

Effectiveness of the California Mastitis Test (CMT), Reductase Test, and Alcohol Test for Dairy Cows Subclinical Mastitis Detection

Eriza Rosalina Rochmah¹, Dadik Raharjo^{2*}, Sri Hidanah³, Mustofa Helmi Effendi², Adiana Mutamsari Witaningrum², Sunaryo Hadi Warsito³

¹Bachelor Program of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia

²Division of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia

³Division of Animal Husbandry, Faculty of Veterinary Medicine, Universitas Airlangga, Jl. Dr. Ir. H. Soekarno, Kampus C Mulyorejo, Surabaya 60115, East Java, Indonesia

ABSTRACT

Mastitis is an inflammation of the mammary glands caused by pathogenic bacteria. Mastitis is divided into two types: clinical mastitis, which has symptoms of swelling and changes in the shape of the mammary gland, and subclinical mastitis, which does not indicate clear symptoms. Subclinical mastitis is a very high case in Indonesia, around 97-98%, while clinical mastitis is 2-3%. Effectiveness of the California mastitis test (CMT), reductase test, and alcohol test to detect subclinical mastitis in dairy cattle. The number of samples taken was 60 samples of fresh dairy milk from 15 cattle in Medowo Village, as much as 50 ml for each sample. The results showed that the test was very effective in identifying subclinical mastitis in dairy cattle. 15% positive results were obtained for subclinical mastitis using CMT, 10% of the milk quality was not good during the reductase test, and 18.33% positive with the alcohol test from a total of 60 samples obtained

ARTICLE INFO

Original Research

Received: October 2nd, 2023

Accepted: November 27th, 2023

Published: December 1st, 2023

*Corresponding Author:

dadik_tdc@yahoo.co.id

DOI:

<https://doi.org/10.20473/agrovet.v7i1.51443>

Keywords: Laserpuncture, male Japanese quail, weight gain, growth rate

Introduction

Milk is a source of animal protein that is often consumed by Indonesian people. Milk is a highly nutritious animal food that is easily absorbed by the blood. The high nutritional value content can cause milk to be an excellent growth medium for microorganisms, both beneficial and harmful microorganisms for humans (Górska-Warsewicz et al., 2019). To get good fresh milk, you must pay attention to several factors that influence the quality of milk, such as the cleanliness of the cage, the health and cleanliness of the livestock, the cleanliness of the milker and the milking equipment used. Apart from that, there are also contamination requirements, maximum microbial content, antibiotic residues, and maximum contamination from dangerous metals that must also be considered (Sjarif et al., 2019). Dairy

cows are a type of milk-producing livestock. The milk produced by dairy cows exceeds what is needed for their calves.

Dairy cows are more efficient at converting feed into animal protein and calories. Until now, no one has matched the efficiency of dairy cows with a production of 4,500 liters of milk per year. Domestic milk needs can only be met around 23.73% (Leone et al., 2022). The low national fresh milk production is due to low livestock productivity and also a small population (Duguma, 2022).

Factors that can influence cow's milk production are: type of cow, lactation level, frequency of milking, age, heat cycle, dry period, feed, environment and disease. A disease that often attacks milk-producing livestock is mastitis (Abebe et al., 2016).

Mastitis is inflammation of the udder gland caused by pathogenic bacteria or fungi, with varying degrees of severity and duration of illness. Cheng and Han (2020) also stated that factors such as poor environmental sanitation and cleanliness during milking play an important role in the incidence of subclinical mastitis. According to Martin *et al.* (2023) milking sanitation and hygiene includes: Cleanliness of the stall, the stall must be routinely cleaned of dirt which is a source of contaminants and odors; Preparing the tools and milking tools, the tools to be used must be washed in clean water with detergent, then rinsed using hot water. Milkmaids must be healthy and free from infectious diseases and wash their hands before milking; Preparing a dairy cow, the cow's tail must be tied. Before milking, the udder and the area around the udder must be cleaned using a clean cloth dampened with warm water; The milking process, when milking manually, the milking hands must be clean and there are no sources of contamination in the surrounding environment (Burtscher *et al.*, 2023).

Mastitis is divided into two types, namely clinical and subclinical mastitis. Clinical mastitis has symptoms of clearly visible inflammation in the udder and changes in the appearance of the milk, while subclinical mastitis does not show symptoms or changes in the udder and the appearance of the milk tends to remain the same (Ibrahim *et al.*, 2023). Subclinical mastitis can be detected by examining the number of somatic cells in the laboratory. Subclinical mastitis is a very high incidence in Indonesia, around 97-98%, while 2-3% of cases of clinical mastitis are detected. Both clinical and subclinical mastitis have a very detrimental impact on dairy livestock because they can reduce or affect the quality and quantity of milk production. Mastitis can also shorten the milk production period, requiring extra costs for treatment and a high number of livestock being abandoned early or dying due to the disease (Heikkilä *et al.*, 2012).

