

IDENTIFICATION OF *Streptococcus suis* BACTERIA ON LAWAR PLEK SOLD IN GIANYAR REGENCY IN 2025

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ABSTRACT

Background: *Streptococcus suis* is a zoonotic pathogen in pigs. These bacteria can cause meningitis and various other diseases. In 2023, Gianyar Regency had the highest number of suspected Meningitis *Streptococcus suis* in Bali. The habit of Balinese in consuming foods made of raw pork meat and blood is suspected to be the cause of *Streptococcus suis* infections in Bali. One of the Balinese traditional food made of raw pork meat and blood is lawar plek.

Methods: This study aims to identify the contamination of *Streptococcus suis* in lawar plek sold in Gianyar Regency. This study used a cross-sectional design with purposive sampling to determine the sample size. A total of 28 samples of lawar plek were collected from four sellers in each sub-districts in Gianyar Regency. Identification of bacteria was carried out through isolation of a blood agar plate, gram staining, catalase test, and Polymerase Chain Reaction test. Data analysis was conducted descriptively to obtain samples contaminated by *Streptococcus suis*.

Results: The results of isolation showed colonies in grayish white, round with flat and convex edges, and measuring 1-4 mm in size. Microscopic observation showed coccus gram-positive bacteria in 22 samples (78.6%) and bacilli gram-positive bacteria in 6 samples (21.4%). Catalase test showed positive results on 18 samples (81.8%) and negative results on 4 samples (18.2%). Polymerase Chain Reaction test on four samples obtained negative results.

Conclusion: According to the results of identification, contamination of *Streptococcus suis* bacteria in lawar plek samples from sellers in Gianyar Regency was not found.

Keywords: Identification, *Streptococcus suis*, Lawar Plek

INTRODUCTION

Streptococcus suis is a gram-positive coccus bacterium, approximately 1.0-1.5 µm, and a facultative anaerobe.¹ These bacteria are known as zoonotic pathogens in pigs, so they can be transmitted to humans. The infection of *Streptococcus suis* in humans can cause various diseases, such as meningitis, pneumonia, endocarditis, arthritis, and sepsis.¹ The first case of *Streptococcus suis* infection in humans was reported in Denmark in 1968.² According to existing literature, it is estimated that 1,600 cases of *Streptococcus suis* infection in humans have occurred, spread across more than 30 countries and regions.³

In 1998, there was a *Streptococcus suis* outbreak in China, which infected 25 people, 14 of them died, and 80,000 pigs were also affected. The second outbreak occurred in 2005, with a total of 215 infection cases in humans, resulting in 38 deaths, and more than 600 pigs were infected.³ Meanwhile, in Thailand, in 2002, 2005, and 2007, 43 infection cases in humans were recorded, 16 of them had meningitis, and eight of those who recovered had hearing impairment.² Most cases occurred in pig farmers, those

who work in pig slaughterhouses, and individuals who consume processed pork.⁴

The first case of *Streptococcus suis* infection in Bali was reported in 1994. In the same year, 2,200 pigs and hundreds of monkeys died in Sangeh, Ubud, and Alas Kedaton.⁵ In the last decade, the number of suspected cases of Meningitis *Streptococcus suis* (MSS) in Bali showed a significant increase. In March 2017, 38 cases of suspected MSS in Badung, Tabanan, and Jembrana Regencies were recorded, with three cases confirmed as *Streptococcus suis*.⁶ Latest cases were reported by the Health Service of Bali Province in April 2023, with 27 cases of suspected MSS in Gianyar Regency, and two of them tested positive for *Streptococcus suis*.⁷ The habit of the Balinese in consuming traditional foods made of raw pork meat and blood is suspected to be the cause of the increase in *Streptococcus suis* infection cases.⁴

