# Detection of mastitis in dairy cows in East Java Province, Indonesia

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## **Abstract**

The high demand for these products is caused by consumer knowledge of the need for protein as well as milk's whole nutritional profile. Mastitis is one of the medical reasons for poor milk quality and quantity. Depending on how severe, persistent, and diseasecausing they are, there are several causes of mastitis, an inflammation of the inner udder tissue, including pathogenic bacteria. This disease is especially dangerous since it decreases the amount of milk available, drives up the cost of prescription drugs, and damages farmers financially. The detection procedure involved the use of milk samples from dairy cows in East Java. The aim of the study was to detect the incidence of subclinical mastitis in dairy cows in East Java, Indonesia. The California Mastitis Test (CMT) reagent was then added, and the reaction was cycled for ten to fifteen seconds. Out of 332 cows with CMT (1,328 nipples), 59 dairy cows (17.77%) had subclinical mastitis. The emergence of cases of subclinical mastitis in dairy cows in the East Java region requires serious treatment and attention. The large number of dairy cows kept in the East Java area has not been balanced with good maintenance management. Farmers in general still use traditional rearing systems without paying attention to the importance of maintaining the cleanliness of the dairy cattle barn environment.

Keywords: Dairy cows, California Mastitis Test, disease, health, mastitis

## Introduction

One of the livestock products that Indonesians require is milk [1]. Dairy cows are one of the livestock animals that produce milk. The purpose of raising dairy cows is to produce milk to meet human animal protein needs [2]. There is a significant necessity for milk consumption. The high demand for these products is caused by consumer knowledge of the need for protein as well as milk's whole nutritional profile [3]. In Indonesia, where there are many needs and demands for milk, the quantity and quality of milk are still insufficient [4]. Various types of diseases have the potential to attack dairy cows. For this reason, farmers should have sufficient knowledge about the health of livestock.

Livestock health is very important because if the livestock is not healthy it will cause many losses including growth disorders, reproductive disorders, and even, most fatally, death of the livestock itself [5]. Of the many livestock diseases, especially dairy cattle, one of them is udder inflammation or what is known as mastitis. This illness is the medical reason for the decreased production and quality of milk [6]. Mastitis is an internal udder tissue infection that has a range of severity levels and causes [7]. Mastitis can have a wide range of effects, both in terms of the disease's length and its after effects [8]. When it comes to dairy cow's, mastitis is frequently caused by a bacterial infection as opposed to other causes like cendawan or kapang [9].

Subclinical mastitis and clinical mastitis are the two categories into which mastitis is subdivided [10]. Clinical symptoms of mastitis, such as swelling, udder hardening, pain, heat, redness, and diminished udder function, are a constant feature of the condition [11]. Subclinical mastitis, on the other hand, is mastitis that does not cause noticeable modifications to the udder or the milk it produces [12]. However, because milk production might progressively diminish, farmers frequently discover these indications too late [13]. According to the Central Statistics Agency, in 2021, East Java Province's fresh milk production occupies the top position in Indonesia, namely in 2019 it was 521,123.43 tons, in 2020 it was 542,860.27 tons, and in 2021 it was 556,431.78 tons [14].

Due to farmer's habits of paying less attention to the care of dairy cows with subclinical mastitis, which will eventually develop into clinical mastitis, reports of cases of subclinical mastitis are also found in East Java, a region with a high population of dairy cattle and a high milk production rate [1]. Bacterial infections are primarily to blame for mastitis. Bacteria might be communicable or they can come from the environment [15]. Mastitis that develops as a result of bacterial contamination from the surrounding environment, such as water, cage mats, and dung, is known as environmental mastitis [16]. Mastitis that spreads to healthy udders while being milked from infected udders is known as contagious mastitis [17]. The sponge used to clean the nipple has the potential to spread infection used to milk cows, whether by hand or machine [18].

Monitoring the quantity of somatic cells by laboratory tests can be used to detect the prevalence of subclinical mastitis [19]. The California Mastitis Test (CMT) is a quick and straightforward procedure [20]. The aim of the study was to detect the incidence of subclinical mastitis in dairy cows in East Java, Indonesia.

