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Detection of Escherichia Coli as an Indicator of Infection of the whole Table Eggs with Salmonella Spp. and Ensuring their Safety

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Abstarct
It is possible to use microorganisms to indicate how microbiologically safe something is. The present study aims to identify Escherichia coli as an indicator of the presence of Salmonella spp. in whole eggs and analyze the steps that may be done to prevent the spread of infection. A total of 90 brown poultry eggs were collected randomly from various locations in the Wasit province of Iraq, then directly submitted to the laboratory. The eggs were kept in the refrigerator at a temperature of 4°C until they were examined for microbial contamination. The tests included the detection of E. coli and Salmonella spp. in both the shell and the internal section of the eggs and the suggestion of steps to control contamination with enteric pathogenic bacteria. E coli was present on the shell and the internal part of the table eggs by 67.8% and 32.2%, respectively, compared to the prevalence of Salmonella spp. on the shell and the internal part of the table eggs by 25.6% and 7.8%, respectively. The findings are conclusive ($P < 0.05$), and the variables E. coli shell and Salmonella spp. shell are connected to one another in some way. The findings are significant ($P < 0.05$), and the variables E. coli internal egg and Salmonella spp. internal egg are linked to one another. According to the findings, there is a connection between the presence of E. coli and Salmonella species in whole table eggs.

Keyword: Contamination, Eggshell, Escherichia coli, Enterobacteriaceae, Salmonella spp.

Introduction

Since the prehistoric humans first gathered eggs from the nests of wild birds, they have been recognized as a nutritious food source. They provide a unique and well-balanced source of nutrients for humans. Eggs have a prominent place in diets due to their accessibility, low cost, ease of preparation, and low-calorie content [1]. Furthermore, hygiene in poultry manufacturing and processing is related to the presence or absence of pathogenic microorganisms (*Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Campylobacter*) as well as rotting organisms (*Pseudomonas*). On each occasion, their presence can also result in substantial economic losses to society and industry, either by causing food-borne illness in humans or by causing the spoilage of commodities that have been improperly managed and stored [2]. It is important to note that foods derived from animals, especially eggs

and egg products, poultry, and poultry products, are frequently implicated in varying instances and incidences of individual salmonellosis. Microbiological standards for food safety may also include tests for indicator species that suggest the possibility of a microbiological danger. *E. coli* in drinking water, for instance, indicates the possibility of fecal contamination and, subsequently, the incidence of additional enteric pathogens such as *Salmonella typhi* in water [3]. The many functional substances in eggs produce a perfect environment for enhancing normal bacterial flora, excluding pathogenic species. The fact that eggs can be contaminated directly (through the shell) or vertically (trans-ovarioles) renders them a potential reservoir of pathogens contributing to the causing of food-borne infections [4]. Matthews et al. [2] stated that once eggs are preserved, their shelf life can be prolonged if stored appropriately. Eggs should be stored in a cool, dark, and unscented environment, such as a refrigerator. As an indication, microorganisms could be used in microbiological standards. The presence of indicator bacteria might also suggest potential dangers posed by microorganisms. For instance, typical *E. coli* in a food chain indicates live fecal contamination. These standards are likely used to classify existing items as suitable or to predict the exact duration of food shelf life. *Salmonella* is a pathogenic bacteria found in the digestive systems of mammals, birds, animals, and insects. Moreover, *Salmonellae* are rod-shaped, facultative anaerobe, flagellated, and Gram-negative bacteria. Salmonellas primarily inhabit the digestive tract. They are often asymptomatic and are spread through various animal feeds, wild animals, rodents, pets, birds, and insects. They can be transferred through feces to fields, rivers, and foods, and then to other animals (involving individualizes) [5]. Hence, the bacterium is consumed orally through contaminated food or water. Cooling can stop the growth of bacteria but does not kill them. Salmonella spp. are killed by heat treatment; 60°C of heat is sufficient to eliminate the bacterium. *Salmonella* spp. can be found on the exterior of an eggshell before cleaning or within an infected chicken's egg. Before packing, federal requirements require commercial egg producers to sterilize all eggs [6]. Roberts referred that the yolk makes up approximately (31.5%) of a chicken's egg, while the albumen makes up approximately (58–62%), and the shell layer accounts for approximately (10.5%) of an egg. It is sold on the market as an egg product, egg shells, or elements derived from eggs. The yolk and the white, also known as the albumen, are the two components that make up the interior of an egg laid by a chicken. In addition, the egg's interior is characterized by the presence of the yolk [7].

