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DETECTION OF *STAPHYLOCOCCUS AUREUS* CAUSING SUBCLINICAL MASTITIS IN SOW FARMS IN PUHU, PAYANGAN, GIANYAR

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Abstract

Subclinical mastitis is an inflammatory disease of the nipple or udder caused by bacterial infection, including *Staphylococcus aureus*, and occurs without obvious clinical symptoms. This study aims to determine the quality of sow milk and detect the presence of *Staphylococcus aureus* in sow milk samples during lactation. Sow milk samples were tested using the California Mastitis Test (CMT) method to identify the presence of subclinical mastitis, and samples positive for subclinical mastitis were grown on Mannitol Salt Agar (MSA) media for isolation. Afterwards, the bacterial isolates were subjected to microscopic examination as additional methods to confirm the characteristics and species of bacteria. The results proved that 29 out of 34 samples tested were positive for subclinical mastitis and the presence of *Staphylococcus aureus* as the main pathogen was detected. Subclinical mastitis is still commonly found in Puhu Village with *Staphylococcus aureus* being the main cause, which can lead to decreased milk production and impaired health of the sows. Regular check-ups, udder health surveillance and good hygiene management are needed to monitor and prevent the spread of subclinical mastitis affecting sows in lactation.

Keywords: *Staphylococcus aureus*, subclinical mastitis, sow

INTRODUCTION

Mastitis is an inflammatory disease of the udder caused by bacteria. Bacteria will infect wounds in the udder area, one of the bacteria that can cause mastitis is *Staphylococcus aureus* (Windria et al., 2022). Based on its clinical symptoms, mastitis is divided into two, namely clinical mastitis and subclinical mastitis. Clinical mastitis shows symptoms in milk changes, while subclinical mastitis does not show symptoms and changes in milk (Sevitasari et al., 2019). The California Mastitis Test (CMT) is a simple test method to detect early symptoms of subclinical mastitis (Afrilia et al., 2021).

Staphylococcus aureus is normal flora in the upper respiratory tract and skin of pigs, but can also be a pathogen that causes mastitis (Purwanti et al., 2018). *Staphylococcus aureus* will enter the udder through the nipple, especially if the udder has a wound. After entering, it will form a layer that protects the bacteria from the host immune response and antibiotic therapy so that the infection becomes difficult to treat (Lukowska et al., 2022). In addition, *Staphylococcus*

aureus also produces enzymes and toxins that cause inflammation without obvious clinical symptoms, but still reduce the quality and quantity of milk production (Paryati, 2010).

Running a pig farm must be accompanied by a good maintenance system with the aim of providing comfort to pigs which will affect growth and productivity (Luju et al., 2023). Maintenance systems that can reduce the risk of mastitis transmission include replacing sows routinely, providing separate pens for sows, making separate entrances for pregnant sows, implementing a quarantine system, slaughtering pigs in slaughterhouses, drinking water from safe sources, and having clear boundaries for everyone who comes to the barn (Bulu et al., 2020). To treat pigs affected by mastitis, antibiotics can be given. The CMT was conducted with the aim of detecting subclinical mastitis in pigs early because subclinical mastitis does not show symptoms in sows, which may make it difficult for farmers to recognize the presence of the disease in their pigs. And looking for the cause of subclinical mastitis can attack sows in lactation without any symptoms appearing (Elyusie and Purbaningsih, 2010).

MATERIALS AND METHODS

Ethical Code

This study was approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, under approval number: B / M. 146 / UN14.2.9 / PT.01.04 / 2024. All research procedures were conducted in accordance with applicable animal welfare guidelines and ethical standards.

Object of Research

The research object used lactating sow milk samples in pig farms in Puhu Village. Puhu has 150 sows in lactation, and this study used the slovin formula to determine the number of samples needed with the results of 34 samples.

Research Design

This study is an observational study with cross-sectional design using purposive sampling method on milk from sow in lactation period in Puhu Village, Payangan District, Gianyar Regency.

Research Variables

This study used milk from lactating sows in a pig farm in Puhu Village, Payangan sub-district, Gianyar Regency which was tested by CMT, bacterial growth on MSA media, and microscopic test.

Data Collection Methods

Samples were fresh milk directly milked from sows in lactation. Pig udders were cleaned with water and 70% alcohol. Milking is done using the five fingers of the hand then the milk that comes out first will be discarded and the milk that comes out after will be immediately collected on the CMT paddle for sample examination (Dermawan et al., 2023).

California Mastitis Test (CMT)

Testing of milk samples using the California Mastitis Test (CMT) reagent in a 1:1 ratio between CMT reagent and milk samples. The milk sample in the CMT paddle will be mixed with the CMT reagent and stirred for approximately 10 - 15 seconds. Reading the reaction results can be done in a bright place to see whether there are clots in the milk sample (Rust et al., 2023).

Sample Dilution

Dilution is done with the aim of reducing the number of bacteria in the milk sample to facilitate the counting of the number of bacterial colonies on the culture medium. If the milk sample is not diluted, the bacteria will cover the culture medium because it is too dense which can make the process of counting bacterial colonies difficult. Dilution was done in a ratio of 1:10 which means 1 ml of pig milk sample was diluted with 9 ml of sterile solvent.

Mannitol Salt Agar (MSA) Bacterial Isolation

MSA is a culture medium used for the isolation and identification of Gram-positive bacteria. MSA is used to identify bacteria that can ferment mannitol and to distinguish specific species within the genus *Staphylococcus*, such as *Staphylococcus aureus*. Inoculation of subclinical mastitis positive pig milk samples on MSA media using the streaking method to separate colonies. After that, MSA media will be incubated at 37 degrees for 24 - 48 hours.

