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Identification of *Salmonella sp.* Contamination in Chicken Eggs Sold at Sanglah Market

Phoebe Ezkiella Nathanael[1], Ni Nyoman Sri Budayanti[2], Ni Nengah Dwi Fatmawati[3], Ida Bagus Nyoman Putra Dwija[4]

[1]Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia,
[2,3,4]Department of Microbiology, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia

Corresponding Author: Phoebe Ezkiella Nathanael

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ABSTRACT

Salmonella sp. is one of the bacteria that causes gastroenteritis that has millions of infections yearly. Generally, this bacterial infection is the result of consuming food which is contaminated with these bacteria, one of which is chicken eggs. These bacteria can contaminate chicken eggs while the eggs are forming or after the egg is formed. The aim of this research is to determine the contamination rate of *Salmonella sp.* in chicken eggs sold at Sanglah Market. A descriptive study with simple random sampling approach was carried out to identify Salmonella sp. contamination in chicken eggs sold at Sanglah Market. A total of 27 chicken eggs were used as samples taken from 9 chicken egg traders at Sanglah Market. Samples were then tested for the presence of *Salmonella sp*. by culturing on *Salmonella-Shigella Agar* media at Laboratorium Biomedik Terpadu Divisi Bakteriologi dan Virulogi FK Universitas Udayana and then later identified using the BD Phoenix™ M50 instrument at Nikki Medika Laboratory Denpasar. Of the 27 eggs that were examined for the contents and the shells of each egg, no samples of chicken eggs were identified that were contaminated with *Salmonella sp.* (0%). However, in 4 samples *Providencia rettgeri* and *Proteus mirabilis* were identified using the BD Phoenix™ M50. *Salmonella sp*. contamination was not found in the shells and contents of chicken eggs sold at Sanglah Market with a contamination prevalence of 0%.

Keywords: chicken eggs, *Salmonella sp.* contamination, *Salmonella sp*

INTRODUCTION

The global mortality rate of *Salmonella sp.* infection reaches 57,000 deaths every year. In Indonesia itself, it is estimated that food poisoning cases due to *Salmonella sp*. are around 60,000 to 1.3 million with the deaths of at least 20,000 people per year. Generally, this bacterial infection in humans can cause diarrhea, fever and stomach cramps. These symptoms last for 4-7 days. If these bacteria infect humans under 5 years or over 65 years, the risk of invasive *Salmonella* infection is high.[1]

Salmonella sp. is a gram-negative bacteria that are motile. These bacteria can survive in dry conditions for several weeks and several months in water. These bacteria can cause gastroenteritis through cross-infection between animals and humans or from humans to other humans via faecal-oral. Generally, this bacterial infection is the result of consuming food contaminated with Salmonella bacteria, one of which is chicken eggs.[2]

Chicken eggs are highly nutritious, contains 6.5 grams of protein with 9 essential amino

acids. The high protein content and affordable price compared to other animal food products make eggs more commonly consumed by the community. Chicken egg consumption itself has increased by 1.71% per year, since 2017. In 2023, the estimated consumption of chicken eggs in Indonesia is 5.59 million tons.[3] Unfortunately, the high protein content in chicken eggs can become a breeding ground for bacteria. The presence of pores in the egg shell allows microbes from air, water and chicken feces to enter the egg.[4] S*almonella sp.* bacterial contamination. in chicken eggs can occur through 2 mechanisms, vertically and horizontally. Vertical contamination happens when the egg are contaminated while they are still in the process of forming. Horizontal contamination happens after the egg is formed.[5] Naturally eggs have physical barrier as the first line of defense against unwanted microbes. These physical barriers are cuticle which are hydrophobic and have pores, eggshell membrane, and the eggshell. Eggs also have protein such as lysozyme, ovotransferrin, and ovocalyxin-26, that are antibacterial.[6]
Contamination of *Salmonella* bacteria from eggs to humans is generally through inadequate cooking and washing of eggs and also through consumption of foods containing undercooked eggs, such as mayonnaise, custard, homemade ice cream and salad dressings. Therefore, eggs to be consumed must meet the criteria for being suitable for consumption, which according to government regulation must not contain *Salmonella sp.*[7]
A study conducted in 2022 in a sub-district in North Minahasa, found that 3 egg shells were positively contaminated with Salmonella sp bacteria. of 18 samples studied from 3 different farms.[8] Meanwhile, a study in Bali in 2021 found positive egg shells for *Salmonella sp*. as much as 11.1% (2 out of 18 traditional markets). This shows that the quality of eggs sold in several places still does not comply with government regulations. Sanglah Market is one of the traditional markets in the city of Denpasar and there has been no research regarding *Salmonella sp* bacterial contamination. on the eggs done.[9] Therefore, authors want to conduct research on bacterial contamination of *Salmonella sp.* on chicken eggs sold at Sanglah Market Denpasar in 2024.