Detection of subclinical mastitis that is widely used in the field is the California mastitis test (CMT) (Kivaria *et al.*, 2007). CMT is a method for diagnosing subclinical mastitis which is considered simple and fast so it can be carried out in the field. Reductase and alcohol tests are also carried out on fresh cow's milk to check the quality and suitability of fresh milk. Based on the research background above, the author intends to determine the incidence rate of mastitis cases in dairy cows using the California mastitis test

(CMT), reductase test and alcohol test, as basic research on subclinical mastitis cases.

Materials and methods

Research design

This research was carried out directly at the dairy cow pen in Kandangan Village, Medowo District, Kediri Regency on 8 April 2023 and at the Veterinary Public Health Laboratory of Airlangga University on 12–17 April 2023. Samples were taken during morning milking. Milk samples came from a dairy farm in Medowo Village, Kandangan District, Kediri Regency. Kandangan was chosen as the sampling location because the dairy farms in the area best met the criteria determined by the researchers. The sampling method used is the purposive sampling method where 60 milk samples will be taken from 15 cows. Selecting cows that meet the criteria includes cows aged 3–8 years and cows that are actively milked.

Milk sampling

Milk samples were taken aseptically from the cow's udder by first cleaning it using clean water and then drying it. Sampling for mastitis detection is carried out by the cow owner or pen kid. The first jet of milk is discarded and the second jet of milk is used and collected in the test paddle as much as 2 ml. Taking samples of milk for alcohol testing and reductase testing, 50 ml of each nipple is collected in a sterile container and has been labeled, then stored in a thermos containing ice so that the temperature is stable at 5–10° C to avoid the development of bacteria and damage to the milk when it arrives at the laboratory.

California mastitis test (CMT)

Testing milk samples uses CMT reagent with a ratio according to the rules for use on the packaging, namely 2 ml of milk sample plus 2 ml of CMT reagent. The mixture is shaken horizontally for 10-15 seconds. The reading of the reaction results is carried out in a bright place and the presence or absence of clots or sediment is seen, then determined based on the CMT scoring, namely negative (-) there are no clots or sediment, meaning the milk is of very good quality, positive 1 (+) there is little sediment, which means the milk is of good quality, positive 2 (++) there is a clear precipitate but there are no gel lumps yet, meaning the quality of the milk is quite good, positive 3 (+++) gel lumps are starting to form, indicating the milk is of

poor quality, positive 4 (++++) lumps. The gel is very thick causing the surface to become convex, meaning the milk is of very poor quality (Rust *et al.*, 2023).

Reductase test

The reductase test uses 0.5 ml of methylene blue solution which is put into a sterile reductase tube plus 20 ml of milk sample which is then homogenized and closed in the tube. The homogenized tube was put into an incubator at a temperature of 37° C and checked every 30 minutes until the blue color was gone. The degree of color change is a measure of the number of bacteria present. The reading of the reaction results can be seen from the time (hours) required for the blue color to disappear (Novita *et al.*, 2022).

Alcohol test

The alcohol testing procedure requires 3 ml of milk to be put into a test tube with the help of a pipette and 3 ml of 70% alcohol added. Then, the tube is shaken gently and observed. The alcohol test is positive if there are grains or lumps of milk attached to the walls of the test tube, while negative if there are no grains or lumps attached to the walls of the test tube (Zebib *et al.*, 2023).

Result

California mastitis test (CMT) results

Based on the results of subclinical mastitis detection using CMT, it can be seen in Table 1 that of the 60 samples obtained and tested, 15 cow's milk samples, or around 25% were identified as subclinical mastitis with scoring CMT 1 (+), CMT 2 (++) and CMT 3 (+++). A positive score of 1 (+) has the possibility of increasing to positive 2 (++), positive 3 (+++) and positive 4 (++++) and so on, a normal score (-) also has the possibility of becoming positive 1 (+), positive 2 (++), positive 3 (+++) and positive 4 (++++) (figure 1).

Table 1. California mastitis test (CMT) results

Samples	Negative	CMT 1	CMT 2	CMT 3	Total CMT
60	45	5	4	6	15
Percentage (%)	75%	83.33%	6.67%	10%	25%



Figure 1. CMT examination results

In this study, the california mastitis test (CMT) was used as a method for detecting subclinical mastitis in the field. Assessment of the CMT results is subjective which is carried out visually when rotating the milk sample that has been reacted with the reagent and when it is tilted or dropped to see the presence of thickening and the sample remaining on the paddle base. The presence of reactions in the milk and reagents in the form of sediment and thickening indicates that the sample indicates subclinical mastitis.

