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Salmonella sp. Contamination Detection in Layer Chicken Eggs Traded in Traditional Market of Medan Johor District

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ABSTRACT

Salmonella sp. can contaminate layer chicken eggs and cause salmonellosis. This study aims to determine whether there is contamination by *Salmonella* sp. in layer chicken eggs traded in traditional markets of Medan Johor District. Sampling locations were determined by purposive sampling, including Kwala Bekala Market, Johor Market, and Tikung Market, with 90 samples. The working procedures included isolating bacteria from egg yolks using the pour plate method on SSA media, observing the bacteria morphology, calculating total *Salmonella* sp. colonies, Gram staining, and biochemical tests. The research results obtained indicated that the average total colonies of *Salmonella* sp. in Kwala Bekala Market were 1.45 x 10^5 CFU/g, in Johor Market they were 5.8 x 10^4 CFU/g, and in Tikung Market they were 3.6 x 10^4 CFU/g. The colony morphology of *Salmonella* sp. is circular and colourless, with a black spot in the center. The *Salmonella* sp. bacteria are Gram-negative with a red color, and are bacilli. Biochemical tests showed indole (-), MR (+), VP (-), SCA (+), and TSIA (+) test results. Based on the study results, it can be concluded that 4.44% of the positive samples were contaminated with *Salmonella* sp. in the traditional market of Medan Johor District and exceeded the BMCM in SNI 7388:2009.

Keywords: contamination, egg, *Salmonella* sp., traditional market

INTRODUCTION

Most food poisoning outbreaks in Indonesia, namely 58.49% of cases, are caused by microorganism contamination, both confirmed and suspected (Syafriyani and Djaja, 2020). Salmonella sp. bacteria is a pathogenic microorganism that must be watched out for because it can contaminate poultry products. WHO (2018) states *Salmonella* sp. can go through the entire food chain from animal feed primary production to households or food services. *Salmonella* sp. is a genus of pathogenic bacteria that can interfere with human health.

Layer chicken eggs are in great demand by the public compared to other poultry eggs. Egg breeds of chickens belong to perishable food. Microbiological damage to eggs can be caused by bacterial contamination because they contain high nutrients, so microorganisms can grow and develop properly. FAO (2002) states contamination of *Salmonella* sp. bacteria in poultry can occur in production environments, either vertical contamination (for example, through eggs that trigger career DOC) or horizontal contamination originating from the environment, feed contaminated with *Salmonella* sp. bacteria or in the process of slaughtering and handling poultry products.

Chusniati et al. (2009) state *Salmonella* sp. bacteria in eggs can cause food-borne disease under certain conditions or the number of bacteria that exceeds the limit of consumers. *Salmonella* sp. includes pathogenic bacteria that can cause food poisoning. Symptoms of salmonellosis after consuming food contaminated with Salmonella sp. include nausea, fever, headache, stomach cramps, gastroenteritis and vomiting for 2-7 days (Rahayu and Nurwitri, 2012). Under certain conditions, infection with *Salmonella* sp. can develop into systemic infections leading to bacteremia, meningitis and endocarditis with high morbidity and mortality (Graham et al., 2000). People at high risk of salmonellosis are pregnant women, infants and toddlers, the elderly and people who are sick (Bell et al., 2016).

Gastroenteritis (diarrhea) is one of the symptoms of bacterial infection of *Salmonella* sp. in the human body. BPS (2020) states it is known that 70,243 cases of gastroenteritis occur in North Sumatra Province. Medan City is in the second position with the highest number of gastroenteritis cases, reaching 8,047. One of the areas in Medan

City is Medan Johor District, with a population of 157.703 people in 2021 (BPS, 2022).

Research by Usman et al. (2014) found 2 out of 10 samples of raw eggs and 2 out of 10 samples of undercooked eggs at coffee shops in Medan Maimun District positive for *Salmonella* sp. Research on *Salmonella* sp. has also been performed by Yansri et al. (2021) showed that 2/18 (11.1%) chicken egg shells consumed from traditional markets of Bali were contaminated with *Salmonella* sp. In the research of Wahyuningsih et al. (2019), they obtained 1/30 samples of layer chicken eggs traded in the Purwokerto Wage Market buoyant for *Salmonella* sp. found in the shell and yolk.

