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Original Article

Detection of *Salmonella* sp. in broiler chickens in closed houses using the polymerase chain reaction method

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ABSTRACT

This study aimed to detect the presence of *Salmonella* sp. in broiler chickens raised in closed house systems using the Polymerase Chain Reaction (PCR) method. Liver and intestinal samples were collected from chickens showing clinical signs such as diarrhea, anorexia, and lethargy, along with pathological lesions observed during necropsy. Bacteriological identification involved isolation using selective media, Gram staining and biochemical tests. Two samples (3A and 3B) showed colony morphology and biochemical characteristics consistent with *Salmonella* sp. Confirmation using PCR targeting the *inv*A gene (primers 139 and 141, expected amplicon size 284 bp) yielded negative results in both samples, while the positive control successfully amplified the target. The inconsistency between bacteriological and molecular results may be attributed to several factors: absence or mutation of the *inv*A gene in the tested isolates, primer mismatch with local Salmonella strains, or inadequate DNA quality and concentration. These findings suggest that reliance on a single molecular marker may be insufficient for accurate detection and underscore the importance of optimizing PCR conditions. The study highlights the need for locally adapted primers and complementary diagnostic approaches to improve the reliability of *Salmonella* sp. detection in poultry, particularly in intensive production systems like closed house environments.

Keywords: Bacteriological test, broiler chicken, closed house, PCR test, *Salmonella* sp

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Introduction

Broiler chickens are a type of chicken breed specifically bred for meat production. This strain is selected for its rapid growth, reaching harvest weight within 28-35 days (Oke *et al*., 2024). The Cobb 500 strain is a commercially popular broiler breed due to its high yield and efficient growth performance (Hammemi *et al*., 2024). Cobb 500 broilers perform differently in various climatic conditions, with the highest production index observed in highland climates (Gholami *et al*., 2020).

The broiler chicken farming industry in Indonesia continues to grow rapidly to meet the population's demand for animal protein. One widely used technology is the closed house system, which provides optimal environmental control to increase productivity and livestock health. This system still carries the risk of infectious diseases that can disrupt production

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performance. There are two types of housing systems for broiler chickens: the open house system, which depends on environmental factors and the surrounding natural conditions, and the closed house system, which allows for the microclimate to be adjusted according to needs (Honig *et al.*, 2024).

Closed houses are specifically designed to protect chickens from direct contact with the external environment, which can potentially spread disease, and to minimize stress factors in broiler chickens (Tanquilut *et al.*, 2020). Chickens can become infected with Salmonella through various routes of exposure, including carrier animals such as rats and insects. Contaminated feed and drinking water can also be a source of transmission through airborne aerosols (Shaji *et al.*, 2023). This also does not rule out the risk of exposure to *Salmonella* sp. bacteria in broiler chickens through contaminated feed, water, equipment, or cross-contamination from workers and the surrounding environment, which can then lead to Salmonellosis in broiler chickens (Morita *et al.*, 2022).

Salmonellosis is an infectious disease, one of which affects broiler chickens, caused by bacteria of the genus Salmonella, with over 2,500 serotypes. Common symptoms of Salmonellosis in broiler chickens include white or green diarrhea, anorexia, cachexia, and malabsorption (Spalević *et al.*, 2023). Pathological conditions of Salmonellosis in broiler chickens typically include hepatomegaly, hemorrhage, and sinusoidal congestion in the liver (Shchebentovska *et al.*, 2021). In the intestines, catarrhal inflammation, degeneration, and intestinal bacteria are found causing inflammation (Saleem *et al.*, 2022). *Salmonella* sp. infection in chickens can begin in the digestive tract, penetrate the intestinal wall, and enter the bloodstream.
This condition can cause systemic infection, spreading to internal organs such as the liver, spleen, and cecum. Severe symptoms such as septicemia and high mortality can occur, especially in young chickens, which can then affect production declines and cause economic losses for farmers (Hu *et al.*, 2024). False-negative results in bacteriological tests are still possible, especially when the bacterial count in the sample is very low or if sample handling procedures are not carried out properly. In some cases of infection, specific antibodies may not be detected despite the presence of the pathogen, necessitating confirmatory testing using the Polymerase Chain Reaction (PCR) method (Adzitey *et al.*, 2013).

