Dynamics of milk leukocytes in response to intramammary infusion of amoxicillin plus sulbactam during bovine subclinical mastitis

U.K. De, R. Mukherjee

Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

ABSTRACT: The therapeutic potential of amoxicillin plus sulbactam and its effect on dynamics of milk leukocytes in bovine sub clinical mastitis were investigated in this study. Therapeutic efficacy was measured by somatic cell count and total bacterial count of the milk, whereas, the dynamics of milk leukocytes were assessed by measuring phagocytosis, hydrogen peroxide production, myeloperoxidase and lactoperoxidase enzyme levels in the milk leukocytes. Forty-five crossbred cows were randomly divided into three equal groups. Group I consisting of 15 cows served as healthy control, whereas 30 cows (sub clinical mastitis) were randomly divided into Groups II and III on the basis of positive reactions in the California Mastitis Test. Group II cows received 300mg of amoxicillin plus sulbactam twice daily for three days and Group III received sterile 5 ml phosphate buffer saline (pH 7.4) for three days. Both treatments were administered via the intramammary route. Observations were made up to 15 days after initiation of treatment. The results revealed a pronounced drop in somatic cell count and total bacterial count, whereas significant ($P < 0.05$) enhancement of phagocytic activity (42.20%), hydrogen peroxide production (29.46%), myeloperoxidase (49.27%) and lactoperoxidase (147.10%) enzyme levels in the milk leukocytes in Group II cows during post treatment periods were observed. Such changes were statistically non-significant in Group III cows. The results of the present study indicate that intramammary use of amoxicillin plus sulbactam augments the bactericidal function of milk leukocytes during bovine sub clinical mastitis and demonstrate the strong therapeutic potential against bovine subclinical mastitis.

Keywords: amoxicillin; leukocytes; subclinical mastitis; sulbactam

Bovine mastitis, usually caused by single or multiple bacterial infections, is an important animal health disease leading to significant economic losses to the dairy industry (Seegers et al. 2003). The intramammary administration of antimicrobials offers a convenient option for the treatment of mastitis in dairy animals to achieve optimum drug concentrations at the site of infection without systemic absorption (Gruet et al. 2001). The outcome of bacterial infection is greatly dependent on the function of polymorphonuclear cells, which play a vital role in udder defence (Burvenich et al. 1994; Kehrli and Shuster 1994; Sordillo et al. 2002). After phagocytosis, leukocytes exert their bactericidal effect through a respiratory burst that produces oxygen radicals that are the key components of the oxygen-dependent killing mechanism (Sordillo et al. 1997). Myeloperoxidase is a lysosomal enzyme of leucocyte granules and forms a system of defence against bacterial infection of the mammary gland (Cooray and Bjorek 1995). The myeloperoxidase-hydrogen peroxide system is a constituent of the oxygen-dependent antimicrobial activity of leukocytes (Mehrzad et al. 2001). Similarly, lactoperoxidase plays the most prominent role in the antimicrobial defence of leukocytes, and requires sufficient concentrations of hydrogen peroxide and thiocyanate ion (Korhonen 1980; Seifu et al. 2004). The efficiency of the lactoperoxidase system in inhibiting the growth of pathogens is mostly related to the amount of hydrogen peroxide available (Tenovuo and Knuuttila 1977). Impaired functioning of immune cells is attributed to the pathogenesis of mastitis, which is observed in the

post parturient period (Dosogne et al. 1999). The clinical efficacy of antibiotics is dependent upon a number of factors including not only intrinsic antibacterial properties but also a positive interaction with host defences (Finlay et al. 2003). Mastitis is mostly treated by intramammary antibiotics; however, these antibiotics may affect leukocyte function and body defences (Hoeben et al. 1998). Hence, antibiotics which would not have adverse effects on polymorphonuclear cell functioning, but might improve the competence of these immune cells particularly in mastitis therapy might be ideal agents for the treatment of mastitis. In this work we examined therapeutic efficacy and milk leukocyte dynamics after administration of amoxicillin plus sulbactam against bovine sub clinical mastitis.

