

Pilot study on the influence of premilking iodine-based teat disinfection on milk iodine content

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Abstract

Premilking cleaning and disinfection of teats were shown to be effective to prevent environmental mastitis. However, premilking teat disinfection is not a commonly used practice in Germany because of the risk of disinfection product residues in the milk.

The objective of this study was to evaluate the effects of five differently concentrated iodine-based, foaming, premilking teat disinfectants on the iodine content of raw milk. The concentrations of the teat disinfectants were 250, 500, 1000, 2000 and 3000 ppm iodine. For each concentration five cows were treated and used for sample collection. Per udder two teats were dipped in the iodine disinfectant before milking; the other two teats were left untreated as a negative control (split-udder design). The contact time amounted to thirty seconds. Afterwards, all teats were cleaned with a dry paper towel. 15 mL milk from one treated and one untreated teat were manually milked into the test tubes before the milking cluster was attached. No significant differences in iodine concentration of the milk samples from the treated and the untreated teats were detected for all five disinfectant concentrations.

Keywords: premilking, disinfection, predipping, iodine, residues

Introduction

Premilking teat disinfection is practised in several countries to reduce the microbial load of the teats prior to milking as well as to reduce intramammary infections (IMI) caused by environmental pathogens. Several disinfectants like hypochlorite, chlorine dioxide, chlorhexidine, alcohol, dodecyl-benzol-sulfonic acid and iodine reduce the microbial load of the teats significantly [1, 2, 3, 4, 5]. The rate of new intramammary infections and the incidence of clinical mastitis caused by environmental pathogens such as *Escherichia coli* and *Streptococcus uberis* are significantly lower when premilking teat disinfection is performed [6, 7, 8, 9, 10]. Nevertheless, the rate of new intramammary infections caused by cow-associated pathogens, like *Staphylococcus aureus*, and coagulase negative staphylococci or *Corynebacterium bovis* is not affected by premilking teat disinfection [6, 7, 9, 11]. Nonetheless, this is not a commonly used practice in Germany because it is hypothesised that premilking teat disinfection may cause residues of the disinfectant

in raw milk. Furthermore, no product on the German market is currently licensed to be used for premilking teat disinfection. This present study was conducted to determine the effect of iodine-based teat disinfectants on the milk iodine content.

Material and Methods

Herd and animals:

The study was conducted on one commercial dairy farm with 100 cows in the German federal state North Rhine-Westphalia. The cows were housed in a free-stall barn with partly sawdust bedded cubicles and partly deep bed stalls. The cows were milked twice a day in a herringbone milking parlour with six milking places on each side. Normally no premilking teat disinfection was practised. After milking, all teats were dipped in a product containing lactic acid and chlorine dioxide. The 25 cows included in the study were chosen randomly as samples were always taken from the same milking place and cows entered the milking parlour in a random order. As this study is considered to be a pilot study, 25 cows were chosen for sample collection. All of the cows were free of clinical mastitis and teat skin lesions.

Premilking teat disinfectant:

For this study, a foaming teat disinfectant based on iodine at concentrations of 250, 500, 1000, 2000 and 3000 ppm iodine was tested. The used iodine was a fatty acid ethoxylate iodine complex. These disinfectants were produced by the Ferdinand Eimermacher GmbH & Co KG in Nordwalde, Germany.

Udder preparation:

To evaluate the effect of the iodine concentration in the teat disinfectant on the iodine residues in raw milk, a split-udder design was used. Two of the teats of an animal (either front left and hind right or front right and hind left) were dipped once in the disinfectant before milking using a conventional foam dip cup. With this foam dip cup, 0.4 mL of the disinfectant was applied to each teat. For each concentration group the same disinfectant solution was used. The other two teats were used as a negative control. For every concentration of the teat disinfection product five cows were treated and used for the sample collection. After an exposure time of 30 seconds, the normal udder preparation was practised starting with the foremilk. Therefore, the first three

streams of milk were manually milked and rejected, beginning with the untreated teats to minimise the transfer of the disinfectant. This resulted in a total exposure time of 35 seconds. After that, all teats were cleaned with a dry paper towel.

Sample collection:

After cleaning the teats, 15 mL of milk per quarter of one treated and one untreated teat (either both front or both hind teats) were manually milked into a plastic test-tube (VWR International GmbH, Germany). The milk samples were sent to the laboratory without cooling as cooling was unnecessary for the analysis.

