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Isolation of *Salmonella* sp. from Eggs Commonly Sold in Tondano Market, Minahasa, North Sulawesi

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Abstract. Various types of eggs is one of the foodstuffs that are in great demand by the community, especially the people of North Sulawesi because it is cheap and easy to obtain. also, eggs have nutritional content of animal protein which is good for humans. Several studies on eggs have been conducted and the results show that microorganisms such as bacteria can contaminate eggs *Salmonella* sp. This study aims to detect *Salmonella* sp. bacteria in various types of eggs in the Tondano Market, Minahasa, North Sulawesi. This research uses descriptive method (qualitative method) to describe an event objectively. Samples taken as many as 3 samples, the first point is located outside the market, 3 samples of purebred chicken eggs, duck eggs and quail eggs are taken then the second point inside the market is taken 3 samples of purebred chicken eggs, duck eggs and quail eggs from the same trader. Planting on the media is done by separating the eggshell and its contents then the eggshell as much as 2 grams is mashed and moistened with 5 ml of Buffered Peptone Water (BPW) 0.1% after which it is placed into 10 ml Rappaport Vassiliadis (RV) media, followed by scraping on Sallmonella Shigella Agar (SSA) media after which it is inoculated on Xylose Lysine Deoxycholate agar (XLD) media (first point and second point), there were 4 positive eggshell samples, namely duck eggs (TB 1), quail eggs (TP 1), broiler eggs (TA 2) and quail eggs (TP 2) only slightly contained *Salmonella* sp. bacteria while 2 samples were negative for *Salmonella* sp. namely broiler eggs (TA 1), and duck eggs (TB 2).

***Keywords*:** *Salmonella* sp., purebred chicken eggs, duck eggs, quail eggs

I. INTRODUCTION

Food and drink are essential for humans to survive, grow, develop and produce. If there is no food then human life must be disrupted which results in being unproductive. Food in the form of animal food is also very important to meet human needs. For example, animal products included in animal protein supplements have nutrients that are beneficial to health because they contain nutrients needed by the body, one of which is eggs [1].

In addition, the concentration of eggs is often determined by environmental conditions, including the low quality of the eggs and the lack of screening of eggs before they are consumed and sold by humans. A number of studies in this field have been conducted and the results prove that microorganisms such as bacteria can affect eggs quality [2].

Egg contamination by *Salmonella* sp. can occur in one of two ways: vertically or horizontally. Vertical contamination, also known as transovarial contamination, occurs when *Salmonella* sp. infects infected meat, while horizontal contamination occurs when feces, straw, and eggs are present. *Salmonella* sp. infection in humans occurs when people consume contaminated food. *Salmonellosis* manifests clinically as gastroenteritis. It is accompanied by abdominal pain, diarrhea, high fever, and nausea, and vomiting for at least one month [3].

The sale of various kinds of eggs is widely developed in North Sulawesi Province, especially in Tondano Market, West Tondano Subdistrict, Minahasa Regency. The marketing and sale of various kinds of eggs in Tondano Market has been going on for a very long time. Based on the findings of the research conducted at Tondano Market, several environmental factors were identified, such as deteriorating market conditions, poor sales locations, and unsterile expansion locations and lack of education on washing eggs before consumption so that they do not have continuous supervision of the quality of eggs produced so that the risk of *Salmonella* sp. bacteria can be in the eggs, which in turn can cause health problems if the processing is not appropriate.

The global prevalence of *Salmonella* sp, according to Institute for Health Metrics and Evaluation statistics (2019), is 4.22 per 100.000 people, with a mortality rate of 1.72. In 2019, the prevalence rate in Indonesia was 5.82 per 100,000 population with a mean of 2.42. In 2018, the prevalence of *Salmonella* sp. in North Sulawesi was 2.9%, followed by 2.7% in North Minahasa Regency.

Salmonella sp. can cause diseases that can endanger human health. Therefore, researchers are interested in conducting research on the presence of *Salmonella* sp. In purebred chicken, duck, and quail eggs sold at Tondano Market, West Tondano District, Minahasa Regency, North Sulawesi. The difference between this research and previous research is the research location and sampling location as well as the methods used in this research.

II. METHODS

Time and Location

The research method used is descriptive method (qualitative method). The descriptive method aims to describe or describe a situation as a whole [4]. This research took place in the Tondano Market, Minahasa Regency and continued research at the Manado State University Laboratory and the Manado Public Health Laboratory Center Laboratory and this research started from the preparation of the proposal until completion, which was 4 months starting from August to November 2023.

