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CLINICAL SYMPTOM ANALYSIS OF CHARACTERISTIC BACTERIA CAUSING SUBCLINICAL MASTITIS IN DAIRY COW AT PENGALENGAN, BANDUNG REGENCY

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ABSTRACT

Mastitis is an udder infection that affects many dairy farms in Indonesia. Many causes can be identified to see the etiology. This study was conducted to determine the bacteria that causes mastitis and attacks dairy farms in the Bandung Regency Region. Based on the results of isolation and identification by examination of Blood Agar Media (BAP), Catalase Test, Coagulase Test, Gram staining, and DNAse Test of milk samples taken from three farm areas in Warnasari and Babakan Kiara in Pengalengan District and Citawa District Kertasari District, the bacteria that cause mastitis are Staphylococcus aureus COPS *and* CONS, Staphylococcus pyogenes, Staphylococcus epidermidis, *and* Staphylococcus saprophytes. *The characteristics of Clinical symptoms analysis from the bacteria causing subclinical Mastitis as seen as udder redness, swelling, and fever. There are tissue necrosis in the nipple and part of the udder. The milk parenchyma becomes fribillated, and the milk produced becomes mixed with blood, drips continuously, and appears watery in the presence of flakes, clots, and mucus. Implementation Early, prompt, and accurate diagnostic examinations must be carried out to prevent the occurrence of subclinical mastitis so that the objective can benefit the farmers for the future livelihood of the farm.*

Keywords: *Mastitis, Staphylococcus, Hemolysis, Examination, Characteristics*

INTRODUCTION

Mastitis, or inflammation of the udder, is a disease that often affects cattle farms, especially dairy cows. The incidence of sub-clinical mastitis in dairy farms in Indonesia is up to 68.18% (Khasanah et al. 2021). In this level of subclinical mastitis disorder, farmers still do not realize the consequences that will be caused later. This is because the clinical symptoms caused by the causative agent have not yet appeared (hal 1). Some of the bacteria that often cause mastitis in dairy cows include Staphylococcus spp., Streptococcus spp., Escherichia coli, Bacillus spp., Corynebacterium spp., Corynebacterium bovis, Pseudomonas aeruginosa, Klebsiella pneumonia, and Actinomyces spp (DEMİRBİLEK 2020). Among the various causative agents of mastitis in dairy cows, the main cause of mastitis is caused by the Staphylococcus spp. and Streptococcus spp. bacterial groups, whose prevalence reaches more than 52.50% (Riyanto et al. 2017).

As a result of this subclinical infection of Staphylococcus aureus bacteria in dairy cows, there are no visible symptoms. This is because subclinical infection of the bacteria only results in changes in the quality of milk produced. In general, the quality of milk produced due to this infection appears to decrease. The parameters used to see changes in milk quality are the CMT test, pH, number of somatic cells, fat, and protein that decrease in quality (Ali et al. 2022). Several factors can influence subclinical mastitis infection in dairy cows. These factors vary greatly depending on the conditions of dairy farm management. Some of these factors include

cow age, where the older the cow, the more susceptible it is to subclinical mastitis infection. Older cows have a loose nipple spinchter, allowing microorganisms to easily enter and cause infection. Next is milk production, where the higher the milk production of a cow, the longer it takes for the nipple spinchter to close completely, which can increase the risk of subclinical mastitis infection. Another influencing factor is the breed of cow, where crossbreeds have a greater risk than local cows (Hal 2).

Environmental and management factors are other factors that strongly influence the incidence of mastitis. Components of these factors, such as feed, housing, number of cattle in one pen, ventilation, pen sanitation, and milking methods, can also affect the incidence of subclinical mastitis in dairy cows (Amri et al. 2020). To identify the cause of mastitis that appears in dairy cows, isolation and identification of the characters that cause mastitis are needed. What bacteria cause the infection, so that subsequently the characteristics of the cause will be obtained, which will give rise to the characteristics of clinical symptoms that can later be seen after the subclinical infection turns into a clinical infection.