Analysis of the iodine content:

The milk samples were analysed in accordance with the Official Collection of methods § 64 LFGB, method: L 00.00-93 2008-12. For iodine extraction 1 mL of a sample was mixed with 5 mL ultrapure water and 1 mL tetra-methyl-ammonium-hydroxide prior to incubation at 90°C for three hours. After cooling down, ultrapure water was added up to a volume of 10 mL and the solution was homogenised by shaking. Then the samples were centrifuged in two steps: First, at 6,000 rpm and afterwards 1.5 mL of the supernatant were centrifuged at 14,000 rpm. 0.5 mL of this clear supernatant was mixed with 0.05 mL tellurium-standard-solution and 4.45 mL water. The tellurium-standard-solution contains 1.2508 g telluriumdioxide (TeO₂) which is suspended in hydrochloric acid and then diluted with water up to a volume of one litre. The calibration solutions to establish the calibration curve contained 5, 20 and 50 µg iodine/l, respectively and were prepared immediately before measuring. The iodine contents of the milk samples were determined by the inductively coupled plasma-mass spectrometry (ICP-MS ICAP Q, Thermo Fisher Scientific GmbH).

Statistics:

This study used a split-udder design. A teat was matched with its contralateral teat to eliminate individual animal effects [12]. SPSS 23.0 software (IBM, USA) was used for data analysis. Descriptive statistics (mean, standard deviation, minimum and maximum) were calculated. Prior to statistical analyses the plausibility and completeness of data were verified. The associations between the dependent variable iodine concentration in milk and the variables dipp/no dipp and iodine concentration in the dipp solution were analysed with a linear mixed regression model for repeated measurements. The subject was the cow, the examined unit was the teat pair and the fixed effects were dipping/no dipping and the iodine concentration of the disinfectant. Statistical significance was accepted at $p < 0.05$. To visualise the results, box plots were used.

Results

In total, the iodine content of 50 milk samples was analysed. The iodine content in milk of the untreated teats ranged from 42 µg/l (= minimum) to 284 µg/l (= maximum) (Table 1). Differences

Table 1: Descriptive statistics

	Minimum in µg/l	Maximum in µg/l	Mean in µg/l	Standard deviation
Untreated	42.0	284.0	119.6	55.2
250 ppm	75.0	153.0	119.6	26.7
500 ppm	37.0	124.0	91.0	28.8
1000 ppm	44.0	160.0	96.6	42.4
2000 ppm	93.0	212.0	156.2	40.3
3000 ppm	115.0	251.0	160.4	48.7

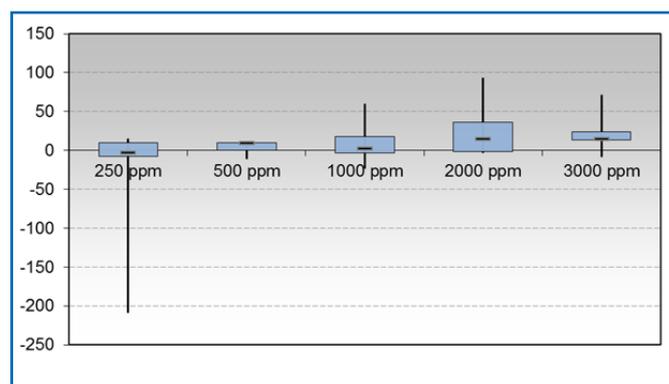


Figure 1: Boxplots of the differences between the iodine concentrations in milk from treated and untreated quarters

between the treated and the untreated teat of a teat pair were calculated (Table 2). To visualize the results, box plots were used (Figure 1). No statistically significant differences of the milk iodine concentrations were found between treated and untreated teats ($p = 0.626$). Furthermore the iodine concentration of the teat disinfectant, calculated for all five concentrations, was not associated with the milk iodine contents ($p = 0.052$).

Discussion

The aim of this study was to determine the effect of iodine-based teat disinfectants on the milk iodine content. The premilking disinfection of the teats with iodine-based disinfectants had no significant effect on the iodine content of the milk. Furthermore, the iodine concentration of the disinfectant did not significantly affect the differences between the iodine concentration of the milk from treated and untreated quarters.