The maximum contamination limit of *Salmonella* sp. bacteria in chicken egg consumption is negative/25 g (SNI, 2009). Based on the magnitude of the risk caused by *Salmonella* sp. bacteria, it is necessary to research to detect the presence or absence of contamination of *Salmonella* sp. bacteria in layer chicken eggs, especially those traded in the traditional market of Medan Johor District. Information regarding the presence of *Salmonella* sp. layer chicken egg products traded in the traditional market of Medan Johor District can increase public awareness in buying, processing and consuming chicken eggs.

MATERIALS AND METHODS

Time and place of research

The research was conducted in the traditional market of Medan Johor District consisting of Kwala Bekala Market, Johor Market and Tikung Market. *Salmonella sp*. was tested at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara.

Research materials and tools

The materials used in this study were 90-layer chicken egg samples taken from three traditional markets of Medan Johor District, sterile aquades, 70% alcohol, *Salmonella* Shigella Agar (SSA), Nutrient Agar (NA), Sulfide Indole Motility (SIM), Simmon's Citrate Agar (SCA), Methyl Red - Voges-Proskauer (MR-VP), Triple Sugar Iron Agar (TSIA), crystal dye violet, iodine, acetone alcohol, safranin, immersion oil, alphanaphthol 5%, methyl red indicator, Kovac's reagent, and KOH 40%.

The equipment used in this study is an autoclave, incubator, erlenmeyer, hot plate, digital scale, colony counter, microscope, preparation glass, bunsen burner, micropipette and tip, test tube rack, test tube, beaker glass, straight and loop needle, magnetic stirer and petri dish.

Research methods

This study used purposive sampling techniques and was a survey study in three traditional market locations in Medan Johor District. The first stage in this study is the observation of each traditional market to determine the cleanliness condition of the market consisting of dirty markets (Kwala Bekala Market), markets with medium sanitary conditions (Johor Market) and clean markets (Tikung Market). Furthermore, samples were taken from all layer chicken egg traders in three predetermined traditional markets.

The sampling process is carried out randomly with different levels of eggshell hygiene. The amount of dirt attached to the eggshell is used to measure eggshell cleanliness (Setiawati, 2016). Sample selection was done by observing the shell's cleanliness and comparing it with eggs sorted from dirty, medium, and clean shells.

Observations of eggshell cleanliness refer to Moreng and Avens (1985) in Mutiar et al. (2022) which classifies egg quality based on shell hygiene into three categories: clean refers to eggs that are clean-shelled and do not have the slightest stain, medium is eggs with shell droppings of no more than 3.1% of the total surface of the shell, and dirty is an egg with a shell that has a stain of about 6.25% of the surface area of the shell. The selected traditional markets and the number of sample data are presented in Table 1.

Table 1. Number of samples from each traditional market

Market name	Market hygiene conditions	Number of egg traders	Number of samples
Kwala Bekala	Dirty	7	42
Johor	Medium	2	12
Tikung	Clean	6	36
Total		15	90

There are seven chicken egg traders at Kawala Bekala market with a sample size of 42, chicken egg traders at Johor market two people with a sample size of 12, and chicken egg traders at Tikung market six people with a sample size of 36, then the sample code will be given as shown in Table 2.

Table 2. Sample coding

Market name	Merchant code	Taking	Sample code
Kwala Bekala	A	I	A1, A2, and A3
		II	A1, A2, and A3
	B	I	B1, B2, and B3
		II	B1, B2, and B3
	C	I	C1, C2, and C3
		II	C1, C2, and C3
	D	I	D1, D2, and D3
		II	D1, D2, and D3
	E	I	E1, E2, and E3
		II	E1, E2, and E3
	F	I	F1, F2, and F3
		II	F1, F2, and F3
	G	I	G1, G2, and G3
		II	G1, G2, and G3
	Total		42
Johor	H	I	H1, H2, and H3
		II	H1, H2, and H3
	I	I	I1, I2, and I3
		II	I1, I2, and I3
	Total		12
Tikung	J	I	J1, J2, and J3
		II	J1, J2, and J3
	K	I	K1, K2, and K3
		II	K1, K2, and K3
	L	I	L1, L2, and L3
		II	L1, L2, and L3
	M	I	M1, M2, and M3
		II	M1, M2, and M3
	N	I	N1, N2, and N3
		II	N1, N2, dan N3
	O	I	O1, O2, and O3
		II	O1, O2, and O3
	Total		36

Sampling

Sampling of layer chicken eggs was carried out at 09.30 - 11.30 WIB. Samples were taken from three traditional markets with different market sanitation conditions. Samples were taken from as many as three layers of chicken eggs from each egg trader with twice the samplings.