Materials

The materials in this study are purebred chicken eggs, duck eggs, quail eggs, distilled water, Salmonella Shigella Agar (SSA), Buffered Peptone Water (BPW) 0.1%, Rappaport Vassiliadis (RV), Xylose Lysine Deoxycholate agar (XLD), aluminum foil/paper and cotton. Tools used such as petri dishes, labels, measuring cups, beaker glass, bunsen, test tubes, mortars, microscopes, autoclaves, ovens, hot plates, scales, round ose, glass objects, drop pipettes and stirrers [2].

Samples and Sampling Techniques

The population of this study were purebred chicken eggs, duck eggs and quail eggs from different seller distributors in the Tondano market, Minahasa Regency.

Samples were taken as many as 3 samples, namely samples of purebred chicken eggs, duck eggs and quail eggs traded in the Tondano Market, West Tondano District, Minahasa Regency, North Sulawesi. Samples were taken from two points in the market located outside and inside the Tondano market. For sampling, it was taken randomly and then tested at the laboratory for microbiological analysis.

Data Collection Technique

The data collection techniques used are as follows: the results of the isolation of purebred chicken eggs, duck eggs and quail eggs containing or not *Salmonella* sp.

Observation Method

Making direct observations to the place that is the object of research to see up close to the object to be studied from the beginning to the end of the research, with the intention and purpose that researchers can obtain complete data such as an overview of the surrounding environment in the Tondano Market, Minahasa Regency.

Activities to document all stages from the beginning to the end of the research to obtain documents as evidence as well as accurate data related to supporting research.

Research Procedure

Sterilization of Tools and Materials

Beaker cups, Petri dishes, reaction trays, stirrers, and beakers wrapped in paper were done first. Finally, they were sterilized using an oven at 160°C for approximately 2 hours. Other logarithmic materials, such as ose needles, were sterilized in a flame bath for approximately one minute. The media was sterilized using an autoclave at 121°C [5].

Samples Preparation

Samples of purebred chicken eggs, duck eggs, and quail eggs were separated from the shell, then were placed in different sample places. These shells were grinded and put in a beaker.

The three types of eggshells were each weighed 2 grams and mixed with 5 ml of 0.1% Buffered Peptone Water (BPW) before incubating at 35°C for 24 ± 2 hours. Eggshell samples were taken 0.1 ml and put into 10 ml Rappaport Vassiliadis (RV) medium incubated at 42°C for 24 hours.

In this study, colony accounting was 5 dilutions, namely -1, -2, and -3. The findings of this study indicate that as the population growth rate increases, the number of colonies that can be reached also increases. This is due to the large number of microbes contained in each volume of inoculant filled due to the completion of the bacterial isolation process on selective medium.

Bacterial Identification

Recognizing the characteristics by bacteria needs to be done by isolating bacteria on selective medium. The procedure works if the growth results are obtained positive then it can be continued to scratch on Sallmonella Shigella Agar (SSA) media incubated at 37°c for 24 ± 2 hours.

Colonies on *Sallmonella Shigella Agar (SSA)* were inoculated on *Xylose Lysine Deoxycholate Agar (XLD)* and incubated at 35°C for 24 ± 2 hours. Taking colonies towards *Salmonella* sp. The occurrence of changes, namely positive results characterized by the formation of red colonies with black cores [6].

Data Analysis Technique

The data will be analyzed manually and presented in a table to determine the prevalence of *Salmonella* sp. In several types of eggs in the Tondano market, Minahasa Regency.

III. RESULTS AND DISCUSSION

Results

The samples in the study were broiler, duck and quail eggs from Tondano Market, Minahasa Regency on November 27, 2023. The three kinds of eggs were taken at 2 market points, namely outside the market (first point) and inside the market (second point) (Figure 1).

At the first point (outside the market), 3 samples were taken, namely 1 chicken egg, duck egg and quail egg each. While at the second point (inside the market), 3 samples were taken, namely 1 chicken egg, 1 duck egg and 1 quail egg. Then the six egg samples were taken and tested at the Manado State University Laboratory and the Manado Public Health Laboratory Center Laboratory.

Isolation and identification of *Salmonella* sp. Bacteria from 6 eggshell samples were carried out through the streak method on Sallmonella Shigella Agar (SSA) media which was previously enriched using Rappaport Vassiliadis (RV) broth media for 24 hours at 37˚C.