METHOD

Isolation and identification of milk samples taken from dairy farms have been carried out. A total of 48 milk samples were collected from smallholder cattle farms belonging to the South Bandung Dairy Farming Cooperative (KPBS) in Pengalengan. The milk collected was taken from three areas, namely Warnasari and Babakan Kiara areas in Pengalengan sub-district and Citawa area in Kertasari sub-district, Bandung district. Each region took 16 milk samples. Furthermore, milk samples were sent and examined at the Microbiology Laboratory of the Faculty of Medicine, Padjadjaran University.

The milk taken was screened using the CMT test, so milk samples that were positive for 2 and 3 were grouped as research samples. Furthermore, the research samples were isolated and identified. Selective media and tests carried out are Blood Agar Media (BAP), Catalase Test, Coagulase Test, Gram staining, and DNAse Test. A detailed examination and test results can be seen in Table 1.

RESULTS AND DISCUSSION

Based on the results of the laboratory examination, it was found that the bacteria causing subclinical mastitis in dairy cows were gram-positive bacteria in as many as 46 samples, while the isolates did not grow in as many as 2 samples. Furthermore, isolates that had beta (β) hemolysis had 25 samples and gamma hemolysis (γ) had 21 samples. Catalase positive was 45 samples, catalase negative was 3 samples, coagulase positive was 24 samples, and coagulase negative was 24 samples, while DNAse positive was 12 samples and negative was 34 samples.

Figure 1. Positive Catalase Test Results One of the samples (personal document)

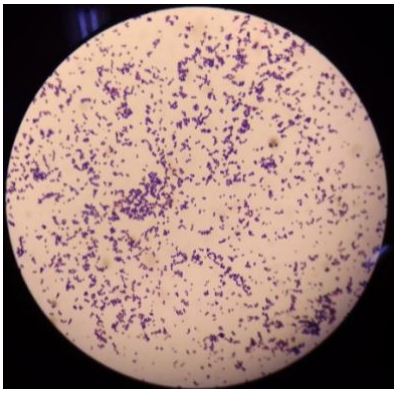

Figure 2. Gram staining results, Staphylococcus aureus bacteria samples (Personal documents)

Table 1. Observation Data Of Dairy Milk Samples Of Kpbs Members In Warnasari Area, Babakan Kiara Pengalengan Subdistrict And Citawa Area, Kertasari Subdistrict, Bandung Regency

No.	Milk Sampling	BAP	Gram Staining	Katalase Test	Koagulase Test	DNAse Test
1.	**Warnasari**					
	W1	γ	+	+	-	-
	W2	γ	+	-	-	-
	W3	β	+	+	-	-
	W4	β	+	+	+	+
	W5	γ	+	+	-	-
	W6	β	+	+	-	-
	W7	γ	+	+	+	-
	W8	β	+	+	+	+
	W9	γ	+	+	+	-
	W10	β	+	+	-	-
	W11	γ	+	+	+	-
	W12	γ	+	+	+	-
	W13	γ	+	+	-	-
	W14	γ	+	+	+	-
	W15	γ	+	+	-	-
	W16	β	+	+	-	-
2.	**Babakan Kiara**					
	B1	β	+	+	+	-
	B2	-	-	-	-	-
	B3	γ	+	+	-	-
	B4	γ	+	+	+	-
	B5	γ	+	+	-	+
	B6	β	+	+	-	+
	B7	γ	+	+	-	+
	B8	γ	+	+	+	-
	B9	β	+	+	+	-
	B10	γ	+	+	+	-
	B11	β	+	+	+	+
	B12	γ	+	+	-	-
	B13	β	+	+	+	-
	B14	β	+	+	+	-
	B15	β	+	+	+	+
	B16	β	+	+	+	-
3.	**Citawa**					
	C1	γ	+	+	+	-
	C2	-	+	+	-	-

