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Identification of *Salmonella* Sp. in Duck Eggs Sold in Larangan Village

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Abstract
Background: Duck eggs are one of the foodstuffs that are very susceptible to damage caused by microbes. The types of microbes that contaminate eggs are mainly from the Gram-negative bacteria type, including Salmonella. *Objectives*: The purpose of this study was to determine the salmonella sp bacteria in duck eggs sold in Larangan village *Materials and Methods*: The examination method used was observation of laboratory tests, isolation methods, and identification of bacteria using biochemical tests. The samples used were duck egg yolks, using Brain Heart Infusion Broth (BHIB) media, Salmonella Shigella Agar (SSA), gram staining, Triple Sugar Iron Agar (TSIA), Simon citrate, and Sulfide Indol Motility (SIM). *Results*: The results of the research that has been done on BHIB media obtained 6 samples showed bacterial growth, on SSA media 6 duck egg samples showed bacterial colony growth, on gram staining obtained gram-negative and rod-shaped bacteria in 6 samples, on TSIA media obtained positive results on 6 media, simon citrate test showed positive results on 6 media, SIM test on motile test 6 media obtained positive results, on sulfide test 5 media obtained positive results, while indole test 6 media obtained negative results. *Conclusions*: Based on the results of research on the identification of salmonella sp bacteria in duck eggs sold in Larangan village, Larangan sub-district, Brebes regency, Central Java, it was found that 6 duck egg samples contained salmonella sp bacteria.

Keywords
Duck Eggs; *Salmonella;* Contamination

1. Introduction

Egg is one of the most practical food ingredients to use, does not require difficult processing. Its use is mostly for side dishes but often for traditional medicines. Some egg food ingredients have important value because they are a source of protein and fat needed by the human body (Warsito, 2015). Some animals can produce eggs, but only certain types of eggs are commonly traded and consumed by humans, namely chicken eggs, duck eggs, quail eggs and fish eggs (Widarta, 2018).

Duck eggs have bluish green shells. The size of duck eggs is much larger than quail eggs and slightly

larger than chicken eggs. The color of duck egg yolks is orange to reddish. (Prasetya et al 2015). The nutritional content of a duck egg is 69.7% water, 13.7% protein, 4.4% fat, 1.2% carbohydrates, 10% shell and 1% organic matter (Yolanda, 2021)

Salmonella Sp. bacteria are usually found in foods that contain high enough protein as a good medium for the growth of microorganisms. One type of food that contains high protein is eggs. In certain circumstances or in amounts that exceed the limit, microorganisms in the eggs can cause poisoning for those who consume them. The possibility of poisoning will be higher in consumers who consume raw eggs (Setiawan, 2008 in (Darmayani, Rosanty, and Vanduwinata 2017).

According to Sa'adah's research, (2021) entitled Identification of *Salmonella* Sp. Bacteria in Chicken Eggs and Duck Eggs Sold at the Antasari Traditional Market, Banjarmasin. Based on the results of the study, 1 incomplete duck egg and 2 intact duck eggs from 3 traders at the Antasari traditional market were found to be contaminated with Salmonella sp. bacteria.

There is a growing understanding in the community of Larangan Village that consuming raw duck eggs can increase protein levels in the body and make stamina fresher. Not a few people like to consume raw eggs, for health reasons, such as drinking them directly, mixed with herbal medicine or milk. This makes the consumption of raw duck eggs quite often done by the people of Larangan Village.

Based on this background, the researcher is interested in conducting a study entitled "Identification of *Salmonella sp*. In Duck Eggs Sold in Larangan Village, Larangan District, Brebes Regency, Central Java". This study is important to do because previously there has been no study on the identification of *Salmonella* sp bacteria in duck eggs sold in Larangan Village. This data can be used as additional information and educational efforts for the public in general to be more careful in consuming duck eggs.

2. Materials and Methods

The sampling location was in Larangan village and testing location was in the Mitra Karya Mandiri polytechnic laboratory. The research time was June - July 2024

2.1. Tools and materials

The research utilized several tools including Petridish, Ose, Bunsen, Test tube, Test tube rack, Incubator, Autoclave, Beaker glass, Erlenmeyer, Volume pipette, Analytical balance, Cotton, Object glass, Cover glass, Stirring rod. Duck egg sample, Brain Heart Infusion broth (Merck), Salmonella Shigella Agar (Himedia), Triple Sugar Iron Agar (Himedia), Simon Citrate Agar (Himedia), Sulfide Indol Motility (Himedia), Aquadest, Crystal Violet, Lugol, 95% Alcohol, Safranin, Ehrlich.

