

IDENTIFICATION OF *Escherichia coli* BACTERIA IN QUAIL EGGS THAT FOR SALE IN THE CENTRAL MARKET OF THE CITY OF GORONTALO

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ABSTRACT

Eggs are one of the animal products that come from poultry and have been known as a source of high quality protein food. From the many eggs we know, one of the eggs that comes from poultry is quail eggs. Quail eggs have complete nutritional content ranging from protein, fat, vitamins and minerals. Even so, quail eggs are also animal food that is easily decreased in quality due to bacterial contamination, contamination of quail eggs by bacteria can affect the quality of the contents of the eggs, bacteria that cause egg contamination, one of which is *Escherichia coli*.

This study aims to determine the presence of *Escherichia coli* bacteria in quail eggs that are sold in the central market of Gorontalo city.

The research method used is descriptive research type with a quantitative research approach, cross sectional research design, a sampling place for the central market of Gorontalo City and a place for researching samples of the Microbiology Laboratory of Gorontalo State University, the number of samples used was 30 samples of quail eggs. Quail eggs were positive for *Escherichia coli* bacteria with a percentage of 6.7%, and negative egg yields from *Escherichia coli* bacteria were 28 or 93.3%. It can be concluded that from 30 samples of quail eggs there were 2 quail eggs that were positive for *Escherichia coli* bacteria, the bacteria contamination factor in eggs was influenced by external factors. Environmental sanitation conditions were not clean, egg storage temperature, egg storage time,

Keywords: *escherichia coli*, food, quail eggs.

INTRODUCTION

Food is a need that is really needed by humans in life, be it vegetable or animal food. One of the animal foods is poultry. The Indonesian State brings in other breeds of livestock from abroad. Until now, many types of livestock live in Indonesia, including cattle imported from America, Australia or from Europe. To fulfill food needs and so on, some humans raise livestock to take its benefits, as a source of food. Animal food sources produce the main products in the form of meat, milk and eggs. [8]

One of the eggs that comes from poultry is quail eggs. The nutritional value of quail eggs is not inferior to other poultry eggs, so that it can increase the variety in the provision of animal protein sources [11].

Quail eggs have complete nutritional content ranging from protein, fat, vitamins and minerals. Even so, eggs are also prone to quality degradation caused by microbial contamination, physical damage and evaporation of water and carbon dioxide, ammonia, nitrogen and hydrogen sulfide

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gases from the eggs, the longer the eggs are stored the evaporation that occurs will make the egg weight shrink and egg whites become thinner [24]. If left in open air (room temperature), quail eggs can only last 10-14 days, after which time the eggs experience changes in the direction of damage such as changes in chemical composition and dilution of egg contents [3].

Contamination of microorganisms on eggs can be caused by various factors, for example, soil, water or feces that stick to the eggshells. Under certain conditions and in excess of the limit, these microorganisms can cause diarrhea in people [13]. Some of the microorganisms that cause contamination of eggs, one of them is the *Escherichia coli* bacteria.

From previous research, it was said that eggs that were dirty with the environmental conditions in which the eggs were also a factor in the death of egg embryos. Experts reported that about 0.5% - 6% of eggs from poultry contained *Escherichia coli* and about 1.75% of the embryos that died contained pathogenic *Escherichia coli* serotypes. Hatching eggs that come from a dirty environment with a thin shell quality will be easily entered by *Escherichia coli* bacteria and can reach the egg yolk. Another source of infection is the ovaries or oviducts which are infected by these bacteria [21].

Escherichia coli bacteria is a type of bacteria that is often pathogenic to humans and animals when it is present in excessive amounts in the intestine. These bacteria can cause diarrhea, diarrhea caused by the *Escherichia coli* bacteria is characterized by wide clinical manifestations starting without showing clinical symptoms until visible diarrhea is bloody or without blood and is characterized by abdominal pain, fever or

no fever, liquid diarrhea, as well as bleeding, vomiting and nausea [1].