When udder preparation was finished, the first 15 mL milk of one treated and one untreated teat were separately milked into plastic test tubes. These first streams of milk from the treated teats were considered to contain the highest amounts of iodine because they had been taken immediately after disinfection and udder preparation and therefore had the highest probability of disinfectant contact. Furthermore, several studies observing the iodine content of bulk tank milk have already been conducted [8, 13, 14]. Analysing the iodine content of the bulk tank milk may be an acknowledged method to investigate the influence of premilking teat disinfection on raw milk. However, focussing only on the iodine content of the bulk tank milk, the high dispersion rate - caused by the high milk yield - of possible iodine carryovers is disregarded. Falkenberg et al. [15] showed that the iodine content of milk is negatively correlated to milk yield. Therefore, the present study was designed to evaluate possible iodine residues in the first milk streams as they have the highest risk of contamination and iodine contents are not diluted by a high milk yield.

To prevent cross-contamination of the raw milk with other iodine sources, a product based on lactic acid and chlorine dioxide was used for the postmilking teat disinfection. The iodine content of the milk samples was analysed in agreement with the Official Collection of Methods in accordance with § 64 LFGB, method: L 00.00 93. The minimum quantification limit is 0.5 µg/l. Therefore, the used method is quite sensitive. In previous studies similar milk iodine contents of 110 to 122 µg/l were detected in German bovine milk samples from different supermarkets [16, 17]. Taking into account that the disinfectant contains other substances than iodine (for example tensides), the analysis of possible iodine residues in the milk functioned as a marker

Table 2: Means and standard deviation of the differences between the treated and the untreated teat of a teat pair

	Minimum of the differences between the treated and the untreated teat of a teat pair in µg/l	Maximum of the differences between the treated and the untreated teat of a teat pair in µg/l	Mean of the differences between the treated and the untreated teat of a teat pair in µg/l	Standard deviation of the mean
250 ppm	-209	15	-39.0	85.4
500 ppm	-11	11	3.8	8.4
1000 ppm	-24	60	10.6	28.1
2000 ppm	-3	93	27.6	35.6
3000 ppm	-9	71	22.6	26.5

for the general carryover of disinfectant from the teat skin into the milk. Iodine was used as the marker because it is the active ingredient in the disinfectant and can be analysed quite sensitively.

The results of the present study show that there are no significant differences between the iodine contents of milk from treated and from untreated teats for the used disinfectant. Nonetheless, as this study was conducted as a pilot study, the results can not be generalised. Several other studies published comparable results [8, 13, 14]. Blowey and Collis [8] used a 1500 ppm iodine-based teat disinfectant for twelve weeks and found that there were no significant differences in the iodine content of bulk milk in all three herds, but the absolute iodine contents slightly increased in two herds (herd 1: From 633 ng/mL to 742 ng/mL; herd 2: From 1480 ng/mL to 1507 ng/mL, respectively). In the third herd the iodine content of bulk milk for the treated and the untreated group was nearly the same with 272 ng/mL and 273 ng/mL, respectively.

Hillerton et al. [13] investigated in a first trial over 20 weeks the iodine contents of bulk tank milk in three herds using a 2500 ppm teat dip. In the treatment groups in all three herds higher iodine contents in milk were obtained in comparison to the negative control groups, but these differences were not significant. In a second trial the authors tested a 2500 ppm teat disinfectant in nine matched pairs of dairy herds (one herd of the pair using the premilking teat disinfectant, while the other herd of the pair was the negative control) including over 2400 cows in milk over 24 weeks. The results of this trial were comparable to the first trial: The premilking teat disinfection had no significant effect on the iodine content of the bulk tank milk. The mean iodine content in milk of the untreated teats in the 250 ppm group of this present study was even numerically higher than the mean iodine content in the milk of the treated teats. Hillerton et al. [13] also found differences in the iodine content between their three study herds of the first trial: On day 7 the control group of herd A reached 70 µg/l while both groups (treated and control) of herd B reached 820 µg/l. These results underline the importance of other factors influencing the iodine excretion into the milk.

One of the most important factors influencing milk iodine content is feeding. There is a linear correlation between the oral intake of iodine and the excretion into the milk [14, 18]. Schöne et al. [18] detected that 30 to 40 % of the oral intake of iodine was excreted into the milk. Skin lesions on the teat end also resulted in higher milk iodine contents when predipping was applied [19]. It is assumed that a disinfection product, which is applied to lesions or injuries, cannot be removed completely by the normal udder preparation procedure [19]. To rule out this biasing factor, all included cows were examined for teat lesions. Only cows having no teat lesions were used for this study. A hyperkeratosis score was not performed because there was no evidence in literature that hyperkeratosis influences the iodine residues

in milk. Furthermore, the present study did not detect any significant influences of the five iodine concentrations on the milk iodine content ($p = 0.052$). Nonetheless, as $p = 0.052$ is borderline, a further study would be needed to prove this. The possible influence of higher iodine concentrations of the disinfectant on the milk iodine content is also mentioned by Falkenberg et al. [15]. They evaluated the effect of a foaming polyvidone iodine dip containing 2700 ppm iodine and found significantly higher iodine concentrations in milk.