C3	β	+	+	+	-
C4	β	+	+	-	-
C5	γ	+	+	-	+
C6	β	+	+	-	+
C7	β	+	+	-	+
C8	β	+	+	-	-
C9	-	+	-	+	-
C10	γ	+	+	-	-
C11	β	+	+	+	+
C12	β	+	+	-	-
C13	β	+	+	+	-
C14	β	-	-	-	-
C15	β	+	+	+	+
C16	γ	+	+	-	-

Figure 3. Beta Hemolysis testing results of milk samples (personal documents)

Figure 4. Positive Coagulase Test results of milk samples (Personal documents)

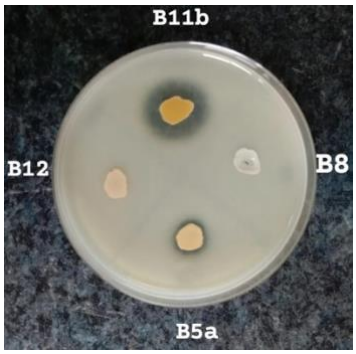

Figure 5. DNAse test results of positive (+) and negative (-) milk samples (Personal documents)

From the observations made, the data obtained showed that gram-positive bacteria, coccus, beta hemolysis, DNAse (+), coagulase (+), and positive catalase are Staphylococcus aureus COPS (Foster 1996), while gram-positive bacteria, coccus, beta hemolysis, negative coagulase is Staphylococcus aureus CONS, and gram-positive bacteria, coccus, beta hemolysis, negative catalase is Staphylococcus pyogenes (Rai et al. 2017). Gram-positive bacteria with gamma hemolysis that are DNAse positive are Staphylococcus epidermidis and Staphylococcus. Saprophyticus (Syamsu et al. 2023). These groups of bacteria usually invade the udder, causing mastitis, but can also invade the skin and urinary tract tissues ((Rai et al. 2017) (Bookshelf 2020).

Discussion

A. Characteristic Clinical Symptom based on the Laboratory Result

Based on the results of laboratory examinations and testing of hemolysis factors, catalase, coagulase, gram staining, and DNAse factors on subclinical mastitis milk samples taken from smallholder farms in the Warnasari area, Babakan Kiara Pengalengan District, and Citawa Area Kertasari District, Bandung Regency, the analysis of the clinical symptom characteristics the bacteria causing subclinical mastitis, can be described as follows as below:

1. Haemolysis Factor

Hemolysis factors are virulence factors possessed by the Staphylococcus aureus group of bacteria. Usually, the hemolysis factors possessed are alpha, beta, and gamma hemolysis (Boerlin et al. 2003). β-hemolytic hemolysin is a virulence factor called complete hemolysis. Alpha hemolytic hemolysin is called an incomplete virulence factor, while gamma hemolysis is called a virulence factor without hemolysis. The genes encoding these virulence factors are hla, hlb, and hlc and can be detected using qRT-PCR (Zang et al., 2016). The character of Staphylococcus aureus bacteria that cause mastitis, which have virulence factors that can lyse red blood cells, shows that these bacteria have considerable destructive abilities. Alpha-haemolysin, or alpha-toxin, is considered the main pathogenic factor due to its hemolytic, dermonecrotic, and neurotoxic effects. While beta-haemolysin contains sphingomyelinase, which is more active against sheep and bovine erythrocytes (Ariyanti, Salasia, and Tato 2011), One way hemolysin lyses erythrocytes is by forming pores and hydrolyzing the phospholipid bilayer. The presence of hemolysin factors will cause a decrease in wall stability, leading to blood cell division (Uertes et al., 2010).

