Contamination of Local Laying Hen's Egg Shell with *Salmonella* Serotypes

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Summary

Fifty locally laying hen's eggs random samples were collected from different markets of Baghdad city in order to investigate the presence of *Salmonellae Spp.* in shell of those eggs. The samples were collected during the period from March 2012 to May 2012.The samples were directly transferred to the Food hygiene laboratory and analyzed immediately without further storage.The isolation and identification methods include: (pre-enrichment) culture stage by peptone water then, (Selective enrichment) culture stage by selenite broth after that culturing on sold (Selective media) which was Bismuth Sulphate agar. The biotyping by using API strip according to the API 20E miniaturized identification system for *Salmonella SPP.*. The isolated Salmonella strains were transferred on TSI agar to undergone sereotyping at the Institute of Public Health,Baghdad,Iraq. Data revealed that 15 out of the total 50 (30%) of the eggs samples were contaminated with *Salmonella spp*. *Salmonella typhimurium* and *Salmonella enteritidis* were the two serotypes that have been found in this study. Nine from 15 (60%) of the isolates was belong to *Salmonella enteritidis* serotypes while 6 from 15 (40%) of the isolates was belong to *Salmonella typhimurium*serotype.

Keywords: salmonella, egg shell, serotypes, Hen's Egg.

Introduction

Salmonellosis is a worldwide health problem; Salmonella infections are the second leading cause of bacterial foodborne illness (1). Approximately 95% of cases of human salmonellosis are associated with the consumption of contaminated products such as poultry, meat, eggs, milk, seafood, and fresh produce. Typhoidal Salmonella is more commonly found in poorer countries and can affect as many as 21.5 million persons each year, where unsanitary conditions are more likely to occur (2). The type of Salmonella usually associated with infections in humans, nontyphoidal Salmonella, is usually contracted from sources such as: Infected tainted fruits and vegetables, meat, egg products, and milk products when not prepared, handled, or refrigerated properly (3). Salmonella *Spp* bacteria can survive several weeks in a dry environment and several months in water; thus, they are frequently found in polluted water, contamination from the excrement of carrier animals being particularly important. Aquatic vertebrates, notably birds and reptiles, are important vectors of salmonella, Poultry, cattle and sheep frequently being agents of contamination (4). This study was aimed to inquire the contamination of locally eggs shell with salmonella serotypes.

Materials and Methods

A total of 50 samples of locally eggs were collected randomly from local markets in Baghdad city during the period from March to May 2012.The samples were directly transferred to the laboratory and analyzed immediately without further storage. A surface of egg's shell was scanned by cotton swab which was saturated with peptone water and immersed in 100 ml of peptone water and incubated for 24 hour at 37°C for pre-enrichment. One ml of pre-enrichment broth was transferred to 10 ml of selenite broth (Selective enrichment) and incubated for 48 hour at 43°C. One loop-full of selenite broth was streaked on to Bismuth Sulphate agar (Selective media) after 24 and 48 hours of incubation and incubated at 37°C for 24 hours (5). One colony from the suspected Salmonella colonies which gave the typical morphology of Salmonella (pure culture) was transferred by

streaking to Nutrient agar and incubated at 37°C for 24 hour, one large colony inoculated into 5 ml 0.85% NaCl solution to inoculate the API strip according to the API 20E miniaturized identification system for *Salmonella SPP*. for bio-typing (6 and 7). The isolated Salmonella strains were transferred simultaneously to TSI agar by stabbing and streaking and incubated at 37°C for 24 hour to undergone stereotyping at the Institute of Public Health, Baghdad, Iraq.

Results and Discussion

Data revealed as in table (1) that (30%) of the eggs samples were contaminated with *Salmonella spp. Salmonella enteritidis* was one of the serotypes that have been found in this study. (60%) of the isolates was belong to this serotype It is the cause of the food-borne salmonellosis pandemic in humans, in part because it has the unique ability to contaminate eggs without causing discernible illness in the birds infected. The infection route to humans involves colonization, survival and multiplication of the pathogen in the hen house environment, the bird and, finally, the egg (8). *Salmonella enteritidis* has been the major cause of the food-borne salmonellosis pandemic in humans over the last 20 years, during which contaminated hen's eggs were the most important vehicle of the infection. Eggs can be contaminated on the outer shell surface and internally (9).