MATERIAL AND METHODS

Selection of animals and experimental protocol

Forty-five crossbred lactating cows aged between three to seven years, in the first to fourth lactation period with average milk yield of 14.76 ± 0.64 litres per cow per day, were selected from the livestock research farm of our institute. These cows were maintained in the animal shed of the institute under identical environmental conditions and were randomly divided in three equal groups. Fifteen clinically healthy cows with fifteen healthy quarter (single quarter from each cow) negative for California Mastitis Test and somatic cell counts of less than 0.3 million cells/ml of milk sample served as the normal healthy control (Group I). Fifteen cows in Group II and 15 cows in Group III (30 cows) positive for subclinical mastitis in a single quarter per cow, on the basis of the California Mastitis Test, with somatic cell counts > 0.9 million cells/ml of milk and positive for intramammary infection, were subjected to the experimental drug treatment. Three hundred milligrams of amoxicillin sodium I.P. (200 mg) plus sulbactam sodium U.S.P. (100 mg) (Amoxirum forte, M/S Virbac Animal Health, Mumbai, India) were infused per quarter via the intramammary route in Group II cows, after diluting the drug in 7 ml sterile distilled water. This regimen lasted for three consecutive days. Similarly, 5 ml sterile PBS was infused via the intramammary route in Group III cows for three days twice daily. Observations were made up to 15 days post treatment.

Milk sampling, somatic cell count and total bacterial count

Eighty millilitres of milk from each quarter were collected in sterile vials after cleaning the teat orifice with 70% ethyl alcohol and after discarding a few streams of milk. The milk was collected before treatment (day 0) and thereafter on days 3, 7 and 15 after initiation of treatment for somatic cell count (Schalm et al. 1971) and total bacterial count (Kolmer et al. 1951). The organisms were identified on the basis of colony morphology, characteristic haemolytic pattern on 5% blood agar plates, Gram staining and biochemical tests.

Isolation of polymorphonuclear cells from milk samples

The isolation of milk leukocytes from milk samples was carried out as per the standard method (Daley et al. 1991). Viability of the cells was determined using the trypan blue exclusion technique and the cell suspension was adjusted to 1×10^6 cells/ml in phosphate buffer saline for determination of phagocytic activity, hydrogen peroxide production and for the myeloperoxidase assay.

Phagocytic activity

The phagocytic assay was performed as per the method described by Boyne and Arthur (1979). In brief, a suspension containing 0.2 ml milk leukocytes (10^6 cells/ml), 0.2 ml Candida albicans (2×10^6 cells/ml), 0.2 ml Hank's balanced salt solution and 0.15 ml fresh autologous pooled bovine serum was incubated for 65 min at 37 °C with intermittent shaking. Then, 0.25 ml methylene blue (2×10^{-4} mol) was added followed by further incubation for 10 min. A sample of the final incubation mixture was then placed in a haemocytometer and phagocytic activity was calculated by counting the number of leukocytes containing ingested *C. albicans* (alive or dead). Activity was measured just prior to treatment (day 0) and on day 3 after initiation of treatment.

Hydrogen peroxide production assay

Hydrogen peroxide production by the milk cells was assayed after stimulating the cells with lipopolysac-

charide (50 µg/ml; Sigma, St Louis, MO, USA) as described previously (Pick and Keisari 1980). Hydrogen peroxide production was assayed before treatment (on day 0) and on day 3 after initiation of treatment.

Myeloperoxidase and lactoperoxidase assay

Myeloperoxidase was assayed using *o*-dianisidine (Sigma, St. Louis, MO, USA) as an electron donor (Bretz and Baggiolini 1974) and enzyme concentration was calculated by using the molar extinction coefficient for oxidized *o*-dianisidine. The lactoperoxidase activity in milk was estimated according to the method described by Marshall et al. (1986). In brief, 1.0 ml of milk was diluted five-fold in 0.1M-acetate buffer (pH 4.5). From diluted milk samples, 30 µl were rapidly added to 2.95 ml of 1.0mM-2, 2'-azino-di-3-ethylbenzthiazoline-sulphonic acid (ABTS) in acetic buffer in a cuvette. The baseline absorbance at 412 nm was adjusted to zero before addition of 30 µl 10mM hydrogen peroxide solution in acetate buffer. The increase in extinction was followed for 5 min and units were expressed as the amount of enzyme required to oxidize 1 mol ABTS/min. The molar extinction coefficient of ABTS is 32 400 × 10^3 mol/dm/cm. Myeloperoxidase and lactoperoxidase activities were assayed before treatment (day 0) and on day 3 after initiation of treatment.

Statistical analysis

The data on the somatic cell counts and total bacterial counts were transformed to $\log_{10}$/ml and analysed using a repeated measurement model with cows as the subject and the period as the repeated measurement. The data for the phagocytic activity, hydrogen peroxide, myeloperoxidase and lactoperoxidase activities were analysed using one-way analysis of variance to determine the level of significance between the groups, and Duncan's multiple range test was applied to determine the level of significance within the group at different time intervals using a statistical software package (SPSS Version 10.1).

