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Detection of *Klebsiella* spp. Causing Subclinical Mastitis in Sows on Farms in Puhu Village, Gianyar, Bali

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Abstract. Subclinical mastitis is a disease that quite often affects sows during lactation without any apparent clinical symptoms, one of which is caused by *Klebsiella* Spp. The purpose of this study was to determine the quality of pig milk and whether *Klebsiella* spp. was detected in the pig milk. Thirty-four samples were tested using the California Mastitis Test (CMT) method, and samples positive for subclinical mastitis were further tested using MacConkey media. Additional tests were conducted, including the TSIA biochemical test and microscopic examinations of the growing bacterial isolates, to confirm the bacterial identity. The results of this study showed that 29 out of 34 positive samples had subclinical mastitis with different levels of severity. According to the laboratory results, the presence of Klebsiella spp (85%) bacteria was identified. The results of this study indicate that subclinical mastitis caused by *Klebsiella* spp remains a significant threat in pig farms in Puhu Village, Payangan Subdistrict, Gianyar Regency, particularly when cage sanitation and management are not optimal. Early detection and improved husbandry systems are needed to prevent similar incidents.

Keywords: subclinical mastitis; sow; *Klebsiella* spp.: California Mastitis Test

I. INTRODUCTION

Sow farming is one of the primary sources of livelihood in rural Bali, particularly in Gianyar Regency, where it plays a vital role not only in generating household income but also in fulfilling cultural and religious obligations. Pork is widely consumed and plays a crucial role in various traditional ceremonies, making pig production a strategic sector for ensuring food security and cultural continuity. As demand for pork continues to rise, the scale of pig farming in the region has expanded, resulting in increased populations of sows in lactation [1]. However, this intensification has also introduced new challenges, esppecially in herd health management [2].

One of the most significant health issues in pig farming is mastitis, an inflammatory condition of the mammary gland that can result in reduced milk production, economic losses, and increased piglet mortality. Mastitis is broadly categorized into clinical and subclinical types. While clinical mastitis is relatively easy to detect through obvious symptoms such as swelling, redness, and abnormal milk consistency, subclinical mastitis often goes unnoticed due to its asymptomatic nature. Despite the lack of visible signs, subclinical mastitis can have a severe impact on milk yield and quality, posing a significant threat to piglet survival, particularly during the first three days of life when they are highly dependent on colostrum and milk for nutrition and immune protection [3-5].

Subclinical mastitis is primarily caused by bacterial infections, with environmental bacteria being the most frequent culprits. Among them, *Klebsiella* Spp has been increasingly identified as a significant pathogen.

Klebsiella spp. is a Gram-negative bacterium commonly found in wet, unsanitary environments, such as dirty bedding, feces, and stagnant water. The bacterium can gain entry into the udder through the teat canal, where it invades mammary tissue, causing inflammation and disruption of milk synthesis. The pathogenicity of *Klebsiella* is further heightened by its ability to form a thick capsule that protects it from phagocytosis and enhances its resistance to various antibiotics, making infections difficult to treat [5-6].

In the context of pig farms in Payangan District, Gianyar Regency, several risk factors associated with the high prevalence of mastitis have been identified, including poor barn drainage, inadequate manure management, overcrowding, and lack of proper hygiene protocols. Field observations indicate that some pens experience waterlogging during cleaning or rainfall, leading to moist litter conditions that promote bacterial growth. Feces are often scattered and not collected in designated areas, which increases the risk of bacterial contact with the sow's udder. The situation is exacerbated by high piglet populations, which sometimes exceed 20 per sow, thereby increasing the potential for teat damage and bacterial invasion if not carefully managed [7].

Despite its high prevalence and impact, subclinical mastitis remains underdiagnosed in many small to medium-scale pig farms due to limited access to diagnostic tools. Therefore, the use of simple yet reliable methods such as the California Mastitis Test (CMT) is essential. CMT is a field-friendly diagnostic technique that detects increased somatic cell counts in milk, indicating inflammation. While it does not directly identify bacterial species, it serves as an effective screening tool before further laboratory investigation [8-9].

