Journal of Elementology ISSN 1644-2296

Miseikiene R., Tusas S., Biziene R., Kerziene S., Miciński J., Matusevicius P. 2020.
Influence of teat disinfection with iodine preparation on bacterial contamination of teats, hygenic quality and content of iodine in milk.
J. Elem., 25(1): 225-236. DOI: 10.5601/jelem.2019.24.2.1879

RECEIVED: 11 June 2019
ACCEPTED: 2 October 2019

ORIGINAL PAPER

INFLUENCE OF TEAT DISINFECTION WITH IODINE PREPARATION ON BACTERIAL CONTAMINATION OF TEATS, HYGENIC QUALITY AND CONTENT OF IODINE IN MILK*

Ramute Miseikiene[1,3], Saulius Tusas[1], Renata Biziene[3], Sigita Kerziene[4], Jan Miciński[5], Paulius Matusevicius[2]

[1] Institute of Animal Rearing Technologies
[2] Department of Animal Nutrition
[3] Institute of Biology Systems and Genetic Research
[4] Department of Physics, Mathematics and Biophysics
Lithuanian University of Health Sciences, Kaunas, Lithuania
[5] Department of Sheep and Goat Breeding
University of Warmia and Mazury in Olsztyn, Poland

Abstract

The objective of the study was to investigate the influence of pre-milking teat disinfection on total bacterial contamination of teat skin, and to analyze the effect of pre- and post-milking teat disinfection on somatic cell count in milk. Three groups of cows in five dairy farms were used. The total bacterial contamination on cow teat skin before and after teat disinfection was determined. The number of colony forming units (cfu) was calculated per 1 ml a total for 2700 samples. The most bacteria on teat skin were effectively reduced after pre-milking teats disinfectionm almost in all groups of 5 farms (from 1.1 to 4.5 times). Summarized results showed that the number of bacteria counted on teat skin and milk somatic cell count (SCC) were significantly higher from teats with no pre- and post-milking teat disinfection in comparison with teats with pre- and post-milking teat disinfection or no pre-milking teat disinfection and post-milking teat disinfection only ($p \leq 0.05$). Pre-milking teat disinfectant with peracetic acid and hydrogen peroxide was effective in reducing total bacterial contamination on cow teat skin. The results also indicate that application with pre- and post-milking teat disinfectants provided an impact on the reduction of SCC in milk. The lowest iodine content was 0.1291 mg L^{-1} (group T_1, whereas the highest was 0.2963 mg L^{-1}). In our research the differences in the iodine content between farms were noted as well. For example, the highest iodine content in milk appeared at F_{IV} farm. Statistical differences appeared at $p \leq 0.05$.

Keywords: bacteria, cow, pre- and post-milking disinfection, somatic cell count, free iodine.

Jan Miciński, PhD DSc Prof., Department of Sheep and Goat Breding, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5/150, 10-719 Olsztyn, Poland, e-mail: jan.micinski@uwm.edu.pl

* The research was carried out within the statutory subject of the Department – payment number: 11.610.012-300.