RESULTS

Somatic cell counts and total bacterial counts

Data on the somatic cell counts and total bacterial counts were transformed to $\log_{10}$/ml and are presented in Table 1. Before initiation of the treatment, the average somatic cell count and total bacterial count in milk samples from Groups II and III (infected with subclinical mastitis) quarters were significantly ($P < 0.05$) higher than those from Group I healthy quarters. Intramammary infusion of the antibiotic (amoxicillin plus sulbactam) significantly ($P < 0.05$) reduced the mean somatic cell count ($\log_{10}$/ml) value by 5.63%, 7.75% and 12.60% on days 3, 7 and day 15 after initiation of treatment, respectively, compared with the day 0 count. Similarly, total bacterial count was reduced significantly ($P < 0.05$) by 9.05%, 16.78% and 40.55% on days 3, 7 and day 15 after initiation of treatment,

Table 1. Somatic cell counts ($\log_{10}$/ml) and total bacterial counts ($\log_{10}$/ml) in normal healthy quarters (Group I), amoxicillin plus sulbactam-treated quarters (Group II) and untreated control quarters (Group III) on different days (mean ± SEM)

Groups (n = 15)	Days post treatment			
	day 0	day 3	day 7	day 15
Somatic cell count				
Group I	$5.430 \pm 0.030^{a,A}$	$5.425 \pm 0.032^{a,A}$	$5.443 \pm 0.020^{a,A}$	$5.469 \pm 0.016^{a,A}$
Group II	$6.038 \pm 0.014^{a,B}$	$5.698 \pm 0.022^{b,B}$	$5.570 \pm 0.025^{c,B}$	$5.277 \pm 0.035^{d,B}$
Group III	$6.051 \pm 0.016^{a,B}$	$6.068 \pm 0.013^{a,C}$	$6.055 \pm 0.018^{a,C}$	$6.054 \pm 0.021^{a,C}$
Total bacterial count				
Group I	$2.592 \pm 0.062^{a,A}$	$2.534 \pm 0.067^{a,A}$	$2.657 \pm 0.052^{a,A}$	$2.622 \pm 0.059^{a,A}$
Group II	$3.723 \pm 0.020^{a,C}$	$3.386 \pm 0.020^{b,B}$	$3.098 \pm 0.023^{c,B}$	$2.213 \pm 0.032^{d,A}$
Group III	$3.674 \pm 0.024^{a,BC}$	$3.683 \pm 0.026^{a,C}$	$3.678 \pm 0.028^{a,C}$	$3.682 \pm 0.023^{a,B}$

*superscripts in each row (a, b, c, d) and each column (A, B, C) differ significantly ($P < 0.05$)

Table 2. Bacterial isolates from milk samples of subclinical mastitis-infected Group II and Group III quarters

Pathogens	Number of isolates in different groups		
	Group II	Group III	Total
Staphylococcus aureus	2	1	3
Staphylococcus sp.	4	4	8
Streptococcus sp.	3	4	7
Micrococci	5	4	9
Coliform bacilli	1	2	3

respectively, in Group II quarters after intramammary antibiotic treatment as compared to the day 0 value. However, no statistically significant changes in somatic cell count and total bacterial count were observed in Group III quarters up to day 15. Out of 30 milk samples collected from subclinically affected animals, *Staphylococcus aureus* (3/30), other *Staphylococcus* sp. (8/30), *Streptococcus* sp. (7/30), Micrococci (9/30) and Coliform bacilli (3/30) were isolated. The distributions of organisms are presented in Table 2.

Phagocytic activity

The average % of phagocytic milk leukocytes in Group II (11.533 ± 0.638 to 16.400 ± 0.761%) and Group III (11.533 ± 0.965 to 11.066 ± 0.572%) subclinically infected quarters was significantly ($P < 0.05$) lower than in healthy Group I quarters (19.200 ± 1.291 to 20.533 ± 1.450%). The phagocytic activity significantly ($P < 0.05$) increased (42.20%) on day 3 after initiation of treatment as compared with the day 0 value in Group II quarters treated with antibiotic. No statistically significant changes

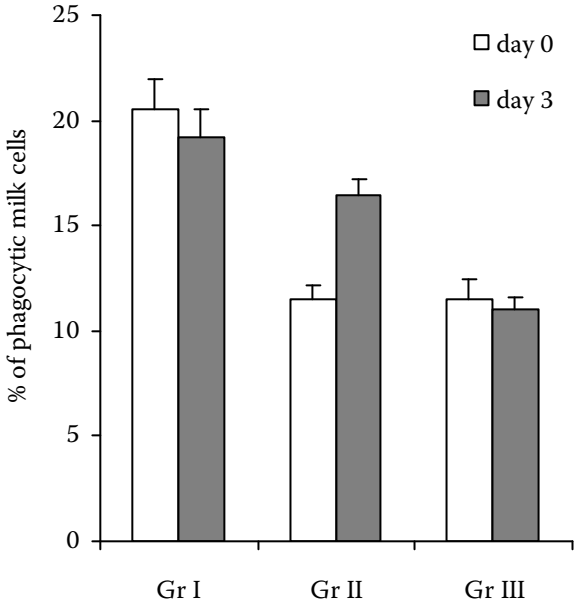

Figure 1. Phagocytic activity (% of phagocytic milk cells) of milk leukocytes in normal healthy quarters (Group I), amoxicillin plus sulbactam treated quarters (Group II) and untreated control quarters (Group III) on day 0 and day 3

