AN OPEN LABEL STUDY TO ASSESS THE EFFICACY OF CEFTIOFUR IN TREATMENT OF CLINICAL MASTITIS IN BUFFALOES

Ashok Boora[1,*], Sarita Yadav[1], Parvina Devi[1], Kunwar Pal Singh[1], Pawanjit Singh Cheema[2], Vijay Muley[4], Virender Sehrawat[3], Ketan Dhamanaskar[4] and Inderjeet Singh[1]

ABSTRACT

The study objective was to evaluate the efficacy of Ceftiofur hydrochloride intramammary (IMM) formulation (Spectramast LC, Zoetis India) with or without parenteral Cefoperazone/Sulbactum antibiotic on bacteriological cure, clinical cure and pathogen cure in lactation clinical mastitis (CM) when compared with control group treated with Amoxicillin-Sulbactum. The study was conducted from September 2015 through December 2017 on lactating buffaloes suffering from clinical mastitis (n=307) (Treatment group, T1 = 156 and Control group, T2 = 151) mostly at farmer's doorstep and also the participation of organized buffalo herds located at Hisar (n=2), Sirsa (n=1) from Haryana and at Nabha (n=1) from Punjab after follow up 1 and 2 at day 10 and 21 respectively. Infected quarters in Grade I and II lactation CM Treatment group (T1) were treated from day 0 to day 4 i.e. for 5 consecutive days with Ceftiofur hydrochloride IMM formulation or in Grade III lactation CM, IMM Ceftiofur hydrochloride along with parenteral Cefoperazone/Sulbactum. Control group (T2) received treatment from day 0 to day 2 i.e. for 3 consecutive days with Amoxicillin-Sulbactum antibiotic. Of 307 buffaloes infected with CM at day 0 pre-treatment, 93.49% of milk samples came culture positive whereas 52.12% (n=160/307) and 29.64% (91/307) of culture positive milk samples were there at day 10 and day 21 post-treatment respectively. Coagulase-negative *Staphylococcus* was the most prevalent causative agent followed by other gram positive, mixed infection, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* spp, other coliform, *Pseudomonas*, other gram negative and *Enterococcus* spp. While apparent bacteriological cure rate of IMI was 50.37% (at animal level) at day 10 post-treatment in the Treatment group receiving Spectramast LC, it was 77.78% at day 21 post-treatment in the same group. The bacteriological cure rate of 45.45% and 57.58% were observed at day 10 and day 21 post-treatment in Control group respectively. Buffaloes receiving Spectramast LC (Treatment group) were 1.12 times (at day 10) and 1.35 times (at day 21) more likely to cure than Control group. Treatment group showed numerically higher clinical and pathogen cure than Control group.

Keywords: *Bubalus bubalis*, buffaloes, lactation, clinical mastitis, antibiotic sensitivity, Ceftiofur

[1]Central Institute for Research on Buffaloes, Indian Council of Agricultural Research, Hisar, India

[2]Disease Investigation Laboratory, Lala Lajpat Rai University of Veterinary and Animal Sciences, Campus Sirsa, Sirsa, India

[3]Animal Husbandry and Dairying, Rohtak, India

[4]Zoetis India Limited, Santacruz (East), Mumbai, India

INTRODUCTION

Mastitis, an inflammation of the mammary gland is characterized by physical and chemical changes in the milk and pathological changes in the glandular tissue (1). Bovine mastitis being one of the most economically significant diseases for the Indian dairy industry causes financial losses to the extent of Rupees 7165.51 crores (2). Mastitis-related economic losses include decreased milk yield, reduced reproductive performance, dairy animal replacement cost and increased treatment expenses (3 to 5). In India, 50% of milch animals are affected with mastitis, out of which clinical mastitis accounted for 10% (6). There are various risk factors that influence the incidence of clinical or subclinical mastitis such as age, parity, stage of lactation, milk yield, history of previous mastitis, morphology of udder and teat, udder hygiene, floor type, season of year and milking practices (1, 7). Bovine mastitis is associated with a wide spectrum of pathogenic agents but *Staphylococci*, *Streptococci* and Coliform group are the most prevalent bacteria implicated in bovine mastitis globally (8, 9).The identification of IMI requires the bacteriological culture (BC) of mastitis pathogens from milk samples, a gold standard for diagnosis of IMI .However, Somatic cell count(SCC) an indicator of intramammary infection (IMI), has been used extensively as a screening test to monitor the udder health status in dairy herds (10, 11).