One of the Balinese traditional foods made of raw pork meat and blood is lawar plek. Different from the common lawar, lawar plek is made of finely chopped raw pork, then mixed with small pieces of young jackfruit, *rames* (partially boiled minced pork skin), green peppercorn, and Balinese spices (*base genep*). A

small amount of fresh pork blood is added to enrich the taste and give the dish its distinctive red color.^{8,9} Lack of public information and knowledge related to the potential for pathogen contamination in processed raw pork can increase the risk of *Streptococcus suis* transmission. Besides the impact on individual health, this condition may cause public concern.¹⁰

According to the background above, the author is interested in conducting a study to identify the contamination of *Streptococcus suis* bacteria in lawar plek sold in Gianyar Regency. This study aims to identify the contamination of *Streptococcus suis* bacteria in lawar plek sold in Gianyar Regency.

MATERIAL AND METHOD

Study Design and Samples Collection

This study used a cross-sectional design with a purposive sampling technique as the method for determining the number of samples. Twenty-eight samples of lawar plek were collected from seven sub-districts in Gianyar Regency by taking four samples in each sub-districts. The inclusion criteria of the samples included: (1) Lawar plek sold by sellers in Gianyar Regency, (2) Lawar plek processed on the same day as the sample was taken by the researcher, and (3) Lawar plek that uses pork as the main ingredient. Meanwhile, the exclusion criteria of the samples included: (1) Lawar plek contaminated after sampling due to the actions or negligence of the researcher. The sampling was carried out aseptically in two months, from August 2025 to September 2025. The collected samples were then taken to the Microbiology Laboratory of the Faculty of Medicine, Universitas Udayana, for the bacterial identification process.

Bacterial Identification Procedures

Bacterial identification was carried out to determine the contamination of *Streptococcus suis* in each lawar plek sample. The identification procedures involved several stages of testing, including bacterial isolation in blood agar plate media, gram staining, catalase testing, and molecular testing using the Polymerase Chain Reaction method. Bacterial isolation was carried out in a blood agar plate with quadrant streak technique. The media was incubated in anaerobic conditions at 37°C for 18-24 hours. The characteristics of colonies observed included color, shape, size, edge, surface, and type of hemolysis formed around the bacterial colonies. Gram staining was carried out to identify the microscopic characteristics of bacteria. Bacterial colonies were observed under a light microscope at 100x magnification. The microscopic characteristics observed included the color and shape of bacteria in each isolate. The catalase test was carried out using hydrogen peroxide (H₂O₂) to determine the ability of bacteria to produce the catalase enzyme. The positive results in the catalase test were indicated by the formation of oxygen

bubbles, while the results are considered negative if the formation of bubbles does not occur.

Polymerase Chain Reaction was used to confirm the existence of *Streptococcus suis* bacteria. The PCR procedures consisted of three stages, including DNA isolation, amplification of DNA fragments, and agarose gel electrophoresis. DNA isolation was carried out using the boiling method by heating the bacteria at 100°C. The results of DNA isolation were then amplified through three cycles, including denaturation (95°C), annealing (52°C), and extension (72°C), which were repeated 30 times. In this study, genes that became the target of amplification were the recombination/repair protein (recN). Amplification was carried out using the primers SSRecN-F (5'-CTACAAACAGTCCTCTTC T-3') and SSRecN-R (5'-ACAACAGCCAATTCATG GCGTCATT-3') with an amplicon of 336 base pair (bp). The results of amplification were then analyzed with agarose gel electrophoresis to detect the existence of a DNA fragment corresponding to the target size.

Data Analysis

Data analysis was conducted descriptively to obtain the number of samples contaminated by *Streptococcus suis* bacteria. Data from the results of the study from each sample were presented using descriptive statistics with narratives and tables.

RESULT

This study was conducted in two months, from August 2025 to September 2025. Lawar plek samples were taken from four sellers in each sub-districts in Gianyar Regency that met the inclusion criteria. Gianyar Regency consists of seven sub-districts, so the total samples collected were 28. All samples were then identified to determine the contamination of *Streptococcus suis* bacteria. The identification was carried out based on the procedures explained previously.