in phagocytic activity were observed in Group III quarters on day 3 after initiation of treatment as compared with the 0 day values (Figure 1).

Hydrogen peroxide production assay

The hydrogen peroxide production capacity of milk leukocytes from the SCM infected quarters of Groups II and III was significantly ($P < 0.05$) lower than Group I healthy quarters before treatment. The values for hydrogen peroxide production did not change significantly in Group I quarters during the study period. However, intramammary treatment with antibiotic enhanced hydrogen peroxide pro-

Table 3. Myeloperoxidase (μmoles/1×10^6 cells) activity of milk cells and lactoperoxidase (μ/ml) activity of milk in normal healthy quarters (Group I), amoxicillin plus sulbactam treated quarters (Group II) and untreated control quarters (Group III) on day 0 and day 3 (mean ± SEM)

Groups (n = 15)	Days post treatment			
	myeloperoxidase (μmoles/10^6cells)		lactoperoxidase (μ/ml)	
	day 0	day 3	day 0	day 3
Group I	$3.414 \pm 0.271^{a,A}$	$3.032 \pm 0.217^{a,A}$	$0.280 \pm 0.029^{a,A}$	$0.284 \pm 0.029^{a,A}$
Group II	$6.233 \pm 0.357^{a,B}$	$9.304 \pm 0.422^{b,B}$	$1.471 \pm 0.124^{a,B}$	$3.046 \pm 0.180^{b,B}$
Group III	$6.624 \pm 0.416^{a,B}$	$6.503 \pm 0.338^{a,C}$	$1.719 \pm 0.144^{a,B}$	$1.606 \pm 0.093^{a,C}$

*superscripts in each row (a, b) and each column (A, B, C) differ significantly ($P < 0.05$)

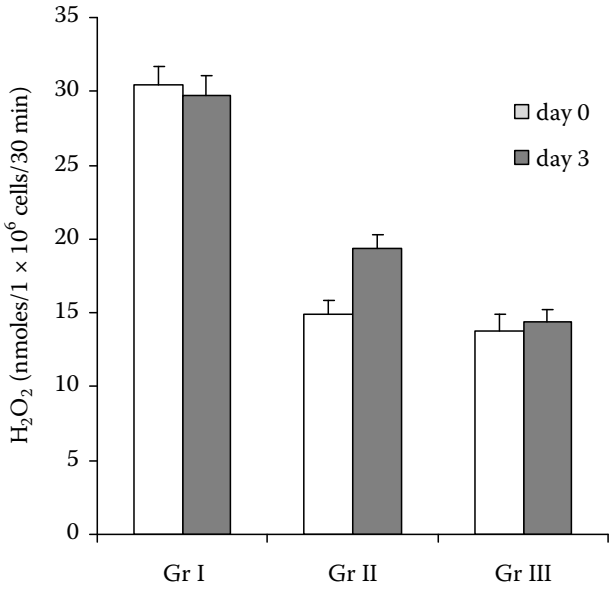

Figure 2. Hydrogen peroxide production (nmoles/1×10^6 cells/30 min) of milk leukocytes in normal healthy quarters (Group I), amoxicillin plus sulbactam treated quarters (Group II) and untreated control quarters (Group III) on day 0 and day 3

duction significantly ($P < 0.05$) by 29.46% on day 3 after initiation of treatment in Group II quarters as compared to the day 0 value. The changes in hydrogen peroxide production noted in Group III quarters (disease control) were non-significant (Figure 2).

Myeloperoxidase and lactoperoxidase assay

The myeloperoxidase and lactoperoxidase activities in Group II and III infected quarters was significantly ($P < 0.05$) higher than Group I healthy quarters before treatment. Myeloperoxidase and lactoperoxidase values did not change significantly in Group I quarters during the study period. However, intramammary antibiotic treatment further ($P < 0.05$) increased the myeloperoxidase (49.27%) and lactoperoxidase (147.10%) activity on day 3 after initiation of treatment as compared to the day 0 value in Group II quarters. No significant changes in myeloperoxidase and lactoperoxidase activities were observed in Group III (disease control) quarters (Table 3).

