International Food Research Journal 22(3): 1037-1042 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Efficacy of premilking and postmilking teat dipping as a control of subclinical mastitis in Egyptian Dairy cattle

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Article history

Received: 4 April 2014
Received in revised form: 27 October 2014
Accepted: 20 November 2014

Keywords

Subclinical mastitis
Teat dipping
Microbiological quality
Raw milk

Abstract

In order to compare the efficiency of two common dairy herd processes for reduction of mastitis potential, 30 dairy cows (Holstein-Friesian) divided into three groups (ten cows each): the first was set as a control, the second was processed using premilking teat dipping and the third with postmilking teat dipping. Teat dipping was done using iodophore 0.5 % solution. Results showed the superiority of postmilking teat dipping procedure in most tests. The number of samples tested positive using California mastitis test were two in case of postmilking teat dipping, 5 in case of premilking teat dipping and 10 in the control group. Somatic cell count revealed also that postmilking teat dipping procedure has reduced number of positive samples in comparison to premilking teat dipping procedure. Bacteriological examination of samples revealed also the superiority of postmilking teat dipping than premilking teat dipping. *Staphylococcus aureus* and coliform organisms counts were reduced about 3 and 2.5 logs in case of postmilking teat dipping against about 1 and 0.5 log in case of premilking teat dipping, respectively. *Streptococcus agalactiae* has been isolated from 2 samples in the postmilking teat dipping group, 4 samples in the premilking teat dipping group and 6 samples in the control group. Obtained results were scientifically assessed and discussed in comparison to worldwide research reports. Ultimately, obtained results suggested the preferential priority of postmilking teat dipping procedure in controlling of subclinical mastitis in Egyptian dairy herds.

Introduction

Mastitis, in either of its two forms; clinical (CM) and subclinical (SCM), represents a prominent hazard to dairy producers (Oliver *et al.*, 2003; De Vliegher *et al*., 2005a; Osteras *et al*., 2008). This hazard may exceed the limit of economical losses due to dropped milk production and culling out of lactating dairy cows (Waage *et al.*, 2001; De Vliegher *et al*., 2005b), as it may represent a food safety issue due to contamination of milk with different mastitis pathogens that may represent an imminent human pathogenic burden.

Thus, proper control of mastitis in dairy herd is considered an indispensable process to ensure both animal health and food (milk) safety. For this, numerous control programs have been developed over the last few decades (Fetrow *et al*., 1991), and despite the massive development in mastitis control techniques, mastitis still constitutes the main problem of dairy production (Bhutto *et al.*, 2012). Among these controlling regimes, teat dipping has acquired great importance as an essential mastitis preventive tool (Hassan *et al*., 2009). Teat dipping has been demonstrated to be highly effective at preventing new intramammary infections with different mastitis pathogens (Hogan *et al.*, 1987). While premilking teat dipping is necessary to reduce the microbial population and minimize new intra mammary infections, postmilking teat dipping has been used mainly in highly infected herds (Paape *et al*., 2001; Bergonier and Berthelot, 2003; Contreras *et al.*, 2003), and it has been revealed also as a very effective tool to prevent mastitis incidence. However, recent researches have revealed that not all types of mastitis causing pathogens are responding the same to teat dipping (Osteras *et al.*, 2008).

Microbial causative agents of mastitis have many types and generally they may be classified into contagious and environmental pathogens. While contagious pathogens (which usually spread from cow to cow or from quarter to quarter) are responding well to teat dipping, environmental pathogens are not (Osteras *et al*., 2008). Moreover, many previous reports have concluded that teat dipping should not be approved as mastitis controlling program except after a detailed identification of the common mastitis pathogens in the dairy herd has been conducted (Osteras *et al.*, 2006).

