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AN ASSESSMENT OF THE MICROBIOLOGICAL RISKS INVOLVED WITH QUAIL EGG QENA CITY, UPPER EGYPT.

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ABSTRACT

Contamination of Japanese quail eggs (Coturnix coturnix) with microorganisms that possibly affect quail eggs quality and pathogen transmission induced food borne infection or intoxication to consumers which cause public health hazards. A total of 150 quail eggs were collected randomly from different quail farms in Qena city (Upper Egypt), every five eggs were represented as one egg sample (n=30). Each egg shells and contents were examined for their microbiological contents. The results showed that average values of aerobic plate, enteroccocci, total coliforms, fecal coliforms, *E.coli*, and total yeast and mold counts / ml of egg shells samples were $1x10^4$, $7 x10^2$, $5 x10^2$, 1 x10, .784 x10 and $9 x10^3$ cells/ ml, respectively. However in egg contents, the corresponding counts were lower than that of egg shells samples ($7 x10^3$, $1 x10^3$, 1.9 x 10, *< 10, - and $6 x10^3$cells/ml). Moreover, *E. coli* was found to be the most prevalent strain recovered from shell but not be detected in contents. The contamination *by S. aureus* were in 12 (40%) and 5(17%) in shells and contents samples, respectively. Also two egg shell samples were Listeria gray positive, and *salmonella* negative. Furthermore the identification of molds revealed that 16(53%) and 8(27%) fungi species belonging to 6 genera were isolated from quail egg shells and contents, respectively. *Penicillium* was the most prevalent genus encounted in egg shells and contents samples comprising 6(20 %) and 4(13%), respectively, of the total fungi.

Key words: *Japanese quail eggs, Microorganisms.*

INTRODUCTION

Today, eggs remain a stable food within the human diet, consumed by people throughout the world. They are consumed worldwide in various dishes and considered very nutritious and a cheap source of protein. Though eggs are considered as complete food for growth and sustenance, studies indicated that microorganisms often contaminate eggs (MAFF, 2000 and Osei-Somuah *et al.,* 2003).

Quail is the smallest avian species farmed for meat and egg production (Panda and Singh, 1990) and their eggs are used in the same manner as those of chicken. Quail eggs taste like chicken eggs but they provide a good alternative for some people who are allergic to chicken eggs (Shanaway, 1994). In addition, because of their small size and attractive appearance, quail eggs are used whole or sliced in salad and casseroles or served boiled or whole with a sauce (Martin *et al.,* 1999).

Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting *materials), eggs become contaminated with different types of microorganisms* (Ellen *et al.,* 2000 and Smith *et al.,* 2000). Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells (Harry, 1963 and Schoeni *et al.,* 1995), and also through the transovarian route (Bruce and Drysdale, 1994). Some other factors such as environmental temperature and humidity influence the bacterial penetration and thus, enhance the infection and spoilage (Theron *et al.,* 2003).

Food-borne diseases caused by microorganisms are a large and growing public health problem. Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce cases of food-borne infection or intoxication to consumers, which constitute public health hazards. Several pathogenic microorganisms have been isolated from the surface of quail egg shells and contents. Among them, Listeria monocytogenes, Yersinia enterocolitica, Escherichia coli O157:H7, Salmonella and Campylobacter were detected (Adesiyun *et al.,* 2005).

Likewise, aflatoxins contaminate a vast array of foods and agriculture commodities, and produced by certain species of fungi. Such mycotoxins pose profound challenges to food safety widespread in many countries, especially in tropic and subtropical regions where temperature and humidity conditions are

optimum for growth of moulds and production of toxins. The possible transmission of such toxic residues to edible eggs results in potential hazards to human health (Martins *et al.,* 1998). Aflatoxin are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (Yaling *et al.,* 2008).

Because of the continuous consumer demands worldwide for eggs, periodical assessment is required to offer safe and good quality eggs for consummation. The present investigation, was, therefore, planned to assess shell and interior quality of consumed eggs at retail levels in Qena city (Upper Egypt). Microbiological quality and presence of food pathogens were investigated.

MATERIALS and METHODS

- Samples collection:

One hundred and fifty quail eggs (30) group (each group consists of 5 eggs) were collected from Qena (Upper Egypt) farms and were tested microbiologically. Preparation of egg shell samples:

- Rinse solution method was carried out as reported by Perales and Audicana (1989).
- Preparation of egg content samples:

The eggs were prepared for evacuation of their contents according to Speck (1976).

- Experimental techniques:

Ten fold serial dilutions up to 10^{-6} were aseptically prepared from the rinse solution of egg shells, as well as, the homogeneous egg contents samples. Then subjected to the following examinations:

1- Aerobic plate count (A.P.H.A., 1992).

2- Enterococci count using KF Streptococcal agar (Deible and Hartman, 1976).

3- Total coliforms, faecal coliforms and E. coli using three tubes Most Probable Number (MPN) technique was employed (FAO, 1992). MPN was streaked on to Eosin Methylene Blue (EMB, OXOID, England). Typical isolates of *E.coli* were confirmed based on their IMVIC pattern according to Koneman *et al.* (2005).