2.2. Methods

1. Brain Heart Infusion Broth Media Culture

   In the enrichment of duck egg yolk samples, 1 ml was taken sterilely using a pipette and then inserted into a test tube containing 9 ml of BHIB, then homogenized and incubated in an incubator at 37℃ for 24 hours. The presence of bacterial growth in the media is indicated by the media becoming cloudy (Rahman et al., 2022)

2. Planting samples on Salmonella-Shigella agar media

   From BHIB media then planted on solid media, namely Salmonella Shigella Agar (SSA) in a zigzag manner, then incubated in an incubator for 24 hours at a temperature of 37℃. Positive results from the isolation of *Salmonella* sp. using Salmonella Shigela Agar (SSA) media are marked by black colonies. (Amiruddin et al., 2017)

3. Gram Staining and Biochemical Reaction Planting

   After being left in the incubator for 24 hours, observe the growth of colonies found on solid media by looking at the shape, color, size, edges, and surface of the colony. Then take the suspected Salmonella colony from the SSA media, do gram staining, and continue planting on the Biochemical media, namely: Triple Sugar Iron Agar (TSIA) media. Colonies suspected of being Salmonella bacteria in SSA media were transferred using a sterile needle into TSIA slant agar media in a test tube by scratching the slant and piercing the vertical part, covering it tightly with sterile cotton, and then incubating it at 37℃ for 24 hours. The characteristics of Salmonella bacteria include the vertical part changing color to yellow with or without black (H2S), and the slant remaining red (does not change) (Supriani, 2019).

   Simon Citrat Colonies suspected of Salmonella bacteria on SSA media were taken using a sterile needle loop, planted on the Simon Citrate media in a zigzag manner on the slanted media surface, covered tightly with sterile cotton and then incubated in an incubator at 37℃ for 24 hours. The results were declared positive if the color of the media changed to blue on the surface of the media, negative results if the color of the media remained green. Sulfide Indol Motility Colonies suspected of being Salmonella bacteria in SSA media were taken using a sterile needle loop, cultured into the media by piercing the loop in a perpendicular position to the bottom of the media, covered tightly with sterile cotton and then incubated in an incubator at 37℃ for 24 hours. Positive Sulfur results: black fog occurs on the media. Negative Sulfur: no black fog occurs on the media. Positive Indole: A red ring is formed after hatching the Ehlrich reagent. Negative Indole: no red ring occurs after dropping the Ehlrich reagent. Positive Mortility: white fog occurs where the colony is pierced. Negative Mortility: no white

fog occurs where the colony is pierced. (Supriani, 2019)

3. Results and Discussion

3.1. Brain Heart Infusion Broth Result

From the duck egg yolk sample inoculated on BHIB media, the following results were obtained:

Table 1. BHIB media results table

No	Sample Code	BHI broth
1	1A	No turbidity occurs
2	1B	No turbidity occurs
3	2A	No turbidity occurs
4	2B	There is turbidity
5	3A	No turbidity occurs
6	3B	No turbidity occurs
7	4A	There is turbidity
8	4B	No turbidity occurs
9	5A	There is turbidity
10	5B	There is turbidity
11	6A	There is turbidity
12	6B	There is turbidity

The use of BHIB media functions as a fertilizing medium for bacterial growth and the presence of bacterial growth in the media is indicated by the media becoming cloudy (Rahman et al., 2022). BHIB is a liquid medium for various aerobic and anaerobic microorganisms. Inoculation of BHIB media is carried out by observing the turbidity which indicates bacterial growth in the sample. The bacteria found in BHIB media include Salmonella (Andrianto, 2012 in (Darmayani et al., 2017)).

3.2. Salmonella Shigella Agar Results

After planting on BHIB media, the sample was then cultured in SSA media, then incubated for 24 hours. The presence of Salmonella sp colonies on the media can be seen with the characteristics of colorless to pink, clear to opaque colonies with black spots in the middle (Saraswati 2012). Based on the results of observations of colony growth on SSA media, the following is known :

Table 2. SSA media results table

No	Sample Code	SSA Media
1	1A	No growth occurs
2	1B	No growth occurs
3	2A	No growth occurs
4	2B	transparent colony
5	3A	No growth occurs
6	3B	No growth occurs
7	4A	Black Colony

No	Sample Code	SSA Media
8	4B	No growth occurs
9	5A	Black Colony
10	5B	Black Colony
11	6A	Black Colony
12	6B	Black Colony

