ISSN 1810-3030 (Print) 2408-8684 (Online)
Journal of Bangladesh Agricultural University
Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

Subclinical mastitis in dairy cows: somatic cell counts and associated bacteria in Mymensingh, Bangladesh

S. M. M. R. Sumon[1,2], M. A. Ehsan[1] and M. T. Islam[1]

[1]Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202
[2]Department of Medicine, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh MujiburRahman Agricultural University, Gazipur-1706

ARTICLE INFO

Article history:
Received: 18 October 2017
Accepted: 17 December 2017

Keywords:
Subclinical mastitis, Somatic cell count, Bacteria, Prevalence, Dairy cow

Correspondence:
M. T. Islam
(taohid@bau.edu.bd)

Abstract

Subclinical mastitis is an economically important disease of dairy cows and has a prominent place amongst the factors that limit milk production. This study was undertaken to determine the association of somatic cell counts (SCC) and occurrence of bacteria with SCM in smallholder dairy cows in Mymensingh, Bangladesh. A total number of 240 quarters milk samples from apparently healthy lactating cows were subjected to SCC using NucleoCounter® SCC-100 ™ (Chemo Metec). A quarter was considered SCM positive if the quarter had SCC>100 x 10^3 cells/ml. All subclinical mastitis positive quarter milk samples were subjected to bacteriological examination and isolates were classified into major, minor, uncommon and mixed pathogens. The overall quarter-level prevalence of subclinical mastitis of dairy cows in Mymensingh district was 25% (95% CI, 19.52% to 30.48%). The most frequently isolated bacterial species were *Staphylococcus aureus* (18.33%) followed by coagulase-negative staphylococci (10%), *Enterobacter* spp. (6.67%), *Escherichia coli* (5%), *Bacillus* spp. (5%) and *Pseudomonas aeruginosa* (5%). Different bacterial isolates were associated with 90% cases of subclinical mastitis as mono infections or mixed infections. Mono and mixed infections significantly influenced SCC and were the most prominent factors responsible for increasing SCC. Mean SCC was the highest for *Bacillus* spp. (713.67 x 10^3 cells/ml) followed by *Enterobacter* spp. (395.75 x 10^3 cells/ml), *Escherichia coli* (386.00 x 10^3 cells/ml), *Staphylococcus aureus* (373.82 x 10^3 cells/ml), coagulase-negative staphylococci (182.67 x 10^3 cells/ml) and *Pseudomonas aeruginosa* (138.67 x 10^3 cells/ml). Major pathogens induced higher SCC (380.72 x 10^3cells/ml) than minor and other pathogen groups.

Introduction

Mastitis is an inflammation of the mammary gland which, together with physical, chemical and microbiological changes, is characterized by an increase in the number of somatic cells in the milk and by pathological changes in the mammary tissue (Gianneechini *et al.*, 2002). About 140 microbial species, subspecies and serovars have been isolated from the bovine mammary gland (Radostits *et al.*, 2007). Among infectious agents, bacterial pathogens are considered to be the major threat to mammary gland. In Asia, major mastitis causing organisms are *Staphylococcus aureus*, *Streptococci*, *E. coli*, *Corynebacterium* spp. and *Klebsella* spp. (Sharma *et al.*, 2012). Different pathogens can cause chronic, subacute, acute, peracute and subclinical forms of the disease (Radostits *et al.*, 2007). In clinical mastitis all the five cardinal signs (redness, swelling, heat, pain and loss of milk production) of udder inflammation are present (Bachaya *et al.*, 2011). There are no visible abnormalities of the milk or udder instead there is a high somatic cell count in subclinical mastitis (Radostits *et al.*, 2007). Although a large number of bacteria have been isolated as causal agents of SCM in dairy cows throughout the world *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Bacillus* spp., and *Corynebacterium* spp. have mostly been isolated from SCM cases of cows from Bangladesh (Kayesh *et al.*, 2014; Rahman *et al.*, 2010).