As antibiotics use for treatment of mastitis may lead to microbial resistance, antibacterial susceptibility should be cautiously and continuously monitored. Estimation of trends over time is crucial to provide long-term efficacy of the antibiotic and to guide the veterinarian in choosing the most appropriate antibiotic for mastitis treatment, especially because antibiotic treatment is usually started before antibiotic susceptibility testing (12).The antibiotic susceptibility results shown in this study may not necessarily reflect the current antibacterial susceptibility of the mastitis causing bacteria, but should provide as a reference baseline.

The objective of this study was to evaluate the efficacy between Spectramast LC, Zoetis with or without parenteral Cefoperazone/Sulbactum antibiotic on bacteriological cure, clinical cure and pathogencure (number of animals remained free from mastitis pathogens after the treatment) in lactation clinical mastitis (CM) when compared with control group given Amoxicillin-Sulbactum. Identification of the major bacterial pathogens were also carried out.

MATERIALS AND METHODS

Study population

The study evaluating lactation therapy formulation was conducted as a randomized controlled open label study that ran from September 2015 through December 2017 on lactating buffaloes suffering from clinical mastitis (n=307) mostly at farmer's doorstep and also the participation of organized buffalo herds located in Hisar (n=2), Sirsa (n=1) from Haryana and in Nabha (n=1) from Punjab. The study was approved by the institutional ethics committee of Central Institute for Research on Buffaloes (ICAR-CIRB), Hisar. The herd size was between 450 to 1000. The eligibility for selection of the animals in trail was assessed according to the inclusion/exclusion criteria shown in Table 1. The grades of mastitis along with associated clinical signs were given in Table 2. Buffaloes which were diagnosed with lactation clinical mastitis in one or more glands at any stage

of lactation were randomized to Treatment group (T1, n=156) and Control group (T2, n=151).Infected quarters in Grade I and II lactation clinical mastitis treatment group were treated from day 0 to day 4 i.e. for 5 consecutive days with intramammary infusion of Ceftiofur hydrochloride formulation alone whereas Ceftiofur hydrochloride formulation along with parenteral Cefoperazone/Sulbactum in Grade III lactation clinical mastitis. The buffaloes in Control group received treatment from day 0 to day 2 i.e. for 3 consecutive days with Amoxicilin-Sulbactum. Treatment protocol for treatment and Control group were given in Table 3. Data was finally analyzed for the study after follow up 1 and 2 at day 10 and 21 respectively.

Sample collection

The study investigators monitored and evaluated the study animals up to 21 days post therapy and collected milk samples from affected quarter at different time period: Pre-treatment sampling at day 0 immediately before therapy, post-treatment sampling twice at day 10 (±1) and day 21 (±1) post-therapy. The California mastitis test (CMT) was conducted buffalo-side or on the farm from clinically affected quarter prior to sampling at day 0. The CMT score was ranked zero (negative) when the mixture of milk and reagent remained normal, whereas CMT score of 3, the maximum, was observed when gel was formed and mixture was tough to shake. The udder preparation before sample collection consisted of teats cleaning and drying with a paper towel and thereafter disinfecting teats especially teat ends using 70% ethanol soaked gauze. The first few streams of foremilk were discarded to flush out any bacteria that might be present in the teat canal and then 10 ml of milk was collected from affected teat aseptically in sterilized disposable plastic tubes with 15 ml capacity.