Work procedure

1. Sample preparation

The eggshell of layer chickens is cleaned with an alcoholic cotton swab. Next, the egg is broken using a sterile knife at the end of the shell, then 1 ml of egg yolk is taken using a micropipette and put into sterile plastic and coded (Velina et al., 2019).

2. Dilution

A sample of 1 ml was taken using a micropipette. The sample was inserted into the first dilution tube (10^{-1}) filled with 9 ml of sterile aquades homogenized with vortex for 5-10 seconds. Next, a solution of 1 ml of 10^{-1} dilution with a micropipette is inoculated into a second dilution tube (10^{-2}) containing 9 ml of sterile aquades, then homogenized with vortex for 5-10 seconds. Other test tubes are carried out in the same procedure to a dilution level of 10^{-3}.

3. Isolation of *Salmonella* sp.

Salmonella sp. bacteria are isolated by pour plate method, where 1 ml of cell suspension from every 10^{-3} dilution is taken with a micropipette and put aseptically into a sterile petri dish, ±15 ml of warm Salmonella Shigella Agar (SSA) media (temperature 45-50 °C) is poured into a petri dish that has contained the bacterial suspension and closed. Petri dishes are gently rotated to homogenize the mixture of media and suspension. The solidified media in the petri dish was incubated at 37 ˚C for 24 hours (Ashrafudoulla et al., 2021).

4. Calculation of total colonies of *Salmonella* sp.

The Standard Plate Count (SPC) calculation technique determines the total number of colonies of *Salmonella* sp. on the petri dish. The criterion for the number of colonies calculated is 30-300 colonies of bacteria in one petri dish. The formula for calculating the total number of bacterial colonies according to Fardiaz (1993) in Joni et al. (2018) as follows:

$$\text{Total colony (CFU/g)} = \text{number of colonies on a dish} \times \frac{1}{\text{Dilution factor}}$$

5. Purification

If the results obtained on Salmonella Shigella Agar (SSA) media show that the growing bacterial colony has the characteristics of *Salmonella* sp. then proceed with the purification stage to obtain a pure culture. Purification is carried out by the scratch-cup method. Colonies suspected of *Salmonella* sp. with ose needles aseptically, then etched into Nutrient Agar (NA) media. NA media inoculated with test bacteria is then incubated at 37 ˚C for 24 hours (Safitri et al., 2019).

6. Gram staining test of bacteria

Suspected bacteria of *Salmonella* sp. purified on Nutrient Agar (NA) media are then

identified by the Gram staining test of bacteria. A round loop needle is heated over the bunsen until incandescent, then dripped on the preparation glass and made a review preparation from the bacteria provided. The review preparation is dripped with gentian violet, allowed to stand for one minute, rinse with aquades, and then dried. After that, the preparation is added iodine (lugol) 1-2 drops for 30 seconds, then rinsed with acetone alcohol for 15 seconds, then rinsed with aquades. Next, the review preparation is given one drop of safranin solution for 1 minute, rinsed with aquades and dried. Then, immersion oil was dripped on the review preparation and observed with a 100x magnification microscope.

