Microbiological evaluation of milk samples positive to *California Mastitis* Test in dairy buffalo cows (*Buballus bubalis*)

R. Bonini Pardo[1], G. Mendoza-Sánchez[2], A. Nader Filho[3], Taís A.B. Santos[4], H. Langoni[5], H. Tonhati[6], E.B.S. Ferreira[7], D.L. Ravena[7], M.E.A. Oliveira[8], D.J. Sturion[9]

[1]Departamento de Medicina Veterinária Preventiva (Post graduation Program), FCAV/UNESP, Jaboticabal/SP - Faculdade de Medicina Veterinária, FIO/FEMM, Ourinhos/SP – Faculdade de Tecnologia em Alimentos, FATEC, Marília/SP

[2] Departamento de Zootecnia, FCAV/UNESP, Jaboticabal/SP (Post graduation Program)

[3] Departamento de Medicina Veterinária Preventiva, FCAV/UNESP, Jaboticabal/SP

[4] Departamento de Produção Animal (Post graduation Program), FMVZ/UNESP/CAPES, Botucatu/SP

[5] Departamento de Higiene Veterinária e Saúde Pública, FMVZ/UNESP, Botucatu/SP

[6] Departamento de Zootecnia, FCAV/UNESP, Jaboticabal/SP

[7] Veterinary Medicine students, UNIMAR, Marília/SP

[8] Faculdade de Medicina Veterinária, UNIMAR, Marília/SP

[9] Faculdade de Medicina Veterinária, FIO/FEMM, Ourinhos/SP

Corresponding author: R. Bonini Pardo. Departamento de Medicina Veterinária Preventiva (Post graduation Program), FCAV/UNESP, Jaboticabal/SP. P.O.box 42, Zip code: 19500-970, Marília/SP/Brasil - Tel. 00551434251904 - Fax: 00551434251904 - Email: rbpardo@life.com.br or rbpardo@gmail.com

ABSTRACT: In order to observe the microbiological status of CMT positive samples, 734 apparently health mammary quarters from buffalo cows were submitted to physical evaluation, strip cup test and CMT. After milk samples inoculation in 10% ovine blood agar base media and in MacConkey agar and incubation under aerobic condition for 72 hours at 37ºC, identification was proceeded. According to CMT, 227 quarters (30,93%) were positive, among them 73 (32,16%) presented 1+ reaction, 53 (23,35%) were 2+ and 101 (44,49%) were 3+. Microbiological exams of such samples were positive in 147 (64,76%) out of 227 CMT positive samples and among the remaining 72 (31,72%) were negative and 8 (3,52) were contaminated. In the 147 microbiological positive samples 204 bacteria were found in pure or associated growth and the most frequent agents were: *Corynebacterium* sp (59,25%); *Staphylococcus* sp (17,65%) among which 86,11% were coagulase negative and 13,89% were coagulase positive; and *Micrococcus* sp (6,37%). The results revealed that, excluding the eight contaminated samples, 147 (67,12%) quarters out of 219 CMT positive could be considered as bacteria-carrier and that even in a smaller percentage false-positive results can cause problems in a sanitary program for mastitis control in dairy buffalo cows.

Key words: CMT, Microorganisms, Dairy buffalo cows, Subclinical mastitis.

INTRODUCTION - Early detection of mastitis is the most important condition to diminish production losses and to increase the recovery chances of affected mammary tissue. Nevertheless, the absence of macroscopic alterations in tissues or secretions in cases of subclinical mastitis makes it impossible to identify compromised mammary quarters before milking once the routine diagnosis methods include only physical and secretion exams, respectively: palpation, inspection and strip cup test. Thus, different indirect tests have been developed to identify milk subclinical alterations related to vessel permeability, tissue damage, somatic cell counts, pH, viscosity, chloride concentration and catalasis, but most of them are directed to bovine milk (Kumar and Takur, 2001).

The *California Mastitis Test* (CMT) developed by Schalm and Noorlander (1957) has been routinely used to evaluate the content of leucocytes and epithelial cells, defined as the somatic cells (SC), in milk of bovine females. It is based on the interaction between an anionic surfactant and the DNA of the somatic cells present in the milk sample submitted to evaluation. The thickness of the gel produced is often scored as negative, trace, 1+, 2+, or 3+ and the probability of mastitis enhances in a direct proportion (Thieres et al., 1999; XIA, 2006).

Considering it has an easy, fast and low cost execution there are authors that believe that CMT can assume an important role as a field diagnosis of buffalo subclinical matistis (Vianni et al., 1990). However, even for bovine milk samples, some scientific papers emphasize that besides the fact the score determinations depend on personal classification, CMT also presents a high occurrence of false-positive results when compared to microbiological evaluation, which can compromise its confidence in field routine. Such problems could be related to physiologic variation on milk cell content during different lactation stages, seasons of the year, cow's age and also intervals between milking procedures (Salonemi, 1995; Nader Filho et al., 1995). These limitations have also been observed in several studies involving CMT and microbiological evaluations of buffalo cows' milk. Thus, the present study was performed to observe the microbiological status of CMT positive samples collected from apparently health dairy buffalo cows.