PCR is a fast and accurate detection method, especially in detecting *Salmonella* sp., and is able to differentiate *Salmonella* sp. serotypes, thus supporting more effective control strategies (Tan *et al.*, 2022). The *inv*A gene is also conservative and found in almost all Salmonella serotypes, so it has been widely used as a molecular marker in the detection of *Salmonella* sp. This study aimed to detect the presence of *Salmonella* sp. in broiler chickens raised in closed house systems using the Polymerase Chain Reaction (PCR) method. Detection of the presence of *Salmonella* sp. in broiler chickens in closed house cages using the PCR method targeting the *inv*A gene is used as a confirmation method to support more accurate detection and more effective disease prevention and control strategies in broiler chicken farms in closed houses.

Materials and methods

Research design

Sampling was conducted at a broiler chicken farm with a closed house system in Jatidukuh Village, Gondang District, Mojokerto Regency, East Java. This research was conducted from March to May 2025. Isolation and identification using bacteriological tests were conducted at the Bacteriology and Mycology Laboratory, Microbiology Division, Faculty of Veterinary Medicine, Airlangga University, Surabaya, and Polymerase Chain Reaction (PCR) tests were conducted at the Institute of Tropical Disease, Airlangga University (ITD).

The research samples were the livers and intestines of broiler chickens from a closed-house broiler farm with a population of approximately 30,000 chickens. A total of 12 organ samples were taken: 6 liver samples and 6 intestinal samples, representing 10% of the 120 broiler chickens showing clinical symptoms of Salmonellosis. Sample selection was random, considering chickens exhibiting symptoms such as green or white diarrhea, loss of appetite, and lethargy.

Sample collection from closed house farm

The research samples were taken from a broiler farm using a closed house system. Twelve broiler organ samples were taken, representing 10% of the 120 broiler chickens exhibiting specific clinical symptoms. The samples were taken from the liver and intestines of broiler chickens, each of which exhibited pathological changes, such as organ swelling, organ wall thickening, and hemorrhage. The sampling technique ensured cleanliness and sterilization of the tools used, such as sample pots, to prevent cross-contamination. Each sample was immediately placed into a sterile sample pot filled with Brain Heart Infusion Broth (BHIB) media and labeled for easy identification. After collection, the samples were stored in a cooler box prior to processing.

Isolation and identification

The organ samples used were first separated based on their type. The liver was then ground and soaked in 9 ml of Tetrathionate Broth (TTB) for 12 hours. The intestines were first cleaned of fecal debris and then scraped from the intestinal mucosa. The soaking medium was inoculated onto selective Salmonella Shigella Agar (SSA) and its growth was observed. The *Salmonella* sp. isolates that grew were characterized by their transparent, round shape with a black center, resembling a fish eye due to the formation of H_2S gas.

Gram staining was performed, and the results showed that *Salmonella* sp. is a Gram-negative bacterium with a characteristic long, rod-shaped, and pink color. Biochemical tests were then conducted on *Salmonella* sp. colonies. through a series of tests, including the Triple Sugar Iron Agar (TSIA) test, the Sulfide Indole Motility (SIM) test, the Simmons Citrate Agar (SCA) test, the urease test, and the fermentation test for sugars such as glucose, lactose, sucrose, mannitol, and maltose. The SIM test for *Salmonella* sp. shows motility and the ability to produce hydrogen sulfide (H_2S) which is indicated by the formation of a black precipitate, but the indole test results are negative, as seen from the absence of a red ring after the addition of Kovac's reagent. The citrate test on SCA media shows a positive result, indicating that the bacteria are able to utilize citrate as a carbon source, while the urease test shows a negative result. In the sugar fermentation test, *Salmonella* sp. can ferment glucose and mannitol, but generally does not ferment lactose; some subspecies can ferment sucrose. After the identification process, the bacteria were inoculated into Nutrient Broth (NB) media and incubated for 12 hours, then mixed with 40–50% glycerin at a ratio of 0.9:0.3. The bacterial suspension was then stored in a freezer at -80°C to maintain its viability.

