery of antibiotics tested. The present study leads to the inference that there is association of mastitis with position of quarter and the pathogens associated with the disease are developing resistant to the commonly used antibiotics. Hence, the animal health workers must recommend antibiotic sensitivity testing so that effective and judicious use of antibiotics can be done. Dairy farmers should adopt scientific methods of animal husbandry in order to achieve profitable and hygienic milk production.

Keywords

Mastitis, Quarter-wise, Species-wise, Bovines, Antibigram, White Side Test, WST, Incidence

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Introduction

Mastitis, a disease of complex etiology, had been recognized for more than a century, and still continues to be an evergreen cause of economic loss to the dairy industry and is the costliest problem all over the world where dairying is practiced Devi and Dutta (2018); Patil *et al.*, (2000); Singh (2003). Mastitis is a complex disease resulting from the interaction of infectious agents and poor managerial practices in dairy animals (Schalm and Woods, 1953). The disease is known to cause a great deal of loss or reduction of productivity. It influences the quality and quantity of milk, and causes culling of animals at an unacceptable age (Mungube *et al.*, 2005).

Therefore, keeping these points in view the importance of subclinical mastitis with its negative impact on dairy industry this study was undertaken to update available information and to further understand epidemiology of mastitis in the area under study. The present study was done to know quarter-wise association of mastitis in bovines and to understand current susceptibility pattern of the isolates to various antibacterial drugs.

Materials and Methods

Ethical approval

The present study was carried on samples collected from animals suspected to be suffering from mastitis. For the collection of clinical milk samples approval of Institutional Animal Ethics Committee was not required as per University rules.

Samples collection and processing

Milk samples were collected from the 469 bovines (1765 quarters/teats), of which 296

were buffaloes (1120 quarters) and 173 were cows (645 quarters) at Disease Investigation Laboratory (DI lab), Bhiwani during the period from July, 2016 to June, 2017 (Table 1).

From each teat 10 ml milk sample was collected in a sterile vial from animals having problems like sudden decrease in milk yield, change in colour, any other physical appearance like change in viscosity etc as reported by livestock owners and were screened by White Side test (WST) as per procedure described by Kahir *et al.*, (2008).

The sample positive for WST was subjected to cultural examination, only one sample per animal was used for cultural examination, for this milk samples of all positive teats from one animal were mixed and then processed for bacteriological examination.

The isolates were identified according to cultural and morphological characteristics complying with methods of Cruickshank *et al.*, (1975). All culture media and antibiotic discs used were manufactured by HiMedia laboratories Pvt. Ltd, Pune.

The *in vitro* antibiotic sensitivity pattern was studied by Kirby-Bauer disc diffusions method with slight modifications (Bauer *et al.*, 1966) using 09 number of standard antibiotic discs (HiMedia laboratories Pvt. Ltd.) such as Amoxicillin, Amoxicillin-sulbactam, Cefoperazone, Ceftriaxone, Ceftriaxone-sulbactam, Chloramphenicol, Enrofloxacin, Penicillin-G, Streptomycin and Tetracycline.

The interpretation regarding the degree of susceptibility (resistant, moderate and highly sensitive) was made as per Clinical and Laboratory Standards Institute (NCCLS, 1999) chart provided by the antibiotic disc manufacturer.

Results and Discussion

Overall and Species wise prevalence

Overall combined prevalence of 42.86 % was observed by WST (Table 1). On Species-wise analysis of data it was seen higher percentage of cows (47.40%) were positive for mastitis with respect to buffaloes (40.20%). 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. Mustafa *et al.*, (2011) also recorded lower prevalence of mastitis in buffalo (20.98%) compares to cows (24.71 %). The lower prevalence rate in buffalo has been ascribed to more perfect sphincter mechanism, which prevents entry of infection into teat canal (Uppal *et al.*, 1994). Similar, to the present study higher quarter prevalence in cows haven reported by various workers (Jingar *et al.*, 2014; Kumar *et al.*, 2015; Swami *et al.*, 2017; Yadav *et al.*, 2019)

Quarter-wise prevalence

Number of quarter involved per animal

Data of the affected animals was analysed with respect to number of quarters involved per animal and it was observed that maximum number of animals were having only single quarter i.e., 35.47% of cows and 41.18% of buffaloes while affected animals having all four quarters infected were lowest. The annotations of the present report are in concord with those of Patel and Trivedi, (2015); Yadav *et al.*, (2019); Thakur *et al.*, (2020); who in their independent studies reported that maximum number of animals had single quarter infection, followed by two quarters, three quarters and all four quarters infection in decreasing order. However, Dasohari *et al.*, (2017) observed that the maximum number of animals was having two quarter infection followed by single, three and

four quarters infection. The fact that single quarter infection had higher incidence is an indication that possibly one quarter is usually first infected and the others become affected through contamination and other means especially during the milking procedures (Shittu *et al.*, 2012). The difference in quarter wise prevalence might be due to the fact that predisposing factors like injury, defective sphincters, and so forth could vary from quarter to quarter Iqbal and Siddique (1999).