SCM is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds (Bachaya *et al.*, 2011). It is responsible for 70% of economic losses and has a prominent place amongst the factors that limit milk production (Heleili *et al.*, 2012). In Bangladesh, the annual economic losses due to reduced milk production by SCM have been estimated to be Taka 122.6 (US $ 2.11) million (Kader *et al.*, 2003). Besides causing huge losses to milk production, the sub-clinically affected animals remain a continuous source of infection to other herd mates.

SCC can be used to monitor the status of SCM in herds or individual cows which is an important component of assessing milk quality, hygiene and mastitis control (Sharma *et al.*, 2011). The SCC of healthy quarters is usually below 100,000 cells/ml or even lowers (Hamann, 2005; Leitner *et al.*, 2003). The high SCC is mostly related to the presence of microorganism in the udder but also the type of microbes could affect the SCC in milk (Ariznabarreta *et al.*, 2002). The major pathogens generally cause the greatest SCC increase and infection by minor pathogens usually causes considerably less SCC elevation and rarely result in

clinical mastitis (Supr *et al.,* 2011; Sargeant *et al.,* 2001). In some cases, high SCC is detected in milk samples without presence of microorganisms and it does not indicate that the gland is healthy (McDougall *et al.,* 2001). However, cows with low SCC can also harbor the mastitis pathogen (Katsande *et al.,* 2013). Therefore, bacteriologic culture is necessary to accurately diagnose the source of the mastitis even though SCC profiles suggest lower probability of SCM. This paper describes the association of SCC and occurrence of bacteria with SCM in smallholder dairy cows in Mymensingh, Bangladesh.

Materials and Methods

Collection of samples

A cross sectional study was conducted on 13 dairy farms, one is Bangladesh Agricultural University (BAU) Dairy Farm and other 12 from surrounding villages (Boira, Charkalibari, Dhigharkanda and Salakanda) of Mymensingh sadar upazila around BAU. A total of 240 quarter milk samples were collected from each quarter of 60 randomly selected apparently healthy lactating cows. Breed of the study cows included indigenous (zebu) and cross-bred cows, i.e. crosses of zebu cows with Holstein-Friesian, Jersey or Shahiwal. Cows with the history of ongoing antibiotic treatment or antibiotic treatment within 96 hours were not included for sampling. Grossly dirty teats and udder were thoroughly washed and dried using a single, dry paper towel per cow with particular emphasis on the teat end. The teat end and orifice was carefully and vigorously scrubbed with a cotton pad moistened with 70% ethyl alcohol. Separate swab was used for each teat being sampled, even within the same cow. The teat end was cleaned until the swab was completely clean and white. Three or four streams of milk were discarded from the quarter being sampled to minimize chances of sample contamination from bacteria in the teat end. After that, collection vial was held at a 45° angle to avoid debris (hair, manure, dirt) contamination and about 10 ml of milk from each quarter was collected aseptically in sterile screw-capped collection vials. They were marked as right front (RF), left front (LF), right rear (RR) and left rear (LR) and transported immediately to the laboratory in ice-box at 4°C.

Detection of subclinical mastitis

Subclinical mastitis was determined by SCC of milk using NucleoCounter® SCC-100 ™ (ChemoMetec). Somatic cell count was performed for all collected quarter milk samples as per instruction of manufacturer. About 50 µl of milk sample and 50 µl of reagent C (1:1 dilution) were taken in an eppendrop tube using micropipette and mixed properly. Then SCC-Cassette was loaded with the diluted milk sample by immersing the tip of the cassette into the solution and pressing the piston. The SCC cassette was placed in the instrument and pressed the "Run" key. After 30 seconds the somatic cell count was presented on the instrument display. Individual quarter having SCC > 100 x 10^3 cells/ml were considered as SCM positive to reduce the occurrence of false-negative results (Malek dos Reis *et al.,* 2011).