Microbiological identification

Milk samples were sent to microbiological laboratory at CIRB and were used bacterial isolationas per the study procedures. Isolation of bacteria was carried out on the basis of standard procedures (13). A loopful of each milk sample was streaked on 5% defibrinated sheep blood agar and MacConkey's agar plates (Himedia) and incubated at 37°C for 24 to 48 h. Plates with the growth of three or more colony types were considered contaminated and discarded. Representative colonies were purified through subculturing on nutrient agar and subjected to initial identification by colony morphology and gram staining. Further identification of the bacteria was done as per the guidelines of national mastitis council (14). Gram-positive bacteria were differentiated as staphylococci, CNS, micrococcus and streptococci using catalase reaction (3% hydrogen peroxide), oxidase, haemolysis pattern, coagulase tube reaction in rabbit plasma (Himedia). Gram-negative bacteria were identified and differentiated by oxidase test, KOH test, IMViC tests (indole, methyl red and Voges–Proskauer reactions and Simmons's citrate), nitrate reduction, triple sugar iron agar test as well as by growth features on MacConkey agar, eosin methylene blue agar and green pigmentation on nutrient agar by pseudomonas (13).

Definition of infection

Bacteriological cure

The treated quarters were qualified as bacteriologically cured if an udder pathogen present in pre-treatment milk sample before antibiotic therapy was (15) absent in any of the post- treatment milk samples. Treated quarters with growth of one original pathogen in post-treatment

Table 1. Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Buffaloes with clinical mastitis either in single or multiple quarters shall be enrolled	Planned participation in another clinical trial during the present trial period.
Consent agreeing to comply with study requirements	Previous received antibacterial or anti-inflammatory therapy less than 21 days prior to enrolment.
	Systemic illness
	Significant underlying chronic disease.
	Animals with injured or damaged teat ends/teats/ quarters

Table 2. Grades of mastitis based on associated clinical signs.

Grades of mastitis	Clinical signs
Grade I	Abnormal milk
Grade II	Abnormal milk, Inflamed udder
Grade III	Abnormal milk, Inflamed udder, Systemic disease

Table 3. Treatment protocol for treatment and control group.

Groups	Number of animals	Grades of mastitis	Spectramast* LC	Pathocef**	Amoxirum forte***	Supportive therapy****
T1	156	I	Yes	No	No	No
		II	Yes	No	No	Yes
		III	Yes	Yes	No	Yes
T2	151		No	No	Yes	Yes

*Intramammary infusion of Ceftiofur hydrochloride formulation (125 mg Ceftiofur hydrochloride in 10 ml, Spectramast LC, Zoetis) from day 0 to day 4, i.e. for 5 consecutive days.

**Intramuscular or intravenous administration of Parenteral combination of Cefoperazone with Sulbactum at dose rate of 10 mg/kg bwt for 3 days at 24 h interval.

***Intramuscular or intravenous administration of Amoxycillin sodium and Sulbactum at dose rate of 7 to 10 mg/kg bwt for 3 days at 24 hours interval.

****Supportive therapy (NSAID and fluid therapy) shall be given as per requirement.

T1 = Treatment group

T2 = Control group

Table 4. Overall distribution of bacterial species and prevalence of clinical mastitis during three sampling time and subsequently in the Control (T2) and Treatment group (T1) of buffaloes.

Bacteriological diagnosis	**Day 0 pre-treatment**		**Day 10 post-treatment**		**Day 21 post-treatment**	
Overall (n = 307)	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**
Total gram positive	198	64.50	104	33.88	49	15.96
Total gram negative	47	15.31	49	15.96	37	12.05
Mixed	42	13.68	7	2.28	5	1.63
Culture positive	287	93.49	160	52.12	91	29.64
Culture negative	20	6.51	147	47.88	216	70.36
Total	307	100.00	307	100.00	307	100.00
Control group (T2) (n=151)						
Total gram positive	95	62.91	48	31.79	39	25.83
Total gram negative	27	17.88	25	16.56	18	11.92
Mixed	15	9.93	4	2.65	3	1.99
Culture positive	137	90.73	77	50.99	60	39.74
Culture negative	14	9.27	74	49.01	91	60.26
Total	151	100.00	151	100.00	151	100.00
Treatment group (T1) (n=156)						
Total gram positive	103	66.03	56	35.90	10	6.41
Total gram negative	20	12.82	24	15.38	19	12.18
Mixed	27	17.31	3	1.92	2	1.28
Culture positive	150	96.15	83	53.21	31	19.87
Culture negative	6	3.85	73	46.79	125	80.13
Total	156	100.00	156	100.00	156	100.00

Table 5. Overall distribution of bacterial species and prevalence of clinical mastitis (n=307, animal level) at day 0 pre-treatment, day 10 and day 21 post-treatment in both control and treatment group together.