#### **Materials and Methods**

# **Ethical approval**

The research was approved by animal care and use committee, Universitas Airlangga with No: 353/HRECC.FODM/VI/2021.

# Study area

A screening test employing the CMT method was used as the research approach. Descriptive data are supplied. This research was conducted from April to September 2022. Purposive sampling was the sampling technique employed, and 332 cows were sampled for this investigation. The KUD Argopuro Probolinggo Regency, KUD Tani Wilis Tulungagung Regency, and KUD Semen Blitar Regency were the locations where this research was carried out in-person on several dairy farms in East Java.

#### Data collection

In order to perform the California Mastitis Test (CMT), up to 2 ml of milk from each nipple (quarter) is deposited in the proper position of the CMT paddle, Following the addition of 2 ml of CMT reagent to each paddle column containing milk, the CMT paddle is gently shaken in a horizontal, circular motion for 10 to 15 seconds. In a well-lit area, the reading of the CMT reaction results took place for about 20 seconds. On the CMT test, changes in viscosity revealed symptoms of mastitis in cows. The existence or absence of a change in the milk's consistency serves as a sign of this reaction, Then, based on the CMT scoring, it was determined that (-) no precipitation in milk indicates good quality milk, (+1) slight deposition in milk indicates good quality milk, (+2) clear precipitation but no gel has formed indicates good enough quality milk, (+3) the mixture thickens and begins to form a gel, which indicates poor quality, and (+4) the gel formed causes the surface to become convex, which indicates very poor quality [21].

#### Result

Some bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus* sp are significant pathogenic organisms that cause subclinical mastitis from the environment [8]. Normal sources of pathogenic agents include feed, bedding, and excrement [22]. When the source of the infection is close to the cow, environmental mastitis can develop at any moment [23]. When a cow comes into contact with an infected area or source, the bacteria will enter the cow's udder [24].

**Table 1.** Assessment Results of the California Mastitis Test (CMT) in 3 districts in East Java

	Number of dairy	<b>CMT Assessment Results</b>					
Location	cows (number of nipples)	X	_	+1	+2	+3	+4
Probolinggo	109 (436 nipples)	25	210	129	52	20	0
Tulungagung	110 (440 nipples)	17	193	156	57	17	0
Blitar	113 (452 nipples)	15	166	177	72	22	0
Total	332 (1,328 nipples)	57	569	462	181	59	0

Note: Each dairy cow has 4 nipples; x=A cow's teats are inactive or unable to produce milk.

According to the data in tables 1, of 109 dairy cows (436 nipples) checked by CMT on farms in KUD Argopuro, Probolinggo Regency, 20 (18.35%) dairy cows tested positive for subclinical mastitis. 17 (15.45%) of the 110 dairy cows (440 nipples) investigated by CMT on the farm of KUD Tani Wilis in Tulungagung Regency were positive for subclinical mastitis. 22 (19.47%) of the 113 dairy cows (452 nipples) checked by CMT on the farm in KUD Semen Blitar Regency were positive for subclinical mastitis.

**Table 2.** The incidence of subclinical mastitis in lactating dairy cows in 3 districts in East Java based on the results of CMT examination

Location	Dairy Cow Population During Lactation	Number of Dairy Cows Under CMT Inspection	Number of Positive Subclinical Mastitis (%)
Probolinggo	2,819	109	20 (18.35 %)
Tulungagung	2,437	110	17 (15.45 %)
Blitar	1,568	113	22 (19.47 %)
Total	6,824	332	59 (17.77 %)

Note: The total population of lactating dairy cows written is the population of non-pregnant lactating dairy cows.

According to the data in tables 2, the study's findings revealed that 59 dairy cows (17.77%) out of a total of 332 dairy cows (1,328 nipples) examined for CMT had subclinical mastitis. Because subclinical mastitis disease does not produce clinical signs or alter the composition of milk, farmers frequently are unaware that their cattle is infected with it [25]. Economic losses can result from subclinical mastitis because milk output gradually declines [26]. Detection of mastitis using the California Mastitis Test (CMT) can be seen in Figure 1.