The results of observation on 28 samples in blood agar plate media indicated relatively homogeneous colony characteristics, which were grayish white, coccus with flat edges, and a convex surface. The size of colonies varied between 1-4 mm, with most colonies having a diameter of 1-2 mm. A total of 22 samples (78.6%) showed α -hemolysis zone, while 6 other samples (21.4%) were non-hemolytic or showed γ -hemolysis.

The results of microscopic observation on 28 samples showed coccus gram-positive bacteria in 22 samples (78.6%), which were potentially from genus *Streptococcus sp.* or *Staphylococcus sp.* Meanwhile, 6 other samples (21.4%) showed gram-positive bacilli, which were suspected of being genus *Bacillus sp.* (Table 1).

Table 1. Results of microscopic characteristic observations

Gram Staining Result	Frequency (n)	Percentage (%)
Coccus gram-positive	22	78.6
Bacilli gram-positive	6	21.4
Total	28	100

The results of the catalase test on 22 samples showed positive results on 18 samples (81.8%). Meanwhile, 4 other samples (18.2%) showed negative results, indicating that

bacteria in four samples were from the genus *Streptococcus* sp. (Table 2).

Table 2. Results of the catalase test

Catalase Results	Frequency (n)	Percentage (%)
Positive	18	81.8
Negative	4	18.2
Total	22	100

Figure 1 shows the results of the Polymerase Chain Reaction on four samples that are suspected of being contaminated with *Streptococcus suis*. In the negative control (K-), no DNA bands were observed, indicating that there was no DNA contamination in the PCR reaction mixture. In the positive control (K+), DNA bands were found at approximately 300-400 bp, indicating that the PCR

reaction successfully amplified the recombination/ repair protein (recN) gene of *Streptococcus suis*. Meanwhile, in four samples, no DNA bands aligned with the positive control were observed. This result showed that the recN gene was not detected in all test samples, so there were no indications for the presence of *Streptococcus suis* in four samples (Table 3)

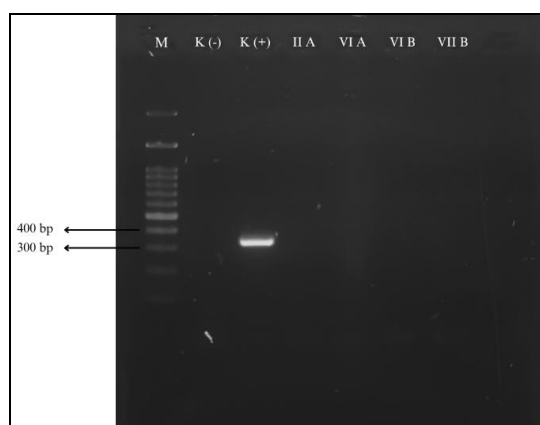


Figure 1. Visualization of polymerase chain reaction test results

Table 3. Results of polymerase chain reaction test

Sample ID	DNA Amplification Results	Remarks
K(-)	No DNA band was observed	Negative
K(+)	A band of approximately 300–400 bp was observed	Positive
IIA	No DNA band was observed	Negative
VIA	No DNA band was observed	Negative
VIB	No DNA band was observed	Negative
VII B	No DNA band was observed	Negative

According to all stages of identification, including the observation of the characteristics of colonies bacterial in blood agar plate media, gram staining, catalase testing, and molecular testing using Polymerase Chain Reaction method.

The percentage of the contamination of *Streptococcus suis* bacteria in lawar plek sample was 0%, while the percentage of samples not contaminated with *Streptococcus suis* was 100% (Table 4).

