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## Quarter-wise incidence of mastitis in bovines and antibiotic sensitivity pattern of associated bacterial pathogens

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### Abstract

The present study was conducted on milk samples collected from the 204 bovines (616 quarters/teats), of which 155 were buffaloes (465 quarters) and 49 were cows (151 quarters) at Disease Investigation Laboratory, Bhiwani. The milk samples collected in sterile vials were screened by White Side test (WST) and positive samples were consequently subjected to bacteriological examination.

Of the total 616 quarters samples tested 59.57% were found to be positive by WST. At species level 59.35% quarters of buffaloes and 60.26% quarters of cows were positive of the tested quarters. While categorizing results on the basis of number of quarter(s) involved per animal, it was revealed that the most of the animals under study were having single quarter infection (47.05%). While observing incidence on front/front quarters and hind quarters basis; much higher overall incidence was observed in hind quarters (78.30%) than front quarters (39.60%); similar trend was noted at species level also. Overall incidence on the basis of position of quarters was the highest in right hind quarters (86.08%) while overall lowest incidence was observed in right front quarter (37.91%). At species level also, right hind quarter was the most affected one for both cows and buffaloes. However, lowest level prevalence was observed in right front quarters in case of buffaloes while deviation was seen in case of cows as it was lowest in left front quarters.

Milk sample from each animal was also subjected to bacteriological examination and 180 (88.23%) samples were observed to be positive. At species level, 45 (91.84%) and 135 (87.09%) milk samples from cows and buffaloes, respectively were positive in cultural examination. Gram's staining of the culture revealed that overall, 67.78% infections were due to Gram positive bacteria while 32.22% were caused by Gram negative bacteria. 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. Antibiogram of the isolates revealed that Enrofloxacin was the most effective (92.22%) followed by Ciprofloxacin (91.11%) while Penicillin-G was found to be least effective (15.56%). From present study it can be concluded that mastitis in clinical and subclinical form has established its roots in dairy animals with higher affinity for hind quarters. The dairy farmers should be educated and made aware to adopt better managemental practices for profitable production of safe and wholesome milk. The pathogenic bacteria are also gradually developing resistance to commonly used antibiotics and hence judicious use of antibiotics and adoption of antibiotic sensitivity testing should be always recommended by veterinary clinicians in order to give effective treatment.

**Keywords:** Mastitis, quarter-wise, white side test, WST, antibiogram

### 1. Introduction

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk and pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large numbers of leukocytes (Radostits *et al.*, 2007) [20]. Mastitis has been 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) [17]. In subclinical mastitis though the symptoms are not evident however, milk yield can drop as much as 20% per infected quarter (Schepers and Dijkhuizen, 1991) [21].

Although there are many studies carried out so far by different researchers on the quarter-wise incidence of mastitis from different regions of India, it is necessary to update the information and to further understand epidemiology of mastitis. Therefore, this study was conducted to estimate quarter-wise association of mastitis in cows and buffaloes and to know the susceptibility pattern of the isolates to various antibacterial drugs.

## 2. Materials and methods

### 2.1 Ethical approval

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

### 2.2 Sample collection and processing

The study was conducted on milk samples collected from the 204 bovines (616 quarters/teats), of which 155 were buffaloes (465 quarters) and 49 were cows (151 quarters) at Disease Investigation Laboratory (DI lab), Bhiwani during the period from July, 2014 to June, 2015 (Table 1). The milk samples were collected in sterile vials from animals having problem related to milk production like sudden decrease in milk yield, change in colour, any other physical appearance like change in viscosity etc as reported by livestock owners were screened by White Side test (WST) and positive samples were processed for bacteriological examination.

The WST was performed as per procedure described by Kahir *et al.* (2008) [12], in brief, after thorough mixing avoiding violent shaking, 50 µl (five drops) of milk were placed on a glass slide with a dark background by micropipette. Subsequently 20 µl of WST reagent (4% NaOH) were added to the milk sample and the mixture was stirred rapidly with a toothpick for 20-25 seconds. A breaking up of milk in flakes, shreds and viscid mass was indicative of positive reaction. On the other hand, milky and opaque and entirely free of precipitant was indicative of negative reaction.

The samples 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) [8]. All culture media and antibiotic discs used were manufactured by HiMedia laboratories Pvt. Ltd. Briefly, each mixed milk sample was taken by means of a sterile inoculating loop and introduced into Brain heart infusion (BHI) broth prepared as per manufacturer's protocol and incubated overnight for substantial growth of microorganisms. Following growth in broth, a loopful of culture was streaked onto nutrient agar muller hinton agar (MHA), eosin methylene blue (EMB) agar, incubated for 24-48 hour at 37°C. The different colonies were marked and noted based on their colony characteristics. The morphological and staining characteristics of the organisms were determined by microscopic examination of Gram stained smears and a tentative analysis regarding the type of isolates was made.