7. Biochemical test

a) Indole test

The indole test uses one pure culture loop from Nutrient Agar (NA) media taken and inserted aseptically into the Sulfide Indole Motility (SIM) media test tube. Next, the test tube is incubated at 37 ˚C for 24 hours. Such incubated media is given 0.2 - 0.3 ml of Kovacs reagent. The indole test result is positive if there is a red layer (ring) on the surface of the media, and the negative result is if a yellow solution forms on the surface of the media. Specific test results of *Salmonella* sp. are a negative indole test (Safitri et al., 2019).

b) MR-VP test (Methyl Red -Voges Proskauer)

Test tubes containing 10 ml of MR-VP media were added pure cultures of NA media, then incubated for 48 hours at a temperature of 35 ˚C. Next, the incubated MR media tubes were given 5-6 drops of methyl red indicator. In the VP test tube, we were given 0.6 ml of alpha naphthol solution and 0.6 ml KOH 40%, homogenized, and allowed to stand. Positive MR-VP (Methyl Red-Voges Proskauer) test result if there is a change in media color to pink or red. Generally, *Salmonella* sp. showed positive results for the MR test and harmful for the *VP* test (VP) (Safitri et al., 2019).

c) Citrate test

Pure culture on NA (Nutrient Agar) media is taken with a loop needle and inoculated aseptically by scratching ose on the inclined part of Simmon's Citrate Agar (SCA) media. Next, the media tube was incubated at 37 °C for 48 hours. If the media changes color from green to blue, it indicates that the test result is positive. Generally, *Salmonella* sp. gave a positive result on the citrate test (Ashrafudoulla et al., 2021).

d) TSIA test (Triple Sugar Iron Agar)

Pure cultures of test bacteria on Nutrient Agar (NA) media are inoculated using loop needles by scraping the slope (oblique) and piercing the middle of the media to tilt TSIA in test tubes. Then, the TSIA media was incubated for 24-48 hours at 37 ˚C (Safitri et al., 2019). The results of the TSIA test reaction are: (a) the presence of glucose fermentation if the slope is red (-) / the base is yellow (+); (b) lactose and sucrose fermentation is characterized by a slope yellow (+) / yellow (+) base; (c) there is no fermentation of sugar, and no gas or H_2S is indicated by the slope in red (-) / the base in red (-); (d) the media appears to rise indicating the presence of gas so that an air chamber is formed at the bottom of the media tube; (e) black color on media shows H_2S (Saimin et al., (2020).

Data analysis

The research data is presented by descriptive analysis.

RESULTS AND DISCUSSION

The results of the calculation of total bacterial colonies that show the characteristics of *Salmonella* sp. in samples of layer chicken eggs traded in the traditional market of Medan Johor district grown on SSA media dishes are presented in Table 3, and the results of macroscopic observations of bacterial colonies growing on SSA media can be seen in Table 4.

Table 3. Total colonies of *Salmonella* sp. and SNI standard

Market name	Sample code	Total colonies *Salmonella* sp.	SNI standard (CFU/g)	Information
Kwala Bekala	D1	$1.3x10^5$ CFU/g	Negative*	>BMCM
	F3	$1.6x10^5$ CFU/g	Negative*	>BMCM
Johor	H3	$5.8x10^4$ CFU/g	Negative*	>BMCM
Tikung	M2	$3.6x10^4$ CFU/g	Negative*	>BMCM

Description: Negative* = in qualitative units
BMCM = Maximum Limit of Microbial Contamination SNI 7388:2009

Table 4. Results of macroscopic observation of bacterial colonies on SSA media

Market name	Sample code	Bacterial colonies				*Salmonella* sp.
		Colour	Shape	Margin	Elevation	
Kwala Bekala	D1	Black	Circular	Entire	Convex	+
	F3	Black	Circular	Entire	Convex	+
Johor	H2	Pink	Circular	Entire	Convex	-
	H3	Black	Circular	Entire	Convex	+
Tikung	L3	Pink	Irregular	Entire	Flat	-
	M2	Black	Circular	Entire	Convex	+
	M3	Pink	Circular	Entire	Convex	-
	J2	Pink	Circular	Entire	Convex	-
	K3	Pink	Circular	Entire	Convex	-
	L3	Pink	Circular	Entire	Convex	-
	O2	Pink	Circular	Entire	Convex	-

Description: + = *Salmonella* sp. bacteria
- = Not *Salmonella* sp. bacteria

Data from macroscopic observations of bacterial colonies contained in SSA media dishes, based on Table 4, show that *Salmonella* sp. bacteria have black colony characteristics, circular shape, entire margin, and convex elevation. Safitri et al. (2019) state *Salmonella* sp. on SSA media has a circular-shaped colony characteristic with a clear colony color marked in black in the middle.