Bacteriological examination

All SCM positive quarter milk samples were analyzed bacteriologically. Isolation and identification of the bacteria were performed on the basis of culture characteristics, morphology and staining reactions and on the basis of biochemical tests (catalase, coagulase, oxidase, indole, methyl red, Voges-Proskauer, and sugar fermentation tests) as described by Quinn *et al.,* (2002). About 100 µl of milk samples from each SCM positive quarter was inoculated into nutrient broth (NB) within 24 hours after collection and incubated under aerobic condition at 37°C overnight for growth of organisms. Inoculums from nutrient broth were then spread into blood agar (BA) and MacConkeyagar. After that, culture and subculture was performed into differential and selective media like Blood agar (BA), Mannitol salt agar (MSA), Eosin-methylene blue (EMB) agar and Triple sugar iron (TSI) agar. Isolates were classified into (1) major pathogens: *Staphylococcus aureus*, *Eschericia coli*, *Enterobacter* spp.; (2) minor pathogens: coagulase-negative staphylococci (CNS); (3) uncommon pathogens: *Bacillus* spp., *Pseudomonas aeroginosa* and unknown and (4) mixed pathogens (Rahman *et al.,* 2010; Radostis *et al.,* 2007).

Statistical analysis

SCC values of subclinical mastitic quarters and bacteriological culture results were entered into Statistical Package for the Social Sciences (SPSS 20.0) for statistical analysis. Independent samples t-test as well as One Way ANOVA followed by Duncan's Multiple Range Test were done to find out the significant variation in SCC corresponding to mammary quarters and different bacteria.

Results

Prevalence of subclinical mastitis and associated bacteria

Results of SCC of 240 quarter milk samples revealed that the overall quarter-level prevalence of SCM was 25% (Table 1). Among SCM positive quarters, 90% were culture positive. The most prevalent bacteria were *Staphylococcus aureus* (18.33%) followed by CNS (10%), *Enterobacter* spp. (6.67%), *Escherichia coli* (5%), *Bacillus* spp. (5%) and *Pseudomonas aeruginosa* (5%) (Table 2). However, overall isolation of major pathogens (30%) were higher than minor pathogens (10%) (Table 3).

Table 1. Prevalence of SCM and status of quarters analyzed bacteriologically

Parameter	No. of Quarters	Percentage
Somatic cell count (SCC) performed	240	
SCM positive (SCC >= 100 x 10^3 cells/ml)	60	25.00
Quarter analyzed bacteriologically	60	
Culture positive	54	90.00
Culture negative	6	10.00

Effect of bacterial isolates on mean SCC

The mean SCC of subclinical mastitic mammary quarters according to the types of individual bacteria and bacterial group are presented in Table 2 and 3, respectively. Significant variation in SCC was observed with mono infections by different bacterial spp. and was the highest for *Bacillus* spp. (713.67 x 10^3 cells/ml) followed by *Enterobacter* spp. (395.75 x 10^3 cells/ml), *Escherichia coli* (386.00 x 10^3 cells/ml), *Staphylococcus aureus* (373.82 x 10^3 cells/ml), CNS (182.67 x 10^3 cells/ml) and *Pseudomonas aeruginosa* (138.67 x 10^3 cells/ml). Among mixed infections, SCC was significantly higher caused by *Bacillus* spp. and *Pseudomonas aeruginosa* (384.33 x 10^3 cells/ml) than other mixed infections. Considering pathogen groups, major pathogens induced significantly higher SCC (380.72 x 103 cells/ml) than minor pathogen groups (182.67b ± 29.79) as shown in Table 3

Table 2. Bacterial isolates and mean SCC of subclinical mastitic mammary quarters according to the types of bacteria causing SCM in dairy cows

Bacterial isolates	No. of quarter	% of SCM positive quarter	SCC (x 10^3 cells/ml) (Mean ± SEM)
Mono infection			
Staphylococcus aureus	11	18.33	373.82^{b}±97.33
Escherichia coli	3	5.00	386.00^{b}± 109.99
Enterobacter spp.	4	6.67	395.75^{b} ± 126.02
CNS	6	10.00	182.67^{c} ± 38.47
Bacillus spp.	3	5.00	713.67^{a} ± 173.63
Pseudomonas aeruginosa	3	5.00	138.67^{c} ± 6.96
Unknown	6	10.00	161.33^{c} ± 21.83
Mixed Infections			
*Staphylococcus aureus*and *E. coli*	3	5.00	146.33^{b} ± 11.61
*Staphylococcus aureus*and *Enterobacter* spp.	2	3.33	315.00^{a} ± 14.50
*Staphylococcus aureus*and *Pseudomonas aeruginosa*	2	3.33	236.00^{a} ± 34.00
E. coli and CNS	3	3.33	237.00^{a} ± 36.00
E. coli and *Bacillus* spp.	2	3.33	189.00b ± 51.00
E. coli and unknown	2	3.33	263.00^{a} ± 57.00
Enterobacter spp. and unknown	2	3.33	341.00^{a} ± 39.00
Bacillus spp. and *Pseudomonas aeruginosa*	3	5.00	384.33^{a} ±48.67