Diarrheal disease is a disease that occurs in people in developing countries such as Indonesia. The target service coverage for diarrhea sufferers of all ages who come to health facilities is 10% of the estimated number of diarrhea sufferers of all ages (the incidence of diarrhea, SU multiplied by the population in one work area in one year). In 2017, there were 4,274,790 SU diarrhea sufferers served in health facilities and an increase in 2018 to 4,504,524 sufferers or 62.93% of the estimated diarrhea in health facilities. The national incidence of diarrhea for all ages is 270 / 1,000 population [10].

Based on observations that have been made, many quail eggs are traded in traditional markets, one of which is the central market. The central market is a traditional market located in Gorontalo City. There are various activities in this market. Egg traders are not placed in a special place but mingle with other food traders, causing cross-contamination. The egg shops in the Central Market have poor environmental conditions, lots of garbage heaps, and dirty and smelly gutters. This is one of the triggers for the growth of microorganisms in eggs, including bacteria. One of the bacteria that causes damage to eggs is *Escherichia coli*.

RESEARCH METHODS

The research method used is descriptive research with a quantitative research approach, a cross sectional research design, a sampling place for the central market of Gorontalo City and a place for researching samples of the Microbiology Laboratory of the State University of Gorontalo, When this research was conducted in August - September 2020, the number of samples used was 30 samples of quail eggs, the sampling technique was Cluster Random

Sampling. descriptive analysis data presentation.

The tools used in this research are autoclave, stirring rod, erlemeyer, test tube, incubator 370C, electric stove, analytical balance, petri dish, test tube, bunsen burner, beaker, oven, measuring cup, spatula, ose needle, vortex, glass object, dropper pipette, microscope.

The materials used in this study were samples of quail eggs, distilled water, NB (Nutrient Broth) media, EMBA (Eosin Methilen Blue Agar) media, cotton, alcohol, Crystal violet, lugol, safranin, oil emersion, SIM (Sulfide Indol Motility) media, MR-VP medium (Methil Red-Voges Proskauer), SCA Media (Simons Citrate Agar), Erlich's reagent, methyl red, KOH.

Data on the identification of Escherichia coli bacteria in quail eggs was obtained through testing in the microbiology laboratory at the State University of Gorontalo. The enrichment stage, gram stain isolation and identification stage used the SIM (Sulfide Indol Motility) biochemical test, MR-VP (Methil Red-Voges Proskauer), SCA (Simons Citrate Agar) with positive and negative categories. The positive category is if the results of the test are proven to contain Escherichia coli bacteria by showing changes in the test media, namely NB (Nutrient Broth) media, turbidity occurs, EMBA media (Eosin Methylene Blue Agar) Escherichia coli that grows gives a distinctive color of black colonies with metallic green, after growing in specific EMBA media, Gram staining was carried out with a solution of Crystal violet for 3 minutes. Drop lugol, for 1 minute. Drop of 96% alcohol, Dropsafranin for 45 seconds, dry with oil Immersion and look under a 100x magnification microscope. The result of

gram staining is if the bacterium Escherichia coli is in the form of a short stem and is red in color

The biochemical test of SIM (Sulfide Indol Motility) media, the indole test if Escherichia coli is positive, shows the results indicated by the formation of a pink indole ring after dropping the Ehrlich reagent, MR-VP (Methil Red-Voges Proskauer) media MR test results if positive Escherichia coli will a red color is formed after the addition of the Methyl Red reagent, and the VP test results if the Escherichia coli test is positive, the Voges Proskauer test (VP) results are negative where there is no change in the color of the media from yellow to pink to red, and the SCA (Simons Citrate Agar) media The results of the citrate test if positive Escherichia coli did not change the media because Escherichia coli did not utilize citrate as a carbon source which was shown to be no change in color on the citrate test media and negative category if the test results are not proven to contain Escherichia coli bacteria, there is no change shown by the test media.