Microscopic Examination

The shape is cocci and clustered to resemble grapes. With Gram staining, *Staphylococcus aureus* will look purple under a microscope. The color that appears is because the cell wall contains a lot of peptidoglycans which can retain crystal violet dye during the staining process. (Dewi, 2013).

Data analysis

Data analysis in this study was carried out by calculating the number of samples using the Slovin formula, which was chosen because it is able to provide a representative sample size of the population with the desired level of accuracy, given the large enough population and data variability that may exist. Data obtained from the CMT, agar media, microscopy, and biochemical tests were processed and presented in a descriptive narrative.

RESULTS AND DISCUSSION

Based on the theory of CMT for subclinical mastitis, it is known that there are different positive results according to the level. Positive 1 (+) there is a slight precipitate, positive 2 (+ +) clear precipitate but no gel clots, positive 3 (+ + +) there are visible gel clots, positive 4 (+ + + +) visible thick gel clots. Based on the CMT results from 34 pig milk samples, 5 milk samples were positive 2 (+ +), 21 samples were positive 3 (+ + +), and 3 milk samples were positive 4 (+ + + +). The results showed that 29 out of 34 pig milk samples were positive for subclinical mastitis with a rate reaching 85% of the total samples (Table 1).

Table 1. CMT Results of Pig Milk Samples During Lactation Period in Puhu Village

Sampling Location	Positive Results level			
	+	++	+++	++++
Banjar Semaon	-	-	12	3
Banjar Ponggang	-	-	8	-
Banjar Kebek	-	2	1	-
Banjar Puhu	-	3	-	-
Total	-	5	21	3
Percentage	-	15%	62%	8%

Based on the results of research that has been carried out on sow milk samples during the lactation period, bacterial isolates were found that show the typical characteristics of *Staphylococcus aureus*. Identification was carried out through the stages of bacterial isolation using Mannitol Salt Agar (MSA) selective media which is specialized for growing *Staphylococcus aureus*. Mannitol Salt Agar (MSA) is a selective and differential medium used

to grow and identify bacteria of the genus *Staphylococcus*, especially *Staphylococcus aureus*. This medium contains a high concentration of NaCl salt (7.5%) that only allows the growth of salt-resistant bacteria, such as *Staphylococcus aureus*. In addition, MSA contains mannitol as a carbohydrate source and phenol red pH indicator. *Staphylococcus aureus* is able to ferment mannitol to produce acid which causes a decrease in pH around the colony, changing the color of the indicator from red to yellow. Therefore, *Staphylococcus aureus* colonies on MSA usually appear yellow with a yellow zone around them, in contrast to coagulase-negative bacteria such as *Staphylococcus epidermidis* which do not ferment mannitol and therefore do not change the color of the media (Ayuti et al., 2023)

Staphylococcus aureus is able to grow on MSA due to its high salt tolerance and ability to ferment mannitol. Mannitol fermentation produces acids that lower the pH of the environment, causing the phenol red indicator to change color from pink (neutral pH) to yellow (acidic pH) (Arbi et al., 2019) (Figure 1).

Figure 1. *Staphylococcus aureus* on Mannitol Salt Agar

Staphylococcus aureus is a Gram-positive bacterium that has a thick cell wall, dominated by a peptidoglycan layer, so it is able to retain crystal violet dye during the Gram staining process and appears purple under a microscope. These bacteria are cocci, arranged in groups resembling strands of grapes. In addition, *Staphylococcus aureus* has the ability to survive in environments with high salt concentrations, is facultatively anaerobic, and is able to ferment mannitol. These characteristics support its ability to adapt to various environments and increase its potential as a major pathogenic agent. Understanding the biological properties of *Staphylococcus aureus* is important in detecting and controlling infections caused by this bacterium, including cases of subclinical mastitis in farm animals (Wardani et al., 2014) (Figure 2).

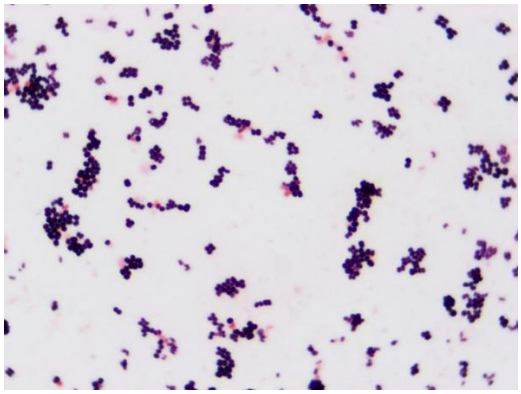

Figure 2. *Staphylococcus aureus* on a microscope at 1000x magnification

CONCLUSIONS AND SUGGESTIONS

Conclusions

The conclusion that can be drawn from the results of the research that has been done and the results of writing a scientific thesis is subclinical mastitis using the CMT. In Puhu Village in 2025 there were 150 sows in lactation period and studied with a total sample of 34 sows milk according to the slovin formula calculation of which 85% or 29 samples were positive for subclinical mastitis. The 29 subclinical positive samples were grown with bacteria on MSA media which resulted in a yellow color indicating that the bacteria were *Staphylococcus aureus*. In Gram staining the results are purple and clustered in the form of cocci when viewed under a microscope.

Suggestions

The advice that can be given to farmers is to always maintain the cleanliness of the cage and conduct routine CMT. If pigs have subclinical mastitis, NSAID drugs such as ketoprofen can be used.

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