To identify the causative agent, further bacterial isolation and identification techniques are required. These include culturing on selective media such as MacConkey Agar, which is selective for Gram-negative and differential for lactose-fermenting bacteria, followed by Gram staining and biochemical analysis using Triple Sugar Iron Agar (TSIA). These methods help differentiate *Klebsiella* Spp. from other potential mastitis pathogens such as *Escherichia coli, Staphylococcus aureus, and Streptococcus Spp.,* which are also commonly found in both pig and dairy herds [10-11]

According to data from the Department of Agriculture and Food Security of Gianyar Regency in 2023, the total pig population in the region reached 39,836 heads, indicating the need for effective animal health surveillance to prevent outbreaks and reduce production losses. With the growing importance of pig farming in the region, efforts to improve animal welfare and disease control are crucial for long-term sustainability.

This study focuses on detecting the presence of *Klebsiella* Spp. as a causative agent of subclinical mastitis in lactating sows in Puhu Village, Payangan District, Gianyar Regency. The research involves CMT screening, microbiological isolation, and laboratory identification of bacterial pathogens. The objective is to establish a scientific foundation for early diagnosis, effective treatment, and prevention strategies against subclinical mastitis. Ultimately, the findings are expected to contribute to improved livestock productivity, enhanced piglet health, and better economic outcomes for local pig farmers.

II. METHODS

A. Research Object

The object of this study involved lactating sows at a pig farm located in Puhu Village, Payangan District, Gianyar Regency. The samples used were milk collected from 34 lactating sows.

B. Research Design

This study employed an observational design with a cross-sectional approach. The purposive sampling method was applied to detect the presence of bacteria causing subclinical mastitis in pig farms in Melinggih Village, Payangan District, Gianyar Regency.

Research Variables

The samples used in this study were milk collected from lactating sows. The quality of the milk was examined to detect potential subclinical mastitis infections. Indicators used included the presence of *Klebsiella* Spp., California Mastitis Test (CMT), microscopic examination, and biochemical analysis. All samples were taken from sows kept on a farm in Puhu Village, Payangan District, Gianyar Regency, to ensure environmental consistency. This study aimed to elucidate the relationship between the milk condition of sows and bacterial infections, and to serve as a reference for the prevention of subclinical mastitis.

C. Sample Collection

Milk samples were collected by manually milking the sows using aseptic techniques. The udder was cleaned with 70% alcohol to remove dirt and contaminants. Milk was then collected by gently pressing the base of the teat until milk was released into sterile containers. Samples were taken from each teat as needed to represent the udder's condition. After collection, the udder was cleaned again, and the sow was released. The samples were stored in a cool box to maintain quality until further analysis using the CMT method.

D. Slovin Formula

The number of milk samples to be calculated in this study uses the following Slovin formula:

$$n = \frac{N}{1 + N.e^2}$$

n = sample size required
N = total population
e = desired margin of error

The results of calculations using the Slovin formula with a total population of 150 lactating sows are as follows:

$$n = \frac{150}{1 + 150\ (0.15)^2} = \frac{150}{4.375} \approx 34$$

Based on the results of calculations using the Slovin formula with a 15% margin of error, the sample size for milk from lactating sows was determined to be 34 samples.

E. California Mastitis Test (CMT)

The CMT was used to detect subclinical mastitis in sow milk. Two milliliters of milk were mixed with two milliliters of CMT reagent on a paddle test. The mixture was gently swirled for 10–15 seconds and observed under bright light. Results were interpreted based on the formation of sediment or gel clots, which indicated the severity of the mastitis infection. A scoring scale ranging from -4 to +4 was used to categorize the results [12].

F. Sample Dilution

For bacterial isolation, milk samples were diluted using the serial dilution method. One milliliter of milk was mixed with 9 ml of sterile saline to produce a 1:10 dilution. This process could be repeated as needed, depending on the desired bacterial concentration. An aliquot of the diluted solution was inoculated onto culture media and incubated at 37°C for 24 hours. The purpose of dilution was to facilitate the isolation and identification of bacteria from the samples [13].

G. Bacterial Isolation on MacConkey Agar

Diluted samples were inoculated onto MacConkey Agar media. This medium is selective for Gram-negative bacteria and differential for lactose fermentation. Inoculation was done aseptically and incubated in an inverted position at 37°C for 48 hours. Bacterial colonies were observed based on color and morphology. For example, Escherichia coli appears as bright pink colonies, while *Klebsiella* Spp. forms pale pink mucoid colonies [14,11,15]

H. Microscopic Examination

Colonies of *Klebsiella* pneumoniae that grew were transferred to glass slides and prepared as thin smears. After drying, the slides were heat-fixed and stained using the Gram stain method. The staining result showed *Klebsiella* pneumoniae in pink, indicating Gram-negative bacteria. Under the microscope, *Klebsiella* pneumoniae appeared as rod-shaped bacilli, usually in pairs or clusters. This identification supported the culture results from the MacConkey media [16-20].