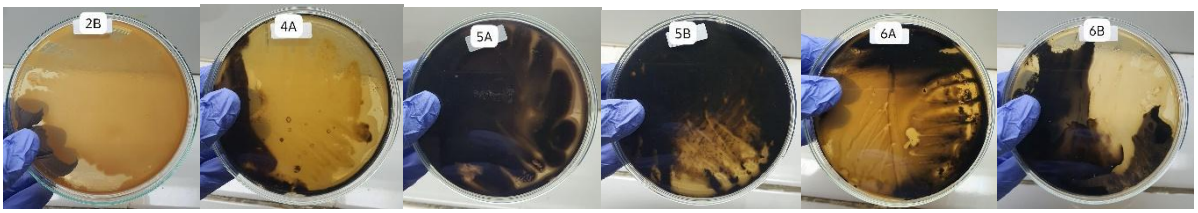


Figure 1. Colony view on SSA

From the research 6 samples were suspected to be positive for salmonella sp . Of the 6 samples, there was growth of colonies suspected of being *salmonella* sp. The 6 duck egg samples suspected of containing salmonella bacteria were then subjected to gram staining and biochemical testing (TSIA, Simon citrate and SIM). The results of the study showed that the colonies appeared to accumulate on the media, this could occur because when inoculating the BHIB media, no dilution was carried out on the duck egg yolk sample.

Based on the results of colony growth on SSA media, there were 6 samples that showed positive results with transparent colonies and black colonies. In samples 4A, 5A, 5B, 6A and 6B. there was black colony growth. According to Maksum Radji's statement (2010) in Wardani and Tanikolan (2021) the black Salmonella colony on SSA media is the result of the presence of H^2S compounds, meaning that the sample is positive for Salmonella bacteria. Bacteria that do not produce lactose such as Salmonella Spp. will be clear and there is a black color in the middle. The black color in the middle is an indication of the presence of H^2S produced by Salmonella spp. which will differentiate it from Shigella (Prayoga & Fatmawati, 2018).

In addition, there is 1 sample, namely sample 2B with transparent colonies. *S.paratyphi A* and *S. berta* are known to be negative for hydrogen sulfide, thus, their colonies do not appear black on media that detect hydrogen sulfide formation (Park et al., 2012). *Salmonella paratyphi* bacteria can produce gas and cannot produce H^2S (Ulya et al., 2020) .

3.3. Gram Staining Results

From the colonies that grew on SSA media, gram staining was carried out with the aim of confirming and observing bacterial morphology. From gram staining, the following results were obtained.

Table 3. Gram staining results table

No	Sample	Gram staining
1	2B	Gram Negative, rod
2	4A	Gram Negative, rod
3	5A	Gram Negative, rod
4	5B	Gram Negative, rod
5	6A	Gram Negative, rod
6	6B	Gram Negative, rod

Based on the gram staining that has been done, the results showed that all samples suspected of being positive for *Salmonella* sp. were rod-shaped and red in color, indicating that the bacteria were gram-negative. In gram staining, gram-negative bacteria will show a red color because gram-negative bacteria have a high lipopolysaccharide content in their cell wall layers so that when the decolorizing stage of staining uses 95% alcohol, the lipopolysaccharide layer becomes colorless because the first staining with gentian violet adheres to the lipopolysaccharide layer and when given the second staining, namely safranin, it produces a red image microscopically which reflects gram-negative bacteria (Yuswananda, 2015).

According to Darmawan (2017) in Safitri et al, (2019) , the pink color indicates the characteristics of Gram-negative bacteria. Salmonella bacteria are Gram-negative bacteria that have two layers of cell walls, namely the outer layer composed of lipopolysaccharides and proteins, and the inner layer composed of peptidoglycan which is thinner than Gram-positive bacteria.

3.4. Results of bacterial biochemical testing

From the colonies that grew on SSA media, further biochemical tests were carried out in the form of TSIA, Simon citrate, and SIM tests. Biochemical tests were carried out to determine the reactions produced by bacteria on the media used (Harti 2015 in (Haryati, 2020)). Based on the tests that have been carried out, the following results were obtained.

Table 4. Biochemical Test Results Table

No	Sample Code	TSIA	Simon citrate	SIM
1	2B	K/A, H^2S+, gas+	+	H^2S-,indole-,motile+
2	4A	K/A, H^2S+, gas+	+	H^2S+, indole-, motile+
3	5A	K/A, H^2S+, gas+	+	H^2S+, indole-, motile+
4	5B	K/A, H^2S+, gas+	+	H^2S+, indole-, motile+
5	6A	K/A, H^2S+, gas+	+	H^2S+, indole-, motile+
6	6B	K/A, H^2S+, gas+	+	H^2S+, indole-, motile+

Information:

TSIA :

K/A: Alkali/Acid , the slanted part is red and the vertical part is yellow.