Bacteriological diagnosis	Day 0 pre-treatment		Day 10 post treatment		Day 21 post-treatment	
Treatment group (n=156)	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**
Staph aureus	24	7.82	6	1.95	4	1.30
CNS	111	36.16	72	23.45	29	9.45
Streptococcus	13	4.23	4	1.30	3	0.98
Enterococcus	2	0.65	1	0.33	1	0.33
Other gram positive	48	15.64	21	6.84	12	3.91
E. coli	27	8.79	23	7.49	16	5.21
Other coliforms	9	2.93	13	4.23	11	3.58
Other gram negative	4	1.30	5	1.63	2	0.65
Pseudomonas	7	2.28	8	2.61	8	2.61
Mixed	42	13.68	7	2.28	5	1.63
No growth	20	6.51	147	47.88	216	70.36
Total	307	100.00	307	100.00	307	100.00

Table 6. Distribution of bacterial species and prevalence of clinical mastitis (n=151, animal level) at day 0 pre-treatment, day 10 and day 21 post-treatment in Control group.

Bacteriological diagnosis	Day 0 pre-treatment		Day 10 post-treatment		Day 21 post-treatment	
Control group (n=151)	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**
Staph aureus	12	7.95	4	2.65	4	2.65
CNS	55	36.42	30	19.87	23	15.23
Streptococcus	5	3.31	3	1.99	3	1.99
Other gram positive	23	15.23	11	7.28	9	5.96
E. coli	16	10.60	11	7.28	6	3.97
Other coliforms	5	3.31	5	3.31	5	3.31
Other gram negative	2	1.32	3	1.99	1	0.66
Pseudomonas	4	2.65	6	3.97	6	3.97
Mixed	15	9.93	4	2.65	3	1.99
No growth	14	9.27	74	49.01	91	60.26
Total	151	100.00	151	100.00	151	100.00

Table 7. Distribution of bacterial species and prevalence of clinical mastitis (n=156, animal level) at day 0 pre-treatment, day 10 and day 21 post-treatment in Treatment group.

Bacteriological diagnosis	**Day 0 pre-treatment**		**Day 10 post-treatment**		**Day 21 post-treatment**	
Treatment group (n=156)	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**	**No.**	**Prevalence (%)**
Staph aureus	12	7.69	2	1.28	0	0.00
CNS	56	35.90	42	26.92	6	3.85
Streptococcus	8	5.13	1	0.64	0	0.00
Enterococcus	2	1.28	1	0.64	1	0.64
Other gram positive	25	16.03	10	6.41	3	1.92
E. coli	11	7.05	12	7.69	10	6.41
Other coliforms	4	2.56	8	5.13	6	3.85
Other gram negative	2	1.28	2	1.28	1	0.64
Pseudomonas	3	1.92	2	1.28	2	1.28
Mixed	27	17.31	3	1.92	2	1.28
No growth	6	3.85	73	46.79	125	80.13
Total	156	100.00	156	100.00	156	100.00

Table 8. Bacteriological cure rate from pre-treatment milk samples at day 0 and post-treatment at day 10, 21 from control (n=132) and Treatment group (n=135) excluding new IMI and NG samples*

Day 0 pre-treatment		**Day 10 post-treatment**			**Day 21 post-treatment**		
Control group (n=132)	**IMI$ cases**	**IMI cases**	**IMI cured**	**cured%**	**IMI cases**	**IMI cured**	**cured%**
Total gram positive	91	45	46	50.55	36	55	60.44
Total gram negative	26	19	7	26.92	12	14	53.85
Mixed	15	1	7	46.67	1	7	46.67
Total	132	65	60	45.45	49	76	57.58
Treatment group (n=135)							
Total gram positive	92	38	54	58.70	8	84	91.30
Total gram negative	16	12	4	25.00	11	5	31.25
Mixed	27	2	10	37.04	2	16	59.26
Total	135	52	68	50.37	21	105	77.78
Overall T1 and T2	267	117	128	47.94	70	181	67.79