F. Data Analysis

Data analysis in this study was conducted using the Slovin formula to calculate the appropriate sample size. This method was chosen because it can provide a representative sample size from a large population with acceptable accuracy, eSppecially considering potential data variability. The results from the CMT test, agar media culture, microscopic examination, and biochemical tests were processed and presented using descriptive narrative analysis, detailing the findings of each examination.

III. RESULTS AND DISCUSSION

Result

Based on the analysis results, *Klebsiella* Spp. was identified as the primary cause of mastitis in pigs in Payangan District, Gianyar Regency. A total of 34 milk samples were collected from sows during the lactation period and tested using the CMT, a widely used screening method for detecting subclinical mastitis based on the increase in somatic cell count, which causes changes in milk viscosity [9], The CMT results indicated that 29 out of 34 samples (85%) were positive for subclinical mastitis. The distribution of positive results was as follows: 5 samples showed a grade 2 reaction (++), 21 samples showed a grade 3 reaction (+++), and 3 samples reached a grade 4 reaction (++++) based on the viscosity and gel formation scale used in the test [8]. These findings suggest a notably high prevalence of subclinical mastitis among the sow population in the observed farm [3]

To identify the bacterial cause, milk samples that tested positive for subclinical mastitis were cultured on selective media. The samples were first inoculated on EMBA to detect Escherichia coli, as mastitis can be caused by environmental pathogens such as E. coli and Klebsiella spp. that originate from dirty barn/flooring conditions. Both pathogens have very similar characteristics, which returned negative results. However, culturing on MCA, which is selective for Gram-negative and lactose-fermenting bacteria, showed growth of mucoid, pale pink colonies characteristic of Klebsiella Spp., suggesting its involvement as the primary pathogen [11].

Further confirmation was achieved through Gram staining and biochemical testing. Microscopic observation under 1000x magnification revealed pink, rod-shaped bacteria consistent with Gram-negative bacilli (Table 2).

TABLE 1
CALIFORNIA MASTITIS TEST RESULT

Sample Collection Location	+	++	+++	++++	-
Banjar Semaon	-	-	12	3	2
Banja Ponggang	-	-	8	-	2
Banjar Kebek	-	2	1	-	1
Banjar Puhu	-	3	-	-	-
Total	-	5	21	3	5
Percentage	-	15%	62%	8%	15%

TABLE 2
GROWTH OF BACTERIAL ISOLATES, BIOCHEMICAL TESTS, AND MICROSCOP

Sample	MAC Agar Media	EMB Agar	TSI Agar	Gram Stain	Microscope	Identified Bacteria
1	Pink Mucioid	Dark	A/A,(+), H2S (-)	-Ve Bacilli	Pink Bacilli	*Klebsiella* Spp.
2	Pink Mucioid	Dark	A/A,(+), H2S (-)	-Ve Bacilli	Pink Bacilli	*Klebsiella* Spp.
3	Pink Mucioid	Dark	A/A,(+), H2S (-)	-Ve Bacilli	Pink Bacilli	*Klebsiella* Spp.

Acid: yellow slant or butt

Discussion

Mastitis is a disease that often affects milk-producing mammals, including pigs, cows, goats, and sheep. Mastitis is defined as inflammation of the udder tissue, usually caused by infection with pathogenic microorganisms, but can also be caused by physical trauma or chemical irritation [8]. Mastitis is divided into two primary clinical forms: clinical mastitis, which exhibits obvious symptoms such as swelling, heat, pain, and changes in milk color, and subclinical mastitis, which does not display obvious clinical signs. However, there is an increase in the number of somatic cells and biochemical changes in milk [2].

Klebsiella spp. and E. coli are known as environmental pathogens that are commonly found in moist and organic environments, such as feces, urine, contaminated waste, and decaying matter. Other bacteria could cause mastitis without any environmental involvement, such as *Salmonella, Staphylococcus, Clostridium,* and *Staphylococcus argenteus*. Poor hygiene practices in the farm environment can lead to contamination of teat ducts, allowing bacteria to invade mammary gland tissue [5]. The ability of bacteria to form a protective capsule increases their resistance to immune defense mechanisms and some antibiotics, often resulting in more severe infections [6].