DNA extraction

DNA extraction was performed using the QIAamp Mini DNA Kit and *inv*A gene DNA primers to detect *Salmonella* sp. Samples were taken from colonies growing on NA media, then placed into a 1.5 ml microcentrifuge tube. A total of 180 µl of ATL Buffer and 20 µl of Proteinase K were added, then the mixture was processed by vortexing and incubated at 56°C until the sample was completely decomposed (for 1–3 hours), with the vortex process being carried out occasionally during incubation. 200 µl of AL Buffer was added then the mixture was homogenized by vortexing for 15 seconds and incubated at 70°C for 10 minutes, then briefly centrifuged to remove droplets from the tube cap. A total of 200 µl of ethanol (96–100%) was added to the mixture, homogenized again by vortexing for 15 seconds and briefly centrifuged to remove droplets from the tube cap. The prepared mixture was then transferred to a QIAamp Mini spin column placed in a 2 ml collection tube and centrifuged at 6000 x g (8000 rpm) for 1 min; the flow-through and collection tube were then discarded. The QIAamp Mini spin column was then transferred to a new 2 ml collection tube, 500 µl of Buffer AW1 was added and centrifuged again at 6000 x g (8000 rpm) for 1 min; the flow-through and collection tube were discarded. The washing process was continued by adding 500 µl of Buffer AW2, then centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min; the flow-through and collection tube were discarded. To ensure that any remaining Buffer AW2 was completely removed, the QIAamp Mini spin column was transferred to a new 2 ml collection tube and centrifuged again at full speed for 1 min. Next, the QIAamp Mini spin column was transferred into a new 1.5 ml microcentrifuge tube, then 200 µl of Buffer AE or

distilled water was added, incubated at room temperature for 1 minute, and centrifuged at 6000 x g (8000 rpm) for 1 minute to elute the DNA. As an optional step, the elution process can be repeated with an additional 200 µl of Buffer AE or distilled water to increase the DNA elution yield.

PCR implementation

The PCR implementation was carried out referring to research by Rahn *et al*. (1992) with the DNA primer sequences used were primer 139 *inv*A gene (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and 141 *inv*A gene (5′-TCATCGCACCGTCAAAGGAACC-3′) with 284 bp amplification. PCR was carried out with a total volume of 20µL with the following information: 2x master mix (Intron) of 12.5 µL, 0.5µL of distilled water, 1µL of forward primer, 1µL of reverse primer, and 5 µL of extracted DNA. The amplification process began with a predenaturation stage at 95°C for 180 seconds with a total of 40 cycles. The denaturation stage took place at 95°C for 2 seconds, also for 40 cycles. The annealing process was carried out at a temperature of 56.3°C for 20 seconds, followed by an elongation stage at a temperature of 72°C for 5 seconds, with each stage carried out for 40 cycles (Moganedi *et al*., 2009; Gallegos-Robles *et al*., 2009).

Reading the results using agarose gel electrophoresis

A total of 0.4 grams of agarose was weighed and added to 20 ml of 1X TBE solution. The solution was then heated in a microwave for ±2 minutes until completely dissolved. 2 µl of Red Safe Gel dye was added and mixed until homogeneous. The solution was poured into molds according to size and allowed to harden. A sample of 5 µl was added to each well. Electrophoresis was carried out at a voltage of 100 Volts for ±30 minutes. The electrophoresis results were visualized using a UV Transilluminator (GelDoc). The formed DNA bands were observed based on the presence and size of fragments compared to a 100 bp DNA marker as a reference. The presence of a band at 284 bp indicates amplification results that match the target *inv*A gene from *Salmonella* sp. (Lee *et al*., 2012; Maurye *et al*., 2021).