INTRODUCTION

Teat skin is a potential reservoir for microbial diversity of milk (Monsallier et al. 2012). The colonization of the teat skin with cow-associated and environmental microorganisms is described as a potential starting point for the invasion into the bovine mammary gland (Vacheyrou et al. 2011. A significant role in the control of udder inflammation of cows is played by the immunological system, whose proper functioning depends on the appropriate environment, including mainly nutrition (Wójcik et al. 2013). The practice of pre- and postmilking teat dipping is one of the critical components of mastitis prevention and control program in a dairy herd because mastitis is one of the most economically significant diseases in the dairy industry for both backyard farmers in developing countries and high producing herds worldwide (Tiwari et al. 2013). While premilking teat dipping is necessary to reduce the microbial population and minimize new intramammary infections, postmilking teat dipping has been used mainly in highly infected herds and it has been revealed also as a very effective tool to prevent mastitis incidence (Kamal, Bayoumi 2015). Pre-milking teat disinfection is practised in several countries to reduce the microbial load of the teats prior to milking and to prevent mastitis caused by environmental pathogens (Böhm et al. 2017). Pre-milking teat cleaning regime involving the washing of teats with an effective disinfectant and then drying was reported to be most effective for removing bacteria and minimizing bacterial growth (Gibson et al. 2008, Hysen et al. 2010). Management practices associated with a low somatic cell count include the use of post-milking teat disinfection, correct udder preparation and milking (Trajkovska et al. 2015). Post-milking teat disinfection is considered to be one of the most effective procedures for reducing the rate of subclinical and clinical cases of mastitis during lactation (El Behiry 2012). The dips are designed to effectively reduce infections caused by environmental bacteria as well as to minimize the spread of infections caused by contagious bacteria. A study of Ruegg (2003) demonstrated that it is necessary to include disinfection with highly effective agents that are active in low concentrations and do not pose a threat of leaving chemical residues in milk (Malinowski 2000). But the efficacy of a teat dip depend not only on the active ingredient and its concentration, but on many other factors. Several disinfectants like hypochlorite, chlorine dioxide, chlorhexidine, alcohol, iodine reduce the microbial load of teats significantly (Gleeson et al. 2009, Enger et al. 2015). Lactic acid and other organic acids are applied as disinfectants for cow teat disinfection because they have a bactericidal effect. Lactic acid may be combined with hydrogen peroxide, and this combination improves teat skin condition and minimizes bacterial colonization on the teat skin surface. Iodine is a broad-spectrum, rapidly acting germicide that is effective against essentially all mastitis-causing bacteria (Gleeson et al. 2009). It has been established that 5% v/v lactic acid is an effective natural teat antiseptic for reducing bacteria on teat skin (Chotigarpa et al. 2019). Teat disinfectants based on iodine provide broad spectrum efficacy with rapid kill, while providing a persistent film on teat skin which offers extended protection owing to hrough the formation of a physical and chemical barrier (Gibson et al. 2008). Teat dipping with iodine-containing solutions is still common in many countries for teat disinfection and to prevent transmission of contagious mastitis pathogens from cow to cow. Peracetic acid is a more effective disinfectant than hydrogen peroxide and has no toxic residuals. The aim of our experiment was to evaluate the effect of pre-milking teat preparation on the number of bacteria on teat skin, and to determine the effect of teat disinfection on the somatic cell count in milk (Riekierink 2012).

MATERIALS AND METHODS

Herds and animals

The study was carried out during an indoor period, at 5 farms, where the herd size varied from 100 to 150 cows. The farms were selected with possibly similar environmental conditions and milk production of around 8000-9000 kg per year. Animals were kept on rubber mats, fed total mixed ration and milked in a herringbone milking parlour twice a day. Cows in the second lactation lasting already for 2-4 months, without signs of clinical mastitis (no swelling, no heat, no pain, no redness of the udder; milk of normal colour, without fibrin clots) were included in the experiment at each of the farms, and the average somatic cell count in milk was from 100.000 to 150.000 thousand ml^{-1}. For the purpose of this study, cows on each farm were divided into 3 experimental groups consisting of ten animals: T_1 (control), T_2 group and T_3 group. Cows of all groups had approximately the same average milk yield, stage of lactation and hygienic quality of raw milk. Differences between the groups were not significant. The research was conducted for 3 months. The milking routine was the same at all 5 farms. All cows were milked in herringbone milking parlours with DeLaval milking equipment.

Udder preparation and disinfection

Cows of the control (T_1) groups from the five dairy herds were divided into non-disinfected pre- and postmilking groups. Each cow received the same pre-milking preparation at all milkings during the experiment. Only basic udder hygiene was maintained in this group, consisting of the wiping of teats with disposable paper wipes. T_2 group cows in the five dairy herds were randomly allocated to non-disinfected premilking and post-milking teat disinfection groups. After milking, teats were immersed in a special cup containing active compounds based on iodine, chlorine or lactic acid.

In T_3 group, both pre- and post-milking teat disinfection were applied. Solutions for pre-milking teat disinfection based on lactic acid, hydrogen peroxide and peracetic acid were used, and post-milking disinfectants containg iodine, hydrogen peroxide or lactic acid were used. Pre-milking teat disinfection was applied to a cow's teats (according to the manufacturer's recommendations) and the disinfectant was left on the teat for approximately 20 s before being wiped off as a step in the preparation for milking.

Pre- and post-milking disinfectants were applied by dipping. All experimental dips were commercial products. Teats were treated using a dip cup.

Table 1 provides description of pre- and post-milking teat disinfection at each farm.

To determine the microbial contamination of teat skin, the samples were obtained prior evening milking. A total of 1350 samples were obtained before treatment (5 farms x 3 groups (T_1, T_2, T_3) x 10 cows x 9 visits) and another 1350 – after treatment (5 farms x 3 groups (T_1, T_2, T_3) x 10 cows x 9 visits).