*New IMI, new intramammary infection, NG, no growth samples.

milk sample compared to two pathogen isolated in pre-treatment milk sample (mixed infection) were not considered as bacteriologically cured. The treated quarters that were initially cultured with no growth before antibiotic therapy at day 0 were not eligible for analysis of bacteriological cure

Clinical cure

A quarter was defined as a clinical cure when its clinical scores (clinical signs such as abnormal milk, inflamed udder and systemic disease) were zero on day 10 and day 21 post-treatment. The treated quarters that were initially cultured with no growth before antibiotic therapy at day 0 were included in clinical cure analysis.

Pathogen cure

When both follow-up samples (at day 10 and day 21) contained no pathogen on culture, the animal was defined as pathogen cure. The treated quarters that were initially cultured with absence of growth before antibiotic therapy at day 0 were eligible for analysis of pathogen cure.

RESULTS AND DISCUSSION

Prevalence of clinical mastitis and udder pathogens at day 0 pre-treatment

Based on the result of microbiological examination (Table 4), the overall prevalence of clinical mastitis in buffalo (at animal level) in the present study was 93.48% at day 0 pre-treatment. From Table 5, it was obvious that the most prevalent causative agent (on animal basis) was coagulase-negative *Staphylococcus,* representing 36.16% of all recovered isolates followed by other gram positive (15.64%), mixed infection (13.68%), *Escherichia coli* (8.79%), *Staphylococcus aureus* (7.82%), *Streptococcusspp* (4.23%), other coliform (2.93%), *Pseudomonas* (2.28%), other gram negative and *Enterococcus* spp (0.65%). No growth was observed in 20 (6.51%) clinical mastitis samples. Gram-positive, gram-negatives and mixed infections represented 64.5%, 15.31 % and 13.68 % of all udder pathogens isolated, respectively (Table 4). In control group, of the 151 samples, 55 (36.42%) isolates belonged to coagulase negative *Staphylococcus* (CNS) spp. followed by 23 (15.23%) isolates of other Gram positive bacteria. (Table 6). On the other hand, in treatment group, of the 156 samples at day 0 post-treatment, 56 (35.90%) isolates belonged to coagulase negative *Staphylococcus* (CNS) spp. followed by 25 (16.03%) isolates of other gram-positive bacteria (Table 7).

Bacteriological cure rate

Of 132 udder milk samples at day 0 pre-treatment in the control group (excluding new IMI and no growth culture samples), 65 samples continued positive with the same mastitis bacteria day 10 post-treatment, yielding a bacteriological cure rate of 45.45% (Table 8). The bacteriological cure rate of 57.58% (76 cases cured) was observed at day 21 post-treatment in Control group. Apparent bacteriological cure rate of IMI was 50.37% (of 135 IMI cases, 68 were cured) at animal level at day 10 and 77.78% (of 135 IMI cases, 105 were cured) at day 21 post-treatment in the Treatment group receiving Spectramast LC. Buffaloes receiving Spectramast LC (Treatment group) were 1.12 times (at day 10) and 1.35 times (at day 21) more likely to cure than Control group. In the Control group, where Amoxicilin/Sulbactum was used, 48.48% of intramammary infections (n=65) were still present 10 days post-treatment whereas only 38.52% (n=52) were presentin the Treatment group (n=135) at day

10 post- treatment. According to gram staining stratification, 46 of 91 (50.55%) Amoxicilin-Sulbactum treated Control group buffaloes with gram-positive mastitis cured whereas 54 of 92 (58.7%) of Spectramast LC (Treatment group) buffaloes with gram-positive cultures experienced bacteriological cure at day 10 post-treatment. Of the buffaloes with gram- negative mastitis, 26.92% of the Amoxicilin -Sulbactum treated Control group cured (n=7/26) whereas a similar percentage (25%) of the Spectramast LC (Treatment group) buffaloes cured (n=4/16) day 10 post-treatment.