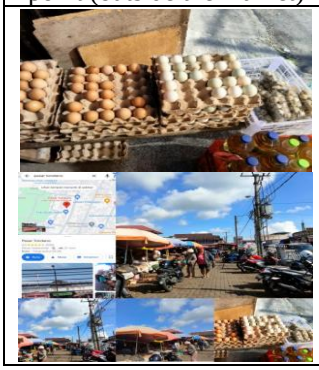
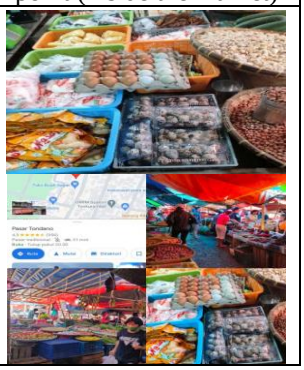
Egg sample at the first point (outside the market)	Egg sample at the first point (inside the market)
	

Fig. 1. Egg Samples Taken at Tondano Market, Minahasa Regency

The isolation results of *Salmonella* sp. Bacteria in 3 eggshell samples at the first point (outside the market) showed that the shell of purebred chicken eggs (TA 1) was negative (*Salmonella-Shigella* bacteria colonies were absent), duck eggshells (TB 1) were positive (*Salmonella* sp. bacteria colonies were present) and quail eggshells (TP 1) were positive *Salmonella* bacterial colonies that grow on SSA are round with flat edges and a black center (*Salmonella* sp. Bacteria were present). While the results of isolation of *Salmonella* sp. bacteria colonies from 3 eggshell samples at the second point (inside the market) obtained that purebred chicken eggshells (TA 2) were Positive (*Salmonella-Shigella* bacteria colonies were present), duck eggshells (TB 2) were Negative (*Salmonella-Shigella* bacteria colonies were absent) and quail eggshells (TP 2) were Positive (*Salmonella-Shigella* bacteria colonies were present) (Fig. 2).

Figure 3.2 *Salmonella* sp. Bacterial Isolates Growing on Sallmonella Shigella Agar (SSA) Media.

Furthermore, the collection of bacteria that developed on Sallmonella Shigella Agar (SSA) media was inoculated onto Xylose Lysine Deoxycholate Agar (XLD) media for 24 hours at 37˚C. There are bacteria on the XLD media. Bacterial colonies that grow on Xylose Lysine Deoxycholate Agar (XLD) media cause a change in the color of the media to a red color so that all colonies that grow on Xylose Lysine Deoxycholate Agar (XLD) media are suspected of being *Salmonella* sp. bacteria (Fig. 3).

Fig. 3. *Salmonella* sp. Bacterial Isolates Growing on Xylose Lysine Deoxycholate Agar (XLD) Media.

Discussion

Salmonella sp. were isolated from samples of broiler chicken, duck, and quail sold in Tondano Market, Minahasa Regency by the streak method and deposited on Sallmonella Shigella Agar (SSA)) and Xylose Lysine Deoxycholate Agar (XLD) media that had been previously enriched. In the pre-enrichment tray, two samples were negative (TA1 and TB2), while the four samples that were positive (TB1, TP1, TA2, and TP2) showed problems after incubating for 24 hours at 37°C. *Salmonella* sp. is a bacterium that is unable to compete effectively with other bacteria in the food chain so it requires nutrients to survive [7]. The protein content shows that *Salmonella* sp. can be found on eggshells in Buffered Peptone Water (BPW) media, providing carbon and nitrogen for bacteria either

Salmonella sp. is coliform bacteria from air, food and waste. Pre-enrichment with Buffered Peptone Water (BPW) is very important to prevent *Salmonella* sp. from forming toxicants or inhibitors, as well as providing nutritional value to *Salmonella* sp.

The enrichment stage was carried out using Rappaport Vassiliadis (RV) media. The four samples that showed positive results were tested, and after incubating for 24 hours at 37°C, the sample was marked by a color change

in the media, the blue color became cloudy. Rappaport Vassiliadis (RV) selective medium contains selective substances such as malachite green and magnesium chloride, combined with a high pH (5.25.2 ± 2). Conditions on RV medium like this will reduce the growth of bacteria other than *Salmonella* sp. caused by the digestive tract. This growth is aided by the presence of soy peptone in RV medium [8]. Soy peptides in RV medium serve as a source of carbon, nitrogen and amino acids for *Salmonella* sp. [9]. Positive results showed that changes to the RV medium made it leaner, more efficient, and in certain cases increased the white precipitate under the medium.