Eggs have a significant nutritional value for humans, but in addition to that, they are a source of food for a variety of other living organisms. After passing through egg shells and membranes, bacterial species can use it as a source of nutrients in their bodies [8]. Eggs and other animal-based foods that have been improperly handled can be a source of foodborne pathogenic bacteria. *Salmonella* is one example of a foodborne pathogen, one of the most prevalent causes of foodborne disease [9]. As the techniques for detecting *Salmonella* spp. in table eggs and other food products are time-consuming and work intensively in the laboratory. They may involve bacteria as an indication in the context of microbiological standards, such as *E. coli*. They are easy to detect in food, can be differentiated from one another, grow on a wide variety of substrates, and thrive on a wide variety of carbohydrates as well as other organic molecules. Hence, the objective of this study was to identify *E. coli* as an indicator of the

presence of *Salmonella* spp. as enteric pathogenic bacteria in the contents and shells of eggs. Collect samples from nine various locations in the province of Wasit and research techniques for preventing the spread of infection.

Materials and Methods

Study design

The experiment was conducted in the microbiological research laboratory of the Biology Department of Wasit University College of Science in Iraq. The 7 months commence in December 2020 and ends in June 2021. Ninety brown poultry eggs were gathered randomly from several supermarkets, micro-markets, and rural egg producing sites in the Wasit province of Iraq. Plan for sampling with nine different locations and (n = 10 per place). Even though eggs are stored and displayed at room temperature at sale stations, it has been ensured that they are no older than 24 h. Following sterilized assembly, eggs were placed in packaging egg containers, with an average of three cartons (30 eggs per carton) being sent to the laboratory immediately. They were stored in the refrigerator at 4°C until they were examined for microbial contamination, at which point they were processed according to modified versions of routine microbiological tests.

Isolation of *Salmonella*

As stated by D'Aoust [10], the pre-enrichment of food samples in a non-selective broth medium is the first step in the general procedure for isolating of *Salmonella*. The next step is enhancing a selective broth medium, then isolating possible colonies on a differentiation plating medium and biochemical testing of potential isolates. Samples being transported in aseptic glass bags (1 egg in each). The egg sample was then properly combined with 150 mL of sterile buffered peptone water in glass bags. Therefore, 25 mL of soak buffered peptone water was transferred to a sterile beaker containing 225 mL of Tryptone Soya Broth (TSB), homogenized, and incubated at 35 ± 1°C for 18–24 h. It was determined by transferring 1 mL of the pre-enrichment broth to 10 mL of Selenite Broth (HiMedia, Mumbai, India) (PH 7.1 ± 0.1) (Enhancement medium) and incubating at 36°C for 24–25 h. A loopful of selenite broth culture was streaked over Brilliant Green Agar (PH 7.0) and Bismuth Sulfate Agar (HiMedia, Mumbai, India) (PH 6.9) and incubated at 35–37°C for 24 h [11]. Finally, pick at least two colonies of *Salmonella* presumptive isolates from each of the selective agar plates to determine whether or not *Salmonella* was present. Before inoculating Triple Sugar Iron (TSI) Agar with a colony's core, a sterile inoculating needle should be used to lightly touch the colony's center (TSI). TSI slants should be streaked and stabbed while being incubated at 36°C for 24 ± 2 hours. The darkening of the butt is a sign of the existence of hydrogen sulfide, which needs an acidic environment to develop. The formation of hydrogen sulfide requires an acidic environment, and the darkening of the butt is an indication of its presence [12].