In the view of the existence of many protocols of teat dipping, premilking and postmilking, and the existence of many reports concerning with the

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efficacy of teat dipping as mastitis control protocol, this study was conducted to evaluate the use of Iodophor 0.5% solution as either premilking or postmilking teat dipping solution for protection of dairy cow from subclinical mastitis. Many parameters were used to assess udder health and efficacy of both procedures in preventing SCM. California Mastitis Test (CMT), Somatic cell count (SCC), chloride % and microbiological examination of quarter milk for the presence of *Staphylococcus aureus (S. aureus)* and *Streptococcus agalactiae (St. agalactiae)* as main causative agents of contagious mastitis and coliform organisms as environmental mastitis pathogens.

Materials and Methods

Animals and experimental design

Thirty clinically-healthy dairy cows were used. All milking cows shared the same diet. Cows were selected to be multiparous and all of them were of Holstein-Friesian crossbred origin. Cows under experimental conditions were hand-milked two times daily following standard hygienic milking procedures. Experimental cows were divided into three groups (10 cows in each). The first serve as control, the second was designed to serve as premilking teat dipping group (PreMTD) and the third as postmilking teat dipping group (PostMTD). Cows' udders in all groups have similar preparatory manipulations, which included premilking washing of the udder with clean water and drying with clean clothes. Just before milking in PreMTD group, nearly the whole external surface of the teat was dipped into dipping solution (Iodophore 0.5%) which was left for about 30 seconds and teat was then dried by another clean clothes. While in the case of PostMTD group, similar teat dipping manipulation was applied after cow has ended milking and dipped teats were not dried off.

Sampling

Initial 15 mL of milk was discarded. The teat ends were then whipped with paper towel soaked in 70% alcohol, dried with a clean towel and then quarter milk sample was collected aseptically (APHA, 2003). Collected samples were transferred to the laboratory of Milk Hygiene and Technology, Faculty of Veterinary Medicine, Zagazig University in an ice box with a minimum of delay.

California mastitis test (CMT)

Two ml of each milk samples were added to a black cup, in which they were mixed with equal amount of Alkyl Aryl sulphonate (CMT reagent). Cup was gently swirled in a horizontal circular motion. Milk flocculation and clotting was noticed and result was recorded after 10 seconds (Schalm and Noorlander, 1957).

Somatic cell count (SCC)

Automatic somatic cell counter was used to determine SCC of milk samples. Initial warming of samples at 35°C for 5 minutes was accomplished in a water bath before mixing and reading (Radostits *et al*., 2000).

Chloride test

10 ml of milk was added to 5 ml of nitric acid 25%, 5 ml of silver nitrate N/10 and 1 ml of ferric ammonium sulphate in a porcelain dish. Titration against ammonium thiocyanate N/10 was continued until a brown color appeared and remained for two minutes and R was recorded (amount of ammonium thiocyanate N/10 used in titration). Appearance of a brown color which remains for two minutes indicates that chlorine content is 0.14% or more according to the method described by Sawyer *et al*. (1994).

Bacteriological examination.

Milk samples were serially diluted using peptone water before 1 ml of appropriate dilution was inoculated onto 3M™ petrifilm™ Staph Express count plates (to enumerate and isolate *S. aureus*, AOAC, 2003) and onto violet red bile lactose agar plate (to enumerate coliform organisms) and evenly distributed. Plates were incubated aerobically at 35°C for 24 h for *S. aureus* and at 35°C for 24 h for coliform. Red-violet colonies were counted as *S. aureus* and any plates with background (any other color) colonies were subjected to, 3M™ Petrifilm™ Staph Express Disk testing to differentiate *S. aureus,* which were incubated at 35±2 °C for 1-3 h. *S. aureus* colonies appear surrounded with pink zone, which were then counted. While for coliforms, dark red/purple colored colonies with a diameter of at least 0.5 mm were counted as coliform organisms. For isolation of *St. agalactiae*, milk samples were centrifuged and a loopful from milk sediment was streaked on the surface of Edward's media containing 5% sterile citrated sheep blood and 1% esculin. Plates were incubated at 37°C for 48 h. Small, round and translucent colonies were picked up and inoculated into 1 ml of tryptone soya broth and incubated for 24 hrs at 37°C then 0.3 ml sterile glycerol was added and preserved at -70°C for further morphological and biochemical identification (Carter and Cole, 1990).