MATERIALS & METHODS

Tools: sterile loops, Bunsen burner, test tubes, pipette, incubator, test tube racks, petri dish, erlenmeyer, syringe, measuring cylinder, mortar, vortex, and BD Phoenix M50.
Materials: 27 chicken eggs sold at Sanglah Market, Tryptic soy broth, and *Salmonella-Shigella Agar*.

Sample

The sample in this study were 27 chicken eggs sold at Sanglah Market. The number of chicken traders at this market is 9. Three eggs were bought at each trader in this market. The method used is simple random sampling. Chicken eggs were then brought to Laboratiorium Biomedik Terpadu Divisi Bakteriologi dan Virulogi Faculty of Medicine in Udayana University. Each egg is labelled by numberin the form of sequential numbers, from number 1 to 27. Number 1 to 3 were from trader 1, number 4 to 6 were from trader 2, and so on. Each egg was checked for it's contents and shell. Egg contents from egg number 1 were labelled as 1A and the eggshells were labelled as 1B. This order was continued until the last egg.

Enrichment

Chicken eggs that have been selected for testing will be separated into egg shells only and egg whites and yolks only. The egg whites and yolks were stirred until evenly mixed. Then, 1 ml of the mixture were taken to put inside of a test tube containing 9 ml of Tryptic soy broth. Next, the tube was homogenized using vortex and then incubated at 37^{o}C for 24 hours. Meanwhile, the egg shells were grounded using a mortar. Then, 4 ml of distilled water were added to make a suspension. Then, 1 ml of

the suspension were taken to put into a test tube filled with 9 ml of Tryptic soy broth. This tube was then homogenized and incubated the same as the contents of the egg.

Isolation

Contents of the incubated Tryptic soy broth tubes were then streaked on *Salmonella-Shigella* Agar, by using sterile loops and scratching them on SSA media. The media was then incubated for 24 hours at 37^{o}C

Biochemical Test

Suspected colonies of *Salmonella sp.* in SSA media were then brought to Nikki Medika Laboratory Denpasar to be identified using BD Phoenix^{TM} M50 instrument.

Data Presentation

Data from this study were analysed descriptively by observing the characteristic of colonies grown in SSA and the identification result from the BD Phoenix^{TM} M50 instrument. Data is going to be presented in the form of percentages of positive samples of *Salmonella sp.* in chicken eggshells and contents.

RESULT

Samples that have been grown on Tryptic soy broth and showed cloudy appearance were then cultured on *Salmonella-Shigella* Agar and incubated for 24 hours at 37^{o}C. From 54 samples, there were only 7 samples which show the growth of clear colonies with black precipitate. These samples were then sub cultured on SSA media for another 24 hours at 37^{o}C. Four of the samples show the growth of clear colonies with black precipitate while the other 3 show clear colonies with no black precipitate. The results of initial isolation and subculture on SSA media can be seen in the table 1.

Table.1. Isolation Results on SSA

No	Sample	SSA	Subculture pada Media SSA
1	3B	Colorless with black precipitate	Colorless with black precipitate
2	4B	Colorless with black precipitate	Colorless with black precipitate
3	7B	Colorless with black precipitate	Colorless
4	9A	Colorless with black precipitate	Pink colonies
5	9B	Colorless with black precipitate	Colorless with black precipitate
6	14A	Colorless with black precipitate	Colorless with black precipitate
7	15B	Colorless with black precipitate	Colorless colonies

Description: SSA, *Salmonella-Shigella Agar;* A, Egg contents; B, Eggshells

These 4 samples were then followed by biochemical tests using the BD Phoenix^{TM} M50 instrument. The results of isolation on SSA media and identification by BD Phoenix^{TM} M50 can be seen in the following table.