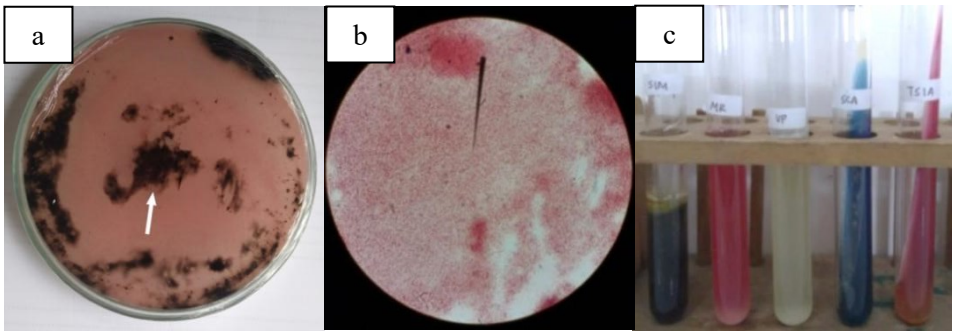

Figure 1. Documentation of research results a) bacterial colonies of *Salmonella* sp. on SSA media; b) Gram staining results with 100x magnification; c) biochemical test results

Data from the Gram staining test of bacteria carried out shows that the bacteria tested are Gram-negative bacteria with red characteristics and bacilli. Therefore, these bacteria are bacteria from the *Enterobacteriaceae* family. It is in line with the statement of Bell and Kyriakides (2002) that *Salmonella* sp. bacteria are Gram-negative bacilli and members of the family *Enterobacteriaceae.* Test for bacteria (*Salmonella* sp.) that have passed the Gram staining test are then carried out biochemical tests, and the data obtained is shown in Table 5.

Table 5. Biochemical test results

Market name	Sample code	Biochemical test					Conclusion
		Indole (SIM)	MR	VP	SCA	TSIA	
Kwala Bekala	D1	-*	+	-	+	+	*Salmonella* sp.
	F3	-*	+	-	+	+	*Salmonella* sp.
Johor	H3	-	+	-	+	+	*Salmonella* sp.
Tikung	M2	-	+	-	+	+	*Salmonella* sp.

Description: SIM = Simmon's Citrate Agar; - (yellow ring), -* (yellow ring, H_2S +)
MR = Methyl Red; + (red)
VP = Voges Proskauer; - (remains yellow/does not change color)
SCA = Simmon's Citrate Agar; + (blue)
TSIA = Triple Sugar Iron Agar; + (slope: red, base: yellow, H_2S -)

Based on the biochemical test results, samples with codes M2, D1, F3 and H3 meet the characteristics of *Salmonella* sp. The test results on SIM media show an adverse reaction of indole with a yellow ring on the surface of the media. D1 and F3 samples showed positive results containing H_2S with black deposits on the SIM media. Afriyani et al. (2016) stated that *Salmonella* sp. bacteria cannot produce indole because the bacteria do not use energy sources from tryptophan. Safitri et al. (2019) suggested that the specific test results of *Salmonella* sp. were negative indole tests. *Salmonella* sp. on SIM media shows the presence or absence of H_2S formation (Amiruddin et al., 2017).

The MR test showed a positive reaction with a change in media color from yellow to red, while the VP test showed an adverse reaction with no change in media color (remained yellow). Afriyani et al. (2016) state that the genus *Salmonella* sp. can ferment glucose and produce large quantities of lactic acid, acetic acid, succinic acid, formic acid, CO2H2, and ethanol. As these acids build up, the pH drops to 5.0 or less. It turns red if a methyl red indicator is added to the low pH culture. It shows that the organism can ferment mixed acids (mixed acid fermenters). Nur et al. (2022) state that if no red color is formed on VP media after adding alpha naphthol and KOH shows negative test results. It indicates that the end product of bacterial fermentation is not acetyl methyl carbinol (acetoin).

Salmonella sp. showed a positive reaction to the citrate test on SCA media and the media changed color to blue. Saimin et al. (2020) state this happens because the single carbon energy source, namely citrate, can be used by bacteria, so bacteria will use ammonium salts and produce ammonia. It causes the acid in the media to disappear, increasing the pH. An increase in pH causes the color of the media to change from green to blue (Ashrafudoulla et al., 2021).