Table 1. Distribution of quarter milk samples according to grades of CMT (N=40).

Group	Positive samples	CMT +	CMT ++	CMT +++
Control	10	4	3	3
Pre-milking teat dipping	5 *	1	2	2
Post-milking teat dipping	2 **	1	1	0

* Significant variation ($p< 0.05$)
** Highly significant variation ($p<0.01$)
N= Number of samples of each group

Results and Discussion

Nowadays, the primary concern of dairy producers is to implement and maintain a good mastitis control program. Despite the widespread of such control programs among dairy herds (teat dipping, dry cow therapy and machine milking maintenance), mastitis cases still to constitute a repetitive problem (Todhunter *et al.*, 1995, Milne *et al.*, 2003). Many factors are influencing the implementation of mastitis control program, mainly, milking attributes (hand or machine milking), environmental conditions and hygienic practices (Sargeant *et al*., 2001, Radostits *et al.*, 2007). Mastitis is mainly resulted from ascending infection by many mastitis pathogens. Different classification was used to differentiate these pathogens, however, two main categories were predominated; contagious and environmental pathogens. *S. aureus* and *St. agalactiae* are considered the main contagious mastitis causative agents, while coliform organisms and other streptococci are those associated with environmental mastitis (Oliver *et al.*, 1993, Smith, 1996).

Teat dipping is among the highly recommended mastitis controlling programs (Oliver *et al.*, 2001), and it is widely used as a simple and cost-effective procedure. Either PreMTD or PostMTD is recommended by dairy specialists for controlling of mastitis in different dairy herds. In this study, an evaluation trail has been conducted to explore which of these methods will suit Egyptian dairy environment. In order to accomplish this evaluation satisfactorily, several parameters were employed to compare the efficacy of both PreMTD and PostMTD.

Results obtained from CMT were in the favor of PostMTD, as in comparison to the control group, only 2 quarter milk samples were yielded positive results in case of PostMTD against 5 samples in case of PreMTD and 10 samples in the control group (Table 1). Moreover, with a closer look to CMT scoring, it is very obvious that PostMTD has scaled down the severity of SCM, as CMT score of 3+ was not recorded in any case of PostMTD. While in case of PreMTD, 2 samples were found to have a CMT score of 3+. Omore *et al*., (1996) and Kivaria *et al*., (2004) proved a positive correlation between CMT score of 2+ or more and intramammary infection with *S. aureus*. Moreover, Sargeant *et al*., (2001) concluded the significant role of CMT to detect intramammary infection with major mastitis pathogens.

Estimation of chloride % in quarter milk samples did not added any significance value for either determining SCM cases nor comparing between PreMTD and PostMTD (data not shown). Chloride % significantly increases with progression of SCM, although many other factors are influencing and may counteract with chloride %. Thus, it was not possible to interpret our findings of chloride % to check the efficacy of both treatments. Sender and Bassalik-chabielska (1990) had declared similar conclusion.

More accurate evaluation's interpretation of both types of dipping was obtained from comparing quarter milk's SCC, as SCC is considered the main specific parameter which correlates significantly with intramammary infection (Zucali *et al.*, 2011). 9 out of 40 quarter milk samples were found positive for SCM according to SCC in case of PostMTD, 16 in case of PreMTD and 18 in case of the control group (Figure 1a). Since SCC is proportionally correlated with the degree of udder inflammation (Guha et al., 2012), SCC can be adopted as a precise and reliable indicator for severity of affection. International Dairy Federation (IDF, 1995) has defined a threshold value of 200000 cells/ml as a guideline for detection of SCM. In the same vein, European countries have restricted the human use of milk with SCC exceeded 400 000 cells/ml. In this study, PostMTD, in addition to reducing cases of SCM more than PreMTD, it seems also to alleviate the severity of these cases. Distribution of mastitic samples according to SCC revealed that samples of PostMTD group lied only at 200000 to less than 500000, while in PreMTD, some mastitic samples have SCC of 500000 to less than 1500000 (Figure 1b). Generally, both treatment