Prevalence based on position of quarter

Incidence was also observed on front quarters and hind quarters basis (Table 2); higher incidence was detected in hind quarters (32.31%) than front quarters (15.46%). At species level also, higher incidences were noted in hind quarters both in cows (37.57%) and buffaloes (29.00%) with respect to front quarters; 17.82% and 14.19% in cows and buffaloes, respectively. The result leads to inference that the hind quarters are more susceptible to mastitis than the front quarters. These findings are in agreement with those Sharma *et al.*, (2012), Yadav *et al.*, (2019) who all published reports with higher incidence(s) in hind quarters. Contrary, to our observations Patel and Trivedi (2015), noted higher prevalence in front quarters than hind quarters.

On analysis of data according to position of four teats/quarters viz., left hind, right hind, left front and right front quarter and it was found that overall basis right hind quarter was having highest prevalence (36.09%) and while over left front quarter (14.35%) was least affected by mastitis. These observations were also valid at species level for both cows and buffaloes. In accord to our observations, Zenebe *et al.*, (2014), also find that right hind quarters have the highest infection rate (63.9%) and lowest infection rate in left front quarters (40.4%). Similarly, reports of Thakur

et al., 2020 from Haryana and Manokaran *et al.*, 2020 were in concurrence with the results of the present study.

This could be attributed to the high production capacity of the hind quarters (Radostits *et al.*, 2007) and due to the larger mass, greater vulnerability to direct trauma,

relatively more closeness to the floor as compared to front quarters, hence high chance of getting faecal, urine and environmental contamination (Hase *et al.*, 2013). Contrary, to our observations Patel and Trivedi, (2015), noted higher prevalence in front quarters than hind quarters.

Table.1 Overall incidence of mastitis in relation to number of quarters involved in affected animals

Species	Total Animals	Positive (%)	Total Quarters	Positive (%)	Number of Quarters Involved animal-wise			
					One (%)	Two (%)	Three (%)	Four (%)
Cow	173	82 (47.40)	645*	177 (27.44)	29 (35.37)	23 (28.05)	18 (21.95)	12 (14.63)
Buffalo	296	119 (40.20)	1120**	233 (20.80)	49 (41.18)	37 (31.09)	22 (18.49)	11 (9.24)
Total	469	201 (42.86)	1765	410 (23.23)	78 (38.81)	60 (29.85)	40 (19.90)	23 (11.44)
645= 4*39+3*21+2*13					1120=4*247+3*34+2*15			

Table.2 Incidence of mastitis in front quarters and hind quarters

Species	Hind quarters	Positive (%)	Front Quarters	Positive (%)
Cow	314	118 (37.57)	331	59 (17.82)
Buffalo	500	145 (29.00)	620	88(14.19)
Total	814	263 (32.31)	951	147 (15.46)

Table.3 Quarter-wise prevalence of subclinical mastitis

Species	Quarters Tested	Positive (%)	LH	Positive (%)	RH	Positive (%)	LF	Positive (%)	RF	Positive (%)
Cow	645	177 (27.44)	158	53 (33.54)	156	65 (41.67)	167	28 (16.77)	164	31 (18.90)
Buffalo	1120	233 (20.80)	257	66 (25.68)	243	79 (32.51)	314	41 (13.06)	306	47 (15.36)
Total	1765	410 (23.23)	415	119 (28.67)	399	144 (36.09)	481	69 (14.35)	470	78 (16.60)
645= 4*39+3*21+2*13					1120=4*247+3*34+2*15					

Table.4 Categorization of bacteria isolated based on Gram's staining

	Cow	Buffalo	Overall
Bacteria			
Gram's positive	23 (62.16%)	83 (59.29%)	106 (59.89%)
Gram's negative	14 (37.84%)	57 (40.71%)	71 (40.11%)
Total	37	140	177