Isolation of *E. coli*

For the objective of isolating *E. coli* bacteria, egg samples were tested according to the method illustrated by Adesiyun et al. [13]. One milliliter of TSB broth, including both the shell and the

content, was transferred individually into 10 mL of lactose broth, which was then obstructed by Durham tubes (for the creation of test gas) and baked in an oven set to 37°C for 48 h. Using a Nichrome inoculation loop, aliquots from tubes containing gas were streaked on the surface of Eosin Methylene Blue and Brilliant Green Agar plates. Then, the plates were placed in an additional incubator at 36–37°C for 24–25 h. According to Alert (2005) [14], the plates were examined microscopically after being stained with Gram stain and put through a series of conventional biochemical testing.

API 20E System for Identification of *Enterobacteriaceae*

The samples of whole table eggs were utilized for the biochemical identification of the bacterial isolates as members of *Enterobacteriaceae*. The isolates in question were E. coli and Salmonella spp. A plastic strip divided into 20 separate parts is utilized in this approach. Each component has a cupule and a small box holding different dehydrated media. The system includes a total of 23 different types of biochemical tests. After the little tubes of strips had been injected with the typical colonies of bacteria that had been obtained from samples of entire table eggs, the strips were placed in an incubator. The bacteria were picked up and suspended in 5 mL of 0.85% sodium chloride before being thoroughly mixed to achieve turbidity levels equivalent to 0.5 MacFarland regular solution. After an incubation time of 20–22 h, the color change and the reactions are recorded, the reagents for the experiment are merged into various sections, and the experiment results are arranged. Each test's positive and negative outcomes were used to obtain the profile number, which was determined by finding the profile number (7 digits)) (in the API) and having seven digits appended to it. After then, it used these numbers to identify different types of bacteria [15].

Statistical Analysis

All statistical analyses were performed with SPSS version 20, and the comparisons between the statements were determined by Chi-square tests with a significance level of $P < 0.05$.

Results and Discussion

The following exemplifies the presence of *E. coli* and *Salmonella* species in entire table eggs (both the interior content and the eggshell). The prevalence of Salmonella spp. on the egg shells is mapped out in Table 1, which may be found below. The overall occurrence of *Salmonella* spp. in egg shells was observed as 25.6% infected with Salmonella spp. out of a total of 90 egg samples that were analyzed. These egg samples came from different batches of eggs. On the other hand, the percentage of samples that did not include *Salmonella* spp. was 74.4%. The findings are inconclusive ($P > 0.05$), and the data imply that the variables Salmonella shell and location are not linked (i.e., they are independent). Moreover, the distribution of *Salmonella* spp. found in the internal eggs is outlined in Table 2. The overall incidence of *Salmonella* spp. in the internal eggs was 7.8%, based on testing and inspection of 90 egg samples. This represents a total of 7.8 infected *Salmonella* spp.

The percentage of samples that did not include *Salmonella* spp. was 92.2%. The findings do not constitute a significant finding ($P > 0.05$). According to the results, the two variables,

Salmonella spp. internal egg and location, are not connected in any way (i.e., they are independent). The spread of *E. coli* on the eggshells is illustrated in Table 3, which may be found below. The overall incidence of E. coli shell was reported at 67.8% positive with *E. coli* out of the total 90 egg samples that were confirmed. In contrast, the proportion of negative *E. coli* was 32.2%. This was determined by comparing the positive and negative *E. coli* results. The findings do not constitute a significant finding ($P > 0.05$). According to the findings, *E. coli* shell and location variables are not related to one another (i.e., they are independent). Thus, the division of *E. coli* that occurs in the internal eggs is detailed in Table 4.

The overall prevalence of *E. coli* internal was claimed to be 32.2% positive with *E. coli*, while the proportion of negative E. coli was 67.8% out of the total 90 egg samples validated. This information was gleaned through the verification of the eggs. The findings do not constitute a significant finding ($P > 0.05$). According to the results, the variables *E. coli* internal egg and location are not related to one another in any way (i.e., they are independent). The correlation between the presence of *Salmonella* spp. on the exterior and the inner surface of the eggs is broken down in Table 5.

The laboratory examined a total of 90 different egg samples. Salmonella spp. shell and *Salmonella* spp. internal egg are associated, and the study findings show that this association is significant ($P < 0.05$). Out of a total of 90 different egg samples, Table 6 analyzes the relationship between the presence of *Escherichia coli* on the outer and interior surfaces of the table eggs. which can be found below. The findings are statistically significant ($P < 0.05$). The variables *E. coli* shell and *E. coli* internal egg are linked.

