



Check for
updates



Research Article

Comparative Study of Natural (Lactic Acid) and Synthetic (Pyodine) Antiseptic Teat Dips for Mastitis Control in Dairy Cattle

Waqas Ahmad¹, Iftikhar Ahmad¹, Muhammad Shuaib², Obaid Ullah³, Sohaib UI Hassan⁴, Danyal Khan¹, Muhammad Shahkar Uzair⁵, Abdul Jabbar⁶, Muhammad Idrees²

¹ Department of Livestock Management, Breeding & Genetics, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

² Arid Zone Small Ruminants Research Institute, Ghulam Banda, Kohat, Pakistan.

³ Faculty of Veterinary Sciences, The University of Veterinary and Animal Sciences Swat, Pakistan.

⁴ College of Veterinary Sciences, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar, Pakistan.

⁵ Department of Livestock Production and Management, Faculty of Veterinary Sciences, The University of Veterinary and Animal Sciences Swat, Pakistan.

⁶ Faculty of Veterinary and Animal Sciences, University of Poonch Rawalakot, Pakistan.

ABSTRACT

Bovine mastitis is one of the most economically important diseases affecting dairy cattle worldwide, leading to reduced milk yield, altered milk composition, and increased treatment costs. The present study aimed to evaluate the effectiveness of natural and synthetic antiseptic solutions used for post-milking teat dipping to control subclinical mastitis in dairy cows and to assess their economic feasibility. A total of 20 lactating dairy cows with healthy udders were selected and randomly divided into five groups (n = 4 cows per group). Group G-1 served as the control, while G-2 and G-3 received lactic acid teat dip solutions at concentrations of 2% and 5%, respectively. Groups G-4 and G-5 received synthetic antiseptic pyodine solutions at concentrations of 2% and 5%, respectively. The experiment lasted 28 days. Milk samples were collected weekly and screened for subclinical mastitis using the California Mastitis Test (CMT) and Surf Field Mastitis Test (SFMT). The results indicated that the highest incidence of mastitis occurred in the control group (G-1), followed by G-4, G-2, and G-5, whereas no cases of subclinical mastitis were recorded in the G-3 group during the entire experimental period. The economic evaluation showed that pyodine-based solutions were less expensive than lactic acid solutions. However, the 5% lactic acid treatment demonstrated superior efficacy in reducing subclinical mastitis. The findings suggest that lactic acid teat dipping can serve as an effective natural alternative for mastitis control in dairy cattle, although further studies with larger sample sizes are recommended.

Keywords: Bovine mastitis; Teat dipping; Lactic acid; Pyodine; Dairy cattle; Antiseptic.



Correspondence

Muhammad Shuaib

shoaibwzr@gmail.com

Sohaib UI Hassan

sohaibvet@aup.edu.pk

Article History

Received: February 20, 2026

Accepted: March 22, 2026

Published: March 30, 2026



Copyright: © 2024 by the authors.

Licensee: Roots Press,

Rawalpindi, Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license:

<https://creativecommons.org/licenses/by/4.0>

INTRODUCTION

Bovine mastitis is one of the most prevalent and economically significant diseases affecting dairy cattle worldwide. The disease leads to substantial economic losses due to decreased milk production, altered milk composition, increased veterinary costs, discarded milk following treatment, and premature culling of affected animals. Both clinical and subclinical mastitis contribute to these losses; however, subclinical mastitis is considered more problematic because it often remains undetected for long periods

while continuously reducing milk yield and quality (Abdalla et al., 2023; Garcia et al., 2022). In many developing countries, the economic burden of mastitis is further aggravated by limited disease monitoring systems and inadequate farm hygiene practices.

Mastitis is primarily caused by a wide range of bacterial pathogens that invade the mammary gland through the teat canal. These pathogens include contagious organisms such as *Staphylococcus aureus* and *Streptococcus agalactiae*, as well as environmental bacteria such as *Escherichia coli*. These microorganisms can enter the udder during or shortly after milking, particularly when the teat canal remains temporarily open. Studies have shown that the teat sphincter may remain open for approximately 15–30 minutes after milking, increasing the risk of bacterial entry and infection during this period (Oliver et al., 2012; Rahman et al., 2023). Therefore, proper post-milking hygiene practices are essential to reduce the risk of intramammary infections in dairy animals.