MATERIAL AND METHODS - This study was performed in a dairy buffalo cows farm located in the west region of Sao Paulo State in Brazil (22º06'31" south latitude, 50º10'18" west longitude, 597 m height). A total of 734 mammary quarters from Jafarabad and Murrah buffalo cows were submitted to inspection and palpation and to the strip cup test to diagnose clinical mastitis. Immediately after, CMT was used to identify subclinical mastitis cases and independently of the results observed, from each quarter an aseptic milk sample was collected for the microbiological examination (National Mastitis Council, 1987). Milk samples sent under refrigeration to the laboratory were inoculated in blood agar base (5% ovine blood) and in MacConkey agar. After incubation under aerobic conditions at 37ºC for 72 hours, growth was observed daily and microorganisms identification was performed according to the colonial characteristics, verification of tintorial and morfological shape under Gram staining methods and biochemical tests according to Quinn et al. (1994). Milk samples from which more than three different colony types were isolated were regarded as contaminated.

RESULTS AND CONCLUSIONS - Physical exams and strip cup test revealed absence of clinical alterations in milk and udder. Out of the 734 apparently health mammary quarters submitted to CMT, 227 (30,93%) presented positive reactions in different scores: 73

(32,16%) 1+, 53 (23,35%) 2+ and 101 (44,49%) 3+. Using CMT to trial subclinical mastitis in buffalo cows mammary quarters, Singh and Singh (1994) observed, in India, a prevalence equal to 24,4%. An occurrence of subclinical mastitis lower than the present one was also described by Mitra et al. (1995) and Naiknaware et al. (1998), 21,96% and 28,63% of all tested quarters, respectively. The percentage of CMT reaction observed in the present study was higher than the subclinical cases published by other brazilian authors: Costa et al. (1997), that observed 14,5% and Olivaira (1997), 16,8%.

Routine microbiological evaluation of such CMT positive samples showed that 8 (3,52%) were contaminated, 72 (31,72%) were negative and that infection was present in 147 (64,76%). In similar studies, Costa et al. (1997) and Naiknaware et al. (1998) found microorganisms in, respectively, 23,7% and 9,87% CMT positive samples.

Even though considered a personal classification, in the present study CMT scores followed microbiological positive exams because bacteria were isolated from 63,01% of 73 samples with score equal to 1+; from 62,26% of 53 with 2+ and from 67,33% of 101 with 3+.

In the 147 CMT and microbiological positive samples, 204 bacteria were found in pure or associated growth and the most frequent agents were: *Corynebacterium* sp (51,47%); *Staphylococcus* sp (17,65%) among which 86,11% were coagulase negative and 13,89% were coagulase positive; and *Micrococcus* sp (6,37%). In brazilian microbiological studies with buffalo subclinical milk samples, Costa et al. (1997) found C*orynebacterium* sp (59,25%) and *Staphylococcus* sp (17,59%); Costa et al. (2000) isolated *Staphylococcus* sp (20,97%), *Corynebacterium* sp (19,76%) and *Streptococcus* sp (16,94%); and Langoni et al. (2001) observed *Corynebacterium bovis* as the most frequent (31,7%).

Out of 227 CMT positive mammary quarters 31,72% were negative to microbiological exams, a finding lower than the 46,1% observed by Langoni et al. (2001) who suggested that such combination can occur due to infections caused by microorganisms with special necessities. According to Lau et al. (1986) and Kapronezai (2004) the same causes observed in bovine false-positive CMT results can be considered to buffalo CMT problems.

Excluding the eight contaminated samples, the present study showed that among 219 CMT positive samples, 147 were also microbiologically positive, which means that the predictive value of the CMT positive results here was 67,12%. The false-positive results observed, even in a smaller percentage, can cause problems in a sanitary program for mastitis control in dairy buffalo cows.

Figure 1.

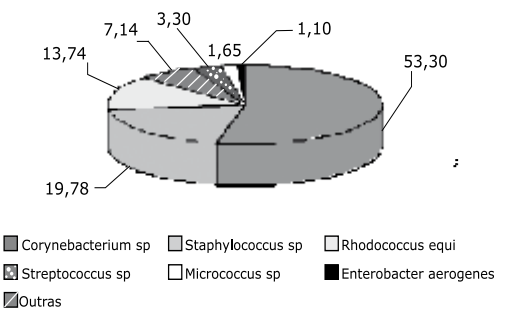
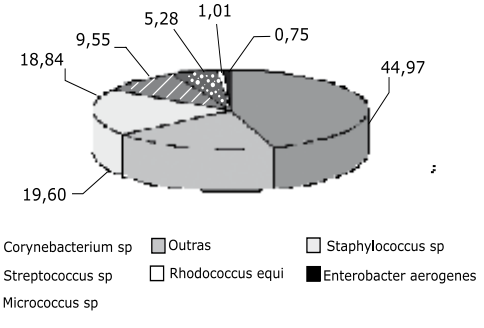


Figure 2.