The connection between *E. coli* shell and the *Salmonella* spp. shell is presented in Table 7, samples were taken from a total of 90 eggs, out of which they were experienced, as shown by the statistically significant findings ($P < 0.05$). The variables *E. coli* shell and Salmonella spp. shell are connected to one another. The relationship between *E. coli* internal egg and *Salmonella* spp. internal egg is depicted in Table 8. In total, 90 egg samples were examined. The variables E. coli internal egg and *Salmonella* spp. internal egg are significantly linked with one another ($P < 0.05$).

E. coli population, as suggested by Ricke et al., can be utilized as a measuring stick for hygienic and quality processing conditions. *E. coli* makes up 90–100% of the coliform population isolated from human and animal feces, making it a classic indicator organism for fecal contamination [16]. Furthermore, it is an organism that is used as an indicator of fecal contamination. Moreover, AL-Ashmawy mentioned that numerous genera of Enterobacteriaceae, one of which is *E. coli*, have been found in the shell and content of several table eggs. These eggs come from a variety of sources. They went on to say that excessive concentrations of outer shell contamination could have a negative impact on the shelf life of the eggs as well as the food safety of the products [1]. Moreover, these increasing numbers of microorganisms on the eggshell were described by Messens et al. [17] who said that this increases the risk of microbial eggshell entrance and egg content contamination. Infection of egg batches with pathogenic *salmonellas* is a problem that has been known for a long time; nevertheless, in the majority of cases, this was due to ruining the egg shell exterior with fecal

debris after the egg laying in the nesting. After the egg batches have been cracked, the shell could later infect the interiors of the eggs.

After cracking a large number of eggs, it is challenging to accomplish this because it is difficult to avoid several illnesses caused by shell fragments. Our findings are in line with the research that has been cited previously.

Adams et al. [5] confirmed that the microbes responsible for the deterioration of the eggs will encounter many different types of practical barriers before the eggs become spoiled and contaminated. First, the significant barrier to entry is provided by the shell covering in addition to the layers of the membranes. Second, the antimicrobial constituents of the egg white content render egg white an unfavorable medium for the growth of microorganisms. These factors combine to make the egg white an unsuitable environment for the proliferation of microorganisms. Although *Salmonella* spp, transovarial transmission is possible, consequently, it enables germs to avoid being stopped by the barriers that the shell and adjacent membrane layers present;

this does not mean they can do so. Berlanga provided evidence that bacteria could be exploited as an indicator of microbiological criteria. The presence of indicator bacteria can also suggest the presence of other dangers posed by microorganisms. For example, the detection of typical *E. coli* inside a food chain indicates the presence of live fecal contamination. These standards are usually employed to deal with products considered to be highly satisfactory or to anticipate the particular amount of time that food can be stored [18].

Papademas and Bintsis showed that *Enterobacteriaceae* is a further suggestible check for post-pasteurization contamination. Subsequently, the examination distinguishes entirely the Gram-negative rods, non-spore forming, heat sensitive, and offers a good indication that contamination has occurred [19]. Britz and Robinson referred that have to be considered that infection with *Enterobacteriaceae* exhibits that furthermore severe pathogens might have infected the outcome too. It is thought by several researchers that the elevated the number of symptoms, the more the opportunity that infective microbes will exist [20]. As stated by National Research Council [21], the presence of *E. coli* in the food supply indicates that fecal infection may have taken place and that other microbes that can be found in feces, including pathogens, may also have been present. When it comes to the organisms that are typically used as fecal indicators, *E. coli* can serve as a reliable indicator of a fecal infection. When foods are handled at temperatures above 40°C, the organisms that cause foodborne illness are easily killed. Therefore, the presence of *E. coli* in a food system that operates using heat is evidence of a procedure breakdown or, more generally, post-processing contamination from equipment or personnel or touch with infected fresh foods.