H^2S+: There is a black precipitate on the media

Gas+: There are bubbles or slightly raised media

Simon citrate:

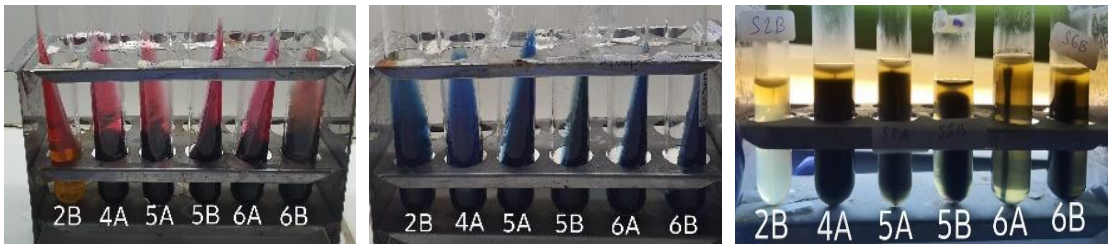
+ media color changes to blue

SIM:

Sulfide/H^2S+: There is a black haze on the media

Motile+: white fog appears where the colony is punctured.

Indole -: no red ring formed



Triple Sugar Iron Agar Simon Citrate Sulfide Indol Motility

Figure 2. Biochemical test results

Based on the results of the TSIA test in table 4, 6 samples suspected of containing salmonella appear to have their vertical parts turned yellow, indicating that the bacteria being tested can ferment glucose and the slopes are red, indicating that these bacteria do not ferment lactose and sucrose. 5 of the 6 samples suspected of containing salmonella in the TSIA test showed positive H^2S results, which are indicated by the presence of black sediment.

The results of the simon citrate test showed positive results in 6 samples suspected of containing salmonella, which was indicated by a change in the media to blue, indicating that the bacteria could use citrate as a carbon source.

The results of the SIM media test, in the sulfide test there were 5 positive sulfide samples marked by the black color formed on the media. The indole test on all samples obtained negative results because no red ring was formed after the Ehrlich reagent was dropped, this indicates that the bacteria do not use tryptophan as an energy source so that the bacteria are unable to produce indole (red ring). The motility test on 6 samples suspected of containing salmonella obtained positive results marked by the formation of colonies that spread along the puncture marks on the media. Motility test on Salmonella sp. positive because *Salmonella* sp. bacteria are bacteria that can move and have flagella.

In TSIA media, the characteristics of Salmonella bacterial colonies are that the vertical part changes color to yellow with or without black (H_2S), and the slanted part is red (does not change). (Safitri et al., 2019). In the TSIA test, the color of the slope area media is red because the bacteria are alkaline. This change in media color indicates that these bacteria do not ferment lactose and

sucrose. In the base area media, it changes to yellow, indicating that the bacteria can ferment glucose. Positive gas formation, the result of fermentation of H2 and CO_2. Positive H^2S formation is indicated by the presence of black sediment. (Tantri, 2016).

Salmonella bacteria in the Citrate test showed a positive reaction. Microbial growth is seen on the sloping surface and the media will turn blue (Saraswati, 2012). The results of this test are positive for *Salmonella* sp. because *Salmonella* sp. can use citrate as a carbon source (Tantri, 2016). Salmonella paratyphi bacteria can use citrate as a sole carbon source. This is because *Salmonella paratyphi* bacteria have the enzyme citrate permease which can facilitate citrate transport in bacterial cells (Cappucino et al, 2013 in (Ulya et al., 2020)).

Sulfur can be reduced to H^2S (Hydrogen Sulfide) if hydrogen sulfide is produced then a black color will form in the media. Rachel Watson (2012) in (Allung, 2019)). Positive motile is indicated by the formation of colonies that spread along the puncture marks on the media (Karunia et al., 2021). Motility test on *Salmonella* sp. positive because *Salmonella* sp. bacteria are bacteria that can move and have flagella (Tantri, 2016).

4. Conclusions

Based on the results of research on the identification of salmonella bacteria in duck eggs sold in Larangan village the results showed that 6 duck egg samples contained bacteria *Salmonella* sp.

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Author Contributions: NAP: conceived the original idea. UNK: provided suggestions. AJ: reviewed the suggestions and evaluated the research results until the drafting of the manuscript.

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