Data analysis

The data obtained through isolation, identification and PCR methods are presented descriptively.

Result

Isolation of Salmonella sp.

Samples were isolated using Salmonella Shigella Agar (SSA) selective media to differentiate them from other bacteria. Of the 12 samples, two showed black bacterial colonies, suggesting *Salmonella* sp. of the 12 samples taken from 6 liver organs and 6 intestinal organs, there were 2 samples that showed dominant pathological changes. The isolation results showed that of the 12 samples tested, 2 of them showed positive results for the presence of *Salmonella* sp. (Table 1). The positive results were indicated by the growth of black colonies on SSA media. These black colonies are a characteristic of the growth of *Salmonella* sp. on SSA selective media. The growth of these distinctive black colonies is clearly visible in Figure 1. In the image, the growing colonies appear rounded with flat edges and have a deep black color in the center, which is a strong indication of the presence of *Salmonella* sp.

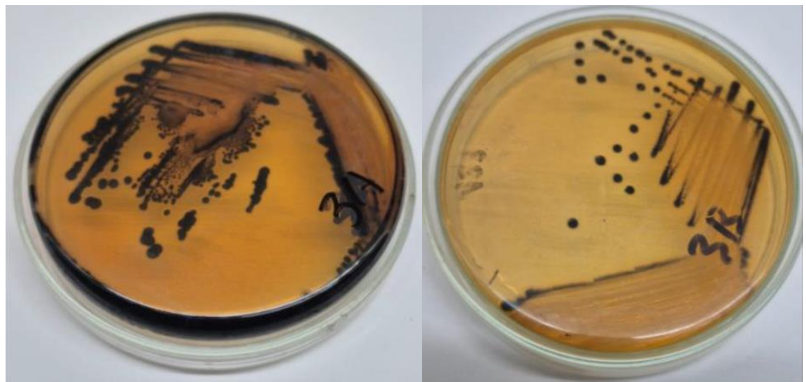

Figure 1. Media 3A and 3B showed bacterial growth on SSA media, *Salmonella* sp. colonies appeared black on SSA media

Table 1. Results of Salmonella Shigella Agar (SSA) Media Isolation

Organ	Sample code	Isolation results	Information
Liver	1A	Negative	No black colonies growing
Intestines	1B	Negative	No black colonies growing
Liver	2A	Negative	No black colonies growing
Intestines	2B	Negative	No black colonies growing
Liver	3A	Positive	Black colonies grow, suspected *Salmonella* sp.
Intestines	3B	Positive	Black colonies grow, suspected *Salmonella* sp.
Liver	4A	Negative	No black colonies growing
Intestines	4B	Negative	No black colonies growing
Liver	5A	Negative	No black colonies growing
Intestines	5B	Negative	No black colonies growing
Liver	6A	Negative	No black colonies growing
Intestines	6B	Negative	No black colonies growing

Identification of Salmonella sp.

The Gram staining results of *Salmonella* sp. bacterial colonies from SSA media in Figure 2 show that these bacteria are pink and long rod-shaped, which indicates Gram-negative properties. The biochemical test results for *Salmonella* sp. identified on the TSIA medium were positive, SCA positive, urea negative, SIM motile, and indole negative (Table 2).

The sugar test showed positive fermentation for glucose, maltose, and mannitol, but negative for sucrose and lactose. The TSIA test showed a red color change on the slant of the medium and the formation of a black precipitate at the bottom of the medium, indicating the production of hydrogen sulfide (H_2S) gas. The SCA test was characterized by a color change in the medium from green to blue. The resulting blue color is a characteristic indicator of a positive SCA biochemical test result for *Salmonella* sp. The urease test for *Salmonella* sp. showed a negative result, and no color change occurred on the urea agar medium; the medium remained orange.