Clinical cure

While, overall 67% of buffaloes (n=205/307) experienced clinical cure or zero clinical scores (clinical signs such as abnormal milk, inflamed udder and systemic disease) at day 10, clinical cure was 68% (n=209/307) at day 21 post-treatment. Of the Amoxicilin-Sulbactum treated Control group buffaloes, 56% (n=85/151) experienced clinical cure at 10 days post-treatment whereas 77% of Spectramast LC Treatment group buffaloes experienced clinical cure at 10 days post-treatment (n=120/156). Similarly at day 21 post-treatment, clinical cure was 58% (n=88/151) and 78% (n=121/156) for Amoxicilin-Sulbactum treated Control group and Spectramast LC Treatment group buffaloes respectively.

Pathogen cure

New intramammary infection was not considered in analysis of pathogen cure. Overall, 51.22% (n=147/287) of buffaloes experienced pathogen cure at 10 days post-treatment: 50.68% (n=74/146) of the Amoxicilin-Sulbactum treated Control group buffaloes and 51.77% (n=73/141) of Spectramast LC Treatment group buffaloes. Similarly at day 21 post-treatment, pathogen cure rate was 61.64% (n=90/146) and 78% (n=110/141) for Amoxicilin-Sulbactum Treated control group and Spectramast LC Treatment group buffaloes respectively.

Safety of drug

There was no drug - related systemic reactions reported in this study. No clinical signs of udder swelling, pain or redness were observed in the Treatment group.

This study evaluated the efficacy of Spectramast LC, Zoetis with or without parenteral Cefoperazone/Sulbactum antibiotic for the treatment of lactation clinical mastitis (CM) by analysing bacteriological cure, pathogen cure and clinical cure. The bacteriological cure was numerically higher in Spectramast LC treated cases compared with Amoxicillin-Sulbactum antibiotic treated cases, estimated at 77.78% (105/135) and 57.58% (76/132) at day 21 respectively. However, this difference was not statistically significant ($\chi 2$=0.726, P=0.39, at P≤0.01).The findings of Schukken *et al.* (2013) also revealed numerically higher bacteriological cure for Ceftiofur treated cases compared to Cephapirin treated cases. Oliver *et al.* (2004) showed that Ceftiofur therapy was effective in treating lactation subclinical mastitis caused by *Strep. uberis* in dairy cows. However, according to Bradley and Green (2009) pathogen cure is the most useful conclusion for the treatment of clinical mastitis clinically (Bradley and Green). Spectramast LC Treatment group had numerically greater pathogen cure compared with Amoxicilin-Sulbactum at day 10 and 21 post-treatment, but nonsignificant statistically ($\chi 2$=0.946, P=0.33, at P≤0.01). For clinical cure, in which Spectramast LC Treatment group was not significantly better than the Amoxicillin-Sulbactum Control group ($\chi 2$=0.0163, P=0.898, at P≤0.01), the observed proportion of

clinically cured animals for Spectramast LC was 78% compared with 58% for the Amoxicillin-Sulbactum regimen. From the results of this trail, it was concluded that Spectramast LC (Treatment group) showed numerically higher bacteriological, clinical and pathogen cures but statistically inconclusive. These findings may be the ground for establishing protocols for treatment of clinical mastitis in buffaloes.

ACKNOWLEDGEMENTS

The authors would like to thank Director, ICAR- CIRB, Hisar and buffalo farmers for their help and support to achieve objectives of this study. We acknowledge Zoetis India Limited for financially supporting this study.

REFERENCES

Bansal, B. and D. Gupta. 2009. Economic analysis of bovine mastitis in India and Punjab- A review. *Indian J. Dairy Sci*., **62**(5): 337-345.