The TSIA test shows a positive reaction with a change in media color, red on the slope (oblique) and yellow at the base (upright), and no H_2S is formed. It is the opinion Saimin et al. (2020) that if the slope is red (-) and yellow at the base (+) indicates the presence of glucose fermentation. Nur et al. (2022) state the color of the media turns red because bacteria are alkaline. It happens because there is no fermentation of lactose and sucrose by bacteria. While at the base, the media's colour turns yellow, indicating that glucose fermentation by bacteria occurs.

Salmonella sp. contamination in the yolk of layer chickens can come from dirty egg shells. Samples M2, F3, and H3 have eggshells with stains (dirt). Most layer chicken egg traders in the traditional market of Medan Johor District do not clean dirty eggshells. The lack of cleaning of layer chicken egg shells by traders can trigger contamination of *Salmonella* sp. into the egg. Yunilas et al. (2019) suggest that microorganisms can come from feces, urine, slaughterhouse residue, blood, fur, etc. Chusniati et al. (2009), *Salmonella* sp. can colonize the digestive tract and then excrete through feces and attach to the surface of the shell so that these bacteria are transmitted horizontally into the egg. Then, there is a penetration of bacteria into the egg through the eggshell's pores that are not closed by the cuticle so that bacterial contamination occurs inside (yolk and albumen).

Layer chicken eggs with clean shells can also be contaminated with *Salmonella* sp. bacteria. The D1 sample is an egg with a clean shell and was positive for *Salmonella* sp. bacteria. Chusniati et al. (2009) state that the possibility of *Salmonella* sp. contamination grooves occur directly (vertically) into the egg through yolk and albumen from the ovaries of hens infected with *Salmonella* sp. Direct contact of *Salmonella* sp. in the yolk at the time of the egg formation process (ovoposition), namely during the passage of the egg from the ovary to the infundibulum and oviduct, this can cause transovarial infection before the shell covers the egg and before it is protected by antibacterial albumen.

Chicken eggs contaminated with *Salmonella* sp. have different shell colors. D1 samples have brown shells, F3 and M2 have light brown shells, and H3 samples have dark brown shells. Eggs with dark brown shells have fewer pores, so the quality decline is slower and can inhibit the penetration of *Salmonella* sp. into the egg. Jazil (2013), the lighter the brown color of the eggshell, the faster the quality declines. Eggs with dark brown shells have more robust and thicker shells and experience lower quality degradation than eggs with brown and light brown shells (Maimunah and Rokhman, 2018).

Contamination of *Salmonella* sp. bacteria can also be affected by the lack of hygiene and sanitation by traders and places selling eggs. Based on the observations, most egg sellers do not have a place to wash their hands, so traders rarely wash their hands during the selling process in the market, as well as the lack of trash cans at each stall.

Poor market sanitation and poorly maintained facilities can increase the risk of contracting *Salmonella* sp. contaminated traded chicken eggs. Test results of *Salmonella* sp. showed that two samples from Kwala Bekala Market were positively contaminated with *Salmonella* sp. In contrast, in the Johor Market and Tikung Market samples, there was one positive sample of *Salmonella* sp. each. Abebe et al. (2020) show that market conditions and marketing practices strongly influence contamination by several disease agents, including bacteria, viruses, fungi, and parasites. *Salmonella* sp. contamination in eggs can also occur during sales due to a lack of attention to hygiene and sanitation.

Prevent *Salmonella* sp. contamination in livestock products by paying attention to hygiene, choosing the right way to consume processing products, and separating raw products from cooked products. It is recommended to consume cooked eggs (WOAH, 2022). Prevention must also be done by educating the public to apply a clean and healthy lifestyle for food safety. Control should start upstream and work downstream into the product. Therefore, applying Hazard Analysis and Critical Control Points (HACCP) is crucial for contamination control (Lawley, 2008).

CONCLUSION

The level of contamination of *Salmonella* sp. bacteria in layer chicken egg samples from traditional markets in Medan Johor District was 4.44% and exceeded the BMCM in SNI 7388:2009.

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