The *in vitro* antibiotic sensitivity pattern was studied by Kirby-Bauer disc diffusions method with slight modifications (Bauer *et al.*, 1966) [3] using 12 number of standard antibiotic discs (HiMedia laboratories Pvt. Ltd.) such as Amoxicillin, Amoxicillin-sulbactam, Cefoperazone, Ceftriaxone, Ceftriaxone-sulbactam, Chloramphenicol, Ciprofloxacin, Cloxacillin, Enrofloxacin, Gentamicin, Penicillin-G and Tetracycline. Briefly, the inoculum was prepared by transferring 4-5 colonies from the culture to BHI broth and incubated at 37°C for 6-8 hours until moderate turbidity developed. Thereafter, the inoculum was smeared onto the MHA agar plate by soaking with sterile cotton swab and allowed the inoculum to dry. The discs were then placed aseptically equidistant from each other on the surface of the

agar plates. The plates were incubated at 37°C for 24-48 hours for development of inhibition zone. The diameters of zone of inhibition were then measured in millimetre (mm). The interpretation regarding the degree of susceptibility (resistant, moderate and highly sensitive) was made as per Clinical and Laboratory Standards Institute (formerly NCCLS) chart provided by the antibiotic disc manufacturer.

### 3. Results and discussion

The screening of the milk samples with White Side test, revealed that of the 616 quarters tested 367 (59.57%) were positive. At species level 59.35% quarters of buffaloes and 60.26% quarters of cows were positive of the tested quarters (Table 1). The high incidence rate in the present study might be due to the fact that the samples were collected from animals in which problems related to physical properties of milk were observed. Comparable to our observations; in independent studies Bachaya *et al.* (2005) [1] in bovines and Badiuzzaman *et al.* (2015) [2] in crossbred dairy cows have reported quarter-wise prevalence of 58.75% and 59.68%, respectively.

**Table 1:** Overall and species wise incidence of mastitis

	Quarters (Animals)	Buffalo (Animals)	Cow (Animals)
Quarters Tested	*616(204)	465(155)	151(49)
Positive Quarters	367(59.57%)	276(59.35%)	91(60.26%)

\*616 = (81 x 4) + (63 x 3) + (43 x 2) + (17 x 1)

While categorizing results on the basis of number of quarter(s) involved per animal (Table 2), it was revealed that the most of the animals under study were having single quarter infection (47.05%) followed by two quarters (34.31%), three quarters (10.29%) and the least percentage of animals were found to be positive for all the four quarters (8.33%). These findings are in agreement to those of Patel and Trivedi, (2015) [19] who also observed maximum number of cows had only single quarter infection, followed by two quarters, three quarters and all four quarters infection in decreasing order. Similar, reports have been published by Srinivisan *et al.* (2013) [22] and Nithya *et al.* (2017) [18] that among the animals positive for mastitis, most have only single quarter infection.

**Table 2:** Incidence of mastitis based on number of quarters infected per animal

Number of Quarter infected	Number of Animals positive (%)
Single	96 (47.05%)
Two	70 (34.31%)
Three	21 (10.29%)
Four	17 (8.33%)

**Table 3:** Incidence of mastitis in Front quarters and Hind quarters

	Hind quarters	Positive (%)	Front Quarters	Positive (%)
<b>Species</b>				
Cattle	79	60 (75.95)	72	31 (43.05)
Buffalo	239	189 (79.08)	226	87 (38.49)
Total	318	249 (78.30)	298	118 (39.60)

While observing incidence on front quarters and hind quarters basis (Table 3); much higher incidence was detected in hind quarters (78.30%) than front quarters (39.60%). At species

level also, higher incidences were noted in hind quarters both in cows (75.95%) and buffaloes (79.08%) with respect to front quarters; 43.05% and 38.49% in cows and buffaloes, respectively. The result displays that the hind quarters are affected more than the front quarters. Outcomes of the present study are in agreement with those of Kavitha *et al.* (2009) [13] and Srinivisan *et al.* (2013) [22]. This could be attributed to the high production capacity of the hind quarters (Radostits *et al.*, 2007) [20] 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 (Chakrabarti, 2007; Hase *et al.*, 2013) [7, 9]. Patel and Trivedi, (2015) [19], noted higher prevalence in front quarters than hind quarters.

Overall incidence of mastitis on the basis of position of each quarter/teat was found to be highest for right hind quarters (86.07%), which was also evident at species level, with 87.29% and 82.5%, respectively right hind quarters positive for buffalo and cattle, respectively (Table 4). Overall lowest prevalence was observed in right front quarter with only 37.91 quarters positive of the tested quarters. However, among buffaloes also lowest incidence was observed in right front quarters (36.21%) in accord with overall incidence; whereas deviance was seen in case of cows with lowest prevalence in left front quarters (42.86%) though it was only slightly lower than that of right front quarters (43.24%) in cows. Our observations were in concurrence with those of Kisku and Samad (2013) [15] who also documented highest prevalence in right hind quarters and lowest in right front quarter.

Various other workers, from different parts of world like Kavitha *et al.* (2009) [13], Badiuzzaman *et al.* (2015) [2], Nithya *et al.* (2017) [18], have also observed right hind quarter to be most affected with mastitis. However, other workers like Khan and Muhammad, (2005) [14], Srinivisan *et al.* (2013) [22], Tripura *et al.* (2014) [23] have observed highest prevalence in left hind quarter instead of right hind quarter which are not in accordance with the findings of present study.