The SIM test showed a positive result with the presence of motile bacteria, resulting in a cloudy medium. The media color changed to black, and the indole negative result showed no red ring formation after the reagent was added. The glucose test showed a positive result. *Salmonella* sp. are capable of fermenting glucose. This results in a color change in the media from red to yellow. The maltose test shows a positive result, with a color change in the media from red to yellow. The mannitol test shows a positive result, with a color change in the media from red to yellow. A positive result in the mannitol test indicates that the bacterial isolate is capable of fermenting mannitol as an energy source. This characteristic is a biochemical characteristic often found in *Salmonella* sp., which generally ferments mannitol to produce acid. The lactose test shows a negative result, indicated by the media remaining red. The sucrose test shows a negative result, with the media remaining red. The absence of a color change indicates that the tested bacteria do not produce acid from the sucrose fermentation process, thus maintaining a stable pH.

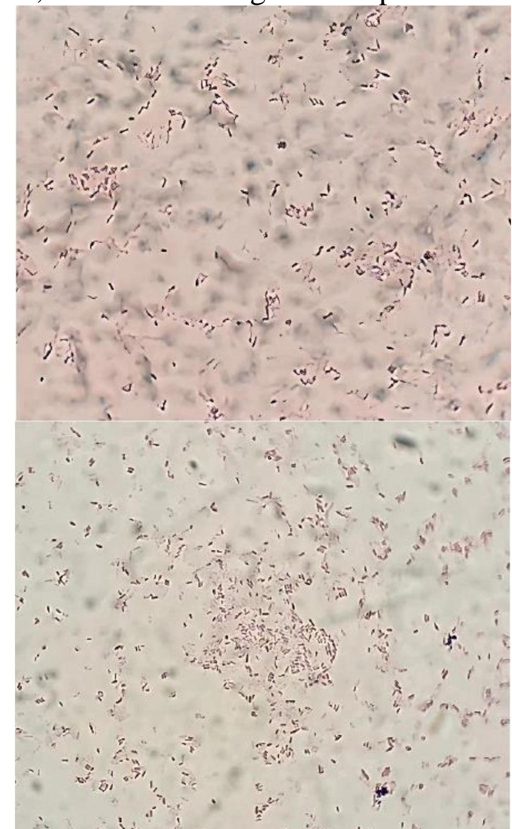

Figure 2. Gram staining results with 1000x magnification. Long rod-shaped bacteria with red color (Gram negative)

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Table 2. Biochemical Test Results of Samples 3A and 3B

Types of Biochemical Tests	Sample 3A	Sample 3B	Information
Triple Sugar Iron Agar (TSIA)	Base/Acid, gas, H_2S (+)	Base/Acid, gas, H_2S (+)	Glucose is fermented, H_2S is formed
Simmon's Citrate Agar (SCA)	Positive (green → blue)	Positive (green → blue)	Citrate is used as a carbon source
Urease Test	Negative (no change)	Negative (no change)	Does not produce urease enzyme
Sulfide Indole Motility (SIM)	Motile (+), Indole (–), H_2S (+)	Motile (+), Indole (–), H_2S (+)	Motility present, non-indole forming, produces H_2S
Glucose Fermentation	Positive (red → yellow)	Positive (red → yellow)	Glucose is fermented
Maltose Fermentation	Positive (red → yellow)	Positive (red → yellow)	Fermented maltose
Mannitol Fermentation	Positive (red → yellow)	Positive (red → yellow)	Fermented mannitol
Lactose Fermentation	Negative (color remains red)	Negative (color remains red)	Lactose fermentation does not occur
Sucrose Fermentation	Negative (color remains red)	Negative (color remains red)	No sucrose fermentation occurs

