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Season wise prevalence of mastitis in bovines with Antimicrobial study

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Abstract
In this Study a total of 378 milk samples (100 from cows and 278 from buffaloes) were screened for mastitis first with california mastitis Test (CMT) and then cultural sensitivity test (CST) was performed on all the positive samples from different villages of Rohtak Districts of Haryana throughout the year. Data was analysed and overall animal season wise prevalence of mastitis was observed. Higher prevalence was observed in summer season in cows (32%) and in rainy season in buffaloes (30.21%) followed by winter season in cows and buffaloes respectively (28%) and (29.85%). Antibiogram was also performed on all the samples and Enrofloxacin (87.30%) was found to be most sensitive antibiotic followed by Ceftriaxone (83.59%) and Gentamicin (83.33%).

Keywords: mastitis, seasons, prevalence, bovines and antibiotic sensitivity

Introduction
India has highest number of cattle in world and stands first in milk production [1]. It possesses the best genetic resources of buffaloes in the world [2]. Mastitis is one of the most important diseases of economic importance affecting dairy animals in India and worldwide. About 135 – 150 bacterial species, sub-species and serovariants have been isolated from bovine mammary glands affected with mastitis [3]. Mastitis affects the quality and quantity of milk. In India, an annual loss due to mastitis in cows and buffaloes has been calculated to the tune of Rs.7165.51 crores of which 57.93% losses are due to sub clinical mastitis [4]. Higher productivity of dairy animals over the period of time and its positive correlation with mastitis incidence makes that treatment of mastitis during lactation is an inevitable tool, and thus it is an important part of economic losses in mastitis affected dairy animals. However, "successful treatment" of all mastitis cases remains the challenging task for dairy farmers and veterinarians across the globe. Complex nature of disease caused very little progress in understanding the reasons for low cure rate. In our study we studied Season wise prevalence of clinical mastitis in bovines the seasons were classified as winter (December to March), summer (April to June), rainy (July to September), and autumn (October to November) and infer out some concusion that how season affect onset of mastitis its prevalence, its progression and how we should manage it after receiving a concrete data followed by Antimicrobial study on causative agents too for better formulation of treatment regime and to create better strategy to prevent this huge loss.

Materials and Methods
The present investigation on clinical mastitis was carried out in cows and buffaloes by conducting California mastitis Test (CMT) and cultural sensitivity test (CST) of the milk samples received in disease investigation Lab Rohtak from July, 2018-June, 2019.

Source of milk samples
A total of 378 milk samples (100 from cows and 278 from buffaloes) were received at Disease Investigation Lab, Rohtak for mastitis were first screened with CMT and then with CST on all the positive samples from different villages of Rohtak Districts of Haryana throughout the year.

Collection of milk samples
Farmers were advised to collect Milk samples under aseptic conditions. The udders of cows and buffaloes were cleaned thoroughly with a cloth containing dilute potassium permanganate

solution. Hands were properly washed with soap and water. The first few milk stripping were discarded and nearly 15-20 ml of quarter milk sample was collected separately in sterilized test tubes. These test tubes were marked as right fore (RF), right hind (RH), left fore (LF) and left hind (LH) and collection was done first from near side and then from off side to avoid contamination of teat apices.

2.1 Prevalence

California mastitis test (CMT): The CMT reagent is detergent based, that reacts with cellular content and inflammatory substance in milk to form a viscous gel or precipitation. The test was performed as per Doxy [5] with CMT reagent solution: 3g of sodium lauryl sulfate was added to 100 ml distilled water and mixed thoroughly. The solution was heated at 50^0C in a water bath to make it clear. Bromcresol purple was added to the solution to make the final concentration as 1:10000 and pH adjusted to 8.

Equal quantities of milk sample and the reagent solution (2-3ml) were mixed in a plastic plate having four cups, representing each milk quarter as RF, RH, LF and LH. The plate was rotated gently with the help of a handle, in a circular fashion for 20 seconds. The positive result was shown by the development of a viscous gel that tended to swirl towards the centre.

Bacteriological examination

The milk samples collected aseptically were shaken thoroughly and 0.01 ml of the milk sample was streaked on Nutrient Agar and MacConkey's lactose agar plates, with the help of a 4 mm diameter platinum loop. The plates were incubated aerobically at 37^0C for 24 to 48 hours. Sub-cultures of the resulting growth were made on MacConkey's lactose agar plates for purification of isolates and identified on the basis of Grams reaction, morphology and colony characteristics.