Observations on the farm support the possibility of environmental contamination. Some pens have poor drainage, leading to standing water during rain or after cleaning processes, which creates ideal conditions for bacterial

Additionally, biochemical testing using TSIA produced positive results for the fermentation of glucose, lactose, and sucrose, along with gas formation, further supporting the identification of Klebsiella Spp. as the etiologic agent of proliferation [10]. In addition, manure is often poorly managed, contributing to contamination of the sow's udder area. In pens with high pig densities of up to 20 pigs per sow, the challenge of maintaining hygiene increases significantly, increasing the risk of cross-infection between litters [7].

If undetected and untreated, subclinical mastitis can lead to severe consequences for the health of sows and piglets. Affected sows often experience pain and avoid lactation, leading to inadequate milk and colostrum intake for piglets. This leads to poor weight gain, digestive problems such as diarrhea, and in many cases, death within the first few days after birth [5,4]. In addition, subclinical mastitis is associated with reduced fertility and overall reproductive performance, which contributes to significant economic losses due to medical expenses, piglet losses, and decreased productivity.

In this study conducted on lactating sows in Puhu Village, Gianyar, 34 pig milk samples were examined using the California Mastitis Test (CMT), of which only 29 samples were positive for subclinical mastitis with reaction variations from + to ++++, indicating a high number of somatic cells in the milk, indicating subclinical udder inflammation [8]. CMT-positive milk samples were then cultured on MacConkey Agar (MCA) medium. The growing colonies appeared mucoid pink in color, typical for gram-negative lactose-fermentative bacteria such as *Klebsiella* Spp. [14,7] The TSIA test showed fermentation of glucose and lactose without H_2S production, confirming the suspicion that the isolate was *Klebsiella* Spp (Figure 1) [21]. Gram stain examination showed short rod-shaped gram-negative bacilli [16,22] (Figure 2). Given the high prevalence rate of 85% (caused by *Klebsiella spp*) observed in this study, it is evident that subclinical mastitis is a significant health problem on this farm and requires immediate attention. It is essential to note that substantial improvements in farm hygiene and housing conditions are crucial to prevent the further Spread of infection.

Preventive measures should include sanitizing pens before and after farrowing, proper floor cleaning and drying, trimming piglets' teeth to prevent nipple injury, and administering anti-inflammatory and antibiotic treatments when necessary and in accordance with veterinary guidelines [7].

Figure 1. (A). *Klebsiella* Spp on MacConkey media; (C). TSIA Biochemical Test (+) *Klebsiella* Spp.

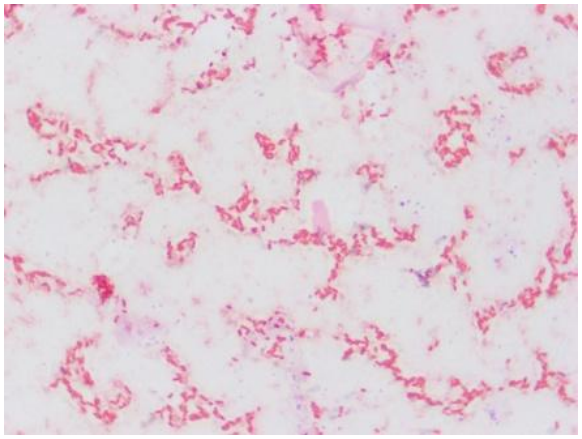

Figure 2. *Klebsiella* spp. on Microscope with 1000x magnification.

CONCLUSION

A study conducted at a pig farm in Puhu Village, Payangan District, revealed that subclinical mastitis was present in 29 out of 34 milk samples from lactating sows, as indicated by the California Mastitis Test (CMT). Subsequent analysis using MacConkey Agar, Gram staining, and TSIA biochemical testing confirmed the presence of *Klebsiella* Spp., identifying it as a significant contributor that causes the infections. To minimize the occurrence of subclinical mastitis, farmers should maintain Proper hygiene in the pig housing area and implement sound management practices, particularly for nursing sows. Regular CMT screening is recommended to allow early detection and treatment, preventing further issues related to milk production and piglet health.

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