Reductase test results

Table 2. Reductase test results

Number of samples	< 1/2 hour	1–2 hour	3–4 hour	>5 hour
60	–	6	8	46
Percentage (%)	–	10%	13.33%	76.67%

From the results shown in **table 2**, 6 samples or around 10% of the samples were declared to be of poor quality and 8 samples or around 13.33% were declared good according to the standard reductase test (figure 2). The results of the reductase test are used as an assessment of the quality of fresh milk. The faster the color of methylene blue changes to a colorless solution, it indicates that the quality of the fresh milk is worse.



Figure 2. Reductase test results

Alcohol test results

Table 3. Alcohol test results

Number of samples	Positive (+)	Negative (-)
60	11	49
Percentage (%)	18.33%	81.67%



Figure 3. Alcohol test results

From the results shown in table 3, the alcohol test shows that the percentage of positive (+) events was 11 samples out of 60 samples, namely 18.33%, while for negative (-) events there were 49 samples or around 81.67% (figure 3). The alcohol test results determine the suitability of the milk. Milk with a positive alcohol test is milk that is not suitable for consumption or sale. A positive alcohol test result is indicated by the presence of white granules or lumps on the walls of the test tube after shaking.

Discussion

Mastitis is a disease that often attacks dairy cattle due to bacterial infection in the udder glands. Subclinical mastitis can only be identified by examining the number of somatic cells carried out in the laboratory because there are no physical changes in the udder (Abed *et al.*, 2021). Poor stall sanitation, unhygienic and incomplete milking management are also factors that cause mastitis. Mastitis also causes a decrease in milk production and quality and increases maintenance costs so that farmers often experience economic losses (Nuraini *et al.*, 2023).

The California mastitis test (CMT) is an alternative early detection method to determine the occurrence of subclinical mastitis which can be carried out directly in the field using a tool called a paddle and using reagents to determine the severity. The higher the results obtained in the CMT test, the higher the number of somatic cells in the milk (Huang and Kusaba, 2022). The research results showed that the incidence of subclinical mastitis in Medowo Village, Kandangan District, Kediri Regency was 25% of a total of 60 milk samples from 15 actively milked cows.

The reductase test is one way to assess the quality of cow's milk. The reductase enzyme is produced by bacteria found in milk and can change the dye methylene blue into a colorless solution. The change in color of methylene blue to a colorless solution indicates a reductase enzyme reaction

produced by bacteria contained in milk. The faster the color of methylene blue changes to a colorless solution, the more bacteria there are in the fresh milk. Apart from that, factors that can influence the reductase rate include the type of animal, lactation level, age of the animal, nutrition provided and health and cleanliness of the udder (Bionaz *et al.*, 2020). Based on the results of the reductase test in table 2, 10% of the samples were of poor quality.

The alcohol test is a simple test carried out to determine the quality of milk that is suitable for consumption. Based on Nurliyania *et al.* (2015), milk that is suitable for consumption is one whose alcohol test results are negative (-) or there is no clotting. If clots occur, this indicates that the water envelope surrounding the milk proteins is weak, forming clumps that stick to the walls of the test tube. The weak bond is caused by the presence of acid bacteria in the milk. The milk tested shows positive results (+) if the acidity is 8–9° SH, except for sour milk, the onset of mastitis and colostrum (Uzatici and Yayintaş, 2017). Based on the alcohol test results in table 3, 18.33% of the samples were unfit for consumption and indicated subclinical mastitis.

The California mastitis test (CMT) is a rapid test for subclinical mastitis that is carried out in the field, then a reductase test is carried out in the laboratory to determine the rough number of bacteria (Atif *et al.*, 2006). If the number of bacteria is too high, it indicates that the quality of the milk is very poor, making it unfit for consumption. Milk from cows suffering from mastitis can be consumed if the alcohol test result is negative (-). Milk with a positive alcohol test (+) indicates that the milk contains acid bacteria which causes the milk to break easily when an alcohol test is carried out.

Conclusion

From the results of research on the effectiveness of the California Mastitis Test (CMT), the reductase test and alcohol test for detecting subclinical mastitis in dairy cows are very effective in identifying subclinical mastitis in dairy cows with 15% positive results for subclinical mastitis using CMT, 10% with poor milk quality when reductase test and 18.33% were positive with the alcohol test from a total of 60 samples obtained. The incidence rate of subclinical mastitis in dairy cows in Medowo Village, Kandangan District, Kediri Regency was 8 cows detected out of a total of 15 cows when research was carried out using the CMT test, reductase test and alcohol test.