DISCUSSION

The results of this investigation into the therapeutic efficacy of amoxicillin plus sulbactam in the treatment of bovine subclinical mastitis indicate a marked reduction in the $\log_{10}$ values of somatic cell counts and total bacterial counts in the quarters of animals subclinically infected with mastitis, as early as day 3. Moreover, a progressive drop could be appreciated from day 7 to 15 after initiation of treatment as compared to day 0 values. Amoxicillin is a member of a semi-synthetic extended spectrum penicillin group of antibiotic and interferes with bacterial cell wall synthesis. Sulbactam is a semi synthetic β-lactamase inhibitor, related chemically as well as in activity to clavulanic acid. Bovine sub clinical mastitis is commonly caused by both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Streptococcus* sp. and coliform bacilli (Shem et al. 2001). It has been observed that amoxicillin alone or in combination with β-lactamase inhibitors is potentially useful for the treatment of mastitis caused by pathogenic organisms (Wilson et al. 1999; De-Oliveira et al. 2000). A very effective clinical recovery of bovine mastitis after intramammary infusion of amoxicillin plus sulbactam was reported (Sharma et al. 2010; Tufani et al. 2010).

The majority of spontaneous cases of mastitis are caused by Gram-positive bacteria; therefore, for intramammary therapy, antibiotics with particular efficacy against these organisms are preferred (Cattell et al. 2001). However, most antibiotics used therapeutically may affect the immune system directly by depressing the respiratory burst of the leucocytes, and indirectly, by changes in the microorganism (Van den Broek 1989; Labro et al. 2000). Weber et al. (1983) recorded low phagocytic and bactericidal activities of milk leukocytes in the lactating phase due to an absence of immunocompetent polymorphonuclear cells chewed with casein and fat globules. Therefore, the antibiotic chosen for the treatment of mastitis should not impede the competence of immune cells but should rather increase the immunocompetence of the resident milk cells.

In the present study, the average % of phagocytic milk leukocytes in Group II and III subclinical mastitis-infected quarters was significantly ($P < 0.05$) lower than that of Group I healthy quarters. Reduced phagocytic activity and production of reactive oxygen species by milk cells after parturition has been described to correlate with a negative metabolic and energy balance that directly affects neutrophil function and increases the risk of mastitis in dairy cows (Cai et al. 1994; Hoeben et al. 2000). Similar

findings were also observed in bovine mastitis (Mukherjee et al. 2005; De and Mukherjee 2009). However, phagocytic activity (42.20%), hydrogen peroxide production (29.46%) and myeloperoxidase (49.27%) enzyme activity increased significantly ($P < 0.05$) in subclinical mastitis-infected quarters following intramammary treatment with amoxicillin plus sulbactam. Several studies reported that amoxicillin and clavulanate, separately or together, possess immunostimulating and antibacterial enhancing activity against a wide range of pathogens (Pascual et al. 1989; Gomez-Lus et al. 1997; Cuffini et al. 1998). Further, these antimicrobials increase phagocytosis and the intracellular killing capacity of polymorphonuclear cells in immunosuppressive diseases where impairment of phagocytosis is an important defect (Finlay et al. 2003; Cuffini et al. 2001a,b). It has been observed that sulbactam increases chemotaxis, respiratory burst, and microbicidal activity of leukocytes in humans during bacterial infection and enhancing the bactericidal function of leukocytes is considered as a secondary antibacterial mechanism of action of sulbactam (Kazmierczak et al. 1989; Santos and Arbo 1989). Phagocytosis and respiratory burst activity of leukocytes can also be substantially augmented by various proinflammatory cytokines such as IL-8, IL-1β, TNF-α, IL-6 etc. (Reato et al. 2004; Dias et al. 2011; Hellberg et al. 2011). No data are available on the effects of sulbactam on cytokine activity; however, studies indicated that amoxicillin treatment up-regulates the expression of IL-8, TNF-α, IL-1β, IL-6 and IL-10 in response to many bacterial infections (Reato et al. 1999; Melhus and Ryan, 2004). Though amoxicillin alone does not possess intracellular bactericidal properties, amoxicillin together with beta lactamase inhibitors increases phagocytosis or microbicidal activities of human polymorphonuclear leukocytes against *Klebsiella pneumoniae* and *Staphylococcus aureus in vitro* and in clinical trials (Anderson et al. 1986; Cuffini et al. 1996). As sulbactam is chemically related to clavulanic acid, it seems that sulbactam may potentiate the activity of amoxicillin. Moreover, a pronounced enhancement of milk leukocyte function might be due to cytokine activity after amoxicillin plus sulbactam treatment and an improvement of its penetration into milk leukocytes.