Table 2. Statistical analytical results of *Staphylococcus aureus* and coliform counts and log reduction of each treatment (N=40)

Microorganism	Group	Mean ± Standard Error (CFU/ml)	Mean ± Standard Error (Log)	Log reduction
S. aureus	Control	$6.6 \times 10^5 \pm 2.3 \times 10^4$	5.82 ± 4.36	--
	Pre-milking dipping	$4.7 \times 10^4 \pm 5.3 \times 10^3$	4.69 ± 3.72	1.13*
	Post-milking dipping	$4.4 \times 10^2 \pm 6.4 \times 10$	2.65 ± 1.8	3.17**
Coliform	Control	$3.0 \times 10^5 \pm 3.8 \times 10^4$	5.47± 4.58	--
	Pre-milking dipping	$8.2 \times 10^4 \pm 7.1 \times 10^3$	4.91± 3.85	0.56*
	Post-milking dipping	$9.8 \times 10^2 \pm 2.5 \times 10$	2.95± 1.4	2.52**

* Significant variation ($p < 0.05$)
** Highly significant variation ($p<0.01$)
N= Number of samples of each group

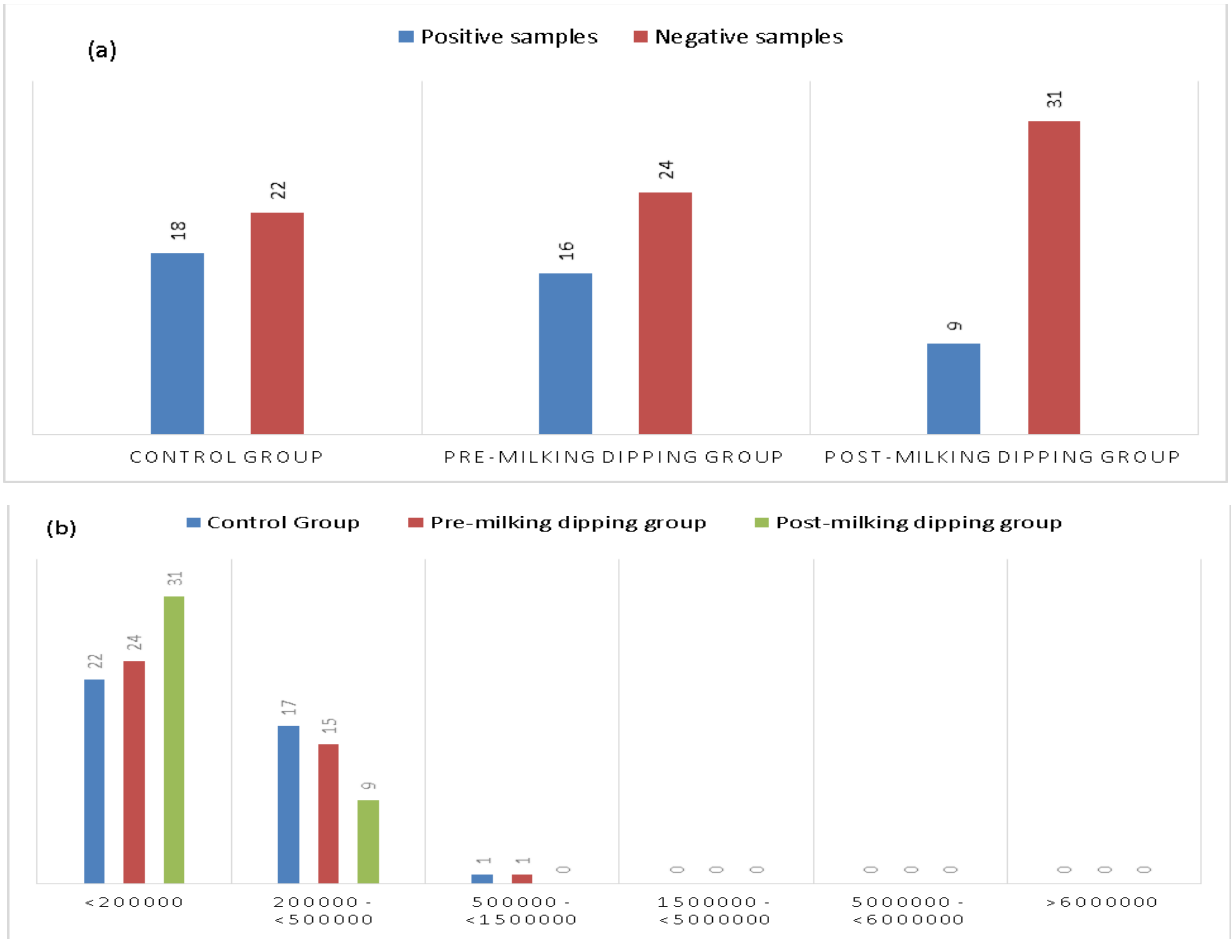

Figure 1. Distribution of examined samples according to SCC (a) positive and negative samples, (b) frequency distribution of samples

methods have their significant role in lowering SCC. This is in accordance with many previous reports which judged the use of idophore in either PreMTD or PostMTD (Zucali *et al*., 2011; Hillerton *et al*., 1993; Goodwin *et al*., 1996).

Microbiological findings were certainly of extreme

significance in dipping techniques' preference. As for the main causative agents of contagious mastitis, PostMTD has achieved prioritized results against PreMTD, although both have significant results. The main count of *S. aureus* of PostMTD, PreMTD and control group quarter milk samples were 4.4 x 10^2, 4.7 x 10^4 and 6.6 x 10^5, respectively (Table 2). Thus, PostMTD has succeeded in achieving more than 3 log of highly significant reduction in *S. aureus* count. While PreMTD has just achieved about 1 log reduction which still to be considered a significant.