Whiley and Ross established that the microorganisms primarily responsible for the contamination of shell eggs and interior components (liquid eggs) are normal Gram-negative bacteria. Microbial putrefaction of handled eggs is not frequently a fault if not these products undertake several forms of mishandling [22]. Accordingly, *Salmonella* can contaminate the egg's inside either horizontally, through the eggshell, or vertically, through direct contact with the egg's interior. Interior contamination may initiate because of the entrance of *Salmonella* during the egg shell (horizontal contamination) or with straight contamination (vertical

contamination) of egg interiors formerly the eggs are covered by the shell originating from infection of the multiplicative organs [23]. Hence, the shells and egg contents were independently tested in the present study. As soon as the egg enters, the microorganisms must cope with bactericide agents in the inner part (albumen and vitelline membrane) formerly passage to the yolk can take place [24].

Polo et al. noted that a strong correlation had been found between groups of overall coliforms, fecal groups (coliforms and *streptococci*), and enteric pathogenic (*Salmonella*) in Portugal's aquatic environments [25]. Similarly, Arvanitidou et al. discovered a high correlation between the prevalence of Salmonella serovars and total coliforms in Greek rivers [26].

Moriñigo et al. found a significant correlation between fecal indicator concentrations and the occurrence of *Salmonella* spp. in waste discharged Spanish freshwater and aquatic native waters [27]. Garbutt noted that enteric bacteria of *E. coli* are a type of coliform group bacteria that frequently occur as a harmless component of the microflora population in the gastrointestinal tract of animals. Certain species are regarded as pathogenic and capable of causing food poisoning [28]. Froning demonstrated that to avoid germs in food, it is commonly recommended to store the food at a low temperature, a minimum of 4°C, below which no evolution occurs. It is also essential to keep the eggs in a spot for as little time as possible so that cross-contamination can occur. Numerous assemblies of egg batches, proper cooling and humidity conditions, a good wash, and careful handling of many eggs to prevent breaking are necessary management variables for reducing bacterial infection in eggs [29].

Nordenskjöld demonstrated that after eggs have been preserved, their shelf life can be prolonged if they are piled under suitable conditions. Egg batches should be stored in a cool, shady, and odor free environment, such as a refrigerator; 1 day at room temperature is equivalent to 5 days [30]. Whiley and Ross showed that chilling many eggs at 4°C for 15 min before the introduction of *Salmonella* significantly reduced the eggshell layers' permeability. It was hypothesized that this was due to decreased growth and a rise in the depressed temperature. This suggests that chilling egg batches during assembly may be an effective method for reducing Salmonella
infection [22]. The rate at which microorganisms penetrate eggs depends on storage temperature, egg age, and contamination level [31]. The entry of microbes into complete egg sets is facilitated by high humidity. Under these conditions, penetration through the shell layer and into the membrane aids the proliferation of microorganisms on the egg surface [32]. According to Jay et al. [31] depending on the egg's storage capacity, the thick white transfers water to the yolk, hence reducing the yolk and reducing the concentration of the white. This makes it possible for the yolk to come into direct contact with the inner membrane, which pathogens can rapidly infect. They highlighted that specific control over eggs could improve their preservation quality during cooling storage. Regular eggshell saturation with odorless and colorless mineral oil maintains moisture, inhibits dehydration and air diffusion, retains carbon dioxide, and retards physical and chemical air changes.

Doyle established that the interior portion of eggs (yolks) is extra treated by pasteurization to eliminate *Salmonella* and thereby extend the shelf life of these items. *Salmonellae* and additional pathogens are more resistant to heat in yolks due to the protective effects of higher

solids and fat concentrations. Pasteurization requirements for yolk components are higher than those for other egg products. A typical approach would involve a temperature rise of 63–65°C for 4–6 min [33]. According to Berlanga [18], the temperatures achieved in the yolk through slight heating practices, such as "quiet boiling" or soft cooking, are possibly insufficient in finishing the microorganism, and the lipid matter of the yolk portion might protect the microorganism from stomach acidity. These findings were published in the journal Microbiology.