Table1

Description of pre- and post-milking teat disinfection

Farm number	T_2 group		T_3 group	
	pre-milking teat preparation/ /disinfection	post-milking teat disinfection	pre-milking teat disinfection	post-milking teat disinfection
	active ingredient		active ingredient	
F-I	x	free iodine 4-8 ppm	hydrogen peroxide	0.5% hydrogen peroxide
F-II	x	lactic acid	peracetic acid and hydrogen peroxide	free iodine 4-8 ppm
F-III	x	lactic acid	peracetic acid and hydrogen peroxide	lactic acid
F-IV	x	free iodine 1400 ppm	Lactic acid 4%	free iodine 1400 ppm
F-V	x	free iodine 5000 ppm	1% hydrogen peroxide	0.75% iodine

x – only basic teat hygiene was maintained (wiping with paper disposable wipes)

Sample collection

The sampling from teats before using the antiseptic/disinfection was performed by triple rotary motions around the surface of the teat close to the tip from the front teat only, and then the samples were placed into disposable transport Transwab®Amies (England). Exposure time of antiseptics on teats was 20 s. Then, the teats of individual cows were dried with paper towels.

After drying, the sampling from teats was performed again. Sterile gloves were used throughout the sampling.

A milk sample (1 ml) was mineralized in a mixture of 4 ml HNO_3 and 1 ml H_2O_2 in airtight high-pressure tanks by heating. The content of iodine was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy in (ICP-AES), Optima 5300 DV according to Flachovsky et al. (2014).

Microbial analysis

All samples were then transported from farm to laboratory under low temperature conditions. The total bacterial contamination of cow teat skin was determined by employing serial dilutions and the plate count method proposed by Tortora et al. (2010). The number of colony forming units (cfu) was calculated per 1 ml (cfu ml^{-1}). The cfu/mL was determined for each individual sample (a total number 2700). Under aseptic conditions, the teat samples were agitated for 10 s to extract the bacteria from the swab. Then, 1 ml of the solution was taken to produce dilutions down to 10^6, and 0.2 ml of the sample was taken for spreading upon the bacteria-specific agar plates.

To determine the quality of raw milk the somatic cell count (SCC) was considered as standard. The samples were taken from each individual cow and transported in sterile 40 ml bottles. To determine somatic cell count the samples were obtained during evening milking. Somatic cells count was performed at SE „Pieno tyrimai“ (Lithuania) by the heavy-duty counter-measurer „Somascope MK2“ („Delta Instruments“, Netherlands), which operates by the fluoro-opto-electronic method.

Statistical analysis

As the natural variables were not normally distributed, making them unsuitable for analysis of parametric methods, we applied a logarithmic transformation of variables. After transforming the variables (bacteria count on teat skin before and after teat disinfection, somatic cell count) they were normally distributed and analyzed using parametric methods. We used General Linear Model Repeated Measures (SPSS Statistics Version 20), which included a survey repeatability (9 times), survey location (5 farms), disinfectants used before and after milking. The criterion Fisher's SD (standard deviation) was used to evaluate differences between the compared groups. Differences were considered statistically significant when $p \leq 0.05$.

RESULTS

The number of bacteria on cow teat skin and somatic cell counts in milk of three dairy cow groups (T_1, T_2, T_3) individually on each farm (F-I, F-II, F-III, F-IV and F-V) are described in Table 2. The use of hydrogene peroxide

Table 2

Average values of bacteria (cfu ml-1) and SCC (thousand ml-1), LSM ± SD

Farm number	Group of cows	cfu $ml^{-1} \cdot 10^6$ on cow teat skin before disinfection	cfu $ml^{-1} \cdot 10^6$ on cow teat skin after disinfection	SCC (thousand ml^{-1})
F-I	T_1	18.94 ± 6.78	12.22 ± 5.34	60.25 ± 31.3
	T_2	26.00 ± 17.8	5.73 ± 2.68 *	65.67 ± 11.18
	T_3	11.70 ± 3.89	4.10 ± 1.70 **	87.93 ± 17.55
F-II	T_1	14.25 ± 2.56	11.33 ± 3.08	172.45 ± 38.9
	T_2	16.80 ± 13.90	0.12 ± 0.01	113.66 ± 16.89
	T_3	2.72 ± 1.77	1.48 ± 1.13	163.93 ± 19.21
F-III	T_1	5.25 ± 2.33	6.81 ± 2.03	138.59 ± 25.94
	T_2	4.68 ± 2.00	1.99 ± 0.95 **	48.74 ± 10.39
	T_3	6.69 ± 2.64	3.51 ± 1.74 **	113.42 ± 45.71
F-IV	T_1	4.67 ± 1.77	5.17 ± 1.86	86.60 ± 11.49
	T_2	5.98 ± 2.08	1.98 ± 0.45 **	93.50 ± 18.99
	T_3	5.26 ± 2.09	4.58 ± 1.86	73.76 ± 14.61
F-V	T_1	0.81 ± 0.28	1.49 ± 0.37 *	551.15 ± 123.34
	T_2	0.83 ± 0.29	1.17 ± 0.32	466.70 ± 78.02
	T_3	0.76 ± 0.35	1.89 ± 0.68	258.93 ± 28.17

* $p \leq 0.05$, ** $p \leq 0.01$ – means in the row differed significantly

(pre- and post-milking teat disinfection) (F-I) reduced microbial counts on cow teats by 78% (T_2, $p<0.01$) and 65% (T_3, $p \leq 0.05$). Cow teat disinfection reduced bacterial contamination on teat skin in T_2 and T_3 groups (F-III) by 58% and 48% ($p \leq 0.01$) respectively. CFU ml^{-1} x 10^6 on cows' teat skin (F-IV) after teat treatment with post-milking disinfectant containing free iodine (T_2) decreased by 66.8% ($p \leq 0.01$). SCC in all groups of cows in five farms varied on average from 60 to 172 SCC (thousand ml^{-1}) except F 5 when SCC in milk ranged from 258 to 551 thousand/ml regardless of the use of disinfectants or not.

The effect of disinfectant active ingredient on teat skin microbial counts when pre-milking teat disinfection was used is presented in Table 3. Based on values of the geometric mean of all groups, the total bacterial count in all groups decreased independently whether pre-milking disinfectant was or was not used (teats were wiped with paper wipes only), but the number of micro-organisms decreased by almost 3.3 times and significant results were obtained when disinfectant based on peracetic acid with hydrogen peroxide was used ($p \leq 0.05$).

Table 4 is a summary of the impact of pre- and post-milking treatment on the bacteria count and reduction of SCC in milk during the study period at all the farms. The results show that the number of bacteria on

Table 3

Influence of disinfectant active ingredient on bacterial contamination (LSM ± SD)

Active ingrediente	cfu ml^{-1} · 10^{6} on cow teat skin before disinfection	cfu ml^{-1} · 10^{6} on cow teat skin after disinfection
Wiping with paper disposable wipes only	8.44 ± 2.09	7.43 ± 0.45
Lactic acid	5.36 ± 3.96	4.29 ± 0.91
Peracetic acid and hydrogen peroxide	8.91 ± 4.78^{a}	2.68 ± 1.10^{b}
Hydrogen peroxide	7.58 ± 3.96	4.23 ± 0.91

^{a,b} – means in the row with different superscripts differed significantly at p≤0.05

Table 4

Effect of teat treatment on bacteria count and somatic cell count (LSM ± SD)

Pre- and post-milking teat preparation/disinfection	cfu ml^{-1} x 10^{6} on cow teat skin after teat preparation	SCC thousand ml^{-1}
No pre- and post-milking teat disinfection	3.94 ± 0.74^{a}	268.55 ± 40.56^{a}
No pre- milking teat disinfection, post-milking teat disinfection only	3.49 ± 0.57^{b}	188.21 ± 33.71^{b}
Pre- and post-milking teat disinfection	3.12 ± 0.53^{b}	147.09 ± 29.73^{b}

^{a,b} – means in the column with different superscripts differed significantly at p≤0.05

teat skin and milk SCC were significantly higher from teats with no pre- and post-milking teat disinfection in comparison with teats with pre- and post-milking teat disinfection or no pre-milking teat disinfection and post-milking teat disinfection only (p≤0.05).

Table 5 shows the content of iodine in the milk collected from cows of different experimental groups from 5 farms. The analysis revealed that the average iodine content was the lowest in cows from T_1 control group

Table 5

The content of iodine in milk of the tested cows (mg L^{-1}) LSM ± SD

Farm number	Group cows		
	T_1	T_2	T_3
FI	^{a} 0.1443 ± 0.027^{a}	^{a}0.1718 ± 0.013^{b}	^{a}0.2022 ± 0.021^{c}
FII	^{b}0.1291 ± 0.031^{a}	^{a}0.1642 ± 0.098^{b}	^{a}0.1931 ± 0.030^{c}
FIII	^{a}0.1513 ± 0.020^{a}	^{a}0.1780 ± 0.021^{b}	^{a}0.2140 ± 0.027^{c}
FIV	^{c}0.1754 ± 0.019^{a}	^{b}0.2279 ± 0.018^{b}	^{b}0.2963 ± 0.028^{c}
FV	^{c}0.1682 ± 0.022^{a}	^{b}0.2836 ± 0.006^{b}	^{c}0.2420 ± 0.023^{c}
Average	0.1537 ± 0.018^{a}	0.2051 ± 0.024^{b}	0.2295 ± 0.019^{c}

