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CONTAMINATION OF THE SHELL AND INTERNAL CONTENT OF TABLE EGGS WITH SOME PATHOGENS DURING DIFFERENT STORAGE PERIODS

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ABSTRACT

Storage of table eggs in refrigeration is a popular habit but it may be lead to different degree of contamination with pathogens and increase the risks of illness in humans. A total of 75 chicken table eggs (grades II) were collected from 5 farms (15 of each) in Alexandria province and which were stored in refrigerator at 5 °C. The collected eggs were divided into three groups (25 of each): 1st group at zero time of storage (at laying day), 2nd group after one week of storage and 3rd group after two weeks of storage. They were analyzed for bacterial and fungal contamination on their shells and internal contents. The results of the current study indicated that the egg shells had the highest while the internal contents had the lowest load of both bacterial and fungal contamination. The isolated bacterial species were identified into *E.coli*, *Enterobacter spp.*, *Enterococcus spp*., *Klebsiella spp.*, *Citrobacter*, *Pseudomonas spp*. and *S. aureus*, while, the isolated moulds species were *Aspergillusspp.*, *Penicillum spp.*, *Cladosporum spp.*, *Fusarium*, *Rhiopus spp.* and *Mucor spp*.

***Key words**: Egg shells, Egg contents, pathogens, storage.*

INTRODUCTION

Egg considered as proteinous food, eggs contain every vitamins and minerals needed by human beings except vitamin C (Mehas and Rodgers, 1994). Fully mixed egg contains about 65% water, 12% proteins and 11% fat (Jay *et al.*, 2005). The availability, modest cost, ease of preparation, popular taste appeal and low caloric value give eggs a deserved place in the diets especially in children diet (Layman and Rodriguez, 2009). Some people take raw eggs as a way of enhancing blood-building process and this is very common among malnourished and anaemic patents (Obi andIgbokwe, 2007). At the same time, the many nutrient substances present in eggs create an excellent environment for the development of bacterial microflora, including pathogenic bacteria (Stępień, 2010).

Fresh egg has three structures, which are an outer waxy shell membrane, the shell and the inner shell membrane and each is effective to some degree of retarding the entry of microorganisms (Jay *et al.*, 2005). In spite of these protective barriers against microbial flora contamination of eggs before laying transovarian and after laying with a variety of organisms from different sources exists, through the vent, from nesting material, floor liter, avian fecal matter, improper handling, washing, the type of detergent used, temperature and pH of the washing solution, storage under very humid condition and inadequate sanitizing of equipment (Kinner and Moats, 1981).

Proper storage of eggs maintains the quality, however, both physical and chemical changes occur as eggs deteriorate. Physically, egg white becomes less viscous and more watery, water from the egg white moves into the yolk thereby making it thinner. Evaporation of water take splace through the shell and CO_2 escapes causing an increase in the pH of the content due to this the protein begins breakdown and other changes occur too (Obi and Igbokwe, 2007).

Owing to poor storage conditions of fresh poultry eggs, more complex spoilage are usually associated with freshly and poorly stored eggs. The relatively high humidity could have contributed to the high microbial growth. The isolated microbes could cause severe health problems like, diarrhea, nausea and abdominal pain, since they are pathenogenic (Adday *et al.*, 2009).

Good egg shell quality is necessary for economical viability of the worldwide egg industry (Roberts, 2004). Bad egg shell quality possibly means injuring of egg shell cause contamination of egg with microorganisms which may lead to spoilage consequently economic losses or perhaps transmission of pathogens inducing cases of food born infection or intoxication to consumers. The major bulk of food born outbreaks is caused by microorganisms that have the capacity to reproduce in food. Food born disease is a public health concern all over the world and can lead to chronic illness and death for the individual (Garbutt, 1997; California Egg Commission, 1999 and Kaneko *et al.*, 1999).

The microflora of the eggshell is dominated by Gram positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defenses of the egg content (De Reu *et al.,* 2008). Bacteria on egg shells have been implicated as a source of bacterial contamination of broken out eggs (Moats, 1980). Poor treatment of fresh egg results in the movement of bacteria into the shell leading to the rotting of egg. Several kinds of bacterial rotting of eggs include green rots (*Pseudomonas* and *Acinetobacter* sp.), black rot (*Proteus, Salmonella* and *Aeromonas spp*.), pink rots (*Pseudomonas* sp.), red spot (*Serratia spp.),* custard rots (*Proteus vulgaris* and *Pseudomonas intermedium*). Several spoilage rots have been associated also with moulds like *Penicillium* and *Cladosporium spp*. A part from the spoilage organisms, several pathogens have been isolated from domestic fowl eggs. These include *Salmonella* and *Escherichia spp*. (Jones *et al.,* 1991).

Fungi comprise a large group of microorganisms, which are ubiquitous in nature. The growth of fungi in food is regarded as an indicator for the presence of mycotoxins (fungal toxic metabolites) leading to a food borne mycotoxicosis (Hassan *et al.,* 1997). The penetration of fungi into eggs leads to spoilage of its as well as some species were incriminated in public health hazard (Ray, 2001).

Aspergillus species may induce pulmonary aspergillosis, pulmonary allergy, skin infection, nasal infection, as well as, nail and external ear infections (external otitis) while, Mucorand Rhizopus species are frequent contaminants of foods. These members may involve the rhino facial-cranial area, the lungs, gastrointestinal tract, skin and possibly other organ systems, as well as, they can induce intra-ocular infection, external otomycosis, orbital cellulitis and deep wound infection (Washington, 1981). Aflatoxin could occur as a natural contaminant of poultry feed (Edds and Bortell, 1983) and the egg may contain aflatoxins due to the chronic exposure of birds to these chemicals via contaminated feed (Jones *et al.,* 1982).

Owing to the continuous consumers demand for fresh egg it is extremely necessary to safe guard consumers against health hazard as using biosecurity in farms. This study was a trail to evaluate the microbial status of chicken table eggs by declaring the microbial contamination on both egg shells and their contents in accordance to their storage periods.

MATERIALS and METHODS

Collection of samples:
A total of 75 chicken table eggs were collected from 5 farms (15 of each) grades II according to the degree of quality reported in E.O.S.Q.C. (2007) No.3169 in Alexandria province during the period from October to November 2014.Collected samples packed each in a sterile plastic bag and were transferred directly to the laboratory for microbiological examination where they divided into 3 groups (25 of each). 1st group of eggs were examined directly while other two groups stored in refrigerator at 5°C until analyzed.

1- Preparation of samples for microbiological examination:
There were three groups of the examined samples: 1st group was examined at zero time of storage (at laying day), 2nd group was examined after one week of storage and 3rd group was examined after two weeks of storage in refrigerator at 5°C.

1.1. Eggshells: Egg shells were examined by surface rinse method as described by (Moats, 1979).

1.2. Egg contents: Eggs were prepared for examination by evacuation of their contents according to (Bailey and Scott, 1998).

2- Microbiological examination:
2.1. Determination of total aerobic plate count according to (APHA, 2001).
2.2. Isolation and identification of aerobic bacteria were carried out according to (Cheesebrough, 2003).
2.3. Determination of total mold count according to (ISO 21527/1, 2009).
2.4. Isolation and identification of mold according to (Koneman and Robert, 1985).

RESULTS

Table 1: Statistical analytical results of total aerobic plate count (CFU/shell or g) in the examined samples of eggshells and egg content during storage periods.
(N = 25 eggs / each group).

Criteria	1st group	2nd group	3rd group
	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Eggshells	$1.3\times10^5 \pm 1.2\times10^5$	$7.1\times10^4 \pm 3.3\times10^4$	$9.4\times10^3 \pm 2.3\times10^3$
Egg contents	$7.9\times10^2 \pm 2.6\times10^2$	$4.2\times10^2 \pm 1.9\times10^2$	$3.5\times10^2 \pm 1.3\times10^2$

1st group =at zero time of storage 2nd group= after one week of storage 3rd group= after two weeks of storage

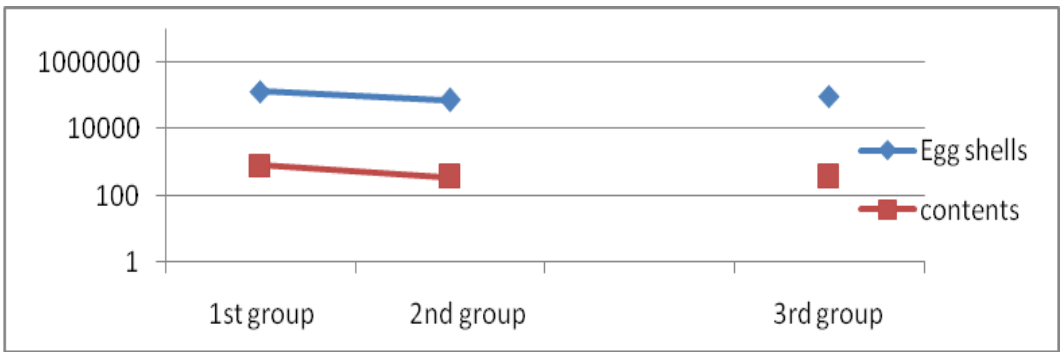

Figure 1: Mean of total aerobic plate count (CFU/shell or g) in the examined samples of Egg shells and egg content during storage periods.

Table 2: Incidence of aerobic bacteria isolated from examined samples of Eggs hells and egg content during storage periods (N = 25 eggs / each group).

Bacterial isolates	1st group				2nd group				3rd group			
	Egg shells		Egg content		Egg shells		Egg content		Egg shells		Egg content	
	No	%	No	%	No	%	No	%	No	%	No	%
E.coli	18	29	6	37.5	12	25	6	37.5	8	20.4	3	30
Enterobacter spp.	8	13	1	6	6	12	2	12.5	5	12.8	2	20
Enterococcus spp.	10	16	2	12.5	9	18	2	12.5	6	15	2	20
Klebsiella spp.	6	9.6	2	12.5	4	8	1	6	3	8	1	10
Citrobacter	4	7	0	0	2	4	0	0	2	5	0	0
Pseudomonas spp.	9	14.4	2	12.5	10	21	2	12.5	10	26	1	10
S. aureus	7	11	3	19	6	12	3	19	5	12.8	1	10
Total No. of isolates	62	100	16	100	47	100	16	100	39	100	10	100

1st group =at zero time of storage 2nd group= after one week of storage 3rd group= after two week storage

Table 3: Statistical analytical results of total mould count (CFU/shell or g) in the examined samples of eggshells and egg content during storage periods.

Criteria	1st group	2nd group	3rdgroup
	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Egg shells	$2.1\times10^2 \pm 0.2\times10^2$	$2.3\times10^2 \pm 0.3\times10^2$	$2.8\times10^2 \pm 0.42\times10^2$
Egg contents	1.3×10± 0.1×10	1.7×10± 0.5×10	1.9×10± 0.3×10

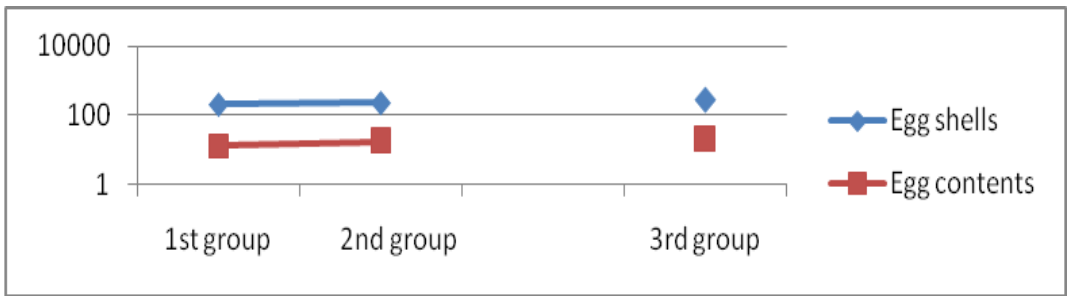

Figure 2: Mean of total mould count (CFU/shell or g) in the examined samples of Egg shells and egg content during storage periods.

Table 4: Incidence of mould spp. isolated from examined samples of Eggshells and egg content during storage periods (N = 25 eggs / each group).

Isolated spp.	1st group				2nd group				3rd group			
	Egg shells		Egg contents		Egg shells		Egg contents		Egg shells		Egg contents	
	No	%	No	%	No	%	No	%	No	%	No	%
Aspergillus spp.	11	31.5	3	37.5	12	31.6	4	44.4	12	29.3	4	33.3
Cladosprium spp.	5	14.3	2	25	6	15.9	2	22.2	6	14.6	2	16.7
Fusarium	1	2.8	0	0	0	0	0	0	0	0	0	0
Mucor spp.	6	17.2	1	12.5	6	15.9	1	11.1	7	17	2	16.7
Penecillium spp.	8	22.8	2	25	9	23.7	2	22.2	11	26.8	3	25
Rhiopus spp.	4	11.5	0	0	5	13.6	0	0	5	12.2	1	8.3
Total No. of isolates	35	100	8	100	38	100	9	100	41	100	12	100

1st group =at zero time of storage 2nd group= after one week of storage 3rd group= after two week storage

DISCUSSION

The presented results in Table 1 and Figure 1 showed the mean values of total aerobic plate count (CFU/g) in the examined samples of egg shells and egg content during storage periods which indicated that the shell having the highest while the internal contents had the lowest load of bacterial count. Higher finding was recorded by El-Leboudy *et al*. (2011). Lower result was reported by El-Kholy *et al*. (1991) but similar to that reported by El-Prince (1988) for total bacterial count of egg shell. The total bacterial counts of the examined egg contents samples were higher than obtained by Ahmed *et al*. (1987) while, nearly similar counts were indicated by Abdel Hady and Emara (1997).

E.O.S.Q.C. (2007) reported that the total aerobic plate count in the fresh table egg must be not more than 25×10^3CFU/g.

The high bacterial count recorded in egg contents of examined samples, is attributed to the bad hygienic measures during production and handling (Board, 1977). At time of laying, the eggs are sterile due to natural chemicals and physical defenses against microbial infection. But on exposure to environmental conditions as temperature, length of storage and dirt in the nest, eggs were contaminated by different types of microorganisms which cause spoilage and public health problems (Kinner and Moats 1981 and Board and Tranter, 1995). De Reu *et al*. (2005a) found a ppositive correlation between the concentration of bacteria in the air of the poultry house and the initial egg shell contamination regarding to total aerobic count, they also showed that floor eggs have a high bacterial load compared to eggs laid in nest and that the egg conveyor belt is a key point for contamination of accumulated eggs. Another study from De Reu *et al*. (2005b) reported that type of housing system can affect bacterial contamination. The wide range of bacterial number on shells may be due to variation in methods of production, handling and storage (Board *et al.*, 1964 and Kraft *et al*., 1967). Housing hens in cages with manure removal belts results in lower bacterial load for both washed and unwashed eggs. High levels of external shell contamination can adversely affect the shelf life and food safety of eggs (Hannah *et al.*, 2011). Increasing numbers of microorganisms on the egg shell consequently increase the risk of microbial egg shell penetration and egg content contamination (De Reu *et al.*, 2006a; Messens *et al.*, 2007). Several factors have been implicated in egg contamination. Among these are faeces of the birds, litter material, egg crates, packing and storage. Others are cloths and hands of poultry workers, dust, the environment, weather conditions, transporting and marketing (Osei-Somuah *et al.*, 2003). The bad storage of eggs under very humid conditions could support the multiplication of these contaminating microorganisms present one ggshell. Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdraw althrough pores of the shells (Neamatallah *et al.*, 2009). Cracked eggs increase the probability of contamination inside the egg (Todd, 1996). This movement from the shell to the yolk was probably due to a fall in the pressure as air escaped through the shell (Obi and Igbokwe, 2007).

Table 2 showed that the incidence of aerobic bacteria isolated from examined samples of egg shells and egg content during storage periods after bacteriological

examination and revealed the isolation of seven bacterial species were identified into *E.coli*, *Enterobacter spp.*, *Enterococcus spp*., *Klebsiella spp.*, *Citrobacter*, *Pseudomonas spp*. and *S. aureus*. These findings similar with those reported by Adesiyun *et al.* (2005 and 2006). (E.O.S.Q.C., 2007) reported that the fresh table egg must be free from *Salmonella spp.* Failure to isolate *Salmonella spp*. in table eggs in the current study may owed to strict control measures applied against these bacteria. Similarly, *Salmonella* was absent in all samples analyzed by Favier *et al.* (2000) and Anon (2004). Other studies reported variable and very low incidence of *Salmonella* in eggs. Begum *et al.* (2010) only could isolate three *Salmonella* strains out of 1100 domestic eggs. Musgrove *et al.* (2005) identified one out of 105 tested samples of egg shells. This variability in *Salmonella* occurrence may be due to sample size, timing of sampling, sites of the egg that were tested, techniques used, investigations of eggs laid by artificially or naturally infected hens (Humphrey, 1994). Storing shell eggs, whether temporarily refrigerated or not, for 9 day or more, resulted in a decrease in bacterial egg shell contamination for both bacterial variables (De Reu *et al.*, 2006b).

The most frequently isolated *Enterobacteriaceae* bacteria on egg shells surfaces were *E. coli*, *Enterobacter spp*. and *Klebsiella spp.* Other Gram-negative bacteria, such as *E. coli, Enterobacter spp., Citrobacter spp., Klebsiella spp., Alcaligenes spp., Aeromonas spp*. and *Pseudomonas spp*. which all have been isolated from whole or cracked eggs with a potential to cause spoilage and enter the food chain through table eggs causing infection in consumers (Musgrove *et al.,* 2004; 2008 and Stępień-Pyśniak, 2010). Examining egg for the presence of members of the family enterobacteriaceae instead for *coliforms* may give a better indication of the likelihood of their presence, as well as providing more accurate information about the handling and storage of the food commodity (Roberts *et al.,* 1995).

Another bacterium infecting food through contact with manure is *Escherichia coli*. This bacterium is found in the normal gut flora in humans and animals and have been isolated from table eggs and their contents (Hope *et al.,* 2002 and Adesiyun *et al.,* 2005). However, there are some strains such as EHEC (0157:H7) which are pathogenic for humans (Garbutt, 1997). *Esherichia coli* population can be used as measures of quality and sanitary processing condition (Kornacki and Johnson, 2001 and Ricke *et al.,* 2001). Among the common contaminant organisms pathogenic to human beings are *Staphylococcus spp*. (Osei-Somuah *et al.,* 2003). *Staphylococcus aureus* has been shown to grow at temperatures as low as 7 °C, but the lower limit for enterotoxin production has been shown to be10 °C (ICMSF, 1980).

From the data presented in Table 3 and Figure 2 it is evident that the mean values of total mould count (CFU/g) in the examined samples of egg shells and egg content during different storage periods indicated that the shell having the highest and the internal contents the lowest load of mould count. Higher results in egg shells were estimated by Ahmed *et al.* (2002); Suba *et al.* (2005) and Salem *et al.* (2009). Other studies indicated lower count in eggs shell samples reported by Ahmed *et al.* (1987); El-Essawy *et al.* (1989). E.O.S.Q.C. (2007) reported that the total mould and yeast count in the fresh table egg not more than 50 (CFU /g).

Jones *et al.* (2004) found fungal contamination of egg shell in the day of egg collections and in the content of unwashed shell eggs. The pathogenic moulds found their way to penetrate and contaminate eggs and may produce their toxin sunder favorable conditions. Therefore, special attention should be directed to safeguard the eggs against their contamination through application of correct farm hygiene programs, good handling and storage methods, as well as, the periodical examination of eggs and poultry feed (Neamatallah *et al.,* 2009).

The presence of the bacterial and fungal isolates in the albumen and yolk of one day old eggs could be due to contamination in the oviduct of the hen with the chicken droppings or contaminated poultry feeds (Obi and Igbokwe, 2007).

On the other side,Table 4 showed the incidence of mould spp. isolated from examined samples of egg shells and egg content during different storage periods. The mycological examination carried out in the current work revealed the isolation of named six genera, *Aspergillus spp., Penicillum spp., Cladosporum spp., Fusarium, Rhizopus spp. and Mucor spp*. which agree with that obtained by El-Essawy *et al*. (1989); Obi and Igbokwe (2007) and Salem *et al.* (2009). Most of the isolated genera have been detected by Ahmed *et al.* (1974), Moursy *et al.* (1982); Torkey (1982); Amer (1990) and Neamatallah *et al.* (2009). The presence of *Aspergillus* in egg samples from the poultry farms indicated the use of contaminated poultry feeds or poultry feeds raw materials or general low hygienic margins in these farms. Occurrence of *Aspergillus* is a threat to health due to the production of aflatoxins that have been found to be carcinogenic, teratogenic and mutagenic in humans and birds.

The presence of the spores of these fungi on and in the eggs could lead to several respiratory diseases like coccidioidomycosis, blastomycosis and histoblastomycosis when the fungal spores are inhaled by the humans and the birds (Obi and Igbokwe, 2007). From the public health point of view, certain strains of moulds were implicated in

food poisoning outbreaks due to production of aflatoxins, as well as some moulds, are capable of forming toxins that cause mycotoxicosis in man leukemia (Ray, 2001).

Result showed the presence of pathogenic microbes in the samples examined and it was concluded that chicken table eggs should not be consumed raw. Thus, the consumption of 7-21 days old eggs without proper cooking increases the probability of occurrence of health problems (Obi and Igbokwe, 2007). Refrigeration is effective for extending the shelf-life of table egg by retarding bacterial growth.

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تلوث القشرة الخارجية والمحتوى الداخلى لبيض المائدة ببعض مسببات الأمراض خلال فترات التخزين المختلفة

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تخزين بيض المائدة بالتبريد هو عادة لدى الكثير من الناس ولكن يمكن أن يؤدي إلى اختلاف درجة التلوث بمسببات الأمراض ويزيد من المخاطر الصحية. فى هذه الدراسة تم تجميع 75 بيض المائدة من 5 مزارع للدواجن (15 لكل منهم) في محافظة الإسكندرية، وقد تم التخزين في الثلاجة في درجة حرارة ٥ درجة مئوية. لذا كانت هناك ثلاث مجموعات من العينات المفحوصة (٢٥ لكل مجموعة): المجموعة الاولى في وقت الصفر من التخزين (بيض طازج)، المجموعة الثانية بعد أسبوع واحد من التخزين والمجموعة الثالثة بعد اسبوعين من التخزين. تم فحص القشرة الخارجية ومحتويات البيض الداخلية من حيث التلوث البكتيرى والفطري. وأشارت نتائج الدراسة إلى ان القشرة الخارجية كانت الأعلى فى التلوث بينما محتويات البيض الداخلية الأدنى من حيث التلوث البكتيرى والفطري . وقد تم التعرف على أنواع البكتيريا وجد انها تنتمى الى العترات الاتية: الميكروب القولونى ، الانتيروباكتر، المكورات المعوية ، الكلبسيلا ، الستروباكتر ،السودموناس والمكور العنقودى الذهبى ، بينما الفطريات المعزولة كانت الاسبرجلس ، الكلادوسبوريوم، الفيوزيريوم ،الميوكر، البنسيللين والريزوبس بنسب مختلفة ، هذا وقد تمت مناقشة الخطورة الصحية لهذه المعزولات مع بيان المصادر المختلفة للتلوث بتلك الملوثات.