Salmonella enteritidis can contaminate the contents of clean, intact shell eggs as a result of infections of the reproductive tissue of laying hens. The principal site of infection would appear to be the upper oviduct. In egg contents the most important sites of contamination are either the outside of the vitelline membrane or the albumen surrounding it. In fresh eggs, only few salmonellas are present and as albumen is an iron-restricted environment, growth will only occur once storage-related changes to vitelline membrane permeability, which allows salmonellas to invade yolk contents, have taken place. When this happens high populations are achieved in both yolk contents and albumen. Some eggs from naturally infected hens have been found to contain large numbers of *Salmonella enteritidis*. The rate of change in membrane permeability is temperature-dependent. In eggs stored at 20 °C, yolk invasion is uncommon until eggs have been stored for 3 weeks. In stimulated kitchen conditions where temperatures reached 30 °C, salmonellas could grow rapidly after a few days (10). *Salmonella enteritidis* has intrinsic characteristics that allow an epidemiological association with hen eggs that are still undefined. There are indications that *Salmonella enteritidis* survives the attacks with the help of antimicrobial molecules during the formation of the egg in the hen's oviduct and inside the egg. This appears to require a unique combination of genes encoding for improved cell wall protection and repairing cellular and molecular damage, among others (9).

Salmonella typhimurium was the second serotype which was isolated in this study, (40%) of the isolates was belong to this serotype, It has been reported to contaminate egg production across the world. *Salmonella typhimurium* is a major food-borne pathogen so it is important to understand how it can impact the microbiological safety of eggs and what serovar-specific control strategies may be appropriate in the future as control over *Salmonella Enteritidis* continues to improve.

Experimentally *Salmonella enteritidis* is more often isolated from egg contents and seems to adhere better to reproductive tract mucosa, whilst *Salmonella typhimurium* appears to provoke a more intense tissue pathology and immune response, and flock infections are more transient. However, it is observed in many cases that the present body of evidence does not identify clear differences between specific behaviours of the serotypes *Typhimurium* and *Enteritidis*, whether in laying hens, in their eggs, or in the laying environment (11).

There are over 2,500 identified serotypes of Salmonella. A smaller number of these serotypes are significantly associated with animal and human disease like *Salmonella typhimurium*. Increasingly, isolates from this serotype is being detected that demonstrate resistance to multiple antimicrobial agents, including third-generation cephalosporins, which are recommended for the treatment of severe infections. Many of the genes that

encode resistance are located on transmissible elements such as plasmids that allow for potential transfer of resistance among strains (1and12). Plasmids are also known to harbor virulence factors that contribute to Salmonella pathogenicity. This serotype of medical importance, including is known to harbor virulence plasmid containing genes that code for fimbriae, serum resistance, and other factors. Additionally, it's containing pathogenicity islands scattered throughout their genomes that encode factors essential for bacterial adhesion, invasion, and infection. *Salmonella typhimurium* have evolved several virulence and antimicrobial resistance mechanisms that allow for continued challenges to our public health infrastructure (6 and13).

Table, 1: The serotypes which isolated and it's percentage

Samples	Isolates	%	Serotype	Isolates	%
50	15	30	*Salmonella enteritidis*	9	60
			Salmonella typhimurium	6	40

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تلوث قشرة البيض المحلي بجراثيم السالمونيلا

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الخلاصة

اجريت هذه الدراسة على 50 عينة عشوائية من البيض المحلي لمدينة بغداد، بهدف التحري عن وجود جراثيم السالمونيلا في قشرة البيض المنتج محليا. وقد جمعت النماذج خلال شهرين ابتداء من آذار2012 ولغاية آيار 2012. نقلت العينات مباشرة الى مختبرصحة الغذاء لغرض اجراء الإختبارات اللازمة وبدون اي خزن اضافي للعينات. كما اعتمدت خطوات معينة في عملية عزل وتشخيص السالمونيلا والتي شملت مرحلة الإغناء الأولي بالزرع في ماء الببتون يليها مرحلة الإغناء الانتقائي بالزرع في مرق السلينيت ثم الاستنبات على الأوساط الانتقائية الصلبة بالزرع على وسط البزموث سلفيت. التنميط المصلي النهائي للعزلات التي أعطت النتائج المطلوبة مع الاختبارات الكيموحيوية على API 20E. وقد أظهرت النتائج ان 15من اصل 50 عينة (30%) من هذه العينات كانت ملوثة بجراثيم السلمونيلا. وان النمط المصلي لـ *Salmonella typhimurium* و *enteritidis* *Salmonella* قد وجدت في هذه الدراسة بنسبة عزل (60%) و ايضا تم عزل نمطين مصليين هما تشخيص 9 عزلات من *Salmonella enteritidis* من اصل 15 عزلة تعود لهذا النمط المصلي بينما تم تشخيص 6 عزلات من اصل 15 (40%) من *Salmonella typhimurium*.

الكلمات المفتاحية: السالمونيلا , قشرة البيض ,انماط , تلوث القشرة.