

STUDIES ON THE PENETRATION AND VIABILITY OF SOME  
PATHOGENIC BACTERIA IN TABLE EGGS; (1) FOLLOWING  
UP SURVIVAL OF E. coli, Proteus vulgaris AND  
Pseudomonas aeruginosa INSIDE THE EGG.

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SUMMARY

Laboratory experiments were done to study the possible circumstances which might accompany some pathogenic bacterial species which penetrate table eggs, being contaminated either naturally or experimentally.

In the first stage, the contents (albumin + yolk) of each of 28 eggs were cultured directly onto brain heart-infusion (BHI), brilliant green sulfa (BGS), MacConkey Agar(MA),mannitol salt agar (MSA), tellurite blood agar (TBA)and 5% sheep blood agar (SBA); in addition to selenite broth (SB). Out of 28 egg samples, 8 bacterial isolates were recovered and identified; as 4 Staphylococcus aureus, 2 Bacillus subtilis and 2 Proteus vulgaris.

In the second stage, another 28 eggs were artificially contaminated by calibrated doses of each of E. coli, Pr. vulgaris and Ps. aeruginosa which were originally isolated from different disease conditions in children. Contamination of each egg was carried out as spots (droplets) on certain areas on the surface. Preliminary results showed a successful penetration of the organisms through the egg within 10, 15 and 30 minutes, and also after 2 and 4 hrs of storage at temperature (12-18 °C), although their numbers were decreasing progressively, espically within the egg's contents, until they could not be recovered after 8 days of storage.



## INTRODUCTION

Eggs are one of the very important and essential sources for human nutrition due to its supreme nutritious quality and value, compared to many other nutrients. It contains balanced ratios of proteins, vitamins, minerals, etc., hence, it is incorporated into many food industries, as well as some pharmaceutical and trade industries, and also has been used up till now in many laboratory diagnostic purposes and -above all- in preparation of vaccines. On the other hand, eggs and their by-products are still being used as major constituents of animal feed, having the consideration of being a major source of calcium. Apart from the above mentioned advantages, there are still many disadvantages, i.e. eggs are considered as, on of, the fastest deteriorating foodstuff, mainly due to the surrounding environmental conditions, therefore, the fresher the eggs were, the highest were their nutritious values<sup>(1. 2)</sup>. Pathogenic microorganisms usually play an important role in egg spoilage, as they need encouraging factors, like faecal contamination of the shell, increase in porosity, increase in relative humidity, etc.<sup>(1. 2)</sup>.

This study concentrated mainly on the probable role (or roles) that those above mentioned factors might contribute to the increase in shell porosity, thereby permitting, in one way or the other, the penetration of bacteria through the egg shell. Moreover, studies involved also, the abilities of such bacteria to thrive and survive inside the egg and cause its spoilage.

## MATERIALS AND METHODS

### 1) The egg:

Usual table hen eggs were purchased from the retail markets and used; their sizes varied greatly and also their weights (ranging between 30 and 50 grams). Times of laying were not known because the eggs were



commercial. The following paragraphs shall include materials and methods of the experiments done using such eggs:

a. In the first experiments, eggs were used for bacterial isolation. They were soaked in 70% ethanol for 5 min., and then left to dry; an opening was made in the tapered end using sterile scissors and forceps, and the contents (albumin + yolk) were dropped into sterile petri-dishes and mixed quickly using a sterile glass rod, then cultured onto different media by means of sterile swabs.

b. In the following experiments, 50% of the eggs were sterilized as above, and the other half were left as they were. The following experiments were done in this respect:

(1) In this experiment, 12 eggs (being sterilized as in -a- above) were used, where a drop (50 ul; equivalent to  $1 \times 10^6$  of cfu/ml estimated by the method of Miles & Misera), for each of E. coli (recovered from infantile diarrhea and serotyped, using Bio-Mareux antisera, as O114: K90) and Proteus vulgaris (recovered from infantile diarrhea at Sadam's Central Hospital for Children) were added separately to each of the tapered end of 3 eggs. The rest of the eggs ( $n = 6$ ) were used for control purposes, i.e. a 50 ul drop of sterile saline was added to the tapered end of each egg. All of the 12 eggs were stored at room temperature (12-18°C).

After 10, 15 and 30 minutes and 4 hours of storage, sampels were taken from the eggs, where a rounded hole (about 2cm in diameter) was made into tapered end of each egg and then the contents were dropped individually into sterile petri-dishes. Swabs from each of the internal surfaces and the mixed contents of each egg were cultured onto different culture media, which were, thereafter, incubated at 37°C for 24-48 hrs.

Direct cultivation was done on the following media: brain-heart-infusion (BHI), brilliant green-sulfa (BGS),



macConkey agar (MA), mannitol salt agar (MSA) and tellurite blood agars (TBA) and selenite broth (SB). From cultured SB media, subcultures were made onto BGS for the isolation of anticipated Salmonella species.