Figure 1. California Mastitis Test (CMT) examination results

## **Discussion**

The emergence of cases of subclinical mastitis in dairy cows in the East Java region requires serious treatment and attention. The large number of dairy cows kept in the East Java area has not been balanced with good maintenance management. Farmers in general still use traditional rearing systems without paying attention to the importance of maintaining the cleanliness of the dairy cattle barn environment. Poor environmental conditions cause bacteria to multiply easily and are susceptible to entering the teat holes of dairy cows [27]. The bacteria that accumulate in the udder is what causes mastitis. Transmission of mastitis from one animal to another and from an infected litter to a normal litter can be through milking hands, cleaning cloths, milking machines and flies [9]. Clinical observation of inflammation of the udder and nipples, changes in the color of the milk produced. Field tests can be carried out using the California Mastitis Test (CMT), namely with a special reagent [20].

The California Mastitis Test (CMT) is a straightforward test that may be used to determine the approximate quantity of somatic cells in milk, which helps detect mastitis [28]. CMT indicates whether a category is high or low rather than providing a numerical number. The results can be categorized into five groups, ranging from the lowest cell count, which is indicated by a mixture of milk and reagent that is nearly clotted, to the highest cell count, which is indicated by the milk and reagent staying liquid [29]. Based on the results of the mastitis test carried out using CMT, the dairy cow in this case was declared positive for subclinical mastitis. In the CMT detection carried out in this study, 59 dairy cows in East Java were detected as having subclinical mastitis out of 332 dairy cows examined.

Mastitis is the term used for inflammation that occurs in the udder, both clinical and subclinical, with an increase in cells in the milk and changes in the physical and composition of the milk, accompanied or without pathological changes in the mammary glands [8]. Losses in cases of mastitis include loss of milk production, reduced quality and quantity of milk, and many cows being abandoned. The decrease in milk production per quarter can reach 30% to 15% per cow per lactation due to mastitis, making it a big problem in the dairy industry [30].

The dry period is the initial time for the bacteria that cause mastitis to infect, because at that time the phagocytic action of neutrophils in the udder is inhibited [31]. Various types of bacteria are the causative agents of mastitis, including *Streptococcus disgalactiae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus zooepedermicus*, *Enterobacter aerogenees*, *Streptococcus uberis*, *Escherichia coli*, and *Pseudomonas aeroginosa* [32]. Yeast and fungi also frequently infect the udder, but usually cause subclinical mastitis [33]. One of the primary causes of mastitis in dairy cows, which results in considerable financial losses from decreased milk output, is *Staphylococcus aureus* [34].

Apart from microorganism factors which include various types, numbers and virulence, livestock and environmental factors also determine whether mastitis occurs easily or not [8]. Predisposing factors for mastitis are seen from the livestock perspective, including the shape of the udder [35]. For example, the udder is very hanging, or the teat hole is too wide.

Age factors and the level of cow's milk production also influence the incidence of mastitis. The older the cow and the higher the milk production, the more her teat sphincter relaxes [36]. Teats with loose sphincters allow cows to be easily infected by microorganisms, because the function of the sphincter is to resist infection by microorganisms [37]. The higher a cow's milk production, the longer it takes for the sphincter to close completely. Cow breed factors also influence the incidence of mastitis [8]. Environmental and management factors are also very influential. These include factors such as feed, housing, number of animals in one cage, ventilation, sanitation of the cage and milking methods [38].

Clinically, mastitis can occur clinically and subclinically. Udder inflammation is said to be subclinical if clinical symptoms of inflammation are not found during udder examination [35]. For clinical ones, clear signs of inflammation are found, such as udder swelling, heat when touched, pain, reddish color and impaired function. Milk changes its properties, becomes broken, mixed with fibrin deposits or lumps, cell debris or protein clumps [39]. The process that takes place subacutely is characterized by symptoms as above, but the degree is milder, the livestock still wants to eat and body temperature is still within normal limits. The process is clinical if the infection in an udder lasts a long time, from one lactation period to the next [40].