In this present study, disinfectants containing between 250 and 3000 ppm were used. 3000 ppm is considered to be the generally recommended iodine concentration in postmilking teat disinfectants [20]. However, in order to decrease the risk of iodine residues in the milk, the use of lower concentrations was considered. In contrast to increasing resistance problems caused by the underdosed use of antibiotics, the continuous use of iodine, even in low concentrations, does not cause any resistance [21, 22, 23]. Studies conducted by McLure and Gordon [22] and Lacey and Catto [23] showed that there is no evidence of resistance of MRSA clinical isolates against iodine. Houang et al. [21] challenged strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella aerogenes* and *Serratia marcescens* over 20 passages with subinhibitory concentrations of povidone iodine. They did not find significant differences either in the minimal inhibitory concentration, the minimal bactericidal concentration or in the killing times between the parent strains and the 20th subcultures.

Moreover, the antimicrobial efficacy of iodine-based disinfectants is caused by the free iodine in an iodine solution. As iodine has to be bound in a complex because it is insoluble in water; there is always complexed iodine and a small part of free iodine - which has been released from the complex - in a solution [24]. The content of free iodine in a solution depends on the total available iodine content [25, 26, 27]. For the range of 250 to 3000 ppm available iodine, the amount of free iodine is nearly the same. Thereby, it can be supposed that the efficacy of the five tested concentrations might be nearly the same. To prove this hypothesis, further studies evaluating the antimicrobial efficacy of these five tested teat disinfectants need to be carried out.

Thus, the results of this study indicate that application of iodine-based premilking teat disinfection represents a safe method with regard to milk residues. Nevertheless, the conducted study has to be considered as a pilot-study since the sample size of five cows per iodine concentration is too small to be able to make any generalisation concerning the results. Moreover, the chronic effects of premilking iodine-based teat disinfection were not investigated.

Conclusion

The present study indicates that unique iodine-based premilking teat disinfection does not significantly increase the iodine content in raw milk. Furthermore, no significant differences between the five tested

iodine concentrations in affecting the iodine content in the milk were detected. Nevertheless, regarding the small sample size a further study would be needed for any generalisation of these results.