^{a,b} – the means values between the tested cows groups (differed significantly at p≤0.05

^{a,b} – the means values between the farms groups differed significantly at p≤0.05

(0.153 mg L^{-1}), whereas the highest one appeared in T_3 group (0.229 mg L^{-1}), i.e. where pre- and after-milking dipping was performed. Statistically significant differences at $p \leq 0.05$ confirmed it. The lowest iodine content was 0.129 mg L^{-1} (group T_1, whereas the highest was 0.296 mg L^{-1}). In oor research, differences in the iodine content between the farms were noted as well. For example, the highest iodine content in milk appeared at F_{IV} farm. Statistical differences appeared at $p \leq 0.05$.

DISCUSSION

Teat disinfection is usually recommended to be applied before and after milking to reduce the number of bacteria on teat skin and in milk. Pre-milking teat dipping is necessary to reduce the microbial population and minimize new intramammary infections because teat surface is a potential direct source of microorganisms for farm milk, while postmilking teat dipping has been used mainly in highly infected herds (Vacheyrou et al. 2011, Verdier-Metz et al. 2012). Microbial load was lower on the skin of teats disinfected before milking compared with teats that were only cleaned (Böhm et al. 2017). Most pre-milking teat cleaning treatments reduce the teat total bacterial count, but cleaning effectiveness is influenced by the type of disinfectant and the application methods (Gibson et al. 2008, Yun, Alam 2016). Our research confirmed it because most bacteria on teat skin were effectively reduced after pre-milking teats dipping in almost all groups (from 1.1 to 4.5 times) except F_5 farm, where pre-milking teat treatment increased bacterial counts in all cow groups (varied from 1.4 to 2.49 times). Teat treatment with pre- and post-milking dips had no positive effect on cow teat skin bacterial count, but it decreased the SCC in this farm. Teat bacteria colonization in two other farms increased and in two farms decreased when only basic teat hygiene was applied (teats wiped with paper disposable wipes). The variation at individual farm may be due to factors that influence microbial load such as the herd size, milk yield, field conditions, general management techniques and housing (Koster et al. 2006). Dairy cow characteristics could interact with farming practices to affect the counts of microbial flora on teat skin (Monsallier et al. 2012). It has also been reported that even after thorough cleaning and wiping significant reduction of contamination of an udder may not occur (Lam et al. 1995).

Our results do not support the general statement that pre- or post-milking teat dipping has a beneficial effect on reducing SCC as the somatic cell count varied in all three groups at each farm. Gleeson et al. (2018) concluded that SCCs were similar in non-disinfected teats and disinfected (pre-milking) teats, and our results confirm it.

The effect of certain disinfection agents on hygienic milk quality and udder health has been evaluated in many studies (Kumar et al. 2012, Böhm et al. 2017). The use of an effective disinfectant is the most important part of effective pre-milking teat-cleaning regimes in addition to washing and drying teats (Suriyasathaporn, Chupia 2011). The overall results considering all farms showed that the active ingredient influences teat skin bacterial contamination. We observed significant results when peracetic acid with hydrogen peroxide based disinfectant was used ($p \leq 0.05$). Similar observations were made by other authors (Murphy et al. 2014, Nasr, Arafa 2015). The efficiency of chemical biocides (peracetic acid) varies and depends on phage- or formulation (Guglielmotti et al. 2011, Mercanti et al. 2012).

Values concerning the iodine content in milk did not differ significantly from the ones presented by other authors (Jahreis et al. 2001, Schöne et al. 2003), recommendations of the EU commission (EU 2003, EFSA 2013), nor did they cause an excessive level of iodine in consumable milk (DACH 2008), which should be no more 0.50 mg L^{-1}.

Brzóska et al. (2015) state that currently the iodine content in cows' milk reaches an optimal level from 0.15 to 0.20 mg L^{-1}. The authors claim that it ranged from 0.103 to 0.236 mg L^{-1}among cows examined in Poland in 2007-2008. They state that using iodine preparations for keeping teat (udder) and milking devices hygienic may be a significant source of iodine in milk. Much attention has been given in the United States to research on the impact of dipping on milk's iodine level (Conrad, Hemken 1978, Iwarsson, Ekman 1978, Berg, Padgitt 1985). The cited authors state that approximately 80% of iodine in milk is inorganic, dissolved in the liquid fraction of milk. The remaining is organic iodine, mainly consisting of hormones (thyroxin, triiodothyronine or thyroglobulin) which are released from the thyroid in the form of hormone-protein complexes.

Brzóska et al. (2015) state that the iodine content in milk from the farms they examined may have increased due to the careless rinsing of milking devices. This can happen especially on milk farms which do not have sufficient water supplies.

Summarizing the results obtained from all groups at all the farms we analyzed, we can conclude that the SCCs decreased by almost 2-fold when pre- and post-milking teat disinfection was used. This agrees with results of several other authors (Bilal et al. 2008, Pavićić et al. 2008, Kućević et al. 2013). Gleeson et al. (2016) stated that individual quarter SCCs were numerically higher for unprepared teats (159.000 cells ml^{-1}) compared with those for prepared teats (133.000 cells ml^{-1}, $p \leq 0.09$). The total bacterial contamination was not significantly higher for unprepared teats (3152 cfu ml^{-1}) compared with milk from prepared teats (1678 cfu ml^{-1}, $p \leq 0.10$). Based on our research results and data from other studies (Baumberger et al. 2016), it can be claimed that farm conditions and additional management practices have a significant effect on the effectiveness of teat disinfection and SCC.

CONCLUSION

We have demonstrated that a pre-milking teat disinfectant with peracetic acid and hydrogen peroxide was effective in reducing total bacterial contamination on cow teat skin. The results also indicate that application with pre- and post-milking teat disinfectants provided an impact on the reduction of SCC in milk. Before choosing disinfectants to be used in teat preparation, it is necessary to identify the microorganisms which prevail on a farm. Application of iodine preparations for pre- and after dipping only slightly influenced the iodine content in milk.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Baumberger C., Guarín J.F., Ruegg P.L. 2016. *Effect of 2 different pre-milking teat sanitation routines on reduction of bacterial counts on teat skin of cows on commercial dairy farms.* J. Dairy Sci., 99: 1-15. DOI: https://doi.org/10.3168/jds.2015-10003

Berg j.n., Padgitt d. 1985. *Iodine concentrations in milk from iodophor teat dips*. J.Dairy Sci., 68: 457-461. DOI: https://doi.org/10.3168/jds.S0022-0302(85)80845-6

Bilal M.A., Muhammad A.A., Younas M., Muhammad G. 2008. *Impact of post milking teat dipping and Staphylococcus aureus vaccination on somatic cell count and serum antibody titre in sahiwal cows.* Pak. J. Agri. Sci., 45(2): 223-226.

Böhm F., Wente N., Krömker V. 2017. *The efficacy of a foaming iodine-based pre-milking teat disinfectant*. Milk Sci Inter., 70: 6-9. https://pdfs.semanticscholar.org/9f68/70ca531795c009ed737d2189c4f3cc9ef83b.pdf

Brzóska f.s., Szybiński z., Śliwiński b. 2015. *Iodine in milk in Poland, and its role in human health prophylaxis.* Wiad. Zoot., 53(4): 41-49. (in Polish)

Chotigarpa R., Lampang K.N., Pikulkaew S., Okonogi S., Silman P., Mektrirat R. 2019. *Antiseptic effect of natural teat dip containing lactic acid against mastitis-causing Escherichia coli.* Vet. World., 12(3): 397-401. DOI: 10.14202/vetworld.2019.397-401

Conrad L.M., Hemken R. 1978. *Milk iodine as influenced by an iodophor teat dip*. J. Dairy Sci., 61: 776-780.

DACH. 2008. *Referenzverte für die Nährstoffzufuhr*. 1. Aufl., 3. Korr. Nachdruck. Umschau Brans GmbH verlagsgesellschaft. Frankfurt/M., p. 179-184.

EFSA. 2013. *Opinion of the safety and efficacy of iodine compounds (E2) as feed additives: clacium iodate anhydrous, based on a dossier submitted by Calibre Europe SPRL/BVBA.* EFSA J., 11: 3100-3134.

El Behiry A.I., Schlenker G., Szabo I., Roesler U. 2012. *In vitro susceptibility of Staphylococcus aureus strains isolated from cows with subclinical mastitis to different antimicrobial agents.* J. Vet. Sci., 13(2): 153-161. DOI: 10.4142/jvs.2012.13.2.153

Enger B.D., Fox L.K., Gay J.M., Johnson K.A. 2015. *Reduction of teat skin mastitis pathogen loads: differences between strains, dips, and contact times.* J. Dairy Sci., 98(2): 1354-1361. DOI: 10.3168/jds.2014-8622

EU. 2003. *Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition.* OJEU, 46, L268/29-L268/43.

FLACHOVSKY G., FRANKE K., MEYER U., LEITERER M. 2014. *Influencing factors on iodine content of cow milk*. Eur J Nutr, 53: 351-365. DOI: 10.1007/s00394-013-0597-4

GIBSON H., SINCLAIR L.A., BRIZUELA C.M., WORTONM H.L., PROTHEROE R.G. 2008. *Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms*. Lett. Appl. Microbiol., 46(3): 295-300. DOI: 10.1111/j.1472-765X.2007.02308.

GLEESON D., EDWARDS P., O'BRIEN B. 2016. *Effect of omitting teat preparation on bacterial levels in bulk tank milk*. Ir. J. Agr. Food Res., 55(2): 169-175. DOI: https://doi.org/10.1515/ /ijafr-2016-0017

GLEESON D., FLYNN J., O'BRIEN B. 2018. *Effect of pre-milking teat disinfection on new mastitis infection rates of dairy cows*. Ir.Vet. J.,71:1-11.DOI: 10.1186/s13620-018-0122-4

GLEESON D., O'BRIEN B., FLYNN J., O'CALLAGHAN E., GALLI F. 2009. *Effect of pre-milking teat preparation procedures on the microbial count on teats prior to cluster application*. Ir. Vet. J., 62(7): 461-467. DOI.org/10.1186/2046-0481-62-7-461

GUGLIELMOTTI D.M., MERCANTI D.J., REINHEIMER J.A., QUIBERONI A.L. 2011. *Review: efficiency of physical and chemical treatments on the inactivation of dairy bacteriophages*. Front Microbiol., 2: 282. DOI: 10.3389/fmicb.2011.00282

HYSEN B., ZAUGG U., SHERIFI K., HAMDI A., GJONBALAJ M., MUJI S., MEHMETI H. 2010. *Influence of management and physiological factors on somatic cell count in raw cow milk in Kosova*. Vet. Archiv., 80(2): 173-183.

IWARSSON K. EKMAN L. 1978. *The effect of a post-milking teat dip on the iodine concentration of bulk herd milk*. Acta Vet. Scand., 14: 338-340.

JAHREIS G., HAUSMANN W., KISSLING G., FRANKE K., LEITERER M. 2001. *Bioavailability of iodine from normal diets rich in dairy products – results of balance studies in women*. Exp. Clin. Endocrinol, Diab., 109(3): 163-167. DOI: 10.1055/s-2001-14840

KAMAL R.M., BAYOUMI M.A. 2015. *Efficacy of premilking and postmilking teat dipping as a control of subclinical mastitis in Egyptian dairy cattle*. Int. Food Res. J., 22(3): 1037-1042.

KOSTER G., TENHAGEN B.A., HEUWEISER W. 2006. *Factors associated with high milk test somatic cell counts in large dairy herds in Brandenburg*. J. Vet. Med., 53(4): 209-214. DOI: 10.1111/ /j.1439-0442.2006.00814

KUČEVIĆ D., PLAVŠIĆ M., TRIVUNOVIĆ S., RADINOVIĆ M., KUČEVIĆ D.S. 2013. *Effect of post-milking teat dipping on hygienic quality of cow's milk*. J. Anim. Sci. Biotechnol., 29(4): 665-673. DOI: 10.2298/ BAH1304665K

KUMAR V.A., VENKATESWARA R.L., KISHAN K.M., SRINU B., MADHAVA R.T. 2012. *Efficacy of udder disinfectants on reduction of bacterial load and certain pathogens of public health significance*. J. Microb. Biotech. Res., 2(1): 147-151.

LAM T.J., VAN VLIET J.H., SCHUKKEN H. 1995. *Udder disinfection and mastitis in cattle: A literature review*. J. Dairy Sci., 120(13): 392-399. PMID: 7610449

MALINOWSKI E. 2000. *The role of udder disinfection and sanitizer types*. Med. Wet., 56(11): 709-714.