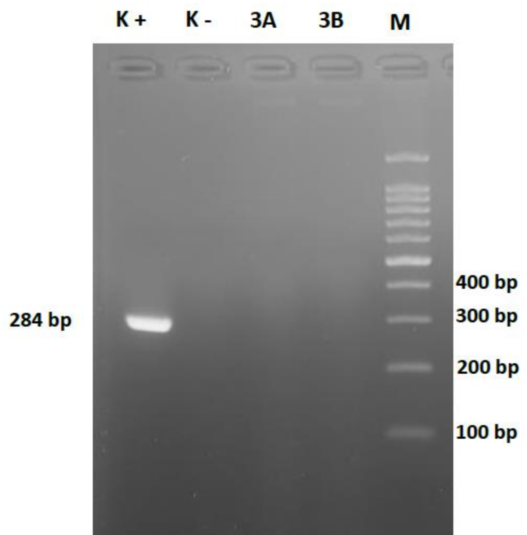

Figure 3. The electrophoresis results of the *Salmonella* sp. PCR test with agarose using a positive control with a clear DNA band at the marker position of around 284 bp and in samples 3A and 3B did not show a DNA band at position 284 bp or at any other position

PCR test

The results of agarose gel electrophoresis with a positive control showed that there was a band at position 284 bp in Figure 3, according to the size of the *inv*A gene amplicon based on primers 139 (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and 141 (5′-TCATCGCACCGTCAAAGGAACC-3′).

Samples 3A and 3B did not show a band at that position, indicating that there was no amplification of the *inv*A gene in both samples

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taken from the liver and intestines of broiler chickens in a closed house system.

Discussion

Isolation of Salmonella sp.

Based on the results of isolation and identification using Salmonella Shigella Agar (SSA) media, 2 of 12 organ samples showed positive results and grew *Salmonella* sp. colonies. Samples 3A (liver organ sample) and 3B (intestinal organ sample) showed the characteristic black *Salmonella* sp. colonies on SSA media caused by the ability of these bacteria to produce hydrogen sulfide gas (H_2S) which reacts with iron ions (Fe_2^+), and forms black iron sulfide (FeS) deposits in the center of the colony. The black color that grows on the surface of the media is a typical indicator of the growth of *Salmonella* sp. bacteria from liver and intestine samples of broiler chickens in a closed house system (Wu *et al*., 2016; Richardson *et al*., 2019).

Identification of Salmonella sp.

The Gram staining results of the isolate suspected to be *Salmonella* sp. showed that the bacteria were Gram-negative, characterized by a pink color and long rod shape when observed under a microscope. According to Ayuti *et al.* (2024), Gram staining is a differentiation technique that separates bacteria based on their cell wall structure, where *Salmonella* sp. has a thin peptidoglycan layer and outer membrane that causes these bacteria to be unable to retain the crystal violet stain after the decolorization process, so it absorbs safranin staining and appears pink. *Salmonella* sp. has a cell wall with a thin peptidoglycan layer and an outer membrane containing lipopolysaccharide, so it is unable to retain the crystal violet stain after the decolorization process with alcohol. The cells will absorb safranin stain as a counterstain, so it appears pink under microscope observation at 1000x magnification. These results are in accordance with the morphological characteristics of *Salmonella* sp. as a Gram-negative bacterium belonging to the Enterobacteriaceae family, rod-shaped, motile, and non-spore-forming (Fàbrega and Vila, 2013).

