Control measures of salmonellosis in eggshell and liquid eggs at sites Local egg production in Wassit Province

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Abstract. The contamination of eggshells and liquid eggs by Salmonella has been recognized as a community health trouble. The current study was undertaken to isolate and identify of Salmonella in eggshells and liquid eggs, study of the susceptibility to antibiotics, and study the factors that help control of salmonellosis for local egg production in Wassit province. The percentages of Salmonella prevalence in eggshells, liquid eggs and whole eggs were 27%, 8%, 35%, respectively. all six sites under subject were indicated to presence of salmonella. This study showed no correlation between the sites of eggs production and presence or absence of salmonella. There were significant differences (p< 0.05) between eggshells and liquid eggs with presence or absence of salmonella in egg samples. Salmonella isolates showed antibiotic susceptibility to gentamycin, moderate sensitivity to chloramphenicol, and resistance to other types of antibiotics. This study involving the investigation of salmonellosis restriction methods during production, storage, transportation and food processing and handling. Additional study is prerequisite to supply additional described checking techniques and knowledge systems to decrease the hazard of salmonellosis after egg ingestion.

Keywords. Pathogenic bacteria; eggshells; Liquid eggs; Antibiotic resistant.

1. Introduction
Salmonella infection of eggs is a complicated problem that is affected by several variables, rendering it complex to including appropriate controlling approaches [1]. A diversity of animals’ act as harbors for transmitting pathogenic Salmonella organism. The individual food poisoning by Salmonella is entirely related to infected poultry and poultry products [2, 3]. Poultry eggs are the eggs generally ingested by persons. Eggs were not generated as a foodstuff for person feeding. Whereas our consumption of eggs could significantly affect the decomposition of this food, there are several influences comprised in alleviating microbial deterioration previously combined into the egg [4].
International, Salmonella is one of the greatest predominant reasons of foodborne disease in the world [1]. Salmonellosis is a public health problem of serious magnitude globally. Particularly, the hazard of disease expands once egg is employed as constituent in arranged foods for the common population [5]. Eggs are susceptible to microbial attack with sequential damage depending upon the eggshell force among its several courses and source of contamination. The most reported source of human salmonellosis is eggs and reservoirs for transmission, poultry are considered to be a significant reservoir which readily transmit the organism to human. Normally infected eggs and egg associated foodstuffs are initial carriers for individual salmonellosis [6]. The egg has three substantial hurdles to the last transference. These are a hydrophobic proteinaceous coating (the cuticle) which covers the shell and the pore openings, the crystal-like shell itself and the membranes which discrete the shell and the albumen [7] The yolk is as speedily delicate as milk, presently with the defense of the shell and antimicrobial constituents of the egg white, the substances of an egg can persist edible for months, still when preserved at room [8].

Egg infection can arise through two ways, vertical and horizontal of bacterial infection. Vertical transference a consequence of generative tissue establishment (ovary and oviduct) formerly shell construction, while horizontal spread take place as a result of outer egg shell infection. The exterior and interior egg infection by Salmonella within chicken yielding is a difficult problem, affected by several variables. Consequently, application of proper restrict techniques is very hard [1].

Obvious the bacteria detected in whole eggs were members of the following genera: Salmonella, Escherichia, Pseudomonas, Proteus, Aeromonas, Enterobacter, Micrococcus, Serratia, Flavobacterium, and Staphylococcus [9]. The genus Salmonella are facultative, chemoorganotrophic, Gram-negative rod-shaped nonspore-forming bacteria belonging to Enterobacteriaceae family [10]. The minimum, optimum, and maximum temperature for growth of Salmonella were 7°C, 36-37°C, and 49.5°C, respectively. The minimum, optimum, and maximum pH for growth of Salmonella were 3.8, 7-7.5, and 9.5 respectively. The requirement of water activity for this pathogenic is more than 0.94. The Salmonella is facultative anaerobic Salmonella is identified to survive properly in foods, on flatness and in the nature. Certain foods, comprising meat seem to be defensive of Salmonella through freezing [11].

Their primary habitat is the intestinal tract of humans and animals. Salmonellosis consequences from the digestion of foods having proper strains of this genus in adequate numbers [12].