Values with different superscripts within a column in different types of pathogens vary significantly (p≤0.05).

Table 3. Mean SCC of subclinical mastitic mammary quarters according to the pathogen groups

Pathogen groups	No. of quarter	% of SCM positive quarters	SCC (x 10^3 cells/ml) (Mean ± SEM)
Major pathogens[1]	18	30.00	380.72^{a} ± 65.35
Minor pathogen[2]	6	10.00	182.67^{b} ± 38.47
Uncommon pathogens[3]	12	20.00	293.75^{ab} ± 82.66
Mixed pathogens	18	30.00	264.17^{ab} ± 22.25

1= *Staphylococcus aureus, Escherichia coli* and *Enterobacter* spp.; 2= CNS; 3= *Bacillus* spp., *Pseudomonas aeroginosa* and unknown. Values with different superscripts within a column vary significantly (p≤0.05).

Effects of mammary quarter location on mean SCC

The mean SCC distribution of SCM positive samples according to the mammary quarter's location are presented in Table 4. The mean SCC was significantly higher for rear quarters (341.25 x 10^3 cells/ml) than front quarters (226.54 x 10^3 cells/ml). Among four quarters, right rear quarter had the higher mean SCC (365.63 x 10^3 cells/ml) than other quarters though it was not statistically significant.

Table 4. Quarter-wise variation in mean SCC in SCM positive cows

Quarter location	SCC (x 10^3 cells/ml) (Mean ± SEM)	p-Value
Right front	239.29 ± 32.93	0.237
Left front	208.70 ± 33.38	
Right rare	365.63 ± 65.50	
Left rare	314.00 ± 51.96	
Front quarters	226.54 ± 23.43	0.050
Rear quarters	341.25 ± 42.03	

Discussion

In this study, the quarter level prevalence of SCM based on SCC was 25% which is almost similar to the prevalence (26.2%) in Uruguay (Gianneechini *et al.,* 2002). Another study conducted by Katsande *et al.,* (2013) showed comparatively lower prevalence (16.3%) of SCM in Zimbabwe. Previous studies in different regions of Bangladesh reported that the prevalence of SCM based on California Mastitis Test (CMT) varies from 29% to 37.6% (Islam *et al.,* 2011; Islam *et al.,* 2010) and based on SCC was 55% (Hoque *et al.,* 2015). However, comparative higher prevalence of SCM indicates lack of awareness about SCM and farmers are not adapted to use different screening tools to detect SCM in timely fashion and maintain the udder and teat hygiene.