The *Salmonella* disease chain in animal meals has been maintained by the use of animal by-products as animal feeds, such as meat or meat products and bone meal. The following sentence highlights this problem: *Salmonella* contamination should be eliminated if these components are subjected to heat treatment during their transformation into feed. On the other hand, they are vulnerable to post process infections either in the plant where they are produced or on the farms where they are grown due to linkages with untreated substances and waste from chickens and rodents [5].

According to Woodring [6], the disinfectants used to treat shell eggs have to be exceptionally bactericidal while simultaneously harmless to humans and animals. In addition, it is effective despite the presence of organic material. It is soluble in water, does not leave a stain, is destructive, and is effective at penetrating materials and overlays. The egg batches have to be clean and undergo an adequate rinsing process; the cleaning process has to be completed using hot water containing an antiseptic that does not have an odor, taste, or color, such as chlorine. Egg batches that have been rinsed need to be dehydrated as soon as possible using the appropriate method. This decreases the number of germs that are found on the eggshell layer. These microbes include bacteria and viruses, which can potentially infect the area that surrounds the egg groups and cause food poisoning.

To avoid ruptures or cracked eggs, the farms that supplied the batches of eggs were required to employ a scoring technique [34]. Appropriate packaging must account for volume, and the contents and the packaging itself must be cleaned of any traces of waste contamination and odors. There is a possibility that an artificial plastic or a carton will serve as the material for the packages. Bags used for egg batches ought to be high quality, resistant to shock, dehydrated, sanitary, and in excellent enough repair to function correctly. The constituents must belong to a class that can protect the egg groups from undesirable odors and diminished quality [30].

Conclusion

The presence of indicator microorganisms may indicate the presence of a microbial risk. The indication provided by microorganisms is applied more frequently to evaluate food safety. It must be present once the pathogen of concern is present, quickly detectable, and readily differentiate itself from other members of the food flora. For a period of time that is a little bit longer than the pathogen of concern,
they must be kept in place. *E. coli* was requested as the primary fecal indicator to ensure the food supply's safety. The presence of particular strains of *E. coli* within a pattern indicates possible fecal infection with enteropathogenic bacteria, such as Salmonella species. Hence, it is necessary to detect *E. coli* in food patterns primarily.

For this reason, it is essential to identify *E. coli* in dietary patterns, the primary example of which is whole table eggs. It is generally believed that the most common reason for foodborne illnesses is improper preparation and handling of food on the part of the consumer. Salmonella infection of egg batches is a complicated issue that is affected by various variables at every stage of the food manufacturing process.

Notably, the risk of disease rises when egg batches are utilized, for example, as a component in prepared nutrients for the general population. This can happen when there is cross-contamination. In addition, there are a requirement for additional research to optimize packing, appropriate temperature, and food processing techniques. In addition, there is a requirement for further research to support more specific control protocols and education programs to reduce the risk of salmonellosis from egg consumption.

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Conflicts of interest

The authors report no financial or any other conflicts of interest in this work.

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Table 1: The occurrence of *Salmonella* spp. on the shell of the eggs (n: 10)..

Location	Salmonella-shell			
	Negative		Positive	
	N	%	N	%
1	8	80.0%	2	20.0%
2	7	70.0%	3	30.0%
3	8	80.0%	2	20.0%
4	8	80.0%	2	20.0%
5	7	70.0%	3	30.0%
6	8	80.0%	2	20.0%
7	6	60.0%	4	40.0%
8	8	80.0%	2	20.0%
9	7	70.0%	3	30.0%
Total	67	74.4%	23	25.6%
Chi-Square Tests			P-value	0.974 N.S

The statements were determined by Chi-square tests with no significance differences of P > 0.05(P-value 0.974).

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Table 2: The occurrence of Salmonella spp. in internal egg content (Liquid eggs) (n=10).

Location	Salmonella- Internal egg			
	Negative		Positive	
	N	%	N	%
1	9	90.0%	1	10.0%
2	9	90.0%	1	10.0%
3	10	100.0%	0	0.0%
4	9	90.0%	1	10.0%
5	9	90.0%	1	10.0%
6	10	100.0%	0	0.0%
7	9	90.0%	1	10.0%
8	9	90.0%	1	10.0%
9	9	90.0%	1	10.0%
Total	83	92.2%	7	7.8%
Chi-Square Tests			P-value	0.975 N.S

The statements were determined by Chi-square tests with no significance differences of P > 0.05 (P-value 0.975).