Bradley, A.J. 2002. Bovine mastitis: An evolving disease. *Vet. J*., **164**(2): 116-128. DOI: 10.1053/tvjl.2002.0724

Bradley, A.J. and M.J. Green. 2009. Factors affecting cure when treating bovine clinical mastitis with cephalosporin-based intramammary preparations. *J. Dairy Sci*., **92**(5): 1941-1953. DOI: 10.3168/jds.2008-1497

Chassagne, M., J. Barnouin and J.P. Chacornac. 1998. Biological predictors for early clinical mastitis occurrence in Holstein cows under field conditions in France. *Prev. Vet. Med*., **35**(1): 29-38. DOI: 10.1016/s0167-5877(97)00092-5

Dohoo, I.R. and K.E. Leslie. 1991. Evaluation of changes insomatic cell counts as indicators of new intramammaryinfections. *Prev. Vet. Med*., **10**(2): 225-237. DOI: 10.1016/0167-5877(91)90006-N

Dua, K. 2001. Incidence aetiology and estimated loss due to mastitis in India- An update. *Indian Dairyman*, **53**: 41-48.

Halasa, T., K. Huijps, O. Osteras and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: A review. *Vet. Quart*., **29**(1): 18-31. DOI: 10.1080/01652176.2007.9695224

Moore, D.A., J.S. Cullor, R.H. Bondurant and W.M. Sischo. 1991. Preliminary field evidence for the association of clinical mastitis with altered interestrus intervals in dairy cattle. *Theriogenology*, **36**(2): 257-265. DOI: 10.1016/0093-691x(91)90384-p

NMC. 1999. NMC *Laboratory Handbook on Bovine Mastitis*. National Mastitis Council Inc., Madison, Wisconsin, USA.

Oliver, S.P., R.A. Almeida, B.E. Gillespie, S.J. Headrick, H.H. Dowlen, D.L. Johnson, K.C. Lamar, S.T. Chester and W.M. Moseley. 2004. Extended ceftiofur therapy for treatment of experimentally-induced Streptococcus uberis mastitis in lactating dairy cattle. *J. Dairy Sci*., **87**(10): 3322-3329. DOI: 10.3168/jds.S0022-0302(04)73468-2

Oliver, S.P. and B.A. Mitchell. 1983. Susceptibility of bovine mammary gland to infections during the dry period. *J. Dairy Sci*., **66**(5): 1162-1166. DOI: 10.3168/jds.S0022-0302(83)81913-4

Quinn, P.J., M.E. Carter, B. Markey and G.R. Carter. 1994. *Clinical Veterinary Microbiology*, Wolfe, London, UK.

Radostits, O.M., C.C. Gay, Blood D.C. and K.W. Hinchcliff. 2000. Mastitis. *In Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*, 9th ed. W.B. Saunders Co., Philadelphia, USA.

Ruegg, P.L. 2003. Investigation of mastitis problems on farms. *Vet. Clin. N. Am. Food A*., **19**(1): 47-73. DOI: 10.1016/s0749-0720(02)00078-6

Schukken, Y.H., M.J. Zurakowski, B.J. Rauch, B. Gross, L.L. Tikofsky and F.L. Welcome. 2013. Noninferiority trial comparing a first-generation cephalosporin with a third-generation cephalosporin in the treatment of nonsevere clinical mastitis in dairy cows. *J. Dairy Sci*., **96**(10): 6763-6774. DOI: 10.3168/jds.2013-6713

Seegers, H., C. Fourichon and F. Beaudeau. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res*., **34**(5): 475-491. DOI: 10.1051/vetres:2003027

Thomas, V., A. de Jong, H. Moyaert, S. Simjee, F.El. Garch, I. Morrissey, H. Marion and M. Valle. 2015. Antimicrobial susceptibility monitoring of mastitis pathogens isolated from acute cases of clinical mastitis in dairy cows across Europe: VetPath results. *Int. J. Antimicrob. Ag*., **46**(1): 13-20. DOI: 10.1016/j.ijantimicag.2015.03.013

Watts, J.L. 1988. Etiological agents of bovine mastitis. *Vet. Mmicrobiol*., **16**(1): 41-66. DOI: 10.1016/0378-1135(88)90126-5