II) In this experiment, 16 eggs (not sterilized as described in -a-) were used, and contaminated as in the first experiment using the same bacterial species, in addition to Pseudomonas aeruginosa (recovered from pus in a child at Sadam's Central Hospital for Children). The same doses of the first experiment were used for the contamination of eggs in this experiment, and at the same sites.

After 0.5 hr, 1 hr, 3 and 8 days of storage of contaminated eggs at room temperature (12-18 °C), each egg was sampled, by an opening (about 2.5-3cm<sup>2</sup>) at the tapered end and evacuating the contents into a sterile petri-dish. Each egg was then cut into two halves by a sterile scissors. Swabs were taken from the internal surfaces of each of the tapered end and the two sliced halves; in addition, to the contents. Swabs were then cultured on different media.

In addition, antimicrobial sensitivity and different biochemical tests were carried out for all the bacterial species used in this study. Tests were done before and after each experiment to check for bacterial identities. All biochemical identification methods were done according to the methods described by Cowan<sup>(4)</sup> and Frankel et al<sup>(5)</sup>.

## RESULTS

### 1) First Experiment:

A. The following bacterial species were isolated from egg samples: Staphylococcus epidermidis (4 isolates), Bacillus subtilis (2 isolates) and Proteus vulgaris (2 isolates).

B. In the second stage, results showed (Table 1) that both E. coli and Pr. vulgaris succeeded in



penetrating the egg shell, although their numbers decreased progressively from an initial dose of  $10^6$  cfu (for each bacterial species) to decreasing numbers fluctuating with the duration of storage. For example, a total of 180 to 200 cfu of E. coli were calculated from cultures of swabs taken from the internal surface of the egg shell after a period of 10 minutes of storage of the contaminated eggs. In addition, no bacteria were isolated from the contents (albumin + yolk) of the same samples eggs. After 2 hrs of storage, the numbers dropped to about 5 cfu (in cultures of swabs taken from interior of egg shells), but rised to 300 cfu in cultures made from egg contents. After 3 hrs of storage, bacterial numbers dropped to about 3 cfu (in the interior egg shell) and 50 cfu (in the contents ).

Conditions seemed to be different in case of Pr. vulgaris, where their total numbers showed a comparatively higher count (in both the interior egg shell and the contents) after 3 hrs of storage, but later on, their numbers decreased compared to the original contamination dose.

Table 1: Numbers of bacterial species recovered from the interior surfaces of precontaminated eggs.

Period of sampling	Bacterial species used			
	<u>E. coli</u> '			<u>Pr. vulgaris</u> '
	Interior of eggshell	contents'	Interior of eggshell	contents''
10 min	180-200"	-	more than 300	70
15 min	H. gr.	-	H. gr.	H. gr
30 min	180-200	150	100	90
2 hr	5	300	250	250
3 hr	2-3	50	300	150

' Initial contamination dose was  $10^6$  colony forming units (cfu) per 50 ul drop for each of the species.

'' comprises both albumin and yolk.

" represents numbers of colonies counted on agar media.



## 2) Second Experiment:

In this experiment, only E. coli was recovered from the internal surface of the egg shell that is opposite to the contamination area; where about 50 cfu (out of  $10^{10}$  cfu original contamination dose) of the same bacteria were counted on agar media after storage of the eggs for 30 min at room temperature (18-20 °C), and concerning the rest of the bacterial species used, no successful recovery was made at any period of storage. Sensitivity test was done before and after the experiment for E. coli used. Results showed that it was resistant to several antibiotics, like tetracycline, erythromycin, sulfamethoxazole, cephalothin, streptomycin and Kanamycin.

## DISCUSSION

The sources of microbial contamination of the eggs are numerous like: dirt and droppings present in nests and cages, also dust accumulated during prolonged storage, etc., therefore, a variety of microorganisms, in number and type, can be found on egg shells. The majority of those species can be included in the gram-positive group, like: Bacillus, Micrococcus, Staphylococcus, Streptococcus, etc<sup>(6, 7)</sup>. On the other hand, each egg has its own defense barriers that inhibit the bacteria from penetrating the egg and reaching the growing embryo (in egg yolk), beginning from the shell which is covered by the cuticle that stands as the uppermost inhibitory barrier due to its role in covering up the shell pores<sup>(6, 8)</sup>, but washing the egg or rubbing it by a coarse material will increase the chances of egg spoilage due to the penetration of bacteria<sup>(9, 10)</sup>.

Accordingly, our results proved efficiently that disinfection of the eggs by ethanol increased the chances of the penetration of the bacteria through the egg shell, so that in the first experiment, the following bacterial species were isolated from the egg



contents: Staph. epidermidis (4 isolates), B. subtilis and Pr. vulgaris (2 isolates each); although such species are considered as normal contaminants of eggs<sup>(?)</sup>.