In-vitro drug sensitivity pattern

Different strains of various organisms isolated from udder infections were subjected to *in-vitro* drug sensitivity testing, using 11 antimicrobials by disc-diffusion method as suggested by Bauer and coworkers [6].The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. The results were recorded as sensitive, intermediate and resistant.

Results and Discussion

In this Study a total of 378 milk samples (100 from cows and 278 from buffaloes) were Screened for mastitis first with CMT and then CST was performed on all the positive samples from different villages of Rohtak Districts of Haryana throughout the year. Data was analysed and overall animal season wise prevalence of mastitis was observed. Prevalence in rainy season was 22% in cows and 30.21% in buffaloes in Spring season prevalence was 18% in cows and 27.69% in buffaloes in Winter season 28% in cows and 29.85% in buffaloes while in summer season 32% in cows and 11.87% in buffaloes (Table1). Higher prevalence was observed in summer season in cows (32%) and in rainy season in buffaloes (30.21%) followed by winter season in cows (28%) and (29.85%) in buffaloes. The season variation is an important factor that directly affects the occurrence of mastitis [7-8].The present study revealed that high incidence of mastitis was recorded during summer season in cows and monsoon season in buffaloes which is in agreement with Shinde and coworkers. [9], Jadhav and coworkers [10] and Ameh and coworkers [11]. The high percentage of humidity in both seasons may cause the most growth of pathogenic agents. Some deficiencies such as selenium and vitamin E deficiency result in an increased incidence of clinical mastitis and dietary supplementation improves udder health, with the effects most evident at calving and early lactation [12]. In USA Olde Riekerink and coworkers [13] showed the increase in the somatic cell count during the cold seasons who established the highest proportion of clinical mastitis during the winter. According to the authors, the incidence of clinical mastitis caused by environmental or infectious pathogens are largely dependent on the production system. In farms where cattle grazed on pastures, the typical environmental coli mastitis exhibited a peak during the winter. Other studies reported a summer increase (from June to August) of clinical streptococcal and E. coli mastitis in farms with rearing in stall all the year round [14]. Taking into consideration the specific epidemiology of each pathogen, the relationship between disease prevalence and climatic/ecological factors could be various. During the summer, the humidity and high ambient temperatures are favourable for the development of coli forms in the bedding [15] whereas for cows grazing during the summer these problems are more frequent during the winter indoor period [16]. In summer, immunosuppression because of heat stress (THI in summer > 72) may also have a role for higher occurrence rate of clinical mastitis in primiparous cows during late lactation. Olde Riekerink and coworkers [13] stated that Clinical mastitis happened in late fall more than in the summer. Cows were in high risk for clinical mastitis in summer more than in winter. The mastitic cows in spring and winter had lower days in milk after calving compared to that of cows in summer during early lactation period. Ghavi Hossein-Zadeh and and coworkers [17] reported the odds of clinical mastitis increased in multiparous cows (odds ratio, OR = 2.83), in winter season (OR = 1.68) and in the first month of lactation (OR = 3.38). The present and preceding studies indicate that the risk of developing mastitis in monsoon and summer season is more as the conditions are favorable for the proliferation of pathogenic bacteria due to adequate humidity and temperature of bacterial growth as it was also favored by our study.

Table 1: Seasonal cases of mastitis in buffaloes and cows at D.I. Lab., Rohtak during July, 2018-June, 2019

Species		Rain	Spring	Winter	Summer	Total
Cattle	Positive	22(22%)	18(18%)	28(28%)	32(32%)	99 (99%)
	Negative	1	0	0	0	1
	Total	23	18	28	32	100
Buffalo	Positive	84(30.21%)	77(27.69%)	83(29.85%)	33(11.87%)	277 (99.64%)
	Negative	1	0	0	0	1
	Total	85	77	83	33	278
Overall	Positive	106(27.96%)	95(25.06%)	111(29.28%)	65(17.15%)	376 (99.47%)
	Negative	2	0	0	0	2
	Examined	108	95	111	65	378

Winter-December, January, February; Summer-April, May, June; Rainy-July, August, September Spring/Autumn-October, November, March

Antibiogram

Antibiotic sensitivity was performed on all the 378 samples, a total of 11 antibiotics were used Amikacin, Amphicillin, Ceftriaxone, ciprofloxacin, Enrofloxacin, gentamicin, cefoperazone, chloramphenicol, levofloxacin, ceftizoxime and Tetracycline (Table.2 and Fig.1). Enrofloxacin (87.30%) was found to be most sensitive antibiotic followed by Ceftriaxone (83.59%) and Gentamicin (83.33%).Ranjan and coworkers [18] also found high sensitivity towards Enrofloxacin (91.67%) where as they observed lower sensitivity towards Ceftriaxone (84.10%). Pankaj and coworkers [19] studied the antibiogram of isolates of mastitis and similiarly revealed 100% sensitivity to Cloxacillin, Ceftriaxone and Cefoperazone and high (90.90-100%) sensitivity towards Enrofloxacin, to Cloxacillin, Ceftriaxone and Cefoperazone and high (90.90-100%) sensitivity towards Enrofloxacin, Cephalexin, Gentamicin and Lincomycin. The antibiogram obtained in current study indicated high sensitivity towards newer and older antibiotics showing rationale use of these antibiotics at Field level.