Antibiotics are widely used to treat bovine mastitis, but there are only a small number of reports of their effects on lactoperoxidase activities in milk. Most antibiotics exhibit inhibitory effects on lactoperoxidase enzyme in milk (Sisecioglu et al. 2011). No data are available on the effect of amoxicillin plus sulbactam on milk lactoperoxidase activity; however, in this study, lactoperoxidase (147.10%) enzyme activity increased significantly ($P < 0.05$) in subclinical mastitis-infected quarters in response to intramammary treatment. The efficiency of the lactoperoxidase system in inhibiting the growth of pathogens is mostly related to the amount of hydrogen peroxide available (Tenovuo and Knuuttila 1977). In this study, hydrogen peroxide production by milk leukocytes was increased in response to amoxicillin plus sulbactam treatment. Here, enhanced lactoperoxidase activity might be due to raised hydrogen peroxide production or an augmented leukocyte response after antibiotic treatment. Karmakar et al (2011) observed increased lactoperoxidase activity after intravenous administration of ceftizoxime in a mastitis model of goat.

It is concluded that no adverse reaction was observed after amoxicillin plus sulbactam treatment in bovine subclinical mastistis, apart from the need to discard milk for five days during the treatment. A significant fall in total bacterial counts and an enhancement of bactericidal function of leukocytes in the treated animals indicates the immunomodulatory potential of amoxicillin plus sulbactam in bovine subclinical mastitis.

Acknowledgements

The authors thank the Director of the institute, Head, Division of Medicine and In-Charge Livestock Production and Management section for providing necessary facilities. U.K. De also thanks the Indian Veterinary Research Institute for their award of a Research Fellowship during his doctoral research.

REFERENCES

Anderson R, Joone G, van Rensburg CEJ (1986): An in vitro investigation of the intracellular bioactivity of Amoxicillin, clindamycin, and erythromycin for Staphylococcus aureus. Journal of Infectious Disease 153, 593–600.

Boyne R, Arthur JR (1979): Alteration of neutrophil function in selenium-deficient cattle. Journal of Comparative Pathology 89, 151–158.

Bretz U, Baggiolini M (1974): Biochemical and morphological characterization of azurophilic and specific

granules of human neutrophilic polymorphonuclear leucocytes. Journal Cellular Biology 63, 251–269.

Burvenich C, Paape MJ, Hill AW, Guidry AJ, Miller RH, Heyeneman R, Kremer WDJ (1994): Role of the neutrophil leukocyte in the local and systemic reaction during experimentally induced E. coli mastitis in cows immediately after calving. Veterinary Quarterly 16, 45–50.

Cai TQ, Weston PG, Lung LA, Brodie B, Mekenna DJ, Wagner WC (1994): Association between neutrophil functions and periparturient disorders in cows. American Journal of Veterinary Research 55, 934–943.

Cattell MB, Dinsmore RP, Belschner AP, Carmen J, Goodell G (2001): Environmental gram-positive mastitis treatment: In vitro sensitivity and bacteriologic cure. Journal of Dairy Science 84, 2036–2043.

Cooray RA, Bjorek L (1995): Bactericidal activity of the bovine myeloperoxidase system against bacteria associated with mastitis. Veterinary Microbiology 46, 427–434.

Cuffini AM, De Renzi G, Tullio V, Paizis G, Carlone NA (1996): Potentiation of human polymorphonuclear leukocyte phagocytosis and intracellular bactericidal activity by amoxycillin/clavulanate. Drugs Under Experimental and Clinical Research 22, 9–15.

Cuffini AM, Tullio V, Ianni Palarchio A, Bonino A, Paizis G, Carlone NA (1998): Enhanced effects of amoxycillin/clavulanate compared with amoxycillin and clavulanate alone on the susceptibility to immunodefenses of a penicillin-resistant strain of Streptococcus pneumoniae. Drugs Under Experimental and Clinical Research 24, 173–184.

Cuffini AM, Tullio V, Giacchino F, Bonino A, Mandras N, Bianchi N, Roana J, Scalas D, Bonello F, Carlone NA (2001a): Improved phagocyte response by co-amoxiclav in renal transplant recipients. Transplantation 71, 575–577.

Cuffini AM, Tullio V, Giacchino F, Mandras N, Scalas D, Belardi P, Merlino C, Carlone NA (2001b): Impact of co-amoxiclav on polymorphonuclear granulocytes from chronic hemodialysis patients. American Journal of Kidney Disease 37, 1253–1259.