Among SCM positive quarters, 90% were culture positive and 10% were negative. Abrahamsen (2012) also reported that the 75.1% of SCM positive quarter milk samples were culture positive. Another study shown that all (100%) SCM positive composite milk samples were culture positive (Kastande *et al.,* 2013). However, SCM positive quarter milk samples with no bacterial growth (22% and 55.1% respectively) were also obtained by Persson *et al.,* (2011) and Gianneechini *et al.,* (2002). The reason behind the elevated SCC in the absence of bacterial isolates may be due to the numbers of bacteria being below detectable levels (10–100 organisms/ml) at collection time or the organisms may have been killed by the cow's immune system. Most frequently isolated bacteria was s*taphylococcus aureus* (18.33%) which suggests that the prevention of spread of contagious bacteria during milking was not effective. Similar to this, *Staphylococcus aureus* was the most frequently isolated pathogen in Sweeden (19%), Jordan (22.2%), and Brazil (30.8%) (Persson *et al.,* 2011; Malek dos Reis *et al.,* 2011; Alekish, 2015). Besides other minor pathogens, *Staphylococcus aureus* was still the most prevalent pathogen in clinically healthy animals and both environmental and contagious forms of mastitis may be caused by *Staphylococcus* a*ureus* (Rall *et al*., 2013; Barkema *et al.,* 2009). Isolation of CNS (10%) is compatible with the results reported in Netherland (10.8%), Sweeden (16%) and Germany (17.17%) (Persson *et al.,* 2011; Schwarz *et al.,* 2010; Sampimon *et al.,* 2009). Although Katsande *et al.,* (2013) and Abrahmsen *et al.,* (2012) reported higher prevalence of CNS (28.7% and 27.6% respectively); these discrepancies may be related to changes in herd management and bacteriological ecology in the herd environment (Pitkala *et al.,* 2004). In this study, the result of isolation of *Escherichia coli* was almost similar to another study (6.67%) in Bangladesh (Kayesh *et al.,* 2015). Although, the frequency of isolation of *Enterobacter* spp., *Escherichia coli*, *Bacillus* spp. and *Pseudomonas aeruginosa* was almost higher than other studies reported in different parts of the world (Umar *et al.,* 2013; Shrestha and Bindari, 2012; Schwarz *et al.,* 2010; lqbal and Siddique, 1999). However, this variation of isolation may be attributed by poor cleanliness, drainage and manure disposal in farms. Besides, milking practices in Bangladesh are also poor, only few farmers practice hygienic procedure before and after milking and post dipping is usually not adapted in most of the farms because calves stay with the cows after milking to suck residual milk. Therefore cow's teats are most prone to contaminated by environmental pathogens.

The main indicator of mastitis is the high SCC but it may vary according to the type of microbes infecting quarter (Sharma *et al.,* 2011). Variation in SCC to infection by different bacteria in this study may be due to the individual immune response and mechanism of development of infection by pathogens. In this study, we have found higher SCC for quarter infected by major pathogens (380.72 × 10^3 cells/ml) compared to minor (182.67× 10^3 cells/ml) and other pathogen groups. Sargeant *et al.,* (2001) reported that quarters infected with minor pathogens tend to have lower SCC values than quarters infected with major pathogens. Malek dos Reis *et al.,* (2011) and Djabri *et al.,* (2002) had also reported that log SCC was highest (5.51–5.79) in sample with major pathogen isolations (5.51–5.79) compared with the minor pathogen (5.04–5.31). The probability of isolating a major pathogen increases as SCC exceeds 200.00 × 10^3 cells/ml. The magnitude of SCC response to major pathogens varies among cows, however, differentiation of types of pathogens seem impossible based on SCC alone (Sharma *et al.,* 2011).

In this study, the mean SCC (341.25 x 10^3 cells/ml) was higher for rear quarters than front quarters. Almost similar to this result, Malek dos Reis *et al.,* (2011) reported that rear quarter had higher log SCC (5.10) compared with front quarters (5.03). Schepers *et al.,* (1997) also reported a higher SCC in rear quarters. Rear quarters might be more susceptible to infections than front quarters because of larger capacity and mass and greater exposure to environmental effects. In addition, teats of the rear quarters are frequently nearer to the floor, especially in older cows, and would thus be contaminated or subjected to injury more readily. Here we included the SCM positive samples only for the bacteriological investigation. It would be worthy if we

could investigate the SCM negative samples for identification of bacteria.

Conclusion

The study indicates a high prevalence (25%) of subclinical mastitis in selected area. *Staphylococcus aureus, Enterobacter* spp., *Escherichia coli*, CNS, *Bacillus* spp. and *Pseudomonas aeruginosa* were associated with SCM as single infections or mixed infections. Of them *Staphylococcus aureus* were the most frequent bacteria causing single infection. It is interesting to note that 10% SCM positive quarters based on SCC had no infection. As in most of the cases of SCM there was higher SCC due to bacterial infection therefore special care should be taken in regards to quarter and teat hygiene to prevent ascending infection through mammary quarters. In addition, regular screening test should be adapted for early detection of SCM otherwise it may develop clinical disease.