The results of the biochemical tests carried out include several important tests, such as Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), urease test, Sulfate Indole Motility (SIM), sugar test (glucose, maltose, mannitol, lactose and sucrose). The Triple Sugar Iron Agar (TSIA) test is a differential medium used to identify enteric bacteria, including *Salmonella* sp. Based on the results obtained, the ability of *Salmonella* sp. to ferment glucose, causing acid production and is indicated by a color change at the bottom of the tube from red to yellow. This species generally does not ferment lactose or sucrose, so the slant of the tube remains red, indicating an alkaline reaction. Some species of the Salmonella genus also produce gas during the glucose fermentation process, which can be observed as bubbles or cracks in the agar medium. According to Sarker *et al*. (2021), another characteristic of *Salmonella* sp. bacteria is that they are not susceptible to glucose fermentation. is its ability to produce hydrogen sulfide (H_2S), which reacts with iron salts in the medium and forms a black precipitate. The combination of these reactions produces a characteristic pattern that indicates a positive result in the identification of *Salmonella* sp. using the TSIA test.

The Simmons' Citrate Agar (SCA) test shows a positive result, indicated by a change in the media color from green to blue. In *Salmonella* sp. isolates, a positive SCA test result indicates that the bacteria have the metabolic ability to utilize citrate as a carbon source. This is an important characteristic that distinguishes the Salmonella genus from several other bacteria in the Enterobacteriaceae family. Bacteria that are able to utilize citrate will convert it into alkaline compounds, such as ammonia, which causes an increase in the pH of the media. This increase in pH changes the color of the bromothymol blue indicator in the media from green (neutral pH) to blue (alkaline pH). This color change indicates that the *Salmonella* sp. test results have successfully broken down citrate and created a more alkaline environment in the media, resulting in the media color changing to blue, indicating a positive test result (Çekin *et al*., 2025).

The urease test results for *Salmonella* sp. showed negative results, indicating that these bacteria do not have the ability to produce the urease enzyme. When inoculated into urea media containing the pH indicator phenol red, no color change occurred

during incubation. The media color remained as it was, namely pale yellow or orange, indicating that the pH did not increase due to the absence of ammonia formation. The absence of ammonia production indicates that *Salmonella* sp. does not hydrolyze urea into ammonia and carbon dioxide, so this test can be used as a differential parameter in the identification of enteric bacteria, one of which is *Salmonella* sp. (Oliyaei *et al*., 2024).

Sulfide Indole Motility (SIM) Test *Salmonella* sp. is able to produce hydrogen sulfide through the activity of the desulfurase enzyme which decomposes sulfur compounds in the media. The positive H_2S formed reacts with iron compounds in the SIM media to form a black precipitate in the form of iron sulfide (FeS). This test also shows positive motile results, so that the bacteria can move away from the inoculation line on semi-solid SIM media. This is seen as blurry growth or the media color becomes cloudy around the inoculation puncture. Negative results in the indole test indicate that the bacteria are unable to produce the tryptophanase enzyme, an enzyme that plays a role in catalyzing the degradation of tryptophan into indole and pyruvic acid. When Kovac's reagent is added to the media after incubation, there is no color change to red in the top layer of the media, which indicates the absence of indole compound formation.

The sugar test showed that *Salmonella* sp. was able to ferment glucose, maltose, and mannitol (as seen from the change in the media color to yellow), but was unable to ferment lactose and sucrose (the media color remained red). This biochemical pattern supports the identification of *Salmonella* sp. according to the general characteristics found in the existing literature.

PCR test

Confirmation of detection using the Polymerase Chain Reaction (PCR) method with primers 139 and 141 targeting the *inv*A gene with an amplification size of 284 bp, showed negative amplification results. Based on agarose electrophoresis readings, DNA bands from samples 3A and 3B did not appear at the 284 bp marker, so the PCR results could not be confirmed positive for *Salmonella* sp. This is thought to occur because the primers used were less specific or because of mutations in the primer binding site, so that the amplification did not run according to the target. There are other possibilities that influence negative PCR results even though isolation and identification show positive results. These factors include the number of bacteria in the sample that is below the PCR detection limit, the presence of PCR inhibitors in the sample, suboptimal quality and efficiency of DNA extraction, and the possibility of infection that only occurs in other tissues that are not sampled. According to Schrader *et al*. (2012), the use of specific target genes such as the *inv*A gene is an important factor in the detection of *Salmonella* sp. through PCR, because if the existing Salmonella strain does not carry the gene or has a mutation, the detection results may still show negative results even though the bacteria are actually present.