Table 4. Results of identification of *Streptococcus suis* on lawar plek samples

Contamination of <i>Streptococcus suis</i>	Frequency (n)	Percentage (%)
Positive	0	0
Negative	28	100
Total	28	100

DISCUSSION

Streptococcus suis is a pathogen in pigs that is zoonotic and can be transmitted to humans. These bacteria are known as one of the causes of meningitis and can lead to various other diseases, such as pneumonia, endocarditis, arthritis, and sepsis in humans.¹ Most cases of *Streptococcus suis* infection occur in individuals with direct contact with pigs or those who consume processed products.¹⁰ In Bali Province, increasing cases of *Streptococcus suis* infection in pigs and humans have been reported in recent years. In April 2023, the Health Service of Bali Province reported 27 cases of suspected Meningitis *Streptococcus suis* (MSS) in Gianyar Regency, and two of them tested positive for *Streptococcus suis* infection.⁷ The habit of Balinese in consuming traditional foods made of raw pork meat and blood is suspected to be the cause of the increase in *Streptococcus suis* infection cases in Bali.⁴

One of the Balinese traditional foods made of raw pork meat and blood is lawar plek. Different from the common lawar, lawar plek is made of finely chopped raw pork, then mixed with small pieces of young jackfruit, *rames* (partially boiled minced pork skin), green peppercorn, Balinese spices (*base genep*), and raw pork blood.^{8,11} Lack of public information and knowledge related to the potential for pathogen contamination in lawar plek can increase the risk of *Streptococcus suis* infection transmission. This study was conducted to identify the contamination of *Streptococcus suis* in lawar plek sold in Gianyar Regency. A total of 28 samples of lawar plek were collected from the sellers in Gianyar Regency. Samples collected were then identified through several testing stages.

Bacterial isolation is a cultivation method in specific media carried out to identify the characteristics of colonies and the morphology of bacteria.¹² In this study, bacterial isolation was carried out in blood agar plate media containing 5% of goat blood. The use of blood agar not only serves as a growth medium but also as a differential medium that allows the identification of bacteria according to the type of hemolysis and characteristics of colony morphology.¹³ *Streptococcus suis* in sheep blood agar media appears grayish white to semi-transparent, the size of a needle tip, a coccus with flat edges, a convex and smooth surface, and is able to display α -, β -, or γ -hemolysis zone.¹⁴

In this study, the results of observation on 28 samples in blood agar plate media indicated relatively homogeneous colony characteristics, which were grayish white, coccus-shaped with flat edges, and a convex surface. Variation is only seen in the size of colonies between 1-4 mm, with most colonies having a diameter of 1-2 mm. The variations in colony size can be influenced by several factors, including differences in growth rate between isolates, differences in the duration of incubation, and the number of inocula.¹⁵ A total of 22 samples (78.6%) showed α -hemolysis zone indicated by the formation of a greenish zone around the colonies due to the production of hydrogen peroxide (H_2O_2) by bacteria that oxidize hemoglobin into methemoglobin.

Meanwhile, 6 other samples (21.4%) showed γ -hemolysis indicated by the absence of color change or a clear zone around the colonies.¹⁶ Overall, this finding indicates that 28 samples observed have the characteristics of a colony that are likely from genus *Streptococcus sp.* or *Staphylococcus sp.*

Gram staining is a differential staining method used to classify bacteria based on differences in their cell wall structure, which is gram-positive and gram-negative.¹⁷ Microscopically, *Streptococcus suis* bacteria appear as gram-positive bacteria arranged in short chains or pairs.¹⁸ In this study, 22 samples (78.6%) showed gram-positive coccus bacteria, which were potentially from the genus *Streptococcus sp.* or *Staphylococcus sp.* Bacteria from the genus *Streptococcus sp.* and *Staphylococcus sp.* have similar colony morphology and microscopic characteristics, so the catalase test is required to differentiate between the two.¹⁹ Meanwhile, 6 other samples (21.4%) showed gram-positive bacilli bacteria. These bacteria are potentially from the genus *Bacillus sp.*, known as a gram-positive and rod-shaped bacterium, as well as having the ability to shape endospores under unfavorable conditions.²⁰