To prevent new infections by bacteria that cause mastitis, several efforts are needed, namely minimizing conditions that support the spread of infection from one cow to another and conditions that facilitate bacterial contamination and penetration of bacteria into the teat canal. The first flow of milk during milking should be collected in a strip cup and observed for the presence or absence of mastitis [8]. It requires dipping or dipping the nipple in 3000 IU biocid (3.3 milliliters/liter of water). Using a different wipe is recommended for each cow and ensure the wipe is washed and disinfected before use [41]. Providing quality nutrition, thereby increasing livestock resistance to bacterial infections that cause mastitis. Supplementation of vitamins E, A, and  $\beta$ -carotene as well as a balance between Co (Cobalt) and Zn (Zinc) needs to be attempted to reduce the incidence of mastitis [42].

Mastitis treatment should use Lincomycin, Erythromycin, and Chloramphenicol [8]. Nipple disinfection with alcohol and intramammary antibiotic infusion can treat mastitis [43]. A combination injection of penicillin, dihydrostreptomycin, dexamethasone and antihistamines is recommended as well [44]. Antibiotics will suppress the growth of bacteria that cause mastitis, while dexamethasone and antihistamines will reduce inflammation [45].

Mastitis caused by *Streptococcus* sp can still be treated with penicillin, because *Streptococcus* sp is still sensitive to penicillin [44]. The use of antibiotics, which may not always be appropriate, will cause new problems, namely the presence of antibiotic residues in milk, allergies, resistance and affect milk processing [46]. Subclinical mastitis caused by Gram-positive bacteria is also increasingly difficult to treat with antibiotics, because many of these bacteria are resistant to various types of antibiotics [47].

## **Conclusion**

In East Java, 59 (17.77%) of the 332 dairy cows who were tested for CMT also tested positive for subclinical mastitis. The emergence of cases of subclinical mastitis in dairy cows in the East Java region requires serious treatment and attention. The large number of dairy cows kept in the East Java area has not been balanced with good maintenance management.

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## References

- [1] A. R. Khairullah, S. A. Sudjarwo, M. H. Effendi, S. C. Ramandininto, M. A. Gelolodo, A. Widodo, K. H. P. Riwu, D. A. Kurniawati, and S. Rehman, "Profile of Multidrug Resistance and Methicillin-Resistant Staphylococcus aureus (MRSA) on dairy cows and risk factors from farmer", Biodiversitas, vol. 23, no. 6, (2022), pp. 2853-2858.
- [2] A. R. Khairullah, D. Rahardjo, J. Rahmahani, Suwarno, W. Tyasningsih, and N. Harijani, "Antibiotics Resistant at Staphylococcus aureus and Steptococcus sp Isolated from Bovine Mastitis in Karangploso, East Java, Indonesia", Indian Journal of Forensic Medicine and Toxicology, vol. 13, no. 4, (2019), pp. 439-444.
- [3] H. Górska-Warsewicz, K. Rejman, W. Laskowski, and M. Czeczotko, "Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet", Nutrients, vol. 11, no. 8, (2019), pp. 1771.
- [4] S. C. Ramandinianto, A. R. Khairullah, and M. H. Effendi, "MecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from dairy farms in East Java, Indonesia", Biodiversitas, vol. 21, no. 8, (2020), pp. 3562-3568.
- [5] S. M. Thumbi, M. K. Njenga, T. L. Marsh, S. Noh, E. Otiang, P. Munyua, L. Ochieng, E. Ogola, J. Yoder, A. Audi, J. M. Montgomery, G. Bigogo, R. F. Breiman, G. H. Palmer, and T. F. McElwain, "Linking human health and livestock health: a "one-health" platform for integrated analysis of human health, livestock health, and economic welfare in livestock dependent communities", PLoS One, vol. 10, no. 3, (2015), pp. e0120761.

[6] A. R. Khairullah, S. C. Ramandinianto, and M. H. Effendi MH, "A Review of Livestock-Associated Methicillin-Resistant Staphylococcus aureus (LA-MRSA) on Bovine Mastitis", Systematic Reviews in Pharmacy, vol. 11, no. 7, (2020), pp. 172-183.