The next stage used Sallmonella Shigella Agar (SSA) media incubated at 37°C for ± 24 hours. Growth on SSA media is characterized by the formation of colonies due to *Salmonella* sp. ability to produce H_2S. Sallmonella Shigella Agar (SSA) media contains sodium thiosulfate which is converted into sulfite gas and H_2S by microorganisms using the enzyme thiosulfate reductase. The production of H_2S gas is detected as a non latent iron sulfide precipitate so that the reaction of H_2S with iron ions or iron citrate is seen in the colonies [10].

Salmonella uses peptone derived from SSA media as its energy source. According to Muktiningsih *et al*. [11], SSA media is a cooling medium for isolating bacteria belonging to the genus *Salmonella* sp. and bacteria members of the genus *Shigella*, but SSA media is not recommended for testing bacteria members of the genus *Shigella* because some *Shigella* strains will be inhibited. *Salmonella* sp. are gram-negative rod-shaped bacteria, have no spores, are facultative anaerobes and move with peritrichous flagella except S*almonella pullorum* and *Salmonella gallinarum* [12].

The final step was to use Xylose Lysine Deoxycholate Agar (XLD) media. Two of the ten samples selected gave four positive results, the first outside the market (2 samples) and the second inside the market (2 samples). Xylose Lysine Deoxycholate (XLD) agar media serves as a selective and differential medium. In addition to xylose, it also contains lysine. Xylose Lysine Deoxycholate (XLD) agar media also allows *Salmonella* sp. colonies to grow because the XLD media resulting in a lower pH. This media can kill Gram-positive bacteria due to its sodium deoxycholate content.

Xylose Lysine Deoxycholate Agar, a medium containing H_2S indicator, is composed of sodium thiosulfate and iron ammonium citrate, so that when hydrogen sulfide is produced, red colonies appear on the surface. Planting on XLD media using the scribble plate method for the growth of *Salmonella* sp. Positive colonies are characterized by colonies that are black or have black cores. Media from the results of the research that has been carried out from 6 samples of eggshells examined 4 positive eggshell samples are only slightly contaminated with *Salmonella* sp. bacteria and 2 eggshell samples are not contaminated by *Salmonella* sp. This shows that the quality of eggs traded in the Tondano Market is not good because of the contamination of *Salmonella* sp bacteria in purebred chicken eggs, duck eggs and quail eggs traded in the Tondano Market.

Government guidelines or directives regarding consumer protection of raw milk regulated in Indonesian National Standard SNI No. 01-6366-2000 regarding the maximum limit of *Salmonella* sp. contamination in milk, meaning that it does not contain *Salmonella* sp. [2].

According to the study, the presence of *Salmonella* sp. bacteria in breeds, ducks, and quail sold in Tondano Market contributed to the lack of sanitation of the market environment, as well as the lack of sterile breed production environment. *Salmonella* sp. contamination is most likely due to poor, dirty, and unsanitary conditions.

Chicken feces that are still attached to the eggshell can trigger eggs to be exposed to *Salmonella* sp. Lack of washing of eggs that are traded or will be processed is the main factor that causes *Salmonella* sp. to be present in broiler chickens, ducks and quails sold at the Tondano Market. From the 6 samples collected, 4 eggshell samples were only positive and slightly contaminated with *Salmonella* sp. bacteria and 2 negative eggshell samples were not contaminated with *Salmonella* sp. As a result, samples to be traded, consumed, and consumed will be relatively safe if not chewed and swallowed within 7 minutes.

Actual food safety indicators should be obtained through a pure or semi pure cooking process, or cooked and then refrigerated for seven days so that the bacteria in the final product decompose and are safe for consumption by the general public. If the mashing process is not optimal, for example, slightly raw or undercooked, then its consumption can trigger the emergence of bacteria that are not perfectly pure and can continue to live.

Salmonella sp. is a bacterium that can live at temperatures ranging from 6.7°C to 45°C, with a maximum survival time of 1 hour at 55°C and 15-20 minutes at 60°C [7, 2].

The research findings of *Salmonella* sp. isolation in eggs in Tondano Market, Minahasa Regency will inspire and instill hope in students and the general public about the importance of maintaining a healthy and safe environment, as well as the importance of storing food in a safe place.

IV. CONCLUSION

Based on the research conducted, six eggshell samples (first and second points) were collected from Tondano Market. Four samples namely Duck Eggs (TB 1), Quail Eggs (TP 1), Broiler Eggs (TA 1), and Quail Eggs (TP 2) were positive and detected without counting bacterial contamination of *Salmonella* sp., while the other two samples (TA 1 and TB 2) were negative or did not contain *Salmonella* sp.

Recommendations: It is expected to contribute to further research on *Salmonella* sp. in various types of eggs, and can consume eggs faster and in a longer period of time to ensure safety.

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