Particularly in alpha hemolysin, this virulence factor is also responsible for osmotic phenomena, cell depolarization, and the loss of important molecules such as ATP, which ultimately leads to cell death or necrosis. Structure Heptamer hemolysin is shaped like a mushroom and measures up to 100 Å in diameter and 100 Å in height. On the outside of the 14-stranded antiparallel β-barrel, there is a hydrophobic belt about 30 Å wide that provides a surface complementary to the nonpolar part of the lipid bilayer. The interfacial bonds consist of salt bonds and hydrogen bonds, as well as hydrophobic interactions, and these contacts provide molecular stability for the heptamer in SDS solution even up to 65°C (Chalmeau et al. 2011). Red blood cells are rich in iron-containing heme. The lysis of these red blood cells will release iron-rich hemes into the environment, which allows bacteria to take up the free iron (Shritaran, 2006; (Griffiths and McClain 1988)). Thus,

the clinical symptoms of mastitis that appear due to this hemolysin factor are the dripping of reddish milk or red mucus during milking, followed by clinical symptoms such as necrosis (Plummer and Plummer 2012). The number of red blood cells in milk may be so large that they give the milk a pink color and cause sediment. However, the number of red blood cells in the milk may be quite low, and the milk may have only a slight reddish or pinkish color. As a result of hyperemia in the udder, udder tissue becomes very red evenly, and extravasation of blood into the interstitium can be seen microscopically (Muhammad and Rashid 2015).

Although it does not cause cell lysis, β-hemolysin is a sphingomyelinase that is highly active against bovine erythrocytes, especially if there is an interaction between α and β hemolysin, so that it can increase attachment to epithelial cells and proliferation of Staphylococcus aureus through damage to the epithelium and subcellular matrix, which increases the potential for chronic mastitis. The destruction of sphingomyelin will increase the permeability of the plasma membrane by progressively lowering the negative pressure on the cell surface, thus facilitating bacterial cell attachment. It is important to note that this toxin is quite stable at high temperatures, making it an important public health issue given the large human consumption of milk and dairy products. For people who work directly on farms infected with mastitis, such as veterinarians and farmers, it can cause pulmonary odema and necrotizing lesions on the skin (Pérez et al. 2020).

2. Catalase Test

A catalase test is a test to be able to distinguish between bacteria that produce the enzyme catalase and those that do not. The Staphylococcus spp bacterial group is a group of bacteria that have catalase enzymes while the Streptococcus spp bacterial group does not have catalase enzymes. This enzyme works by breaking down hydrogen peroxide (H2O2) into water and oxygen (SVA, National Veterinary Institute). The main function of catalase in the cell is to prevent the accumulation of toxic levels of hydrogen peroxide formed as a by-product of tissue metabolic processes, especially the electron transport pathway. Host white blood cells can produce H2O2, and this H2O2, when reacted with the catalase enzyme produced by Staphylococcus aureus, can be easily degraded into H2O and O2 (Sidiq, 2014). Intramammary infection in this species affects the biochemical composition of milk and oxidative stress markers. Indicators Intramammary infections such as mastitis impact the activity of antioxidants such as catalase, lactoperoxidase, or glutathione peroxidase.

Accumulation of H202 in tissues can be harmful to organisms, including Staphylococcus aureus. For the host, certain amounts of H202 become highly beneficial due to its involvement in certain physiological processes, such as unsaturated fatty acid metabolism, phagocytosis, or immune responses. Catalase is an oxidoreductase that catalyzes the degradation of hydrogen peroxide with the formation of water and oxygen. Its activity depends on several factors, and one of them is the presence of an intramammary infection. In raw cow's milk, the average catalase activity is 1.96 U/mL, but there are variations in this value that are highly dependent on diet, lactation stage, or the development of an inflammatory process. In healthy goat milk, the catalase activity value is 3.8 U/mL, higher than the value set for cows (Novac and Andrei 2020). The

clinical symptoms of mastitis that appear when associated with the role of this catalase enzyme are a red udder, swelling, fever, dripping cow's milk, and a and a watery appearance with the presence of flakes and clots (Cheng and Han 2020).