REFERENCES - Costa, E.O.; Wantanabe, E.T.; Ribeiro, A.R.; Garino JR, F.; Horiuti, A.M.; Baruselli, P.S., 2000. Mastite bubalina: etiologia, índices de mastite clínica e subclínica. Napgama, 3:1, 12-5**. Costa, E.O**.; Garin JR., F.; Wantanabe, E.T.; Ribeiro, A.R.; Vezon, P.; Baruselli, P.S.; Paske, A., 1997. Study of mastitis among ten dairy buffaloes herds (*Bubalus bubalis*) in the Vale do Ribeira São Paulo, Brazil. In: World Buffalo Congress, V, 1997, Caserta. Proceedings...635-8. **Kapronezai, J**., 2004. Estudo de provas microbiológicas e celulares em amostras de leite provenientes de fêmeas bubalinas (*Bubalus bubalis*) no Estado de São Paulo. Dissertation (Master Science course). Veterinary Medicine and Zootecny Course, São Paulo University, Brasil. **Kumar, P**.; Thakur, D.K., 2001. Comparative efficacy of indirect tests for the detection of mastitis in buffaloes. Indian Veterinary Journal, 78, 801-3. **Langoni, H**.; Dominigues, P.F.; Molero Filho J.R.; Baldini, S., 2001. Etiologia e sensibilidade bacteriana da mastite subclínica em búfalos (*Bubalus bubalis*). Ars Veterinária, 17:3, 213-7. **Làu, H.D**.; Singh, N.P.; Hess, S.J., 1986. Comparação de testes indiretos no diagnóstico de mastite subclínica em búfalas. Boletim de Pesquisa – CPATU, 77, 5-13. **Mitra, M**.; Gosh, D.; Ali, K., 1995. Prevalence of subclinical mastitis in an organized buffalo farm at Haringhata. Indian Veterinary Journal, 72, 1310-1. **Nader, Filho A**, A.; Amaral, L.A.; Rossi Junior, O.D.; Nascif Junior, I.A., 1995. Influência das fases de ordenha sobre o número de células somáticas do leite bovino. Pesquisa Veterinária Brasileira, 15:4, 117-20. **Naiknaware, H.S**.; Shelke, D.D.; Bhalerao, D.P., 1998 Prevalence of subclinical mastitis in buffaloes in and around Mumbai. Indian Veterinary Journal, 75, 291-2. **National Mastitis Council**. Laboratory and Field Handbook on Bovine Mastitis. Arlington:W. D. Hoard, Sons Co., 1987. 208 p. **Oliveira, M.V.V**., 1997 Exame microbiológico do leite de búfalas e avaliação dos testes Whiteside e Califórnia Mastitis Test no diagnóstico da mastite bacteriana subclínica em bubalinos. Dissertation (Master Science course). Federal University of Pernambuco, Brasil. **Quinn, P.J**.; Carter, M.E.; Markey, B. Clinical Vetereinary Microbiology. 1.ed. London: Wolfe, 1994, 648 p. **Salimenei, H**., 1995. Use of somatic cell count in udder health work. In: Sandholm, M. et al. The bovine udder and mastitis. Finnland:Gummerus Kirjapaino, p. 105-110. **Schalm, O.W**.; Noorlander, D.D., 1957. Experiments and observations leading to development of the California Mastitis Test. Journal of American Veterinary Medical Association, 130, 199-204. **Singh, P.J**.; SINGH, K.B., 1994. A study on economic losses due to mastitis in India. Indian Journal of Dairy Science, 47, 265-72. **Thiers, F. de O.**; Benites, N.R.; Riberio, A.R.; Costa E.O., 1999. Correlação entre contagem direta de células somáticas e o teste de Califórnia Mastitis Test (CMT) no leite de vacas. Napgama, 2:4, 9-12. **Vianni, M.C.E**.; Nader Filho, A.; Rossetti, D.J.G.; Longhi, J.L.; Sicher, M., 1990. Eficiência do Califórnia Mastitis Test (CMT) na estimativa do número de células somáticas do leite bubalino. Ciência Veterinária, 4:2, 3-4. **Xia, S.Z**., 2006. The rheology of gel formed during the California Mastitis Test. Dissertation (Master Science course). Veterinary Medicine and Zootecny Course, São Paulo University. Thesis (Master of Science and Technology). Department of Engineering, The University of Waikato, Hamilton, New Zealand.