Among various preventive measures, teat dipping is widely recognized as one of the most effective and practical strategies for controlling mastitis in dairy herds. Teat dipping involves applying antiseptic solutions to the teat surface immediately after milking to eliminate pathogenic microorganisms and prevent bacterial colonization. Various disinfectant agents have been used for this purpose, including iodine-based solutions, iodophores, chlorhexidine, sodium hypochlorite, and chlorine compounds (Garcia et al., 2022). Iodine-based disinfectants are particularly popular because of their broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative mastitis-causing bacteria.

Despite their effectiveness, prolonged use of iodine-based disinfectants may lead to increased iodine residues in milk, potentially affecting milk quality and raising concerns about consumer health and environmental safety (Ovadia et al., 2018; Rezaei et al., 2021). Consequently, there has been growing interest in developing natural, environmentally friendly alternatives to conventional chemical disinfectants used in dairy farming.

Organic acids, such as lactic acid, have gained attention as potential natural antiseptic agents due to their antimicrobial properties. Lactic acid is known to inhibit bacterial growth by lowering environmental pH and disrupting bacterial cell membranes, thereby preventing the proliferation of mastitis-causing pathogens (Patel and Patel, 2022; Nguyen et al., 2024). In addition to its antimicrobial activity, lactic acid may also improve teat skin condition and reduce microbial colonization on the teat surface.

Although several studies have investigated the role of antiseptic teat dips in mastitis prevention, comparative evaluations of natural antiseptic agents such as lactic acid and commonly used synthetic disinfectants under field conditions remain limited, particularly in developing dairy production systems. Therefore, the present study was designed to evaluate the effectiveness of natural (lactic acid) and synthetic (pyodine) antiseptic solutions used for post-milking teat dipping in dairy cattle to control subclinical mastitis and to assess the economic feasibility of these treatments.

MATERIALS AND METHODS

Ethical approval of the study

This study protocol was approved by the ethical committee of the University of Agriculture Peshawar (No. 7518/LM, B & G/UOA, dated: 29/09/2022).

Study location, and animal selection

This trial was conducted at Livestock Research and Breeding Farm, The University of Agriculture Peshawar. A total of 20 cows were selected for the experimental purpose; all were screened for subclinical mastitis before the trial and had good udder health. Animals were divided into 5 groups (G-1, G-2, G-3, G-4, and G-5), with 4 animals per group. G-1 served as the control group, while G-2, G-3, G-4, and G-5 received lactic acid and pyodine solutions for teat dipping. The study lasted 28 days.

Preparation of antiseptic solution

Pyodine, a synthetic, and lactic acid, a natural antiseptic solution, were purchased from the local market in district Peshawar. Two types of antiseptic solution were prepared at two different concentrations, e.g., lactic acid at 2% and 5%, and pyodine at 2% and 5%. G-1 served as the control group, while G-2 and G-3 received 2% and 5% lactic acid solutions, respectively, and G-4 and G-5 received 2% and 5% pyodine solutions, respectively, for teat dipping.

Collection of milk samples

Milk samples were collected from all experimental animals on a weekly basis during the 28-day experimental period. Before sample collection, the teats were cleaned, and the first few milk streams were discarded to minimize contamination. Approximately 10 mL of milk was collected from each quarter into sterile containers and transported to the Laboratory of Dairy Technology at the University of Agriculture, Peshawar, for further analysis.

Screening for mastitis

Subclinical mastitis was screened using both the Surf Field Mastitis Test (SFMT) and the California Mastitis Test (CMT). For the SFMT, 1 mL of 3% surf solution was mixed with 1 mL of the milk sample in a clean test plate. The mixture was gently rotated, and the formation of gel or coagulation indicated the presence of subclinical mastitis. The intensity of gel formation was used to estimate the severity of infection. For the California Mastitis Test, milk from each teat quarter was collected into the four chambers of a CMT paddle. An equal amount of CMT reagent containing sodium hydroxide (1.5 g), Teepol detergent (0.5 mL), bromothymol blue indicator (0.01 g), and distilled water (100 mL) was added to each chamber. The paddle was gently rotated for approximately 10–15 seconds.

A positive reaction was indicated by the formation of gel or precipitate due to the interaction between the reagent and somatic cells present in mastitic milk. Samples showing visible gel formation were considered positive for subclinical mastitis. Each cow has four teat quarters; therefore, mastitis screening was performed at the quarter level. Weekly observations from all teat quarters were recorded and pooled for statistical analysis to determine the incidence of subclinical mastitis among treatment groups.