- [7] M. Cobirka, V. Tancin, and P. Slama. "Epidemiology and Classification of Mastitis", Animals (Basel), vol. 10, no. 12, (2020), pp. 2212.
- [8] W. N. Cheng and S. G. Han, "Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments A review", Asian-Australasian Journal of Animal Sciences, vol. 33, no. 11, (2020), pp. 1699-1713.
- [9] D. D. Bannerman, "Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows", Journal of Animal Science, vol. 87, no. 13, (2009), pp. 10-25.
- [10] T. Shaheen, S. B. Ahmad, M. U. Rehman, S. Muzamil, R. R. Bhat, I. Hussain, N. Bashir, M. U. R. Mir, B. A. Paray, and M. A. O. Dawoodd. "Investigations on cytokines and proteins in lactating cows with and without naturally occurring mastitis", Journal of King Saud University Science, vol. 32, no. 6, (2020), pp. 2863-2867.
- [11] A. J. Bradley and M. J. Green, "Factors affecting cure when treating bovine clinical mastitis with cephalosporin-based intramammary preparations", Journal of Dairy Science, vol. 92, no. 5, (2009), pp. 1941-1953.
- [12] E. J. Sarba and G. K. Tola, "Cross-sectional study on bovine mastitis and its associated risk factors in Ambo district of West Shewa zone, Oromia, Ethiopia", Veterinary World, vol. 10, no. 4, (2017), pp. 398-402.
- [13] J. F. Johnsen, K. A. Zipp, T. Kälber, A. M. de Passillé, U. Knierim, K. Barth, and C. M. Mejdell, "Is rearing calves with the dam a feasible option for dairy farms?— Current and future research", Applied Animal Behaviour Science, vol. 181, no. 1, (2016), pp. 1-11.
- [14] A. Susanty, N. B. Puspitasari, H. Prastawa, and S. V. Renaldi, "Exploring the best policy scenario plan for the dairy supply chain: a DEMATEL approach", Journal of Modelling in Management, vol. 16, no. 1, (2021), pp. 240-266.
- [15] L. J. Kvist, B. W. Larsson, M. L. Hall-Lord, A. Steen, and C. Schalén, "The role of bacteria in lactational mastitis and some considerations of the use of antibiotic treatment", International Breastfeeding Journal, vol. 3, no. 1, (2008), pp. 6.
- [16] C. Azevedo, D. Pacheco, L. Soares, R. Romão, M. Moitoso, J. Maldonado, R. Guix, and J. Simões, "Prevalence of contagious and environmental mastitis-causing bacteria in bulk tank milk and its relationships with milking practices of dairy cattle herds in São Miguel Island (Azores)", Tropical Animal Health and Production, vol. 48, no. 2, (2016), pp. 451-459.
- [17] H. W. Barkema, M. J. Green, A. J. Bradley, and R. N. Zadoks, "Invited review: The role of contagious disease in udder health", Journal of Dairy Science, vol. 92, no. 10, (2009), pp. 4717-4729.
- [18] S. M. McGuirk, "Disease management of dairy calves and heifers", Veterinary Clinics of North America: Food Animal Practice, vol. 24, no. 1, (2008), pp. 139-153.

[19] M. P. Patil, A. S. Nagvekar, S. D. Ingole, S. V. Bharucha, and V. T. Palve, "Somatic cell count and alkaline phosphatase activity in milk for evaluation of mastitis in buffalo", Veterinary World, vol. 8, no. 3, (2015), pp. 363-366.