Literature

- Galton DM, Petersson LG, Merrill WG, Bandler DK, Shuster DE. Effects of premilking udder preparation on bacterial population, sediment, and iodine residue in milk. *J Dairy Sci.* 1984 Nov; 67(11):2580-2589
- Gibson H, Sinclair LA, Brizuela CM, Worton HL, Protheroe RG. Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. *Lett Appl Microbiol.* 2008 Mar; 46(3):295-300
- Gleeson D, O'Brien B, Flynn J, O'Callaghan E and Galli F. Effect of pre-milking teat preparation procedures on the microbial count on teats prior to cluster application. *Irish Veterinary Journal* 2009, 62(7):461-467
- Ingawa KH, Adkinson RW, Gough RH. Evaluation of a gel teat cleaning and sanitizing compound for premilking hygiene. *J Dairy Sci.* 1992 May; 75(5):1224-1232
- Enger BD, Fox LK, Gay JM, Johnson KA. Reduction of teat skin mastitis pathogen loads: differences between strains, dips, and contact times. *J Dairy Sci.* 2015 Feb; 98(2):1354-61
- Pankey JW, Wildman EE, Drechsler PA, Hogan JS. Field trial evaluation of premilking teat disinfection. *J Dairy Sci.* 1987 Apr; 70(4):867-872
- Pankey JW. Premilking udder hygiene. *J Dairy Sci.* 1989 May; 72(5):1308-1312
- Blowey RW, Collis K. Effect of premilking teat disinfection on mastitis incidence, total bacterial count, cell count and milk yield in three dairy herds. *Vet Rec.* 1992 Feb; 130(9):175-178
- Oliver SP, Lewis MJ, Ingle TL, Gillespie BE, Matthews KR, Dowlen HH. Premilking teat disinfection for the prevention of environmental pathogen intramammary infections. *Journal of Food Protection*, Volume 56, Number 10, October 1993, pp. 852-855(4)
- Oliver SP, Gillespie BE, Lewis MJ, Ivey SJ, Almeida RA, Luther DA, Johnson DL, Lamar KC, Moorehead HD, Dowlen HH. Efficacy of a new premilking teat disinfectant containing a phenolic combination for the prevention of mastitis. *J Dairy Sci.* 2001 Jun; 84(6):1545-1549
- Falkenberg U, Tenhagen BA, Baumgärtner B, Heuwieser W. Prevention of intramammary infections in dairy cows by the use of a premilking teat dip method with a foaming iodophor dip agent. *Dtsch Tierarztl Wochenschr.* 2002 Oct; 109(10):421-460
- Barkema HW, Schukken YH, Lam TJGM, Galligan DT, Beiboer ML, Brand A. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. *J Dairy Sci.* 1997 Aug; 80(8): 1592-1599
- Hillerton JE, Shearn MF, Teverson RM, Langridge S, Booth JM. Effect of pre-milking teat dipping on clinical mastitis on dairy farms in England. *J Dairy Res.* 1993 Feb; 60(1):31-41
- Borucki Castro SI, Berthiaume R, Robichaud A, Lacasse P. Effects of iodine intake and teat-dipping practices on milk iodine concentrations in dairy cows. *J Dairy Sci.* 2012 Jan; 95(1):213-20
- Falkenberg U, Tenhagen BA, Forderung D, Heuwieser W. Effect of predipping with a iodophor teat disinfectant on iodine content of milk. *Milk Science International*, 2002; 57(11/12): 599-601
- Johner SA, von Nida K, Jahreis G, Remer T. Time trends and seasonal variation of iodine content in German cow's milk-investigations from Northrhine-Westfalia. *Berl Munch Tierarztl Wochenschr.* 2012 Jan-Feb; 125(1-2):76-82.
- Köhler M, Fechner A, Leiterer M, Spörl K, Remer T, Schäfer U, Jahreis G. Iodine content in milk from German cows and in human milk: new monitoring study. *Trace Elements and Electrolytes*, Vol. 29 - No. 2/2012 (119-126)
- Schöne F, Leiterer M, Lebzien P, Bemmann D, Spolders M, Flachowsky G. Iodine concentration of milk in a dose-response study with dairy cows and implications for consumer iodine intake. *J Trace Elem Med Biol.* 2009; 23(2):84-92
- Rasmussen MD, Galton DM, Petersson LG. Effects of premilking teat preparation on spores of anaerobes, bacteria, and iodine residues in milk. *J Dairy Sci.* 1991 Aug; 74(8):2472-2478
- Deutsche Veterinärmedizinische Gesellschaft. Leitlinien zur Bekämpfung der Mastitis des Rindes als Bestandsproblem. Verlag der Deutschen Veterinärmedizinischen Gesellschaft, Gießen 2012
- Houang ET, Gilmore OJA, Reid C, Shaw EJ. Absence of bacterial resistance to povidone iodine. *J clin Path.* 1976 Jan; 29: 752-755
- McLure AR, Gordon J. In-vitro evaluation of povidone-iodine and chlorhexidine against methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 1992; 21(4): 291-299
- Lacey RW, Catto A. Action of povidone-iodine against methicillin-sensitive and resistant cultures of *Staphylococcus aureus*. *Postgrad Med J* 1993; 69(3): 78-83
- Wallhäuser KH. Praxis der Sterilisation, Desinfektion – Konservierung, Keimidentifizierung – Betriebshygiene. Georg Thieme Verlag Stuttgart 1995, 5. überarbeitete Auflage
- Gottardi, W. Potentiometrische Bestimmung der Gleichgewichtskonzentrationen an freiem und komplex gebundenem Jod in wässrigen Lösungen von Polyvinylpyrrolidon-Jod (PVP-Jod). *Zeitschrift für analytische Chemie.* 1983; 314:582-585
- Gottardi W. The influence of the chemical behaviour of iodine on the germicidal action of disinfectant solutions containing iodine. *Journal of Hospital Infection* 1985 Mar; 6(1):1-11
- BASF. PVP-Iodine grades, Technical information. http://www.pharma-ingredients.basf.com/Statements/Technical%20Informations/EN/Pharma%20Solutions/03_030744e_PVP-Iodine-grades.pdf