Economic evaluation of various teat dip

The economics of each antiseptic solution were calculated using market prices, per-unit use, and unit costs.

Statistical Analysis

All collected data were analyzed using SPSS 16.0. The incidence of subclinical mastitis across treatment groups was analyzed using the Chi-square (χ^2) test because the data were categorical (mastitis-positive or mastitis-negative). The Chi-square test was used to determine whether there were significant differences in mastitis frequency among the treatment groups across different weeks of the experimental period. The level of statistical significance was set at $P < 0.05$, and results were expressed as frequencies and percentages. Crosstabulation analysis was performed to compare the distribution of mastitis cases among the experimental groups across different weeks of the trial.

RESULTS

Incidence of subclinical mastitis among teat quarters of dairy cows treated with different antiseptic teat dips

The incidence of subclinical mastitis was calculated as the number of positive teat-quarter observations per sampling week (Table 1). During the first week of the trial, mastitis incidence was low across all groups, and differences among treatments were not statistically significant ($P > 0.05$). The control group (G-1) had the highest incidence of mastitis, with 2 positive cases (7.1%), followed by the G-4 group, with 1 positive case (3.5%). No cases of mastitis were recorded in groups G-2, G-3, and G-5. During the second week, mastitis incidence increased in several groups, but the differences among treatments remained statistically nonsignificant ($P > 0.05$). The highest incidence was again observed in the control group (42.2%), followed by the G-4 group (39.2%) and the G-2 group (21.4%). Only one case of mastitis (3.5%) was observed in the G-5 group, whereas none were detected in the G-3 group.

In the third week of the trial, significant differences among treatment groups were observed ($P < 0.05$). The control group (G-1) recorded the highest mastitis incidence (50%), followed by the G-4 group (35.7%). Groups G-2 and G-5 showed moderate mastitis incidence (25%), while no cases were observed in the G-3 group treated with 5% lactic acid. Similarly, during the fourth week, a statistically significant difference among treatment groups was observed ($P < 0.05$).

The highest mastitis incidence remained in the control group (50%), followed by the G-4 group (46.4%). Lower mastitis incidence was observed in the G-5 (7.1%) and G-2 (3.5%) groups, whereas the G-3 group continued to show no positive mastitis cases throughout the study period.

The cumulative analysis across the entire experimental period did not show statistically significant differences among treatments ($P > 0.05$). Overall, the highest cumulative incidence of mastitis occurred in the control group (37.5%), followed by the G-4 group (23.2%), the G-2 group (20.5%), and the G-5 group (8.9%). In contrast, the G-3 group treated with 5% lactic acid showed no cases of subclinical mastitis during the entire experimental period.

Economic evaluation of groups treated by different antiseptic chemicals.

The economic evaluation of the antiseptic treatments is summarized in Table 2. The results showed that the cost of lactic acid solutions was higher than that of pyodine solutions. Among the treatment groups, the G-3 group had the highest monthly treatment cost, followed by the G-2 group. In contrast, the pyodine-based treatments (G-4 and G-5) had considerably lower costs.

Table 1. Weekly incidence of subclinical mastitis among teat quarters of dairy cows treated with different antiseptic teat dips.

Week	Groups	Positive	Negative	Total	Incidence (%)	χ^2	<i>P</i> -value
1	G-1	2	26	28	7.10	2.074 ^a	0.355
	G-2	0	28	28	0.00		
	G-3	0	28	28	0.00		
	G-4	1	27	28	3.5		
	G-5	0	28	28	0.00		
2	G-1	12	16	28	42.2	3.733 ^a	0.155
	G-2	6	22	28	21.4		
	G-3	0	28	28	0.00		
	G-4	11	17	28	39.2		
	G-5	1	27	28	3.50		
3	G-1	14	14	28	50.0	7.593 ^a	0.022
	G-2	7	21	28	25.0		
	G-3	0	28	28	0.00		
	G-4	10	18	28	35.7		
	G-5	7	21	28	25.0		
4	G-1	14	14	28	50.0	11.511 ^a	0.003
	G-2	1	27	28	3.50		
	G-3	0	28	28	0.00		
	G-4	13	15	28	46.4		
	G-5	2	26	28	7.10		
Over all	G-1	42	70	112	37.5	5.464 ^a	0.65
	G-2	23	89	112	20.5		
	G-3	0	112	112	0.00		
	G-4	26	86	112	23.2		
	G-5	10	102	112	8.9		

P < 0.05 indicates statistically significant differences.