Daley MJ, Oldham ER, Williams TJ, Coyle PA (1991): Quantitative and qualitative properties of host PMN cells during experimentally induced Staphylococcus aureus mastitis in cows. American Journal of Veterinary Research 52, 74–79.

De UK, Mukherjee R (2009): Expression of cytokines and respiratory burst activity of milk cells in response to Azadirachta indica during bovine mastitis. Tropical Animal Health and Production 41, 189–197.

De-Oliveira AP, Watts JL, Salmon SA, Aarestrup FM (2000): Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis cases in Europe and United States. Journal of Dairy Science 83, 855–862.

Dias IH, Matthews JB, Chapple IL, Wright HJ, Dunston CR, Griffiths HR (2011): Activation of the neutrophil respiratory burst by plasma from periodontitis patients is mediated by pro-inflammatory cytokines. Journal of Clinical Periodontology 38, 1–7.

Dosogne H, Hoeben D, Burvenich C, Lohuis JACM (1999): Effect of Cephapirin and Mecillinam on the phagocytic and respiratory burst activity of neutrophil leucocytes isolatedfrom bovine blood. Journal of Veterinary Pharmacology and Therapeutics 21, 421–427

Finlay J, Miller L, Poupard JA (2003): A review of the antimicrobial activity of clavulanate. Journal of Antimicrobial and Chemotherapy 52, 18–23.

Gomez-Lus ML, Aguilar L, Martin M, Gimenez MJ, Martínez P, Prieto J (1997): Intracellular and extracellular killing of a penicillin-resistant, serotype-9 strain of Streptococcus pneumoniae by polymorphonuclear leucocytes in the presence of sub-inhibitory concentrations of clavulanic acid. Journal of Antimicrobial and Chemotherapy 40, 142–144.

Gruet P, Maincent P, Berthelot X, Kaltsatos V (2001): Bovine mastitis and intramammary drug delivery: review and perspectives. Advanced Drug Delivery Reviews 50, 245–259.

Hellberg L, Fuchs S, Gericke C, Sarkar A, Behnen M, Solbach W, Laskay T (2011): Proinflammatory stimuli enhance phagocytosis of apoptotic cells by neutrophil granulocytes. Scientific World Journal 11, 2230–2236.

Hoeben D, Burvenich C, Heneman R (1998): Antibiotics commonly used to treat mastitis and respiratory burst of bovine polymorphonuclear leucocytes. Journal of Dairy Science 81, 403–410.

Hoeben D, Monfardini E, Opsomer G, Burvenich C, Dosogne H, Kruif AD, Beckers JF (2000): Chemiluminescence of bovine polymorphonuclear leukocytesduring the periparturient period and relation withmetabolic markers and bovine pregnancy associatedglycoprotein. Journal of Dairy Research 67, 249–259.

Karmakar UK, Datta BK, Patra PH, Prashant M, Suman S, Sar TK, Chakraborty AK, Mandal TK (2011): Disposition kinetics of ceftizoxime in healthy and mastitic goats after intravenous administration. Pharmacologyonline 3, 935–946.

Kazmierczak A, Pechinot A, Siebor E, Cordin X, Labia R (1989): Sulbactam: secondary mechanisms of action. Diagnostic Microbiology and Infectious Disease 12, 139S–146S.

Kehrli ME, Shuster DE (1994): Factors affecting milk somatic cell count and their role in health of bovine mammary gland. Journal of Dairy Science 77, 619–627.

Kolmer JA, Spaulding EH, Robinson HW (1951): Approved Laboratory Technic. Methods for the Bacteriologic Examination of Milk. 5th ed. Appleton-Century-Crofts Inc, New York, USA. 586–593.

Korhonen H (1980): Potential role of the lactoperoxidase system (LP/SCN–/H_2O_2) in mastitis resistance. In: Bassalik-Chabielska L, Ryniewicz Z (eds.): Proceedings of International Conference on Resistant Factors and Genetic Aspects of Mastitis Control. Jablonna, Poland, 2–5 October, 421–438.

Labro MT (2000): Interference of antibacterial agents with phagocyte functions: Immunomodulation or "Immuno-Fairy Tales"? Clinical Microbiology Reviews 13, 615–650.

Marshall VME, Cole WM, Bramley AJ (1986): Influence of lactoperoxidase system on susceptibility of the udder to Streptococcus uberis infection. Journal of Dairy Research 53, 507–514.

Mehrzad J, Dosogne H, Meyer E, Burvenich C (2001): Local and systemic effects of endotoxin mastitis on the chemiluminescence of milk and blood neutrophils in dairy cows. Veterinary Research 32, 131–144.