RESEARCH FINDINGS

Based on the results of research on the identification of Escherichia coli bacteria in quail eggs, the morphology of bacterial colonies on EMBA media was obtained as shown in Table 1.

Table 1.

Observation of the morphology of Escherichia coli colonies growing on EMBA media

| Sample | Shape | Size | Surface | Edge | Color |
|--------|-------|-----------|---------|------|----------------|
| 3A | Round | Mode rate | Smooth | Flat | Metallic green |
| 3D | Round | Mode rate | Smooth | Flat | Metallic green |

Source: Data processed (2020)

Based on table 1, it can be seen that the two colonies suspected of being E. coli


bacteria show the characteristics of a medium-sized round colony, smooth

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surface on the flat edge and metallic green. The existence of *Escherichia coli* colonies on EMBA media produce blackish purple colonies with a metallic green luster are *Escherichia coli* bacteria.

Table 2.

The results of Gram stain of the colony of samples grown on EMBA media

| Sample | Color | Shape | Result | Picture |
|--------|-------|---------------|---------------|--|
| 3A | Red | Stems (basil) | Gram Negative |  |
| 3D | Red | Stems (basil) | Gram Negative | |

Source: Data processed (2020)

Based on the results of gram staining to see the morphology of *Escherichia coli* bacteria from EMBA media microscopically based on differences in the structure of whether gram-positive or gram-negative bacteria with the results obtained that the morphology of bacteria in the form of rods is red which indicates the bacteria is gram negative.

Table 3.

Biochemical Test Results from Colony 3A and 3D samples that grew on EMBA media

| Biochemical Test | Sample | | Escherichia coli standard bacteria |
|-------------------------------|--------|----|------------------------------------|
| | 3A | 3D | |
| <i>Sulfide Indol Motility</i> | + | + | + |
| <i>Methylene Red</i> | + | + | + |
| <i>Voges Proskauer</i> | - | - | - |
| <i>Simons Citrate Agar</i> | - | - | - |

Source: Data processed (2020)

Based on table 3, the biochemical test results of the bacteria isolated on the EMBA media show positive Indol test results (+), the positive results show a color change on the media and a red ring appears on the media surface after adding Ehrlich reagent. MR test was positive (+) with a visible change in the color of the media from yellow to red after adding methylene red. The negative VP test (-) was negative because there was no color change on the media after adding the KOH reagent. and negative citrate test (-) obtained negative results, there was no color change in the media that had been tested (still green). The results above are in accordance with the standards of bacteria *Escherichia coli* in biochemical tests.

Table 4.

Results Percentage of *Escherichia coli* bacteria in quail eggs

| Check up result | Frequency (n) | Percentage (%) |
|-----------------|---------------|----------------|
| Positive | 2 | 6.7% |
| Negative | 28 | 93.3% |
| Total | 30 | 100% |

Source: Data processed (2020)

Table 4 shows that the results of bacterial examination *Escherichia coli* 2

quail eggs stated positive presence of Escherichia coli bacteria or 6.7% of the total number of 30 samples examined.

DISCUSSIONS

Based on the results of research on identification regarding the presence or absence of Escherichia coli bacteria found in quail eggs that are sold in the central market of Gorontalo City. That the quail egg samples were in the Central market of Gorontalo City, 2 or 6.7% of the 30 samples of quail eggs were contaminated by Escherichia coli bacteria.

Observation of quail egg samples isolated on EMBA media obtained results from 30 quail egg samples found 2 positive egg samples of Escherichia coli bacteria with the characteristics of round colonies, convex purple-black with metallic green. Puspitawati (2012) stated that the bacteria inoculated on EMBA media produced colonies that were blackish purple with a metallic green sheen, which were Escherichia coli bacteria [14].

EMBA media contains lactose so that it can distinguish between types of bacteria with the ability to ferment lactose. One of the bacteria that can ferment lactose is Escherichia coli, these bacteria are bacteria that can ferment lactose quickly and can produce lots of acids so that they can produce a metallic green colony color. EMBA contains the carbohydrate lactose, in the presence of these carbohydrates. The color before bacterial fertilization on EMBA media was purplish red. The color change to metallic green in this medium is due to the fact that Escherichia coli bacteria can ferment lactose which increases the acid level of the media [23].