G-1: Control group (no antiseptic teat dip applied); G-2: 2% lactic acid teat dip; G-3: 5% lactic acid teat dip; G-4: 2% pyodine teat dip; G-5: 5% pyodine treat dip.

χ^2 = Chi-square value used to compare mastitis incidence among treatment groups within each week.

Table 2. Economic evaluation of groups treated by different chemicals.

Groups	Total Sol/Day	Total Rs	Cost/animal/day(Rs)	Cost/animal/ Month (Rs)	Cost/group/ Month (Rs)
G-2	100ml	188	47	1316	5264
G-3	100ml	470	117.5	3290	13160
G-4	100ml	2	0.5	14	56
G-5	100ml	5	1.25	35	140

G-1: Control group; G-2: 2% lactic acid teat dip; G-3: 5% lactic acid teat dip; G-4: 2% pyodine teat dip; G-5: 5% pyodine teat dip.

RS = Pakistani Rupees; Sol = Solution volume used per day.

DISCUSSION

The findings of the present study indicate that post-milking teat dipping with lactic acid solutions, particularly at 5%, was effective in reducing the incidence of subclinical mastitis in dairy cattle. The antimicrobial activity of lactic acid can be attributed to its ability to lower environmental pH and disrupt bacterial cell membrane integrity, thereby inhibiting the growth of mastitis-causing pathogens (Kunwar, 2024). Organic acids have been widely reported to exhibit strong bactericidal activity against both Gram-positive and Gram-negative bacteria associated with bovine mastitis (Patel and Patel, 2022; Nguyen et al., 2024).

The importance of teat dipping in mastitis prevention has been well documented (Breen, 2019). During the post-

milking period, the teat canal remains open and susceptible to bacterial invasion, which increases the risk of intramammary infection (IMI) (Haider, et al., 2023). Proper post-milking teat disinfection has been shown to significantly reduce the transmission of contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae* (Oliver et al., 2012; Garcia et al., 2022).

In the present study, no cases of subclinical mastitis were observed in cows treated with 5% lactic acid throughout the experimental period. These results are consistent with earlier studies reporting improved udder health and reduced mastitis incidence following the use of organic acid-based disinfectants (Zhang et al., 2021). Similarly, Schmidt et al. (1984) reported that lactic acid teat dips resulted in a greater reduction in bacterial counts compared with iodophor-based disinfectants.

Lactic acid-based disinfectants have also been reported to inhibit biofilm formation by mastitis pathogens (Koti et al., 2024). Biofilm formation is an important virulence factor that enhances bacterial survival in the mammary gland environment (Costa et al., 2014). Inhibition of biofilm formation, therefore, contributes to improved control of mastitis infections (Isaac et al., 2017). Although iodine-based antiseptics such as pyodine are widely used due to their rapid antimicrobial activity and broad-spectrum efficacy, several studies have reported that frequent iodine use may increase iodine concentrations in milk (Rezaei et al., 2021). Elevated iodine levels in milk may affect both milk quality and human health. Therefore, alternative disinfectants with lower chemical residues are increasingly being explored.

The economic evaluation conducted in the present study revealed that pyodine solutions were more cost-effective than lactic acid solutions. However, despite their higher cost, lactic acid treatments demonstrated greater effectiveness in reducing mastitis incidence. Similar observations have been reported in previous studies where natural teat disinfectants showed superior antimicrobial efficacy but slightly higher production costs (Rahman et al., 2023).

Overall, this study's findings suggest that lactic acid may be a promising natural alternative to conventional synthetic disinfectants for mastitis prevention in dairy cattle. Nevertheless, further studies with larger herd sizes, longer experimental durations, and different farm management conditions are required to validate these results and optimize the cost-effectiveness of lactic acid-based teat-dipping solutions.