The microorganisms mainly dependable for the decomposition of whole eggs (shell eggs and liquid eggs) are normally Gram-negative microbes. Infective damage of handled eggs is not frequently a difficult unless these foodstuffs suffer some form of mishandling. There has as well been a change in customer consumption behaviors with expanding request for raw and natural foods [13]. The expanding publicity of natural home-made foods comprising raw eggs for example mayonnaise, specified sauces and raw egg based dessert similar to ice-cream, tiramisu and even milkshakes possibly rises the hazard of salmonellosis [13]. Therefore, the objective of this study was to isolate and identify of Salmonella as pathogenic bacteria from eggshells, internal egg contents (liquid eggs), studying the effect of antibiotics on Salmonella isolates and identifying the necessary measures to reduce of salmonellosis.

2. Materials and Methods

2.1. Preparation of Shell and Liquid Egg Sampling Methodologies

Twelve eggs from sixth assembly location were closely retained into bubble cartons, arranged into half-cases, and returning to the lab at conventional temperature. Ten of the 12 eggs assembled at individually location were sampled applying a shell washing procedure. Alone egg was retained into a sanitary container with 100 ml of sterile buffer peptone water (pH 7.0 ± 0.2) (non-selective broth medium) and washed by agitating for 20 min. Eggs were saved at lab temperature, but the diluent medium had been incubated at 35±1°C for 24h. Individually egg was handled for the existence of Salmonella on the egg shell and in the interior contents (liquid egg) [6].

2.2. Isolation and Identification of Salmonella
An egg contaminated with Salmonella bacteria cannot be differentiated from a non-contaminated egg through physical or visual check. To accelerate retrieval of microbes from shells, a wash pattern was gained. Separately, the egg was removed and transferred to another sanitary container. It followed by the selective enrichment in which 1 ml of the non-selective broth was transferred to 10 ml of selenite broth (Himedia) (pH 7.0 ± 0.2) and incubating at 35±1 °C for 24h. Selective plating was done by transferring a loop full of selenite broth culture to 10 ml of selenite broth (Himedia) (pH 7.6), brilliant green agar (Himedia) (pH 6.9), and deoxycholate citrate agar (Himedia) (pH 7.0) and all were incubated at 35±1 °C for 24h. For screening, suspected colonies were transferred onto triple sugar iron agar (Himedia) (pH 7.0) and lysine iron agar (Himedia) (pH 6.7), inoculated the media by streaking back and forth on the slant and then by stabbing the butt, for H2S production and lysine decarboxylase and incubated at 37±1 °C for 24h. Characteristic of Salmonella can be observed from other members of the Enterobacteriaceae family through the inability to lactose fermentation, glucose fermentation as well as generating of gas and H2S production from thiosulfate [12]. The colonies of Salmonella isolates were taken for further identification by using of API 20 E technique.

2.3. Preparation of Internal Egg Contents (Liquid Eggs)
On the next morning, individually egg was isolated from the other container and broken unclose on the border of a sterilized beaker. Liquid whole egg was prepared in our laboratory through sanitizing the shell of eggs by 70% alcohol to cease interior content infection from the egg shell external, breaking shells via hand into a sterile container. Entire internal egg contents were separated, for each egg were carefully combined by sterile pipe individually, and the interior of the shell was washed applying sterile phosphate buffer solution (PBS) to eliminate greatest of the sticking albumen. Replicate 1 ml liquid egg white patterns or fresh formulated liquid whole egg patterns were transported to 10 ml of sterile buffer peptone water (pH 7.0 ± 0.2) (non-selective broth medium), and the same procedure as mention above was applicable to isolate and differentiate of Salmonella from liquid egg samples [5, 14].

2.4. Antibiotic Susceptibility Test
The Kirby-Bauer Disc Diffusion Method is used to define which antibiotic is the most active against a specific pathogen. With their respective disc potencies were used: Vancomycin VA30(μg), Tetracycline T(TE)30(μg), Streptomycin S10(μg), Gentamicin GM10(μg), Chloramphenicol C30(μg). After incubation, the plates are tested and the distance of the inhibition zones is calculated to the closest whole millimeter by use of sliding calipers, a ruler, or a template prepared for this purpose. The zone distances for particular antibiotics are interpreted into started susceptible, intermediate, or resistant groups by referring to a revealing table [15].

2.5. Study protocol and ethics
Study protocol approved and ethical issues done by local committee in college of science/ Babylon University, already the committee depends on principles of declaration Helsinki.

2.6. Statistical Analysis
All statistical analysis was performed using Gensat 12 software, and the comparisons between observations by using of chi-square with a level of significance at (p < 0.05).

3. Results
The results of the present study were tabulated as follow:
Figure 1. Triple sugar iron agar (TSI) without *Salmonella*

Figure 2. Triple sugar iron agar (TSI) with *Salmonella*

Figure 3. Identification of *Salmonella* by using of API 20 E technique.