- [20] N. Namira, A. I. Cahyadi, and S. Windria, "Comparison of Subclinical Mastitis Detection Methods: A Literature Review", Acta Veterinaria Indonesiana, vol. 10, no. 1, (2022), pp. 39-50.
- [21] S. A. Kandeel, D. E. Morin, C. D. Calloway, and P. D. Constable, "Association of California Mastitis Test Scores with Intramammary Infection Status in Lactating Dairy Cows Admitted to a Veterinary Teaching Hospital", Journal of Veterinary Internal Medicine, vol, 32, no. 1, (2018), pp. 497-505.
- [22] R. N. Zadoks, J. R. Middleton, S. McDougall, J. Katholm, and Y. H. Schukken, "Molecular Epidemiology of Mastitis Pathogens of Dairy Cattle and Comparative Relevance to Humans", Journal of Mammary Gland Biology and Neoplasia, vol. 16, no. 1, (2011), pp. 357-372.
- [23] J. E. Hillerton and E. A. Berry, "Treating mastitis in the cow a tradition or an archaism", Journal of Applied Microbiology, vol. 98, no. 6, (2005), pp. 1250-1255.
- [24] F. Zigo, Z. Farkašová, J. Výrostková, I. Regecová, S. Ondrašovičová, M. Vargová, N. Sasáková, E. Pecka-Kielb, Š. Bursová, and D. S. Kiss, "Dairy Cow's Udder Pathogens and Occurrence of Virulence Factors in Staphylococci", Animals, vol. 12, no. 4, (2022), pp. 470.
- [25] D. B. Goulart and M. Mellata, "Escherichia coli Mastitis in Dairy Cattle: Etiology, Diagnosis, and Treatment Challenges", Frontiers in Microbiology, vol. 13, no. 1, (2022), pp. 928346.
- [26] N. Mukhamadieva, M. Julanov, D. Zainettinova, V. Stefanik, Z. Nurzhumanova, A. Mukataev, and A. Suychinov, "Prevalence, Diagnosis and Improving the Effectiveness of Therapy of Mastitis in Cows of Dairy Farms in East Kazakhstan", Veterinary Sciences, vol. 9, no. 1, (2022), pp. 398.
- [27] J. Owusu-Kwarteng, F. Akabanda, D. Agyei, and L. Jespersen, "Microbial Safety of Milk Production and Fermented Dairy Products in Africa", Microorganisms, vol. 8, no. 5, (2020), pp. 752.
- [28] J. D. Rust, M. J. Christian, C. J. Vance, M. B. Bolajoko, J. T. Wong, J. Suarez-Martinez, F. K. Allan, and A. R. Peters, "A study of the effectiveness of a detergent-based California mastitis test (CMT), using Ethiopian and Nigerian domestic detergents, for the detection of high somatic cell counts in milk and their reliability compared to the commercial UK CMT", Gates Open Research, vol. 5, no. 1, (2023), pp. 146.
- [29] S. Pegolo, D. Giannuzzi, V. Bisutti, R. Tessari, M. E. Gelain, L. Gallo, S. Schiavon, F. Tagliapietra, E. Trevisi, P. A. Marsan, G. Bittante, and A. Cecchinato, "Associations between differential somatic cell count and milk yield, quality, and technological characteristics in Holstein cows", Journal of Dairy Science, vol. 104, no. 4, (2021), pp. 4822-4836.
- [30] C. Tommasoni, E. Fiore, A. Lisuzzo, and M. Gianesella, "Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives", Animals (Basel), vol. 13, no. 15, (2023), pp. 2538.

[31] D. K. Swain, M. S. Kushwah, M. Kaur, and A. K. Dang, "Neutrophil dynamics in the blood and milk of crossbred cows naturally infected with Staphylococcus aureus", Veterinary World, vol. 8, no. 3, (2015), pp. 336-345.