CONCLUSION

The findings of this study demonstrate that post-milking teat dipping with lactic acid solutions, particularly at 5%, can effectively reduce the incidence of subclinical mastitis in dairy cattle. Compared with pyodine-based treatments, lactic acid showed superior antimicrobial effectiveness, although its solutions were relatively more expensive. The results suggest that lactic acid may serve as a promising natural alternative to conventional synthetic disinfectants for mastitis prevention in dairy herds. Adoption of effective teat disinfection practices can significantly improve udder health, milk quality, and overall dairy farm productivity. However, further studies involving larger herd sizes, longer experimental periods, and evaluations under different farm management conditions are recommended to confirm the long-term effectiveness and economic feasibility of lactic acid-based teat-dipping solutions.

ACKNOWLEDGMENT

We acknowledge the staff of the Department of Livestock Management, Breeding and Genetics, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, who provided technical and laboratory facilities.

AUTHOR CONTRIBUTIONS

Waqas Ahmad: Conceptualization, methodology, formal analysis, writing-review & editing. Iftikhar Ahmad: Supervision, conceptualization, methodology, interpreted the study findings, writing-review & editing. Muhammad Shuaib: Formal analysis, software, writing-original draft preparation, visualization. Obaid Ullah, Sohaib Ul Hassan, Danyal Khan, Muhammad Shahkar Uzair, Abdul Jabbar, Muhammad Idrees: Validation, data curation, writing-review & editing. All authors read and approved the final manuscript.

COMPETING OF INTEREST

The authors declare no competing interests.

REFERENCES

Abdalla, AM., El-Sayed, A., Awad, A. 2023. Recent advances in bovine mastitis control strategies in dairy cattle. *Animals*. 13:1452.

- Breen, J., 2019. The importance of teat disinfection in mastitis control. *Livestock*, 24(3), 122-128.
- Costa, J. C. M., Espeschit, I. D. F., Pieri, F. A., Benjamin, L. A., & Moreira, M. A. S., 2014. Increase in biofilm formation by *Escherichia coli* under conditions that mimic the mastitic mammary gland. *Ciência Rural*, 44, 666-671.
- Garcia, M., Sharma, N., Singh, V. 2022. Mastitis control programs and teat disinfectants in modern dairy farming. *Journal of Dairy Science*. 105:8950–8963.
- Haider, A., Ikram, M., Shahzadi, I., & Asif., RM., 2023. Bovine mastitis. In *Polymeric Nanoparticles for Bovine Mastitis Treatment*, 19: 49-80. Cham: Springer Nature Switzerland.
- Isaac, P., Kumar, A., Sharma, N. 2017. Antibacterial and biofilm inhibition activity of teat disinfectant compounds against mastitis pathogens. *Veterinary Microbiology*. 203:66–72.
- Koti, K., Rodas-Gonzalez, A., Nadon, C., McAllister, T., Yang, X., and Narváez-Bravo, C., 2024. Evaluating disinfectant efficacy on mixed biofilms comprising Shiga toxin-producing *Escherichia coli*, lactic acid bacteria, and spoilage microorganisms. *Frontiers in Microbiology*, 15, 1360645.
- Kunwar, H., 2024. Lactic acid bacteria with antagonistic properties towards dairy food microbial contaminants and mastitis pathogens (Doctoral dissertation, Swinburne).
- Nguyen, TT., Lee, SH., Kim, HJ. 2024. Natural antimicrobial agents for controlling bovine mastitis pathogens. *Veterinary Microbiology*. 285:109906.
- Oliver, SP., Murinda, SE., Jayarao, BM. 2012. Impact of teat dipping on prevention of bovine mastitis. *Veterinary Clinics of North America: Food Animal Practice*. 28:217–229.
- Patel, J., Patel, R. 2022. Role of organic acids in controlling bacterial infections in dairy animals. *Veterinary World*. 15:2458–2466.
- Rahman, M., Islam, M., Hossain, M. 2023. Evaluation of post-milking teat disinfectants for the control of subclinical mastitis in dairy cattle. *Frontiers in Veterinary Science*. 10:118420.
- Rezaei, M., Shahbazi, Y., Fathi, B. 2021. Effect of iodine teat disinfectants on iodine concentration in milk. *Journal of Dairy Research*. 88:141–146.
- Schmidt, A. L., Oliver, S. P., and Fydenkevez, M. E., 1984. Evaluation of experimental teat dip containing sodium chlorite and lactic acid by excised teat assay. *Journal of Dairy Science*., 67(12): 3075-3080.
- Zhang, L., Wang, Y., Li, H. 2021. Effectiveness of barrier teat dips in preventing subclinical mastitis in dairy cows. *Journal of Dairy Science*. 104:5678–5686.