Table 3: The occurrence of *Escherichia coli* in egg shells (n=10).

Location	Escherichia-shell			
	Negative		Positive	
	N	%	N	%
1	3	30.0%	7	70.0%
2	2	20.0%	8	80.0%
3	4	40.0%	6	60.0%
4	3	30.0%	7	70.0%
5	3	30.0%	7	70.0%
6	5	50.0%	5	50.0%
7	1	10.0%	9	90.0%
8	4	40.0%	6	60.0%
9	4	40.0%	6	60.0%
Total	29	32.2%	61	67.8%
Chi-Square Tests			P-value	0.726 N.S

The statements were determined by Chi-square tests with no significance differences of P > 0.05 (P-value 0.726).

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Table 4: The occurrence of *Escherichia coli* in internal egg content (liquid eggs). (n=10).

Location	Salmonella-Internal egg			
	Negative		Positive	
	N	%	N	%
1	7	70.0%	3	30.0%
2	6	60.0%	4	40.0%
3	8	80.0%	2	20.0%
4	6	60.0%	4	40.0%
5	7	70.0%	3	30.0%
6	8	80.0%	2	20.0%
7	6	60.0%	4	40.0%
8	7	70.0%	3	30.0%
9	6	60.0%	4	40.0%
Total	61	67.8%	29	32.2%
Chi-Square Tests			P-value	0.974 N.S

The statements were determined by Chi-square tests with no significance differences of P > 0.05 (P-value 0.974).

Table 5: The relationship between the presence of *Salmonella* spp. on the outer and inner surface of the eggs.

Categorical variables		Salmonella- Internal egg				Total	
		Negative		Positive			
		N	% ¥	N	%	N	%
Salmonella-shell	Negative	67	74.4%	0	0.0%	67	74.4%
	Positive	16	17.8%	7	7.8%	23	25.6%
Total		83	92.2%	7	7.8%	90	100.0%
Chi-Square Tests			P-value			0.001**	

¥% of total, **P<0.01. The statements were determined by Chi-square tests with significance differences of P < 0.05 (P-value 0.001).

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Table 6: The correlation between the existence of *Escherichia coli* on the outer and inner surface of the eggs.

Categorical variables		Escherichia- Internal egg				Total	
		Negative		Positive			
		N	% ¥	N	%	N	%
Escherichia-shell	Negative	29	32.2	0	0.0%	29	32.2%
	Positive	32	35.6%	29	32.2%	61	67.8%
Total		61	67.8%	29	32.2%	90	100.0%
Chi-Square Tests		P-value				0.001**	

¥ % of total, **P<0.01. The statements were determined by Chi-square with significance differences of P < 0.05 (P-value 0.001).

Table 7: The association between the *Escherichia coli* shell and *Salmonella* spp. shell

Categorical variables		Salmonella-shell				Total	
		Negative		Positive			
		N	% ¥	N	%	N	%
Escherichia-shell	Negative	29	32.2%	0	0.0%	29	32.2%
	Positive	38	42.2%	23	25.6%	61	67.8%
Total		67	74.4%	23	25.6%	90	100.0%
Chi-Square Tests		P-value				0.001**	

¥: % of total, **P<0.01. The statements were determined by Chi-square tests with significance differences of P < 0.05 (P-value 0.001).

Table 8: The connection between the *Escherichia coli* internal egg and *Salmonella* spp. internal egg.

Categorical variables		Salmonella- Internal egg				Total	
		Negative		Positive			
		N	% ¥	N	%	N	%
Escherichia-Internal egg	Negative	61	67.8%	0	0.0%	61	67.8%
	Positive	22	24.4%	7	7.8%	29	32.2%
Total		83	92.2%	7	7.8%	90	100.0%
Chi-Square Tests		P-value				0.001**	

¥: % of total, **P<0.01. The statements were determined by Chi-square tests with significance differences of P < 0.05 (P-value 0.001).