EMBA media is a selective medium for the isolation of Escherichia coli bacteria. The media can distinguish Escherichia coli bacteria from other Enterobacteriaceae bacteria. Eosin Methylene Blue Media This agar has a

feature that is the presence of lactose in the media and can be used as a differentiating factor between Escherichia coli bacteria and others [18]

Based on the results of Gram staining on samples 3A and 3D, it can be seen that the bacteria obtained shows the morphology of the bacteria in the form of a red rod which indicates that the bacteria is gram negative. This is because Gram negative bacteria have a thin peptidoglycan layer and are surrounded by a fat-containing layer on the outside. The result of the research states that Escherichia coli is a Gram-negative bacteria with a short rod. Escherichia coli bacteria appear pink in color and rod-shaped which is the morphological characteristics of Escherichia coli bacteria [2].

Escherichia coli bacteria are classified into gram-negative bacteria. This is because Gram-negative bacteria have a thin peptidoglycan layer. Gram-negative bacteria will lose their crystal violet dye after washing with alcohol, and when given a counter dye that is safranin dye will appear red [15].

Biochemical test begins with the SIM media indole test (Sulfide Indol Motility). What is suspected that the Escherichia coli bacteria taken on EMBA media with colony characteristics of blackish purple metallic green showed positive results indicated by the formation of a pink indole ring after being dripped with erlich reagent, indole and positive motility.

The results of the study [1] stated that Indol was positive because the adhesion turned red (red ring) on the surface of the medium. This happens because the Escherichia coli bacteria can produce tryptophase and can hydrolyze tryptophan, which can be determined by adding Ehrlich's solution containing paradimethyl-aminobenzaldehyde.

Stating that the presence of a red ring on the top is due to the Escherichia coli bacteria that can produce indole from the

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breakdown of the amino acid tryptophan using the tryptophase enzyme. In the culture medium, Indol accumulates as a waste product, while other parts of the tryptophan molecule (pyruvic acid and NH_4^+) can be used to meet the nutrient requirements of microorganisms [20].

The results obtained were different from studies where the indole results were negative. This was due to several factors, namely the erlich reagent should be 5 to 10 drops and incubated for 48 hours but in this study only 2 drops were given with an incubation time of 24 hours [17].

Based on the results of the Methyl Red test, positive results are obtained, which is indicated by a color change from yellow to red [9] which states that the Methyl Red test can detect bacteria using the acid pathway because there is a color change in the media from yellow to red after adding Methyl Red reagent. That is, these bacteria produce mixed acid (Methylene glycon) from the glucose fermentation process contained in MR-VP media. The formation of mixed acid on the media will reduce the pH to 5.0 or less, therefore, when the methyl indicator is added to the culture with such a low pH, the indicator will turn red. [1] stated that the addition of the indicator Methyl red was used to determine the presence of mixed acid fermentation.

From the results of the Proskauer Voges Test, which was negative, there was no change in the color of the media from yellow to red. The Voges Proskauer test is used to identify microorganisms that carry out fermentation with a final yield of 2,3 butanediol. When bacteria ferment carbohydrates into 2,3 butanediol as the main product, there will be a buildup of these materials in the growth medium. In this test, 40% KOH and 5% alphanaphtol solution was added at the time of observation. This can determine the presence of acetoin (acetyl methyl carbinol), a starting compound in the

synthesis of 2,3 butanediol [22]. states that the Voges Proskauer Test (VP) is negative for Escherichia coli because Escherichia coli ferments carbohydrates into acidic products and does not produce neutral products such as acetoin. The observations for the VP test were negative, namely in alkaline conditions which were marked by no color change after 3-5 drops of KOH and α -naphthol. Generally, Escherichia coli bacteria give positive results for MR test and negative for VP test [16].