Acknowledgement

The authors acknowledge the financial support from the Ministry of Science and Technology, Govt. of the People's Republic of Bangladesh.

References

Abrahmsen, M. 2012: Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. http://epsilon.slu.se ISSN 1652–8697.

Alekish, M.O. 2015. The association between the somatic cell count and isolated microorganisms during subclinical mastitis in heifers in Jordan. *Vet Med (Praha)*, 60(2): 71–76.

Ariznabarreta, A., Gonzal, C. and Primitivo O.F.S. 2002. Microbiological quality and somatic cell count of ewe milk with special reference to Staphylococci. *J. Dairy Sci.*, 85:1370–1375.

Bachaya, H.A., Raza, M.A., Murtaza, S. and Akbar, I.U.R. 2011. Subclinical bovine mastitis in MuzaffarGarh district of Punjab (Pakistan). *J. Anim. Plant Sci.*, 21: 16–19.

Barkema, H.W., Green M.J., Bradley, A.J. and Zadoks, R.N. 2009. Invited review: The role of contagious disease in udder health. *J. Dairy Sci.*, 92: 4717– 4729.

Djabri, B., Bareille, N., Beaudeau, F. and Seegers, H. 2002. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. *Vet. Res.*,33**:** 335–357.

Gianneechini, R., Concha, C., Rivero, R., Delucci, I. and Moreno Lopez, J. 2002. Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. *Acta vet. scand.*, 43: 221–230.

Hamann, J. 2005. Diagnosis of mastitis and indicators of milk quality. In: "Mastitis in Dairy Production: Current Knowledge and Future Solutions" (ed. H. Hogeveen), Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 82–91.

Hoque, M.N., Das, Z.C., Talukder, A.K., Alam M.S. and Abu Nasar Md. Aminoor Rahman, A.N.M.A. 2015. Different screening tests and milk somatic cell count for the prevalence of subclinical bovine mastitis in Bangladesh. *Trop Anim Health Prod*, 47:79–86.

Heleili, N., Ayachi, A., Melizi, M., Kassah A.L. and Mamache, B. 2012. Prevalence of subclinical bovine mastitis and the in vitrosensitivity of bacterial isolates in Batna Governorate, East of Algeria. *J. Anim. Sci. Adv.*, 2: 576–582.

International Dairy Federation, 1987. Bovine mastitis. Definitions and guidelines for diagnosis. *Bull. Int. Dairy Federation*, 211: 3–8.

Iqbal, J. and Siddique, M. 1999. Some epidemiological aspects of mastitis in cows and biocharacterization of isolated Staphylococci. *Pak Vet J*, 19: 149–154.

Islam, M.A., Islam, M.Z., Islam, M.A., Rahman, M.S. and Islam, M.T. 2011. Prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. *Bangl. J. Vet. Med.*, 9: 73–78.

Islam, M.A., Rahman A.K.M.A., Rony S.A. and Islam, M.S. 2010. Prevalence and risk factors of mastitis in lactating dairy cows at Baghabari milk shed area of Sirajganj. *Bangl. J. Vet. Med.*, 8: 157–162.

Kader, M.A., Samad, M.A. and Saha, S. 2003. Influence of host level factors on prevalence and economics of sub-clinical mastitis in dairy cows in Bangladesh. *Indian J. Dairy Sci.*, 56: 235–240.

Katsande, S., Matope, G., Ndengu, M. &Pfukenyi, D.M. 2013. Prevalence of mastitis in dairy cows from smallholder farms in Zimbabwe. *Onderstepoort J Vet Res.*, 80(1): 523-529. http://dx.doi. org/10.4102/ojvr.v80i1.523

Kayesh, M.E.H., Talukder, M. and Anower A.K.M.M. 2014. Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. *Int. J. BioRes.*, 2 (2): 35–38.

Leitner, G., Eligulashvily, R., Krifucks, O., Perl, S. and Saran, A. 2003. Immune cell differentiation in mammary gland tissues and milk of cows chronically infected with *Staphylococcus aureus. J. Vet. Med.*, 50: 45–52.