On the other hand, the following experiments showed clearly and precisely that cleaning or washing eggs offered ideal conditions for penetration of bacteria. In addition, the results showed astonishing speed of penetration by those organisms into the eggs, so that the total of more than 180 colonies of E. coli and more than 300 colonies of Pr. vulgaris were counted in samples of egg contents (albumin + yolk) of pre-contaminated eggs, only after 30 min of storage at room temperature. Moreover, arrival of Pr. vulgaris organisms to the egg contents was diagnosed after 15 min of storage at the same temperature, while E. coli failed to do so within the same period of storage, thereby highlightening another interesting story: "The differences in speed of motility between different species of bacteria with regard to penetration of the egg shell". Those results may lead us to an interesting question: "Are such differences happening only in the egg contents or possibly in other environments as well?"

This phenomena is worth to study in more details.

The results of most experiments showed also continuous growth of the seeded organisms on all the types of media used, but with a gradual decrease in numbers, which is mostly due to factors that play an important role in the destruction of individual microorganisms, like lysozymes that damage bacterial cell walls, and conalbumin, avidin and alovaflavoprotein which inhibit growth of bacteria and fungi<sup>(?)</sup>.

In the second experiment, where E. coli, Pr. vulgaris and Ps. aeruginosa were used, the results showed again the importance and role of the cuticle in preventing the bacteria from entering the eggs, inspite of prolongation of storage period to 8 days. None of the bacterial species used was recovered at any sampling



period, with the exception of E. coli which grew (50 colonies) in cultures made from swabs taken from the interior of eggshell, opposite to the externally-contaminated spot. Those eggs were stored for 30 min at room temperature (18-20 °C). Pr. vulgaris and Ps. aeruginosa were never recovered, in spite of the fact that Ps. aeruginosa has the ability of lysing and digesting the protein present in 90% of the cuticle contents, thus enabling it to penetrate easily through shell<sup>(11)</sup>.

These experiments were followed by other experiments, concentrating mainly on changes anticipated in egg spoilage after prolonged storage conditions and variable temperatures, in addition to the use of other pathogenic bacteria.

#### ACKNOWLEDGEMENT

The author would like to express his deepest regards and appreciation to Miss Iklas Sabry M'hawas for her beneficial and serious help throughout the research.

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دراسات حول اختراق وفعالية بعض الجراثيم المرضية داخل بيض المائدة: (١) متابعة بقاء جراثيم *E. coli* ، *Pseud. aeruginosa* و *Prot. vulgaris* حية داخل البيض

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### الخلاصة

اجريت بعض التجارب المختبرية لغرض دراسة الظروف المحتملة التي قد تصاحب بعض انواع الجراثيم المرضية التي قد تخترق بيض المائدة الذي يتعرض للتلوث بصورة طبيعية او تجريبية .

في المرحلة الاولى، تم زرع محتويات (الايومين + المح) ٢٨ بيضة بصورة مباشرة على اوساط زرعية صلبة شملت: وسط نقيع القلب والدماغ (BHI)، وسط برليانت كرين الحاوي على السلغا (DGS)، وسط المكونكي (MA)، وسط المانيتول الملحي (MSA) ووسط الدم الحاوي على التلورايت (TBA) ووسط الدم الحاوي على ٥% كريات دم اغنام، هذا اضافة الى وسط مرق السلينات (SB). من تلك النماذج المزروعة عددها (٢٨) تم عزل وتشخيص ٨ عزلات بكتيرية كانت ٤ منها:

*Staphylococcus aureus* واثنان لكل من: *Bacillus subtilis* و *Proteus vulgaris*.

في المرحلة الثانية، استخدمت ٢٨ بيضة اخرى جرى تلويثها مخبريا باستخدام جرعات محسوبة لكل من جراثيم *E. coli* و *Pr. vulgaris* و *Ps. aeruginosa* حيث كانت تلك الجراثيم معزولة املا من حالات مرضية مختلفة في الاطفال. لقد تم تلويث كل بيضة بوضع قطرات من عالق البكتريا المعنية على مناطق محددة على سطح البيضة. اظهرت النتائج الاولى اختراق ناجح لكافة الجراثيم المستخدمة الى داخل البيض بعد ١٠ و ١٥ و ٣٠ دقيقة وكذلك بعد ساعتان واربع ساعات من الخزن بدرجة حرارة الغرفة (١٢-١٨° مئوية) على الرغم من ان اعدادها كانت تتناقص بشكل سريع، وخاصة داخل محتويات البيض، بحيث لم يمكن عزلها من نماذج البيض المخزون لفترة ٨ ايام.