The catalase test is one of the biochemical tests used to determine the ability of bacteria to generate catalase enzyme, which is an enzyme that functions to break down hydrogen peroxide into water and oxygen.¹⁷ The catalase test plays an important role in differentiating *Streptococcus sp.* and *Staphylococcus sp.* species. In *Staphylococcus sp.*, the breakdown of hydrogen peroxide occurs, indicated by the formation of oxygen bubbles, thereby showing positive results. In *Streptococcus sp.*, the formation of bubbles does not occur because there are no activities from the catalase enzyme, thereby showing negative results.¹⁹ In this study, the catalase test was conducted on 22 samples, indicating the conformity with the microscopic characteristics of *Streptococcus sp.* and *Staphylococcus sp.* A total of 18 samples (81.8%) showed catalase-positive results. Meanwhile, 4 other samples (18.2%) showed catalase-negative results, indicating that bacteria in four samples were from the genus *Streptococcus sp.* However, further identification is required to confirm the presence of *Streptococcus suis* in four samples.

Polymerase Chain Reaction is used to identify the presence of *Streptococcus suis* specific DNA in samples that have been tested through morphological and biochemical tests. Gene that became the target of amplification were the recombination/repair protein (*recN*). The use of *recN* as a target gene in the identification of *Streptococcus suis* has a high level of specificity, as *recN* is a housekeeping gene, which is only found in *Streptococcus suis*, so it can minimize the possibility of false positives. Another advantage is that this gene can be found in almost all strains of *Streptococcus suis*, so it has good consistency and sensitivity.^{16,18}

The results of the PCR test in this study showed that in the negative control (K-), no DNA bands were observed, indicating that there was no DNA contamination in the PCR reaction

mixture. In the positive control (K+), DNA bands were found at approximately 300-400 bp, indicating that the PCR reaction functioned properly and successfully amplified the *recN* gene of *Streptococcus suis*. Meanwhile, in four samples, no DNA bands aligned with the positive control were observed. Failure to form DNA bands in the test sample can be caused by several factors, such as the absence of target DNA in the test samples, the poor quality of the DNA template, or excessively low DNA concentrations.²¹ If correlated with the observation results of colony morphology, gram staining, and catalase test, the four samples showed characteristics that correspond with *Streptococcus suis* bacteria. However, the results of negative PCR showed that the *recN* gene was not detected in all test samples, so that molecularly it can be concluded that there were no indications for the presence of *Streptococcus suis* in four samples.

According to all stages of identification, it can be concluded that contamination of *Streptococcus suis* bacteria is not found in all lawar plek samples. A similar study was conducted by Agustini (2024) in 20 lawar plek samples taken from 10 sellers in sub-districts of Sukawati. The results of the study showed that three samples were detected as positive containing *Streptococcus sp.* bacteria. However, based on the results of the sugar fermentation test, the characteristics of bacteria in the three samples are more indicative of *Streptococcus porcinus* than *Streptococcus suis*.²²

The absence of *Streptococcus suis* was not found in all lawar plek samples, which can be influenced by various factors, one of which is related to the limited number of samples. In this study, samples were only collected from 28 sellers, so there is a possibility that the samples taken were not lawar plek contaminated with *Streptococcus suis*. The condition of pigs used can also influence the contamination of *Streptococcus suis*.^{16,23} The pigs used as raw ingredients for lawar plek may be healthy pigs that do not exhibit abnormalities or signs of infection. Moreover, increasing cases of suspected *Streptococcus suis* infection in Bali in recent years have encouraged sellers and personnel at slaughterhouses to pay more attention to personal hygiene.²⁴ These factors may contribute to the absence of *Streptococcus suis* contamination in all samples examined.

This study has several limitations that must be considered as a point of evaluation for further studies. Limited resources, time, and funding are the factors that have limited the number of samples in this study. Therefore, further study is suggested to use a larger sample size to obtain more accurate and representative results for the population studied.

CONCLUSIONS AND SUGGESTIONS

There was no contamination of *Streptococcus suis* bacteria in lawar plek samples taken from sellers in Gianyar Regency. The percentage of contamination of *Streptococcus suis* bacteria in lawar plek samples was 0%.

Therefore, further study is required with a larger sample size to obtain more accurate and representative results for the population studied.

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