Table.5 Antibigram of bacterial isolates

Organism										
	E	Am	P	AS	CS	CPZ	CTR	Ch	T	S
Gram Positive	96 (90.57)	23 (21.70)	13 (12.26)	81 (76.41)	98 (92.45)	93 (87.73)	92 (86.79)	95 (89.62)	67 (63.20)	47 (44.34)
Gram Negative	64 (90.14)	14 (19.72)	7 (9.86)	52 (73.24)	65 (91.55)	62 (87.32)	61 (85.92)	63 (88.73)	42 (59.15)	32 (45.07)
Total	160 (90.39)	37 (20.90)	20 (11.30)	133 (75.14)	163 (92.09)	155 (87.57)	153 (86.44)	158 (89.26)	109 (61.58)	79 (44.63)
E =Enrofloxacin		Am = Amoxicillin		P = Penicillin-G		AS= Amoxicillin-sulbactam		CS= Ceftriaxone-sulbactam		
CPZ= Cefoperazone		CTR= Ceftriaxone		Ch=Chloramphenicol		T= Tetracyclin		S= Streptomycin		

Cultural examination

Microbial cultural examination of 201 milk samples, of which 44 were cow milk samples and 157 were buffalo milk samples was carried out and organisms were characterized based on Gram's staining and antibiotic sensitivity pattern of the isolates was determined.

Total of 177 (88.06%) milk samples were found to be positive in cultural examination. Of the examined samples 37 (84.09%) of cows while 140 (89.17%) of buffaloes, respectively, were positive for microbial isolation (Table 3).

Gram's staining was done and it was observed overall majority of isolates were Gram-

positive bacteria (59.89%) as well as for both cows (62.16%) and buffaloes (59.29%). These observations are in agreement with those of Thakur *et al.*, 2020 who instituted that majority of bacterial isolates associated with mastitis are Gram-positive.

Based on colony and morphological characteristics among the Gram-positive bacteria *Staphylococcus sp.* bacteria were the major pathogens while *E. coli* was the major organism among the Gram-negative bacteria. Zeryehun and Abera (2017) in their study found *Staphylococcus* species predominant species (Table 4).

Bhalerao *et al.*, (2000) and Zenebe *et al.*, (2014) in their studies have also observed overall predominance of *Staphylococcus sp.*

among the organisms isolated from subclinical mastitis positive samples and also *Escherichia coli* as major pathogen among the Gram's negative bacteria which are in agreement with the present study.

The high prevalence of *Staphylococcus sp.* may be associated with its frequent colonization of teats, its ability to exist intracellular and localize within micro abscesses in the udder and hence resistant to antibiotic treatment (MacDonald, 1997). The organism is well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it shed in milk facilitating trans-mission to healthy animals mainly during milking (Radostits *et al.*, 2007).

Antibiogram

In vitro antibiotic sensitivity pattern of isolates was also studied. Ceftriaxone-sulbactam was found to be the most effective (92.09%) among the tested antibiotics followed by enrofloxacin (90.39%) while Penicillin-G was found to be least effective (11.30 %) among the battery of antibiotics tested (Table 5). However, Thakur *et al.*, 2020 and Jeykumar *et al.*, 2013 found enrofloxacin to be the most effective antibiotics while Penicillin G was found to be least effective by *in vitro* sensitivity test. Bhat *et al.*, (2017) also found enrofloxacin to be the most effective against the mastitis causing pathogens. The alarming level of less susceptibility or more resistance of organisms to a particular drug might be due to the prolonged exposure to the same drug and due to indiscriminate use. This high emergence of resistance to commonly used antibiotics is not surprising as there is irrational use of these antibiotics due to lack of cultural antibiotic sensitivity practice before treatment (Jeykumar *et al.*, 2013).

In conclusion the buffalo and cattle are mostly reared for milk production. A disease, mastitis

renders them useless for this purpose. Milk production usually decreases and blood alone or mixed with mucus in milk. It is one of the most important reasons for termination of lactation and unwanted culling of dairy buffalo

The present study lead to the inference that there is association of mastitis with position of quarter and the pathogens associated with the disease are developing resistant to the commonly used antibiotics. The control of mastitis can be achieved by a definite regime of administration of antibiotics and holistic approach to the disease management. Hence, the animal health workers must recommend antibiotic sensitivity testing so that effective and judicious use of antibiotics can be done. Dairy farmers should adopt scientific methods of animal husbandry in order to achieve profitable and hygienic milk production.

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