4- Enumeration and isolation of viable yeast and mold using Sabourauds dextrose agar (Mislivec *et al.,* 1992). The isolated fungi were identified according to different guidelines adopted by Raper and Fennell (1965); Pitt and Hocking (1997) and Klich (2002).

5- Isolation and identification of *Staph. aureus*.

- Enrichment procedure, loopful of the incubated broth was streaked into plates of selective media" Baired Parker agar" (Finegold and Martin, 1982).
- The identification was carried out using Gram staining, production of coagulase ,catalase and fermentation of mannitol (Bennett and Lancette, 1995).

6- Isolation and identification of *Listeria* species.

- Enrichment procedure: 1ml of each rinse solution, as well as, from homogenous egg contents was placed aseptically in the Listeria enrichment broth.
- Plating using Palcam medium (Curtis *et al.,* 1989).
- Identification and species differentiation were carried out (Warburton *et al.,* 2003) including Gram staining,Catalase teste, Carbohydrate fermentation, B-haemolysis on blood agar and CAMP test.

7- Isolation and identification of Salmonella.

- Pre-enrichment
- Selective- enrichment according to Rappaport *et al.* (1956).
- Selective plating using Xylose Lysine Desoxycholate agar (XLD; Oxoid).
- Identification of isolates.
- Morphological examination (A.P.H.A., 1992) motility test (Baron *et al.,* 1994).
- Biochemical identification by hydrolysis of Christensen urea agar (Koneman *et al.,* 1992), triple sugar iron (TSI) agar reaction (Baron *et al.,* 1994), Gelatin liquefaction test (Quinn *et al.,* 1994) Indole test, Methyle red test, Voges Prskauer test, citrate utilization test (Koneman *et al.,* 1992) and sugar fermentation reaction (A.P.H.A., 1992).
- Serological identification according to Kauffmann (1974).

RESULTS

Table 1: Statistical analytical results of various microorganisms recovered from the examined quail egg shells / ml.

Types of microorganisms	No. of examined samples	Positive samples		Counts /ml		
				Min.	Max.	Average
		No./30	%			
Aerobic plate count	30	30	100	2.5×10^3	2.07×10^4	1×10^4
Enterococci count	*30*	*28*	*93*	1×10^2	1.6×10^3	7×10^2
Total c*oliforms* counts	30	22	73	*< 10	1.1×10^3	5×10^2
Fecal coliforms counts	*30*	*4*	*13*	*< 10	2.1 x10	1 x10
E. coli counts	30	2	7	*< 10	3.6 x10	.784 x10
Yeast and mold counts	30	26	87	2.1×10^3	1.6×10^4	9×10^3

*Colonies could not be detect on the plates.

Table 2: Statistical analytical results of various microorganisms recovered from the examined quail egg Contents / ml.

Types of microorganisms	No. of examined samples	Positive samples		Counts / ml		
				Min.	Max.	Average
		No./30	%			
Aerobic plate count	30	30	100	3×10^2	1.74×10^4	7×10^3
Enterococci count	*30*	*22*	*73*	1×10^2	1.24×10^4	1×10^3
Total c*oliforms* counts	30	12	40	*< 10	1.50×10^2	1.9 x 10
Fecal coliforms counts	*30*	-	-	*< 10	*< 10	*< 10
E. coli counts	30	-	-	-	-	-
Yeast and mold counts	30	22	73	1.4×10^3	9.6×10^3	6×10^3

Table 3: Frequency distribution of the positive shells and contents of quail eggs samples based on their total *Coliform, Fecal coliform* and *E. coli c*ounts.

Count/ml	Egg shells						Egg contents					
	Total coliform		*Fecal coliform*		*E.coli*		Total coliform		F*ecal coliform*		E.coli	
	No./22	%	No./4	%	No./2	%	No./12	%	No./	%	No/	%
1-<	-	-	-	-	-	-	-	-	-	-	-	-
10^{1-}<	8	36	4	100	2	100	2	17				
10^{2-}<	2	9					10	83				
10^{3-}<	12	55										
10^{4-}<												

Table 4: Incidence of *Staph. aureus* and *Sal. spp.* isolated from quail eggs samples.

Quail eggs samples	*Staph. spp.*				*Sal. spp.*	
	Staph. Aureus		CNS			
	No./ 30	%	No./ 30	%	No./30	%
Shells	12	40	5	17	-	-
Contents	5	17	2	7	-	-
Total	17	57	7	23	-	-

CNS = Coagulase Negative Staphylococci

Table 5: Incidence of *Listeria species* isolated from quail eggs samples.

Listeria species	Quail egg shells		Quail egg contents	
	No./30	%	No./30	%
L. monocytogene	-	-	-	-
L. innocua	-	-	-	-
l..seeligri	-	-	-	-
L. gray	2	7	-	-

Table 6: Incidence of fungi recovered from the examined quail eggs.