Table.2 Isolation Results on SSA and Identification with BD Phoenix^{TM} M50

No	Sample	SSA	BD Phoenix^{TM} M50
1	3B	Colorless with black precipitate	*Proteus mirabilis*
2	4B	Colorless with black precipitate	*Providencia rettgeri*
3	9B	Colorless with black precipitate	*Providencia rettgeri*
4	14A	Colorless with black precipitate	*Providencia rettgeri*

Description: SSA, *Salmonella-Shigella Agar*; A, Egg contents; B, Eggshells

Result showed that of the 27 chicken eggs tested, none of them were positive for *Salmonella sp.* There are several other bacteria other than *Salmonella sp.* have been identified. 3 Samples were positively identified as *Providencia rettgeri* and 1 of them was *Proteus mirabilis*. Two samples of chicken eggshells, from trader 2 and 3, and 1 sample of egg contents, from trader 7, were identified as contaminated by *Providencia rettgeri* by the BD Phoenix^{TM} M50. *Proteus mirabilis* was found in the eggshell of a chicken egg sold by trader 1.

DISCUSSION

Salmonella-Shigella Agar is a selective media commonly used for isolation of *Salmonella sp.* bacteria. This media can inhibit the growth of gram-positive bacteria because it contains bile salts, brilliant green, and citrate. This media also contains lactose to differentiate *Salmonella sp.* from other enteric bacteria. Enteric bacteria that can ferment lactose will produce acid which will change the neutral red indicator to red. *Salmonella* cannot ferment lactose, so they will show clear colonies. Sodium thiosulfate and ferric acid are also contained in this media to detect the production of hydrogen sulfate (H_2S) which will be indicated by colonies that have black precipitate in the middle. However, it is not recommended to use SSA media as the primary isolation media for *Salmonella sp.*[10]

Other bacteria that have similar appearance as *Salmonella sp.* in SSA is *Proteus sp.* These bacteria is a gram-negative facultative anaerobe which are a normal flora in human guts and commonly found in water and land environment. Like *Salmonella sp.*, these bacteria cannot ferment lactose and produce H_2S.[11] To make sure that the suspected colonies in SSA is *Salmonella sp.*, further identification through biochemical tests are needed. In this study, the instrument BD Phoenix^{TM} M50 is used.

The BD Phoenix^{TM} M50 instrument has 51 wells containing various biochemical substrate used for identification. This instrument can identify organisms based on the microbial utilization and the degradation of specific substrates. For example, changes in the phenol red indicator indicate the utilization of carbohydrate substrates due to the acid formed. If hydrolysis of urea occurs and the formation of ammonia, the pH will rise and change the fluorescent indicator. Black precipitates are an indication of the presence of ferric ions when esculin are hydrolysed.[12]

From this study, results showed by the BD Phoenix^{TM} M50 were none of the 27 chicken eggs sold at Sanglah Market were positively contaminated by *Salmonella sp.* This shows that chicken farmers have implemented strict biosecurity to prevent *Salmonella sp.* infection. This biosecurity can be implemented starting from the level of chicken egg production on the farm. Chicken egg must be produced by chickens that are free of *Salmonella sp*. Chicken feeds are also thoroughly checked to avoid contaminations. Livestock products must also be handled hygienically by maintaining the cleanliness of equipment and personnel involved in the production process.[13]

However, this study found other bacteria on the egg shells and contents of the eggs. *Providencia rettgeri* bacteria were found in 3 samples examined by the BD Phoenix^{TM} M50 machine and *Proteus mirabilis* bacteria in 1 sample. *Providencia rettgeri* is an opportunistic pathogen that can cause urinary tract infections in immunocompromised patients. *Providencia rettgeri* is a gram-negative bacteria that is often found in water and soil.[14] *Proteus mirabilis* is a gram-negative bacterium from the Enterobacteriaceae family that can cause urinary tract infections. *Proteus* is also often found in soil, water, and is a normal flora in the human digestive tract. About 1-2% of the causes of urinary tract infections are *Proteus mirabilis*.[11] This shows that bacterial contamination can occur due to contact between chicken eggs and soil or water contaminated with these bacteria.

CONCLUSION

The prevalence of *Salmonella sp.* contamination in chicken eggs sold at Sanglah Market in 2024 was 0% on both egg shells and contents, where 0 out of 27 samples were identified as not containing *Salmonella sp.* However, this study found that 4 out of 27 eggs contained *Providencia rettgeri* and *Proteus mirabilis* bacteria. *Providencia rettgeri* was found in 2 of the eggshells and 1 in the content of the egg. *Proteus mirabilis* was found in 1 of the egg shells.

Declaration by Authors

Ethical Approval: This study has obtained ethical clearance issued by the research Ethic Commission of Faculty of Medicine, Udayana University, Denpasar (Ethical Clearence No. 1097/UN14.2.2.VII.14/LT/ 2024)

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Conflict of Interest: The authors declare no conflict of interest.

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