Figure 4. Antibiotics were used in susceptibility test
Table 1. Prevalence of Salmonella in eggshells (n:10)

| Site No. | Positive | Negative |
|---------|----------|----------|
| Site1   | 2        | 8        |
| Site2   | 3        | 7        |
| Site3   | 2        | 8        |
| Site4   | 4        | 6        |
| Site5   | 2        | 8        |
| Site6   | 3        | 7        |
| collection | 16   | 44       |

P-value 0.888ns

Pearson chi-square value is 1.70 with 5 d.f. ns:no significant differences (p> 0.05)

Table 2. Prevalence of Salmonella in internal egg contents (liquid eggs) (n: 10)

| Site No. | Positive | Negative |
|---------|----------|----------|
| Site1   | 1        | 9        |
| Site2   | 1        | 9        |
| Site3   | 0        | 10       |
| Site4   | 1        | 9        |
| Site5   | 1        | 9        |
| Site6   | 1        | 9        |
| collection | 5    | 55       |

P-value 0.955ns

Pearson chi-square value is 1.09 with 5 d.f.ns:no significant differences (p> 0.05)

Table 3. Comparison between eggshells and internal egg contents (liquid eggs)

| Site No. | Positive | Negative |
|----------|----------|----------|
| Eggshells | 16   | 44       |
| liquid eggs | 5   | 55       |

P-value 0.008**

Pearson chi-square value is 6.98 with 1 d.f. ** significant differences (p< 0.05)

Table 4. Effect of antibiotics against Salmonella isolates (n:3)

| Antibiotic   | Disk symbol | Disk content (µg) | Effect     |
|--------------|-------------|-------------------|------------|
| Vancomycin   | VA          | 30                | Resistance |
| Tetracycline | T(TE)       | 30                | Resistance |
| Streptomycin | S           | 10                | Resistance |
| Gentamicin   | GM          | 10                | Susceptible|
| Chloramphenicol | C   | 30                | Intermediate|


Figure 5. The percentages of *Salmonella* prevalence in eggshells, liquid eggs and whole eggs, respectively.

![Pie charts showing percentages of *Salmonella* prevalence in eggshells, liquid eggs, and whole eggs](image)

Figure 6. Effect of antibiotics against *Salmonella* isolates (n: 3)

Figures 1 and 2 show the presence and absence of salmonella respectively in the triple sugar iron agar. Tables 1 and 2 show the prevalence of Salmonella in eggshells, liquid eggs, respectively on six location sites which ready to eggs production in province of Wassit. Tables 1 and 2 display no significant differences (p> 0.05) between the sites of eggs production and presence or absence of salmonella. Table 3 refers to significant differences (p< 0.05) between eggshells and liquid eggs with presence or absence of salmonella in egg samples. The percentages of Salmonella prevalence were 27.0 %, 8.0%, 35% in eggshells, liquid eggs sand whole eggs, respectively (Figure 5). Table 1 pointing to 16 cases as positive, and 44 cases as negative on eggshells, whereas there were 5 cases as positive and 55 cases as negative in liquid eggs (Table 2). Figure 5 refers to all six sites under subject were indicated to presence of salmonella. Table 4. Shows that the effect of five antibiotics against of Salmonella isolates, and at the same time which indicates that Salmonella isolates were sensitive to gentamycin, intermediate to chloramphenicol, and resistant to streptomycin, tetracycline, and vancomycin.

According to Samiullah [10], all main food contaminating of Salmonella ssp can penetrate eggshell. The Salmonella first penetrate across the cuticle and shell, then colonize the shell membranes from where it transfers on to albumen and yolk resulting in eventually to whole egg contamination.

Egg is physically protected with hurdles that support save microorganisms from penetrating the interior shell, membranes and internal egg contents [16]. A number of factors similar to relative humidity, generally shell quality, amount of shell pores, temperature, pH and bacterial load directly affect microbial penetration across the eggshell [17]. Poultry assembles, especially hens and fowls are
commonly populated with Salmonella with no noticeable indicators (sub-clinical diseases /healthy transporters) by vertical and horizontal of bacterial infection and spread at initial making amount. The existence of Salmonella in healthful hens’ animals is proposed as the major hazard cause, by permitting pathogenic bacteria (Salmonella) to simply transfer in board eggs and poultry meat to persons [18, 19]. In the present investigation, the general ratio of egg shell infection (external contamination) by Salmonella was higher significantly than it is compared with liquid eggs (internal egg contents) (Table 3). Our results are consistent with results by [6] who proposed that horizontal contamination (egg shells) across infected feces is the major way of egg infection by S. typhimurium in place of laying hens. Barrow and Lovell [20] proposed that most of egg infection as a result of horizontal transmission. Samiullah [10] concluded that eggshell quality, temperature and general flock health status are the causes that greatly affect Salmonella continuance in the poultry and their final transmission to different personal.