Cultural examination revealed that 180 (88.23%) samples were positive for bacterial growth. Among cattle, 45 (91.84%) samples and from buffaloes 135 (87.09%) samples were positive in bacteriological examination (Table 5). Gram's staining of the culture revealed that overall, 67.78% infections were due to Gram positive bacteria while 32.22% were caused by Gram negative bacteria. Both in cattle and buffalo infection with gram positive bacteria was found to be higher than gram negative bacteria. 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. Khan and Muhammad, (2005) [14],

Jeykumar *et al.* (2013) [10], Jyothi *et al.* (2018) [11], Mohanty *et al.* (2013) [16] in their studies conducted in different parts of India 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.

*In vitro* antibiotic sensitivity pattern of the isolates using 12 commonly used antibiotic discs viz., Enrofloxacin, Ciprofloxacin, Gentamicin, Ceftriaxone-sulbactam, Amoxicillin-sulbactam, Chloramphenicol, Tetracycline, Penicillin-G, Amoxicillin, Cloxacillin, Ceftriaxone, Cefoperazone was determined (table 6). Enrofloxacin was found to be most effective (92.22%) in inhibiting the bacterial growth followed by Ciprofloxacin (91.11%) while Penicillin-G was found to be least effective (15.56%). Amoxicillin-sulbactam and Ceftriaxone-sulbactam were also found to be very effective with 90.56% and 87.78% susceptibility, respectively. Annotations of the present study are in accord with those of Jeykumar *et al.* (2013) [10] who also found Enrofloxacin to be most effective followed by Ciprofloxacin and found Penicillin-G to be least effective. Similarly, Bhanot *et al.* (2012) [4] and Bhat *et al.* (2017) [5] also found enrofloxacin to be the most effective against the mastitis causing pathogens among the battery of antibiotics tested. However, other workers have also found other antibiotics to be most effective against mastitis causing bacteria compared to enrofloxacin (Ceniti *et al.*, 2017; Jyothi *et al.*, 2018) [6,11]. Isolates in the present study showed moderate sensitivity or even resistance to the tested antibiotics. The possible reason for this could be indiscriminate and frequent use of these antibiotics in animals which have eventually led to resistance against these antibiotics in the pathogens (Jeykumar *et al.*, 2013; Verma *et al.*, 2018) [10, 24].

#### 4. Conclusions

The forgoing result and discussion lead to conclude that, mastitis in clinical and subclinical form is prevalent in dairy animals with higher affinity for hind quarters. Farmers have lack of knowledge on the control measures, hygiene and sanitation in relation to mastitis. The dairy farmers should be educated and made aware to adopt better managerial practices for profitable production of safe and wholesome milk. WST would provide an easy, economic and rapid test for the diagnosis of subclinical mastitis and farmers should be trained to utilize this test in the farm management practices. The pathogenic bacteria are also gradually developing resistance to commonly used antibiotics and hence judicious use of antibiotics and adoption of antibiotic sensitivity testing should always be recommended by veterinary clinicians in order to give effective treatment.

**Table 4:** Individual quarter-wise incidence of mastitis

Animal	Left Hind		Right Hind		Left Front		Right Front	
	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
Cow	39	27 (69.23)	40	33 (82.5)	35	15 (42.86)	37	16 (43.24)
Buffalo	121	86 (71.07)	118	103 (87.29)	110	45 (40.90)	116	42 (36.21)
Total	160	113 (70.63)	158	136 (86.07)	145	60 (41.38)	153	58 (37.91)

**Table 5:** Categorization of bacteria isolated based on Gram's staining

	Cow	Buffalo	Overall
<b>Bacteria</b>			
Gram's positive	31 (68.89%)	91 (67.41%)	122 (67.78%)
Gram's negative	14 (31.11%)	44(32.59%)	58 (32.22%)
Total	45	135	180

**Table 6:** Antibigram of bacterial isolates

	Antibiotics											
	E	C	G	Cs	As	Ch	T	P	Am	Cx	Ct	Cf
<b>Bacteria</b>												
<b>Gram positive</b>	90.98	88.52	31.97	86.07	89.34	82.79	32.79	17.21	22.13	22.95	62.30	69.67
<b>Gram negative</b>	94.83	96.55	41.38	91.38	93.10	81.03	31.03	12.07	18.97	22.41	79.31	82.76
<b>Total</b>	92.22	91.11	35.00	87.78	90.56	82.22	32.22	15.56	21.11	22.78	67.78	73.89
E = Enrofloxacin			C= Ciprofloxacin			G=Gentamicin			Cs = Ceftriaxone-sulbactam			
As = Amoxicillin sulbactam			Ch= Chloramphenicol			T = Tetracycline			P = Penicillin-G			
Am = Amoxicillin			Cx = Cloxacillin			Ct = Ceftriaxone			Cf = Cefoperazone			

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