Melhus A, Ryan AF (2004): Effects of amoxicillin on cytokine and osteocalcin expression in bone tissue during experimental acute otitis media. Cytokine 25, 254–259.

Mukherjee R, Ram GC, Dash PK, Ram GC (2005): Immunotharapeutic potential of Ocimum sanctum (L) in bovine subclinical mastitis. Research in Veterinary Science 79, 37–43.

Pascual A, Martinez Martinez L, Aragon J, Perea EJ (1989): Effect of amoxycillin and clavulanic acid, alone and in combination, on human polymorphonuclear leukocyte function against Staphylococcus aureus. Europen Journal of Clinical Microbiology and Infectious Diseases 8, 277–281.

Pick E, Keisari Y (1980): A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. Journal of Immunological Methods 38, 161–170.

Reato G, Cuffini AM, Tullio V, Mandras N, Roana J, Banche G, Foa R, Carlone NA (2004): Immunomodulating effect of antimicrobial agents on cytokine production by human polymorphonuclear neutrophils. International Journal of Antimicrobial Agents 23, 150–154.

Reato G, Cuffinia AM, Tullioa V, Ianni Palarchio A, Boninoa A, Foab R, Carlonea NA (1999): Co-amoxiclav affects cytokine production by human polymorphonuclear cells. Journal of Antimicrobial and Chemotherapy 43, 715–718.

Santos JI, Arbo A (1989): The in vitro effect of sulbactam on polymorphonuclear leukocyte function. Diagnostic Microbiology and Infectious Disease 12, 147S–152S.

Schalm OW, Carrol EJ, Jain NC (1971): Bovine Mastitis. Lea and Febiger, Philadelphia. 128–129.

Seegers H, Fourichon C, Beaudeau F (2003): Production effects related to mastitis and mastitis economics in dairy cattle herds. Veterinary Research 34, 475–491.

Seifu E, Buys EM, Donkin EF, Petzer IM (2004): Antibacterial activity of the lactoperoxidase system against food-borne pathogens in Saanen and South African Indigenous goat milk. Food Control 15, 447–452.

Sharma N, Mukherjee R, Ingale SL, Jadhav RK (2010): Therapeutic and anti-oxidant activity of vitamin E and selenium in bovine Staphylococcal mastitis. Indian Journal of Veterinary Research 19, 25–31.

Shem MN, Malole JML, Machangu R, Kurwijila LR, Fujihara T (2001): Incidence and causes of sub-clinical mastitis in dairy cows on smallholder and large scale farms in tropical areas of Tanzania. Asian Australasian Journal of Animal Sciences 14, 372–377.

Sisecioglu M, Uguz MT, Cankaya M, Ozdemir H, Gulcin I (2011): Effects of Ceftazidime Pentahydrate, Prednisolone, Amikacin Sulfate, Ceftriaxone Sodium and Teicoplanin on bovine milk lactoperoxidase activity. International Journal of Pharmacology 7, 79–83.

Sordillo LM, Streicher1 KL (2002): Mammary Gland Immunity and Mastitis Susceptibility. Journal of Mammary Gland Biology and Neoplasia 7, 135–146.

Sordillo LM, Weaver SH, DeRosa D (1997): Symposium immunology. Immunobiology of the mammary gland. Journal of Dairy Science 80, 1851–1865.

Tenovuo J, Knuuttila MLE (1997): The antibacterial action of the various components of the lactoperoxidase system on a cariogenic strain of Streptococcus mutans. Journal of Dental Research 56, 1603–1607.

Tufani NA, Hafiz A, Peer FU, Makhdoomi DM, Qureshi SD (2010): Clinico-therapeutic management of gangrenous mastitis in ovines. Indian Journal of Small Ruminants 16, 145–147.

Van Den Broek PJ (1989): Antimicrobial drugs, microorganisms, and phagocytes. Reviews of Infectious Diseases 11, 213–245.

Weber K, Peterhans E, Wyler R (1983): The chemiluminiscent response of bovine polymorphonuclear leukocytes isolated from milk and blood. Veterinary Immunology and Immunopathology 4, 397–412.

Wilson DJ, Gonzalez RN, Case KL, Garrison LL, Grohn YT (1999): Comparison of seven antibiotic treatments with no treatment for bacteriological efficacy against bovine mastitis pathogens. Journal of Dairy Science 82, 1664–1670.

Received: 2012–04–28
Accepted after corrections: 2012–10–31

Corresponding Author:

Ujjwal Kumar De, Indian Veterinary Research Institute, Division of Medicine, Izatnagar 243122, Uttar Pradesh, India
Tel. +91 9411473760, E-mail: ujjwalde@gmail.com