The results obtained from the citrate test were negative because there was no change in the media. [16] stated that the results of the observation for the citrate test were negative for the Escherichia coli bacteria because the Escherichia coli bacteria could not utilize citrate as a carbon source which was shown to have no color change on the citrate test medium. The citrate test is used to see the ability of microorganisms to use citrate as the only source of carbon and energy.

Escherichia coli is positive if the indole and methyl red tests are positive, while the Voges Proskauer and citrate tests are negative. If one of the interpretations of the results is not correct, then the culture tested is declared not to contain Escherichia coli. This is in accordance with the results of research [19] isolation of Escherichia coli bacteria in eggs in the field laboratory of Shia Kuala University animal husbandry obtained 3 out of 10 egg samples were contaminated by Escherichia coli bacteria with IMVIC test results showing positive Indol (+), positive MR (+), Negative VP (-), and Negative Citrate (-). In contrast to the research carried out to carry out research on the identification of Escherichia coli in refill water in Manado City,

The results showed that the Escherichia coli bacteria was one of the bacteria that could contaminate quail eggs. The results showed that 3 out of 30

samples (10%) of quail eggs were contaminated by *Escherichia coli* bacteria. *Escherichia coli* bacteria are also the cause of failure to hatch quail eggs in the quail farm in Garot Village, Darul Imarah District, Aceh Besar. [21] The results of the study stated that the results of the examination on 20 egg samples from the Animal Husbandry Field Laboratory of Syiah Kuala University found that there were several samples tainted by *Escherichia coli* bacteria but there were also samples tainted with *Salmonella sp* bacteria [19]. Furthermore, research) in 60 egg samples collected from 5 different farms that were analyzed microbiologically, 28.33% of eggs contained coliform bacteria where each farm had egg samples contaminated by *Escherichia coli* bacteria [4].

The presence of *Escherichia coli* bacteria in quail egg samples can be used as an indication of the presence of other pathogenic bacteria in quail eggs. [13] stated the presence of *Escherichia coli* bacteria in quail egg samples. This is due to several factors that trigger microbial growth, namely environmental sanitation factors where sales are made, cage sanitation, and animal waste. Factors for bacterial contamination of eggs can occur vertically or horizontally. Vertical contamination can come from infected mother eggs so that the eggs contain polluting microorganisms, while horizontal contamination occurs when the eggs taste outside the mother's body. This contamination can be caused by various factors, for example, less clean environmental conditions, egg storage temperature, egg storage time, egg storage area.

In research, it is stated that contamination by bacteria in eggs can occur before the shell is formed which will infect the egg components, this can occur when the uterus and ovaries of the

parent have been infected by bacteria [5]. states the state when the mother has a systemic infection of the ovaries by bacteria, this can allow the presence of bacteria in the egg [7].

The thing that affects the presence of bacteria in quail eggs is the location for taking egg samples where the location of the sale of quail eggs is close to the seller of vegetables, spices and in front of the egg sales place there is a ditch and heaps of garbage. From the results of observations that have been made, it is found that egg traders are not placed in a special place but mingle with other food traders so that cross-contamination can occur. The egg shops in the Central Market have poor environmental conditions, lots of garbage heaps, and dirty and smelly gutters. This is one of the triggers for the growth of microorganisms in the eggs. stated that to avoid eggs from being contaminated by bacteria, it is necessary to pay attention to the cleanliness of the surrounding environment [12]. From a dirty, damp and smelly environment it can become a disease to result in death. To avoid the occurrence of bad environmental conditions, cleanliness must be maintained, dirt must be cleaned regularly and trash should not accumulate. In general, disease prevention in livestock can be done by means of sanitation, feeding, providing a comfortable environment for livestock so that the ternah results obtained are free from microognism.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that, quail eggs that are traded in the central market of Gorontalo City from 30 samples of quail eggs studied there were 2 samples of eggs that were positive for *Escherichia coli* bacteria with a percentage yield of 6.7%.

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