- [32] N. Belay, N. Mohammed, and W. Seyoum, "Bovine Mastitis: Prevalence, Risk Factors, and Bacterial Pathogens Isolated in Lactating Cows in Gamo Zone, Southern Ethiopia", Veterinary Medicine (Auckland), vol. 13, no. 1, (2022), pp. 9-19.
- [33] R. Antanaitis, V. Juozaitienė, V. Jonike, W. Baumgartner, and A. Paulauskas, "Milk Lactose as a Biomarker of Subclinical Mastitis in Dairy Cows", Animals (Basel), vol. 11, no. 6, (2021), pp. 1736.
- [34] O. K. Dego, P. A. Pacha, B. E. Gillespie, and G. M. Pighetti, "Experimental Staphylococcus aureus Mastitis Infection Model by Teat Dipping in Bacterial Culture Suspension in Dairy Cows", Animals (Basel), vol. 10, no. 5, (2020), pp. 751.
- [35] A. S. Neculai-Valeanu and A. M. Ariton, "Udder Health Monitoring for Prevention of Bovine Mastitis and Improvement of Milk Quality", Bioengineering (Basel), vol. 9, no.11, (2022), pp. 608.
- [36] T. Zeryehun and G. Abera, "Prevalence and Bacterial Isolates of Mastitis in Dairy Farms in Selected Districts of Eastern Harrarghe Zone, Eastern Ethiopia", Journal of Veterinary Medicine, vol. 2017, no. 1, (2017), pp. 6498618.
- [37] M. E. Alnakip, M. Quintela-Baluja, K. Böhme, I. Fernández-No, S. Caamaño-Antelo, P. Calo-Mata, and J. Barros-Velázquez, "The Immunology of Mammary Gland of Dairy Ruminants between Healthy and Inflammatory Conditions", Journal of Veterinary Medicine, vol. 2014, no. 1, (2014), pp. 659801.
- [38] K. Sato, P. C. Bartlett, L. Alban, J. F. Agger, and H. Houe, "Managerial and environmental determinants of clinical mastitis in Danish dairy herds", Acta Veterinaria Scandinavica, vol. 50, no. 1, (2008), pp. 4.
- [39] W. J. Underwood, R. Blauwiekel, M. L. Delano, R. Gillesby, S. A. Mischler, and A. Schoell, "Biology and Diseases of Ruminants (Sheep, Goats, and Cattle)", Laboratory Animal Medicine, vol. 2015, no. 1, (2015), pp. 623-694.
- [40] J. Nitz, N. Wente, Y. Zhang, D. Klocke, M. T. Seeth, and V. Krömker, "Dry Period or Early Lactation-Time of Onset and Associated Risk Factors for Intramammary Infections in Dairy Cows", Pathogens, vol. 10, no. 2, (2021), pp. 224.
- [41] T. Miyama, J. Byaruhanga, I. Okamura, H. Nagahata, R. Murata, W. Mwebembezi, Y. Muramatsu, and K. Makita, "Prevalence of sub-clinical mastitis and its association with milking practices in an intensive dairy production region of Uganda", The Journal of Veterinary Medical Science, vol. 82, no. 4, (2020), pp. 488-493.
- [42] A. Guha, S. Gera, and A. Sharma, "Evaluation of Milk Trace Elements, Lactate Dehydrogenase, Alkaline Phosphatase and Aspartate Aminotransferase Activity of Subclinical Mastitis as and Indicator of Subclinical Mastitis in Riverine Buffalo (Bubalus bubalis)", Asian-Australasian Journal of Animal Sciences, vol. 25, no. 3, (2012), pp. 353-60.

[43] W. T. Yang, C. Y. Ke, W. T. Wu, R. P. Lee, and Y. H. Tseng, "Effective Treatment of Bovine Mastitis with Intramammary Infusion of Angelica dahurica and Rheum officinale Extracts", Evidence-Based Complementary and Alternative Medicine, vol. 2019, no. 1, (2019), pp. 7242705.

- [44] S. Pyörälä, "Treatment of mastitis during lactation", Irish Veterinary Journal, vol. 62, no. 4, (2009), pp. 40-44.
- [45] X. Li, C. Xu, B. Liang, J. P. Kastelic, B. Han, X. Tong, and J. Gao, "Alternatives to antibiotics for treatment of mastitis in dairy cows", Frontiers in Veterinary Science, vol. 10, no. 1, (2023), pp. 1160350.
- [46] S. Sachi, J. Ferdous, M. H. Sikder, and S. M. A. K. Hussani, "Antibiotic residues in milk: Past, present, and future", Journal of Advanced Veterinary and Animal Research, vol. 6, no. 3, (2019), pp. 315-332.
- [47] Ł. Kaczorowski, J. Powierska-Czarny, Ł. Wolko, A. Piotrowska-Cyplik, P. Cyplik, and J. Czarny, "The Influence of Bacteria Causing Subclinical Mastitis on the Structure of the Cow's Milk Microbiome", Molecules, vol. 27, no. 6, (2022), pp. 1829.