The results of isolation and biochemical identification showed that the sample was positive for *Salmonella* sp., but the PCR test results showed the absence of a DNA band corresponding to the amplicon size of primers 139 of the *inv*A gene (5′-GTGAAATTATCGCCACGTTCGGGCAA-3′) and 141 of the *inv*A gene (5′-TCATCGCACCGTCAAAGGAACC-3′) with 284 bp amplification. Based on the reading of the electrophoresis results on agarose, it was seen that the DNA band from samples 3A and 3B did not appear, compared to the positive control which showed a DNA band corresponding to a size of 284 bp, which is the amplicon size that should have formed according to the design of primers 139 and 141 for the *inv*A gene. The absence of a DNA band in both samples indicated the possibility of nonspecific amplification, so that the PCR results could not be fully confirmed as positive for *Salmonella* sp. even though the previous test had shown a positive indication.

A possible explanation for negative PCR results is that the chickens sampled had bacterial counts below the PCR detection limit. The infection could have been localized in other tissues not sampled, or the presence of bacteria could have been transient, leading to false-positive results in previous tests. The use of primers targeting a specific gene (the *inv*A gene) could also be a contributing factor. If the Salmonella strain present does not carry the target gene or if

there is a mutation in the primer binding site, PCR detection could produce inaccurate results even though the bacteria are actually present in the sample. Schrader *et al.* (2012) and Yan *et al.* (2019) state that the presence of inhibitors in the sample matrix can reduce the sensitivity of PCR tests. Animal samples often contain inhibitors that can mask the target nucleic acid signal, leading to false-negative results.

PCR primers are designed to specifically bind to the target DNA sequence so that only the desired fragment is amplified. If the primers are not specific enough or the PCR conditions are not optimal (e.g., the annealing temperature is too low), the primers can bind to DNA locations that are similar but not the actual target. According to Yanestria *et al.* (2019), this results in the amplification of DNA products that are longer or different from the original target. These non-specific products will appear as larger or additional bands in the agarose gel. Non-specific amplification can also occur if the primers have a tendency to form dimers or hairpins, which interfere with PCR results.

Several other factors can also cause invalid bacterial detection results. One such factor is genetic variation between strains or isolates of *Salmonella* sp., a common finding in molecular studies. These differences can be caused by DNA insertions, such as additional genetic elements, the presence of transposons, or other mutations occurring in the PCR target region. Porwollik *et al.* (2004) and Liaquat *et al.* (2018) explain that if a genetic element is inserted between the DNA sequences where the primers bind, PCR amplification can produce DNA fragments longer than the standard size. This results in invalid results, where the amplicon band in the electrophoresis results appears to be above the expected marker size. This phenomenon is not caused by technical errors but rather indicates natural genetic variation between the Salmonella isolates tested. Interpretation of PCR results must consider the possibility of genetic differences to avoid errors in molecular data analysis.

Conclusion

Based on the research results, both bacterial isolates from the liver and intestines of broiler chickens with a closed house cage maintenance system showed the characteristics of the presence of *Salmonella* sp. through bacteriological tests, such as isolation and identification that showed the growth of *Salmonella* sp. bacterial colonies. Confirmation of the PCR test with primers 139 *inv*A gene (5'-GTGAAATTATCGCCACGTTCGGGCAA-3') and 141 *inv*A gene (5'-TCATCGCACCGTCAAAGGAACC-3') with specific amplification of 284 bp did not confirm the presence of *Salmonella* sp., marked by the undetectable DNA band at the target size in samples 3A and 3B, although the positive control showed clear amplification.

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