Malek dos Reis, C.B., Barreiro, J.R., Moreno, J.F.G., Porcionato, M.A.F. and Santos, M.V. 2011. Evaluation of somatic cell count thresholds to detect subclinical mastitis in Gyr cows. *J. Dairy Sci.*, 94: 4406–4412.

McDougall, S., Murdough, P., Pankey, W., Delaney, C., Barlow, J. and Scruton, D. 2001. Relationship among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. *Small Rumin Res*, 40: 245–254.

Persson, Y., Nyman, A.K.J. and Andersson, U.G. 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet. Scand.*, 53 (1): 36.

Pitkala, A., Haveri, M., Pyorala, S., Myllys, V. and Honkanen-Buzalski, T. 2004. Bovine mastitis in Finland 2001—Prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci.*, 87:2433–2441.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J. and Leonard, F.C. 2002. Veterinary Microbiology and Microbial Disease. Iowa State University Press, Ames, USA.

Radostits, O.M., Gay C.C., Hinchcliff, K.W. and Constable, P.D. 2007. Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.10th ed., Saunders Elsevier, Spain.

Rahman, M.M., Islam, M.R., Uddin, M.B. and Aktaruzzaman, M. 2010. Prevalence of subclinical mastitis in dairy cows reared in sylhet district of Bangladesh. *Int. J. BioRes.*, 1(2): 23–28.

Rall, V.L.M., Miranda, E.S., Castilho, I.G. and Camargo, C.H. 2013. Diversity of *Staphylococcus sp*ecies and prevalence of enterotoxin genes isolated from milk of healthy cows and cows with subclinical mastitis. *J. Dairy Sci.*, 97: 829–837.

Sampimon, O., Barkema, H.W., Berends, I., Sol, J. and Lam, T. 2009. Prevalence of intramammary infection in Dutch dairy herds. *J. Dairy Res.*,76: 129–136.

Sargeant, J.M., Leslie, K.E., Shirley, J.E., Pulkrabek, B.J. and Lim, G.H. 2001. Sensitivity and specificity of somatic cell count

and California Mastitis Test for identifying intramammary infection in early lactation. *J. Dairy Sci.,* 84: 2018–2024.

Schepers, A.J., Lam, T.J., Schukken, Y.H., Wilmink, J.B.M. and Hanekamp, W.J.A. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. *J. Dairy Sci.,* 80**:** 1833–1840.

Schwarz, D., Diesterbeck, U.S., Failing, K., Konig, S., Brugemann, K., Zschock, M., Wolter, W. and Czerny, C.P. 2010. Somatic cell counts and bacteriological status in quarter foremilk samples of cows in Hesse, Germany—A longitudinal study. *J. Dairy Sci.,* 93**:** 5716–5728.

Sharma, N., Rho, G.J., Hong, Y.H., Kang, T.Y., Lee, H.K., Hur, T.Y. and Jeong, D.K. 2012. Bovine Mastitis: An Asian Perspective. *Asian J Ani Vet Adv*, 7: 454–476.

Sharma, N., Singh, N.K. and Bhadwal, M.S. 2011. Relationship of Somatic Cell Count and Mastitis: An Overview. *Asian-Australas J Anim Sci.,* 24: 429– 438.

Shrestha, S. and Bindari, Y.R. 2012. Prevalence of sub-clinical mastitis among dairy cattle in Bhaktapur District, Nepal. *Inter J Agril Biosci,* 1: 16–19.

Supr, K., Haesebrouck, F., Zadoks, R.N., Vaneechoutte, M., Piepers, S. and VliegherS.De. 2011. Some coagulase-negative Staphylococcus species affect udder health more than others. *J. Dairy Sci.,* 94: 2329–2340.

Umar, S., Sarwar, F., Usman, M., Shah, M.A.A., Ghafar, A., Ali, A. and Asif, S. 2013. *In vitro* antimicrobial sensitivity pattern of mastitis causing bacterial pathogens isolatedfrom cattle in arid zones of Punjab, Pakistan. *SciLett,* 1: 17–20.