3. **Coagulase Test**

The coagulase test is used to determine which mastitis-causing organisms form fibrin and which do not. Coagulase-positive bacteria, such as Staphylococcus aureus, cause plasma to form clots, while coagulase-negative bacteria, such as Staphylococcus epidermidis, do not form clots. There are two types of coagulases produced by most Staphylococcus aureus strains: bound coagulase (clumping factor) and free coagulase. Bound coagulase attaches to the bacterial cell wall and can enzymatically convert fibrinogen in plasma into insoluble fibrin and cause bacterial cells to clump. Free coagulase reacts with plasma factor globulin (coagulase reaction factor) to form staphylothrombin. Staphylotrombin then catalyzes the breakdown of fibrinogen into insoluble fibrin (Chamberlain 2009). Coagulase positive Staphylococcus aureus is highly associated with the production of classical enterotoxins such as alpha, beta, and gamma hemolysin (Rajic-Savic et al. 2015). These enterotoxins become very important in relation to food-borne diseases. Coagulase positive Staphylococcus aureus, on average, affects many dairy cows two months after calving, and the incidence can reach 43% (Roberton et al., 1994). According to (Cook 2022), enterotoxins in Staphylococcus aureus are soluble in water, stable to heat, and not destroyed at low pH. Therefore, entrotoxins can still maintain their biological activity even after heating treatment (e.g., pasteurization) and after digestion in the gastrointestinal tract, despite the presence of proteolytic enzymes such as pepsin and trypsin. In humans, infection can cause severe nausea and vomiting accompanied by abdominal cramps, diarrhea, or fever. These enterotoxins not only act as potent gastrointestinal toxins but also as superantigens. If cltotting occurs, then the frequent symptoms of mastitis associated with coagulase enzyme performance are moderate swelling, and the udder is hard, heavy, reddened, and warm to the touch. Cows generally show signs of discomfort, making frequent footsteps and/or kicks during the evaluation. Remarkably, superantigens are thought to develop the capacity to stimulate the entire bovine immune response through superantigen diversity, which suggests an important role in host immune evasion (Wilson et al. 2018).

4. **DNAse Test**

Staphylococcus aureus bacteria could produce the enzyme DNase. This enzyme can hydrolyze nucleic acids in the DNase medium, which is indicated by the appearance of a colorless zone around the colony (Pumipuntu et al. 2017). DNase is an enzyme that can cleave host cell DNA molecules, which will increase inflammation and tissue sepsis. During mastitis infection, the integrity of the BMB (Blood Milk Barrier) is reduced and breaks the gradation between blood and milk, leading to the transfer of immune cells such as leukocytes from blood to milk and causing the loss of milk constituents in the blood circulation. The presence of bacteria invading the mammary gland is shown to open the BMB. Damage to the BMB can lead to an increase in the degree of bacterial infection and promote the development of even greater inflammation (Jingjing et al. 2021).

5. **Gram Staining**

From the results of observations on gram staining, it was found that the bacteria that cause mastitis in the canning area are caused by gram-positive coccus-shaped bacteria that lead to Staphylococcus aureus bacteria. This is supported by the results of bacterial identification testing using the catalase test, coagulase test, and DNAse test. Specificity examination using this gram staining method can reach 86%. The examination will give more specific and sensitive results on milk samples if the samples used are centrifuged first so that the protein and fat will be separated while the bacteria are precipitated under the tube (Suzuki, Kaneko, and Isobe 2022). In addition to the centrifugation method, there is also a Fourier-transform infrared (FTIR) spectroscopy and MALDI-TTOF/MS method developed by Lydia et al. (2014). According to (Becerra et al. 2016), the modification of Gram staining that was carried out was by adding a few minutes to the mechanism of the Gram staining technique of the host tissue clinical biopsy sample for just a few minutes so that it could produce a cost- and time-effective technique for identifying bacteria in clinical biopsies even though they contained connective tissue without having to isolate and identify them first.