MERCANTI D.J., GUGLIELMOTTI D.M., PATRIGNANI F., REINHEIMER J.A., QUIBERONI A. 2012. *Resistance of two temperate Lactobacillus paracasei bacteriophages to high pressure homogenization, thermal treatments and chemical biocides of industrial application*. Food Microbiol., 29(1): 99-104. DOI: 10.1016/j.fm.2011.09.003

MONSALLIER F., VERDIE-METZ I., AGABRIEL C., MARTIN B., MONTEL M.C. 2012. *Variability of microbial teat skin flora in relation to farming practices and individual dairy cow characteristics*. Dairy Sci. Technol., 92(3): DOI 10.1007/s13594-012-0064-7

MURPHY J., MAHONY J., BONESTROO M., NAUTA A., VAN SINDEREN D. 2014. *Impact of thermal and biocidal treatments on lactococcal 936-type phages*. Int. Dairy J., 34(1): 56-61.

NASR S.A.E., ARAFA A. 2015. *Effect of some disinfectants on antibiotic resistance staphylococcus isolated from dairy farms in Egypt*. J.Agric.Vet. Sci., 8(12): 1-7.

PAVIČIĆ Ž., CERGOLJ M., BALENOVIĆ T., EKERT-KABALIN A., VALPOTIĆ H. 2008. *Influence of udder sanitation on hygienic quality of cow milk*. Vet.Archiv., 78(2): 105-112.

RIEKERINK R.H., OHNSTAD I., VAN SANTEN B., BARKEMA H.W. 2012. *Effect of an automated dipping and backflushing system on somatic cell counts*. J. Dairy Sci., 95(9): 4931-4938. DOI: 10.3168//jds.2011-4939

RUEGG P.L. 2003. *Practical food safety interventions for dairy production*. J. Dairy Sci., 86(Suppl.): 1-9. https://pdfs.semanticscholar.org/bd6e/ce0e6f277ae496044643eb02 de1eff525aa3.pdf

SCHÖNE F., LEITERER M., HARTUNG H., KINAST C., GREILING A., BÖHM U., JAHREIS G. 2003. *Trace elements and further nutrition-related constituens of milk and cheese*. Milchwissenschaft, 58: 486-490.

SURIYASATHAPORN W., CHUPIA V. 2011. *Reduction in numbers of bacteria after pre-milking teat dipping in milking dairy cows*. J. Nat. Sci., 10(2): 301-306.

TIWARI J.G., WARYAGH C.H., TIWARI H., WILLIAMS V., DE WET S., GIBSON J., PAXMAN A., MORGAN E., COSTANTINO P., RAJU S., ISLOOR S., MUKKUR T. 2013. *Trends in therapeutic and prevention strategies for management of bovine mastitis: An overview*. J. Vacines Vaccin., 4(2): 176. DOI: 10.4172/2157-7560.1000176

TORTORA G.J., FUNKE B.R.,CASE C.L. 2010. *Microbiology: An Introduction*. 10th ed San Francisco CA, Pearson Benjamin Cummings. https://trove.nla.gov.au/work/6856000

TRAJKOVSKA B., KOCHOSKI L., HRISTOVA V.K., MAKARIJOSKI B., TOMOVSKA J. 2015. *Influence of management practices on somatic cell count and total bacteria count in cow's bulk tank milk*. J. Hyg. Engin. Design., 11: 3-8. https://pdfs.semanticscholar.org/7fbc/96f73909ea88465b86b6561fa1303ae2f253.pdf

VACHEYROU M., NORMAND A.C., GUYOT P., CASSAGNE C., PIARROUX R., BOUTON Y. 2011. *Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms*. Int. J. Food Microbiol., 146(3): 253-262. DOI: 10.1016/j.ijfoodmicro.2011.02.033

VERDIER-METZ I., GAGNE G., BORNES S., MONSALLIER F., VEISSEIRE P., DELBÈS-PAUS C., MONTEL M.C. 2012. *Cow teat skin, a potential source of diverse microbial populations for cheese production*. J. Appl. Environ. Microbiol., 78(2): 326-333. DOI: 10.1128/AEM.06229-11

WÓJCIK R., MAŁACZEWSKA J., SIWICKI A.K., MICIŃSKI J., ZWIERZCHOWSKI G. 2013. *The effect of β-hydroxy-β-methylbutyrate (HMB) on the proliferative response of blood lymphocytes and the phagocytic activity of blood monocytes and granulocytes in calves*. Pol. J.Vet. Sci., 16(3): 567-569. DOI: 10.2478/pjvs-2013-0078

YUEN S.K., ALAM M.R. 2016. *Effect of modified pre-milking sanitizing approaches on raw milk quality obtained from the dairy farmers of tawau area, sabah*. Turk. J. Agri. – Food Sci Techn., 4(1): 5-8. DOI: https://doi.org/10.24925/turjaf.v4i1.5-8.403