Jay [9] demonstrated that the rapidity in which bacteria come in eggs is associated to storage temperature, eggs age, and amount of infection. Movement happened in 24 h. at 30°C but not until two weeks at 7°C. Also, 24 h. old eggs were more resistant than one month old eggs, and the rapidity of movement was supportively associated with the intensity of infection. Braun and Fehlhaber [21] referred that the high humidity helps microorganisms to enter whole eggs. In such situations, growth and increasing of microorganisms numbers on the external of eggs is preferred, subsequently entrance during the shell and interior membrane.

According to Gast and Holt [22], keeping appropriate temperature controls after assembly of eggs is a necessary step in certifying the safety of the egg. Saving eggs in cold storage prevents the growth of Salmonella in the egg. Moreover, safe handling through transportation also demands sufficient temperature control measures. Jones et al. [23] referred that refrigerating has a positive influence on the whole quality of egg and the more speedily an egg is carried near the freezing point.

Table 4. shows the effect of antibiotics against Salmonella. In our experiment, the results display that isolates of Salmonella are sensitive to gentamycin and moderate resistance to chloramphenicol and there is the development of this sensitivity to antibiotics to resistance to affect the safety of the consumer. According to Parry and Threlfall [24], the rise and extend of Salmonella isolates representing resistance to many antibiotics is of worry for the reason that remedies are critical to the effective medicine of aggressive infection. The first indication is the strong relationship amongst the practice of antibacterial factors and the happening of resistance. Other suggestion for the influence of poultry reproduction on person health troubles connected with antibacterial resistance in Salmonella is the relationship amongst diverse harbors (persons and food-animals). Egg producers with their veterinarians are dependable for the handling and supply of antibiotics used on a poultry farm [25, 26]. According to [1], antibiotic treatment is a debated process for the restriction of Salmonella as a result of the early development of antibiotic resistant populations. The occurrence the resistant to antibiotic strains is greatly related to food protection, safety and public healthiness with respect to therapy of the additional aggressive reasons of salmonellosis.

According to Cogan and Humphrey [27], vaccines can reduce public health hazards introduced by Salmonella spp in poultry products by decreasing the colonization of reproductive tissues as well as decreasing fecal shedding, but some disadvantages are live vaccines would be the extent to the environment and/or to people. Vaccination of a flock previously infected may reduce the prevalence of the Salmonella enteritidis serotype in the flock, but will not defend the hens from other serotypes.

In the surface contamination of eggshell with viable pathogens, the existence of chicken fertilizes and other moist organic materials enables the survival and growth of Salmonella by offering the requisite nutrients and an amount of physical defense [28].

According to Xie et al [29], Several forms for example proteins, oils, or an additional polymer have been covered on the shell with the objective of prolonging shelf life by means of decreasing the chemical changes in the egg and avoiding the entrance of microbes. Some new measures have been established to enhance the protection and safety and shelf life of shell eggs. Once eggs are covered by whey protein isolate, soy protein isolate, or wheat gluten, they come to be more hole resistant and have decreased bacterial penetration.
The pasteurization practice for eggs is proposed to finish microorganisms that have the possible to cause disease; microorganisms such as Salmonella subspp. large numbers of eggs removed from their shells, sold to commercial institutions, are pasteurized in raw form and sold as a liquid. According to Jin et al. [5], the high temperature procedures are intended to make a salmonellae-free product and extends the shelf life of the egg. Since, the property characteristics of liquid egg product are very sensitive to high temperature, it is consequently essential to use the least heat treatment that will remains support sufficient protection and safety in the ultimate product.

4. Conclusions

Salmonella as pathogenic bacteria for this research were selected due to their importance as signs of property, quality, procedure hygiene, or protection and safety. Eggs are main resource of the Salmonella spp occurrences of salmonellosis in humans. Decreasing the infection of chickens and eggs in production is a logical stage to decrease human infections. Primary control processes such as checking, hygiene performs, antibiotics, and vaccines can all be used in attempts to rise public safety. Certification for egg producers would be useful. Other control techniques depend on the consumer to gain responsibility for appropriate storage and preparation. Further research is necessary to improve storage, temperature and food handling practices for decreasing the public health risk.

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