B. The Effectivenes Mastitis Management for farmer

Implementation of therapies for mastitis infections in dairy cows, such as antibiotics, immunotherapy, bacteriosin, bacteriofagus, antimicrobial peptides, probiotics, stem cell therapy, natural secretory factors, nutritional improvement, dry and lactation cows therapy, genetic selection, herbal, and nanoparticle-based technology therapy, has been evaluated to see their effectiveness. Although several strategies have been developed over the years to deal with the clinical and subclinical forms of mastitis, they all still do not provide maximum results to eliminate the etiological agents of such mastitis when used as monotherapy. All the things mentioned must be done at the same time as good farm management. According to (Sharun et al. 2021), a diagnosis of mastitis is a primary requirement in parrot farming to produce clean milk, not only for economic and public health reasons but also related to animal welfare. Diagnosis should be made early, prompt, and accurate for mastitis prevention or early detection of mastitis for effective enforcement or therapeutic purposes. Some of the things to note are how the application of conventional and advanced diagnostic methods can be applied to people's farms or small-scale farms. Conventional methods are relatively inexpensive, easy, quickly available, and can be implemented in the field, but they are usually not very specific, while advanced diagnostics are usually more expensive and require more trained technical skills as well as sophisticated infrastructure and facilities, so the results obtained are more accurate and specific to diagnose various forms of subclinical mastitis.

The dairy farmers at the Pengalengan Territory are mostly small farmers with an average of three to five productive females and an average experience of more than 10 years. The greater the experience of the farmers, the better the knowledge and skills in the handling of pears (Markham, Firman, and Hermawan 2024), but not with an understanding of the control of mastitis. The character of the folk farm in the Pengalengan region is a small farm with a cage behind the house and a few lawns; it does not even have grassland at all. The amount of labor could only be filled by the core family members, consisting of parents and children as employees (cattle workers), and the milk marketing pattern produced directly related to the cooperative (Mauludin 2017).

The milk production capacity of pearly cows on peasant farms is also heavily influenced by environmental factors. Around 70% of environmental factors influence milk production. These factors include external and internal factors. External environment factors consist of climate, feeding, and maintenance management, while internal environment factors include the quality and quantity of feeding and biological aspects such as lactation length, cage dryness, empty periods, and calving intervals (Suprayogi, Ihsan, and Ruhyana 2019).

Such farm conditions make the quality of produced milk still have an average Total Plate Count (TPC) higher than the national standard of Indonesia. (SNI). This situation is directly related to the level of cage hygiene, cattle maintenance, and milk disposal techniques, as well as the handling of fresh milk from cooking to delivery to the milk storage cooperative. The facts in the field indicate that the "clean and healthy" cultural performance of the people's pearly cattle farms in Pengalengan is still low and still needs to be improved (Ariningsih, Purwantini, and Irawan 2022). The high TPC indicates that there is still a high level of microbial contamination during decay. Various factors can be the source of damage, such as the equipment, the process, the farmer himself, and other factors. (Waskita 2020). Hygienic cleaning of beef cattle, disposal equipment, farmers, and cages is key to preventing the contamination of fresh cow's milk by microbes. (Ariningsih, Purwantini, and Irawan 2022), in addition to the accurate diagnosis of mastitis that is common in beef farms. Once all the components have been observed by the farmer, subclinical mastitis infections can be controlled, the quantity of milk produced increases with better quality, and of course, this provides a lot of benefit to the farmers for the survival of their farms in the coming years.

CONCLUSION

Based on the results of the isolation and identification of milk samples taken from the regions of Warnasari, Babakan Kiara Pengalengan, and Citawa Kertasari district in Bandung district, we can conclude that the main causes of mastitis in cattle are the bacteria Staphylococcus aureus COPS and CONS, Staphylacoccus pyogenes, Staphenococcum epidermidis, and Staphylloccus saprophytes. The clinical symptoms of mastitis that appear when associated with the identification carried out by the bacteria causing mastitis range from stings to redness, swelling, and fever. There is necrosis of tissue on the putting and a partial sting. The milk was mixed with blood, continually dropling and appearing to be broken, with crumbs, and with mucus. Implementation Early, prompt, and accurate diagnostic examinations must be carried out to prevent the occurrence of subclinical mastitis so that the objective can benefit the farmers for the future livelihood of the farm.

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