S. aureus has accounted for most cases of mastitis (Guha *et al*., 2012) and also for the highest severity of the mastitis affection (Contreras and Rodríguez, 2011). Consequently, reduction of this serious pathogen is of a great outcome. Oliver *et al.*, (2001) revealed a reduction of the intramammary infection of *S. aureus* after implementation of a PreMTD program. Goodwin *et al*., (1996) reported at the contrary a significant reduction for *S. aureus* count with idophore PostMTD program.

In Egypt, *St. agalactiae* has a great importance as a major causative agent of contagious mastitis (El-Jakee *et al.*, 2013), and its severity arises from the development of multidrug resistant strains. Our finding regarding *St. agalactiae* simulate those of *S. aureus*, in which PostMTD was responsible for significant reduction in numbers of samples, which yielded positive for *St. agalactiae* (6.4 and 2 for control, PreMTD and PostMTD groups, respectively). Boddie *et al*., (2000), Oura *et al*., (2002) and Suriyasathaporn (2010) revealed similar findings regarding either PreMTD and PostMTD reduction in *St. agalactiae* prevalence, respectively. In case of environmental mastitis pathogens, and in the contrary of an earlier report (Goldberg *et al.*, 1994), PostMTD was found to have the greater reduction effect against coliform organisms (Table 2). In log, PostMTD was responsible for about 2.5 log reduction of coliform organisms count versus 0.5 log reduction in PreMTD group.

Conclusion

From the aforementioned results and discussion, PostMTD looks promising in controlling of major mastitis pathogens in Egyptian environment. In this aspect, a more rigorous program should be applied with routine follow up to limit the problem of mastitis and in turn to avoid its implications on human health.

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