Table 2: Antibiogram of CMT positive Milk samples at DI lab., Rohtak during the year 2018-19

Total	Amikacin	Amphi+sul	Ceftriaxone	Ciprofloxacin	Enrofloxacin	Gentamicin	Cefopera+Sul	Chloramphinicol	Livo.	Ceftizo	Tetra
378	196	136	316	274	330	315	66	229	172	162	62
%	51.85	35.97	83.59	72.48	87.30	83.33	17.46	60.58	45.50	42.85	16.40

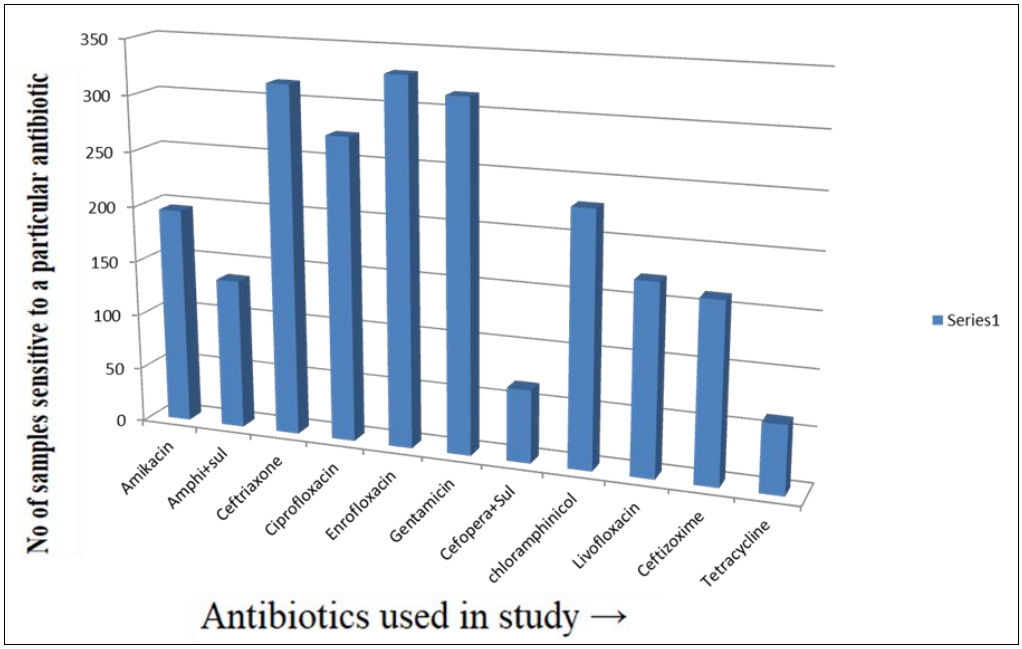

Fig 1: Antibiogram Profile of 378 Samples

Conclusion

The above Study carried out on 378 milk samples (100 from cows and 278 from buffaloes) which were Screened for mastitis first with California mastitis Test (CMT) and then cultural sensitivity test (CST) on all the positive samples from different villages of Rohtak Districts of Haryana throughout the year. Data was keenly studied and analysed for season wise prevalence of mastitis. Higher prevalence was observed in summer season in cows (32%) and in rainy season in buffaloes (30.21%) followed by winter season in cows and buffaloes respectively (28%) and (29.85%). Antibiogram studies were was also performed on all the samples and Enrofloxacin (87.30%) was found to be most sensitive antibiotic followed by Ceftriaxone (83.59%) and Gentamicin (83.33%), Cloxacillin and Cefoperazone were also observed to have good sensitivity. This study clear cut indicates that summer and rainy seasons in both buffaloes and cows were more prone to udder related infections i.e mastitis. So, utmost care should be taken for hygiene and cleanliness in organized and unorganized farms to avoid the milch animals to get infection in these seasons and Antibiogram study help in proper recommendation of antibiotics for early and timely treatment.

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