References

- Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet Res.* 2016; 12(1): 270.
- Abed AH, Menshawy AMS, Zeinhom MMA, Hossain D, Khalifa E, Wareth G, Awad MF. Subclinical Mastitis in Selected Bovine Dairy Herds in North Upper Egypt: Assessment of Prevalence, Causative Bacterial Pathogens, Antimicrobial Resistance and Virulence-Associated Genes. *Microorganisms.* 2021; 9(6): 1175.
- Atif EAG, Hildebrandt G, Kler JN, Molla B, Kyule MN, Baumann MP. Comparison of California Mastitis Test (CMT), Somatic Cell Counts (SCC) and bacteriological examinations for detection of camel (*Camelus dromedarius*) mastitis in Ethiopia. *Berl Munch Tierarztl Wochenschr.* 2006; 119(1–2): 45–49.
- Bionaz M, Vargas-Bello-Pérez E, Busato S. Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. *J Animal Sci Biotechnol.* 2020; 11: 110.
- Burtscher J, Rudavsky T, Zitz U, Neubauer V, Domig KJ. Importance of Pre-Milking Udder Hygiene to Reduce Transfer of Clostridial Spores from Teat Skin to Raw Milk. *Microorganisms.* 2023; 11(5): 1337.
- Cheng WN, Han SG. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments - A review. *Asian-Australas J Anim Sci.* 2020; 33(11): 1699–1713.
- Duguma B. Farmers' perceptions of major challenges to smallholder dairy farming in selected towns of Jimma Zone, Oromia Regional State, Ethiopia: possible influences, impacts, coping strategies and support required. *Heliyon.* 2022; 8(6): e09581.
- Górska-Warsewicz H, Rejman K, Laskowski W, Czacotko M. Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet. *Nutrients.* 2019; 11(8): 1771.
- Heikkilä AM, Nousiainen JI, Pyörälä S. Costs of clinical mastitis with special reference to premature culling. *J Dairy Sci.* 2012; 95(1): 139–150.
- Huang CH, Kusaba N. Association between differential somatic cell count and California Mastitis Test results in Holstein cattle. *JDS Commun.* 2022; 3(6): 441–445.
- Ibrahim N, Regassa F, Yilma T, Tolosa T. Impact of subclinical mastitis on uterine health, reproductive performances and hormonal profile of Zebu × Friesian crossbred dairy cows in and around Jimma town dairy farms, Ethiopia. *Heliyon.* 2023; 9(6): e16793.
- Kivaria FM, Noordhuizen JP, Nielen M. Interpretation of California mastitis test scores using *Staphylococcus aureus* culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dar es Salaam region of Tanzania. *Prev Vet Med.* 2007; 78(3–4): 274–285.
- Leone C, Thippareddi H, Ndiaye C, Niang I, Diallo Y, Singh M. Safety and Quality of Milk and Milk Products in Senegal-A Review. *Foods.* 2022; 11(21): 3479.
- Martin NH, Evanowski RL, Wiedmann M. Invited review: Redefining raw milk quality-Evaluation of raw milk microbiological parameters to ensure high-quality processed dairy products. *J Dairy Sci.* 2023; 106(3): 1502–1517.
- Novita A, Putri ANY, Herialfian, Isa M, Armansyah TRT, Hasan M. Detection of Reductase and Catalase Enzymes in Goats Milk (*Capra aegagrus hircus*) Sold in Banda Aceh. *Int J Trop Vet Biomed Res.* 2022; 7(2): 15–19.
- Nuraini DM, Andityas M, Sukon P, Phuektes P. Prevalence of mastitis in dairy animals in Indonesia: A systematic review and meta-analysis. *Vet World.* 2023; 16(7): 1380–1389.
- Nurliyanita, Suranindyah Y, Pretiwi P. Quality And Emulsion Stability of Milk From Ettawah Crossed Bred Goat During Frozen Storage. *Procedia Food Sci* 2015; 3: 142–149.
- Rust JD, Christian MJ, Vance CJ, Bolajoko MB, Wong JT, Suarez-Martinez J, Allan FK, Peters AR. A study of the effectiveness of a detergent-based California mastitis test (CMT), ... the commercial UK CMT. *Gates Open Res.* 2023; 5: 146.
- Sjarif DR, Yuliarti K, Iskandar WJ. Daily consumption of growing-up milk is associated with less stunting among Indonesian toddlers. *Med J Indones.* 2019; 28: 70–76.
- Uzatici A, Yayintaş ÖT. Determination of the quality of raw milk from black and white cows from biga (Canakkale, turkey). *J Sci Perspect.* 2017; 1(1): 29–42.
- Zebib H, Abate D, Woldegiorgis AZ. Nutritional quality and adulterants of cow raw milk, pasteurized and cottage cheese collected along value chain from three regions of Ethiopia. *Heliyon.* 2023; 9(5): e15922.

How to Cite: