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Microbial Hazards Associated with Consumption of Table Eggs

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Key words:
Balady eggs, Farms eggs, Duck eggs, Egg contents, Salmonella *Staphylococcus aureus* and *Coliforms.*

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ABSTRACT

Egg is considered as a nutritionally complete food and an excellent source of protein. Contamination of eggs with microorganisms that may affect eggs quality and transmit pathogens or intoxication to consumers, causing public health hazards. This study was designed to determine the microbiological status of balady, farms hens' eggs and ducks' eggs with isolation of some pathogens that cause public health hazard. A total of 75 balady, farms hens' eggs and ducks' eggs samples (25 eggs of each) were randomly collected from different farmers and groceries in Alexandria province, Egypt. Eggs contents were microbiologically examined for total bacterial count, *Staphylococcus aureus*, coliforms count and molds count as well as for presence of Salmonellae. The obtained results were revealed that the mean values of Aerobic plate count, *Staphylococcus aureus* count, coliforms count and molds count in balady hens' eggs content were $1.03\times10^2\pm0.4\times10^2$, $4.0\times10^2\pm1.08\times10^2$, 0 and $4.3\times10\pm0.9\times10$ cfu/ml, respectively. As well as, the mean populations of total bacterial count, *Staphylococcus aureus*, *Coliforms* count and molds count in farms hens' eggs content were $1.0\times10\pm0.0$, $4.8\times10^2\pm1.4\times10^2$, $0.4\times10^2\pm0.34\times10^2$ and $2.0\times10\pm0.4\times10$ cfu/ml, respectively; while, the mean values of ducks' eggs content were $1.6\times10^3\pm0.27\times10^3$, $5.6\times10^2\pm1.7\times10^2$, $1.3\times10^2\pm0.57\times10^2$ and $1.2\times10\pm0.2\times10$ cfu/ml, respectively. Salmonellae were not isolated from farms hens' eggs content while, they were isolated from balady hens' eggs content and ducks' eggs content with the incidences of 4 and 8%, respectively. Isolated Coliforms were identified into Citrobacter spp., Enterobacter spp., *Escherichia coli* and Klebsiella spp., while, the isolated molds species were Alternaria spp., Aspergillus spp., Cladosporium spp., Mucor spp., Penicillum spp., Syncephalastrum racemosum and Trichoderma spp. It is concluded that eggs sold in Alexandria city were of relatively good quality, although occurrence of some pathogenic microorganisms. Therefore, it is recommended that hens' and duks' eggs should not be consumed raw.

1. INTRODUCTION

As an egg is originally designed to create a chick, it has a complete life support system with many natural, built-in barriers to prevent bacterial entrance and growth, protecting the developing embryo. These barriers protect, as well, the egg on its way from the hen to the consumers (Neamatallah et al., 2009). Most eggs (about 90%) have been found to be sterile when laid, but they have the potential to become occasionally contaminated (Egg Safety Center, 2010). Unfortunately, egg is also an ideal source of nutrients for proliferation of both spoilage and pathogenic contaminating microorganisms. 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 trans-ovarian and after laying with a variety of organisms from different sources exists, through the vent, from nesting material, soil, dust, 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 (Al-Bahry et al., 2012).

Egg can be contaminated at both egg shell and egg contents through a variety of microbes with a wide range of pathogens especially Escherichia coli, salmonellae, fungi, and Staphylococci which considered as a most common bacteria contaminating egg shells (Ricke et al., 2001).

Staphylococci secrete thermostable enterotoxins elaborated in large numbers of foods and animal products including eggs causing rapid onset of nausea, vomiting, abdominal pain, cramps and diarrhea within 6 hours of ingestion of food. Several outbreaks of Staphylococcus aureus food poisoning have been recorded (EFSA, 2014).

Coliforms count is the traditional indicator of possible fecal contamination, microbial quality, wholesomeness and reflect the hygienic standards adopted in the food operation. Consequently, such eggs with high coliforms constitute an economic and public health importance. Escherichia coli are normal inhabitants of intestinal tracts of birds and human. Escherichia coli can bring about urinary tracts infections, pneumonia meningitis and peritonitis in humans (Musgrove et al., 2008).

Salmonella enteritidis and Salmonella typhimurium are the most frequent Salmonella serotypes found inside shell eggs that caused food poisoning. Also, the organism causes Human Salmonellosis which may range from gastroenteritis to typhoid. (Tan et al., 2012).

Because of the continuous consumer demands worldwide for eggs, periodical assessment is required to offer safe and good quality eggs for consumption. Therefore, the study was done as a trial to evaluate the microbial status and quality of both hens' egg and duck's egg.

2. MATERIALS AND METHODS

2. 1. Sampling:

A total of 75 random samples of edible eggs were collected and represented as 50 samples hens' egg (balady and farm egg, 25 of each) and 25 samples of ducks' egg. Eggs samples were collected from different groceries, supermarkets and street-vendors' farmers in Alexandria city, Egypt from January 2017 to August 2017.

2. 2. Microbiological evaluation of examined table eggs:

2. 2.1. Preparation of eggs contents for microbiological evaluation:

Each collected egg was prepared for evacuation according to the method applied by (Himathongkham et al., 1999). The egg was aseptically swabbed with 70% ethyl alcohol. The blunt end of the shell was flamed and alcohol was then burned off. The blunt end was broken with a sterile forceps or scissors. A pair of sterile forceps were used to gently remove loose pieces of shell from the internal membranes. Membranes were sprayed lightly with 70% ethanol before being completely torn off. Egg contents were pipetted, poured into a sterile jar and homogenized. The magma was blended well manually and subjected for microbiological examination.

2.2.2. Preparation of serial dilutions: **(APHA, 2001)**One ml from the homogenous egg content was added separately to 9 ml of sterile distilled water and thoroughly mixed to make a dilution of 1/10, from which decimal serial dilutions were prepared for:

2. 2.3. Aerobic plate count: (APHA, 2001).

2. 2.4. Staphylococcus aureus count: (ISO, 2003b; Cader et al., 2014).

2. 2.5. Coliforms count (cfu/ml): (APHA, 1985).

2. 2.6. Isolation and Identification of Salmonellae: (Downes and Ito, 2001).

2.2.7. Enumeration of molds: (ISO 21527/1, 2009).

3. RESULTS AND DISCUSSION

Results obtained in Table (1) recorded that, the average values of Aerobic plate count in balady, farm hen egg content and duck egg content were $1.03\times10^2\pm0.4\times10^2$, $1.0\times10\pm0.0$ and $1.6\times10^3\pm0.27\times10^3$ cfu/ml with minimum values of 5.0×10, 1.0×10 and 1.0×10 cfu/ml and maximum values of 1.8×10^2, 1.0×10 and 3.0×10^3 cfu/ml, respectively. The respective incidences of aerobic plate count in the examined egg samples were 12, 8 and 48%. There is a significant difference (at $P < 0.05$) between hens' and ducks' egg content samples in aerobic plate count. The obtained results showed that 100% of examined entire contents of balady, farm hen egg content and duck egg content complied with Egyptian standards with a total bacterial count within the permissible count (25000/g) stated by the (Egyptian Organization for Standardization and Quality Control, 2007) (Table 8).

Aerobic plate count is a prime consideration in examination of food. It reflects the sanitary measures adopted during production, handling and storage

(ICMSF, 2009). Contamination of hens' egg contents can occur in a number of ways. Prior to being laid, chicken eggs may become vertically infected (congenitally infection). Generally, these bacteria migrate from infected organs of the hen (Al-Bahry et al., 2012).

After deposition, eggs may also come into contact with environmental bacteria which may due to temperature, soil, length of storage, dirty nesting materials, cloths, hands of poultry workers, dust, the environment, weather conditions, transporting and marketing (Osei-Somuah et al., 2003). This resulting in eventual penetration of the shell (Al-Bahry et al., 2012). Entrance of microorganisms into egg content may be either by penetration or withdrawal through pores of the shells (Neamatallah et al., 2009).

Table (1) Aerobic plate count of balady, farm hens' and duck egg content (cfu/ml).

Source of egg	No. Of examined samples	Egg Content				
		Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Balady hens' egg	25	3	12	5.0×10	1.8×10^2	$1.03\times10^2 \pm 0.4\times10^2$ (b)
Farm hens' egg	25	2	8	1.0×10	1.0×10	$1.0\times10 \pm 0.0$ (c)
Duck egg	25	12	48	1.0×10	3.0×10^3	$1.6\times10^3 \pm 0.27\times10^3$ (a)

Means with different letters are significantly difference (at $P<0.05$).

Table (2) Staphylococcus aureus count of balady, farm hen and duck egg content (cfu/ml).

Source of egg	No. Of examined samples	Egg Content				
		Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Balady hens' egg	25	4	16	2.0×10^2	7.0×10^2	$4.0\times10^2 \pm 1.08\times10^2$ (b)
Farm hens' egg	25	13	52	1.0×10^2	2.4×10^3	$4.8\times10^2 \pm 1.4\times10^2$ (b)
Duck egg	25	12	48	1.0×10^2	2.1×10^3	$5.6\times10^2 \pm 1.7\times10^2$ (a)

Table (3). Coliforms count of balady, farm hen and duck egg content (cfu/ml).

Source of egg	No. Of examined samples	Egg Content				
		Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Balady hens' egg	25	4	16	2.0×10^2	7.0×10^2	$4.0\times10^2 \pm 1.08\times10^2$ (b)
Farm hens' egg	25	13	52	1.0×10^2	2.4×10^3	$4.8\times10^2 \pm 1.4\times10^2$ (b)
Duck egg	25	12	48	1.0×10^2	2.1×10^3	$5.6\times10^2 \pm 1.7\times10^2$ (a)

Table (4): Frequency distribution of Coliforms isolated from examined egg contents samples.

Coliforms Spp.	Balady egg content		Farm egg content		Duck egg content	
	Frequency	%	Frequency	%	Frequency	%
Citrobacter freundii	0	-	0	0	2	13.33
Citrobacter diversus	0	-	0	0	1	6.67
Enterobacter cloacae	0	-	2	20	0	0
Enterobacter aerogenes	0	-	1	10	2	13.33
Escherichia coli	0	-	2	30	3	20
Klebsiella oxytoca	0	-	3	30	4	26.67
Klebsiella pneumoniae	0	-	1	10	2	13.33
Klebsiella ozaenae	0	-	1	10	1	6.67
Total	0	0	10	100	15	100

Table (5): Incidence of Salmonellae in content of balady, farm hen eggs and duck eggs.

Source of egg	Positive samples	
	No.	%
Balady hens' egg	1	4
Farm hens' egg	0	0
Duck egg	2	8

Table (6): Mold count of balady, farm hen egg and duck egg content (cfu/ml).

Source of egg	No. Of examined samples	Egg Content				
		Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Balady hens' egg	25	7	28	1.0×10	1.4×102	4.3×10 ± 0.9×10 (a)
Farm hens' egg	25	6	24	1.0×10	6.0×10	2.0×10 ± 0.4×10 (b)
Duck egg	25	5	20	1.0×10	2.0×10	1.2×10 ± 0.2×10 (c)

Table (7): Frequency distribution of molds isolated from egg shell, content samples of different eggs.

Mold Spp.	Balady egg content		Farm egg content		Duck content	
	Frequency	%	Frequency	%	Frequency	%
Alternaria alternaria	2	13.34	3	18.75	4	20
Aspergillus flavus	1	6.66	1	6.25	2	10
Aspergillus fumigatus	-	-	-	-	3	15
Aspergillus niger	4	26.66	4	25	2	10
Cladosporium spp.	2	13.34	2	12.50	-	-
Mucor spp.	1	6.66	1	6.25	1	5
Penicillium spp.	5	33.34	2	12.50	6	30
Syncephalastrum racemosum	-	-	-	-	2	10
Trichoderma spp.	-	-	3	18.75	-	-
Total	15	100	16	100	20	100

Table (8) Comparison between the results obtained from microbiological evaluation for examined egg content samples and Egyptian standards (E.O.S.Q.C.) (3169/2007).

Parameter	Egyptian standards (E.O.S.Q.C.) (3169/2007)	Balady egg content		Farm egg content		Duck content	
		Samples failed to conform with Egyptian standards		Samples failed to conform with Egyptian standards		Samples failed to comply with Egyptian standards	
		No.	%	No.	%	No.	%
Aerobic plate count	25000/g	0	0	0	0	0	0
Staphylococcus aureus	Nil	4	16	13	52	12	48
Salmonellae	Nil	1	4	0	0	2	8
Mold count	50 cell/g	4	16	1	4	0	0

The results in Table (2) declared that the mean values of Staphylococcus aureus count of content for balady, farm hen egg content and duck egg content were $4.0\times10^2\pm1.08\times10^2$, $4.8\times10^2\pm1.4\times10^2$ and $5.6\times10^2\pm1.7\times10^2$ cfu/ml with minimum values of 2.0×10^2, 1.0×10^2 and 1.0×10^2 cfu/ml and maximum values of 7.0×10^2, 2.4×10^3 and 2.1×10^3 cfu/ml, respectively. The respective incidences of Staphylococcus aureus in the examined samples were 16, 52 and 48%. There is a significant difference (at $P < 0.05$) between hens' and ducks' egg content in staphylococcus aureus count. While, there is no significant difference between balady and farm hens' egg. Regarding to the (Egyptian Organization for Standardization and Quality Control, 2007) which stated that Staphylococcus aureus must not be found in egg content (Nil), it was noticed that there were samples that failed to achieve the Egyptian Standard levels with incidences of 16, 52 and 48% of balady, farm hen egg content and duck egg content, respectively (Table 8).

Staphylococcus aureus is common to humans and found in infected cut, pimples, boils, eczema, abscesses, wound infections, noses and throats. It can be transmitted to eggs when handled by a person who has S. aureus infection. Also, eggs' contents may be contaminated accidentally by *Staph. aureus* from shell as it might have originated from ova during egg formation, dust and from the surface of inanimate objects (California Egg Commission, 1999).

Furthermore, among the pathogenic food poisoning organisms that affect the public health of humans due to consumption of eggs, Staphylococcus aureus organisms have a serious concern to public health (Wyah, 1992) causing Staphylococcal food-borne disease (SFD). The organism is also inculpated in septicemia, pneumonia, meningitis, endocarditis, toxic shock syndrome and scalded skin syndrome which is caused by exfoliative toxins secreted on the epidermis and mostly affects neonates and young children (Eisenstein, 2008). Ducks' egg contents have the highest count of staphylococcus aureus when it is compared with balady and farm hens' egg contents.

Data summarized in Table (3) indicated that the mean values of Coliforms count of content for balady, farm hen egg content and duck egg content were 0, $0.4\times10^2\pm0.34\times10^2$ and $1.3\times10^2\pm0.57\times10^2$ cfu/ml with minimum values 0, 1.0×10 and 1.0×10 cfu/ml and maximum values 0, 2.2×10^2 and 7.1×10^3 cfu/ml, respectively. The respective incidences were 0, 24 and 52%. There is a significant difference (at $P < 0.05$) between hens' and ducks' egg content in Coliforms count.

The biochemical identification of coliforms organisms in the examined content samples revealed that Escherichia coli was the most common Table (4). Escherichia coli was isolated by incidences 30 and 20% from farm hen egg content and duck egg content while from examined balady hen egg contents Escherichia coli organisms were failed to be detected.

From the data collected in Table (4), it was noted that all the examined balady hen egg contents were free from Coliforms while Citrobacter, Enterobacter and Klebsiella species were isolated from examined farm hen egg content and examined duck egg content. Citrobacter spp. were identified into Citrobacter freundii and Citrobacter diversus and they were isolated with the incidences of (0 and 0%) and (13.33 and 6.67%) from farm hen egg content and duck egg content, respectively. Enterobacter spp. were identified

into Enterobacter cloacae and Enterobacter aerogenes and they were isolated with the incidences of (20 and 10%) and (0 and 13.33%) from farm hen egg content and duck egg content, respectively. Klebsiella spp. were identified into Klebsiella oxytoca, Klebsiella pneumonia and Klebsiella ozaenae and they were isolated with the incidences of (30, 10 and 10%) and (26.67, 13.33 and 6.67%) from farm hen egg content and duck egg content, respectively.

Coliforms count is the traditional indicator of possible fecal contamination, microbial quality, wholesomeness and reflect the hygienic standards adopted in the food operation. The bacteria most frequently isolated from eggs are Gram-negative bacteria such as E. coli, Enterobacter, and Klebsiella spp. (Musgrove et al., 2008).

Escherichia coli can penetrate the shell contaminating the egg contents (Mayes and Takeballi, 1983). That's why Escherichia coli is the major micro-organism isolated from both the surface and the content of the egg, this may be attributed to the fact that Escherichia coli are normal inhabitants of intestinal tracts of birds and human (Singleton and Sainsburg, 1981). They have also been known to contaminate the surface of egg. The mechanical process can spread the bacteria through the eggs while contaminations with the pathogen in the field occur through improperly decomposed manure and poor hygienic practice of farm workers. Escherichia coli can bring about urinary tracts infections, pneumonia, meningitis, peritonitis and induce profuse watery diarrhea in humans (Schiavoni and Vergora, 2000).

Citrobacter spp. can cause a wide spectrum of infections in humans. Among the various sites of infection, the urinary tract is the most common, followed by the respiratory tract, and skin/soft tissues (Pavani, 2012). Enterobacter spp. were incriminated in urinary tract infection and septicemia, while Klebsiella pneumoniae is a world wide spread bacteria that can be responsible for arthritis, meningitis, appendicitis, cystitis and septicemia outbreaks in kids and newborns, but is more frequent responsible for pneumonia and necrotic damage of the lungs (Bernabe et al., 1998). From previous data it is obvious that, ducks' egg contents have the highest Coliforms count if it is compared with hens' egg contents.

Also, data in Table (5) referred that the number of sampled contents which were positive for presence of Salmonellae was 1, 0 and 2 samples in examined balady, farm hen egg content and duck egg content, respectively (out of twenty-five samples for each). The respective incidences of Salmonellae in egg contents were 4, 0 and 8%. (Egyptian Organization for Standardization and Quality Control, 2007) stated that the fresh table eggs must be free from Salmonellae spp. in their contents. Regarding to the Egyptian standards for Salmonella in egg contents (Nil), the percentages of samples that failed to stratify the standards were 4, 0 and 8% in balady, farm hen egg content and duck egg content, respectively (Table 8).

However, poultry is widely acknowledged to be a reservoir for Salmonellae. Egg contents have been shown to be contaminated with salmonellae by 2 routes: trans-ovarian (vertical transmission from infected reproductive tissues) or trans-shell (horizontal transmission from fecal contamination of the shell and through environmental vectors, such as farmers, pet and rodents). Salmonella may be able to contaminate egg contents by migration through the egg shell and membranes. Such route is facilitated by moist egg shells, storage at ambient temperature and shell damage (Zeidler, 2002).

Moreover, Salmonellae spp. remains a potential threat to human health, as well as, broiler chickens. Avian salmonellosis has public health importance associated with gastroenteritis in man resulting from consumption of infected hens' and ducks' eggs. Human Salmonellosis may range from gastroenteritis to typhoid (Abouzeed et al., 2000). The highest incidence of Salmonella in examined contents is found in duck egg contents while the lowest incidence of Salmonellae is found in farm hen egg contents.

The presented results in Table (6) declared that the mean values of Molds count of content for different eggs were $4.3\times10\pm0.9\times10$, $2.0\times10\pm0.4\times10$ and $1.2\times10\pm0.2\times10$ cfu/ml with minimum values of 1.0×10, 1.0×10 and 1.0×10 cfu/ml and maximum values of 1.4×10^2, 6.0×10 and 2.0×10 cfu/ml. The respective incidences of molds were 28, 24 and 20%. There is a significant difference (at $P < 0.05$) between hens' and ducks' egg content in mold count.

Results in Table (7) indicated that the different isolated Mold species which were identified from different examined egg contents. These species were Alternaria alternaria with incidences of (13.34, 18.75 and 20%), Aspergillus flavus with incidences of (6.66, 6.25 and

10%), Aspergillus fumigatus with incidences of (0, 0 and 15%), Aspergillus niger with incidences of (26.66, 25 and 10%), Cladosporium spp. with incidences of (13.34, 12.50 and 0%), Mucor spp. with incidences of (6.66, 6.25 and 5%) and Penicillium spp. with incidences of (33.34, 12.50 and 30%) from examined balady, farm hen egg content and duck egg content, respectively. Also, from recorded results in Table (7) it was obvious that Syncephalastrum racemosum was isolated from examined duck egg content only with the incidence of 10% while, Trichoderma spp. were isolated from examined farm hen egg content only with the incidence of 18.75%. Egyptian Standards stated that fresh table egg contents must not contain mold count more than 50 cell/g (Egyptian Organization for Standardization and Quality Control, 2007). Regarding to these limits 16, 4 and 0% of sampled egg contents in balady, farm and duck egg, respectively failed to achieve the permissible count (Table 8).

This high charge in egg contents could suggest that the eggs were kept under relatively high humid conditions (Joseph and Babatunde, 2006). When the molds contaminate egg shells the shell pores are penetrated and growth occurs on the shell membrane and it will further break down the yolk membrane. So, mold contamination causes severe losses in egg industry in addition to occurrence of public health hazard (USDA/AMS, 2000).

Penicillium spp. could penetrate into the eggs at different temperatures and produce their toxins which increase with the storage time. Also certain types of molds produce mycotoxins which were implicated in human cases of food poisoning and neoplastic diseases including leukemia and other cancers as liver cancer (Foster et al., 1983). Presence of Aspergillus in the egg contents is of a serious health concern because the organism has the ability to cause disease condition called Aspergillosis, which affects respiratory system. Aspergillus spp. may induce skin infection, nasal infection as well as nail and external ear infections (Washington, 1981). From the obtained results balady hen egg contents have the highest mold counts between different eggs while duck egg contents have the lowest mold counts.

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