Isolated strains	Egg shells		Egg contents	
	No. /30	%	No. /30	%
Cladosporium cladosporoides	5	16.7	2	6.7
Penicillium crysogenum	1	3.3	2	6.7
Penicillium cyclopium	2	6.7	2	6.7
Penicillium funiculosum	1	3.3	-	-
Penicillium paxilli	2	6.7	-	-
Trichoderma viride	1	3.3	1	3.3
Trichothecium roseum	2	6.7	-	-
Fusarium oxysporium	1	3.3	1	3.3
Mucor heimalis	1	3.3	-	-
Total	16	53	8	27

DISCUSSION

Although eggs are valuable and even indispensable food, they may play an important role in transmitting different diseases. Human infection due to consumption of infected eggs has been reported in numerous countries all over the world (Ko and Chang, 1995).

Results presented in Table 1 declared that the average counts of aerobic plate, enterococci, total coliforms, faecal coliforms, E. coli and yeast and mold / ml of the rinsing solution of the examined quail eggs shell samples were 1 $x10^4$, 7 $x10^2$, $5x10^2$, 1x 10, .784 x10 and 9 $x10^3$ml , respectively.

The average aerobic plate counts were counts 1 $x10^4$ and 7 $x10^3$ for shell and content of quail eggs were less than the accepted 10 $x10^5$ cfu / ml as recommended by the International Commission on the Microbiological Specification for Food (ICMSF, 1998).

The most important index of microbiological quality is aerobic plate counts, coliforms, yeast and mold counts and detection of specific pathogens and their toxins is recorded by A.P.H.A.(1992) as the microbial quality reflected the care with which quail eggs were handled and stored.

In addition from the data recorded in Table 2, it is evident that the average values of the aforementioned fresh homogenous contents were lower than that of examined quail eggs shells. This finding substantiates what has been postulated by Labaque *et al.* (2003); Jones *et al.* (2004) and Bahobail *et al.* (2012). Moreover, Humphrey (1994) reported that the final microbial load of egg contents depends on temperature and length of storage.

The results given in (Tables 1-2) pointed out that the average values of enterococci $7x10^2$ and $1x10^3$ for shells and contents, respectively, of quail eggs nearly agree with El- Prince *et al.* (2003). Moreover, fecal coliform failed to detect in contents, these results agree to a certain extant with those obtained by Saleim and El- Prince (2000) in chicken eggs and El- Prince *et al.* (2003) in quail eggs. Also the results pointed out that E. coli was the most prevalent bacteria recovered from the egg shell in a percentage of 7% and an average. 784 x10 however *E. co*li could not be detected in egg contents. In contrast, the contamination of egg shell and content with *E. coli* was previously investigated by Abdel -Hady and Emara (1997); Chang (2000); Moustafa *et al.* (2001) and Sabreen (2001) also could isolate E.coli in an incidence of 5% from examined infertile quail eggs while El–Prince *et al.* (2003) found *E.coli* in egg shell in a percentage of 6% from quail eggs and Bahobail *et al.* (2012) found 3 (7%) of (n=45 pooled samples) had *E. coli* in their shells but not in the egg content in chicken eggs It has been stated that avian pathogenic *E.coli* causes airsacculitis, polyserositis, septicemia and other avian species. Avian pathogenic E.coli are found in the intestinal microflora of healthy birds and most of the disease associated with them are secondary to environmental and host predisposing factors (Dho-Moulin and Fairbrother, 1999). They also added that prevention and control of these infections include control humidity and ventilation.

The results in Table 3, revealed that the highest frequency distribution of positive samples of tot*al coliform* in shells quail eggs were 55% lied in the range of 10^3- < 10^4 cfu/ ml while in contents were 83% lied in the range of 10^2->10^3cfu/ml. The rest of the positive samples of total coliforms in shells were distributed as 36 and 9% lied between 10^1-> 10^2 and 10^2 > 10^3cfu /ml. Otherwise the highest frequency

distribution of positive samples of *fecal coliforms* and *E. coli* in shells were 100% lied in the range of 10^1->10^2 cfu/ml.

A health issue associated with quail eggs is their contamination by pathogenic bacteria in this study (Table 4), *Staph. aureus* was recovered from 12 (40%) and 5(17%) of the total examined shells and contents of quail eggs, respectively. As well the *Staph. aureus* scored higher percentage of contamination than that recorded by Korashy *et al.* (2008) and Abdel-Hameed and El–Malt (2009).

Additionally, *Staph. aureus* was detected in 17% of the content examined quail eggs. From Table 4, the low incidence of *Staph. aureus* in the egg contents may be due to the presence of lysozyme in the inner shell membrane which act as an effective agent against Gram positive organisms. Regarding CNS, they were isolated from 5 shell surfaces, (17 %) and 2 egg contents (7%) samples contamination.

In this study, *L. gray* was detected in examined eggs with low ratio 2 samples (7%) of 150 quail eggs (n=30 samples) in their shells but not in the contents (Table 5). El-Malt, and Abdel-Hameed (2009) examined table eggs obtained *L. gray* high ratio 13.33 % in egg shells and 11.71 in egg contents. The presence of *Listeria* species other than *L.monocytogenes* as indicators of the presence that organisms have been proposed Johnson *et al.* (1990). The presence of other *Listeria s*pecies could be attributed to unsanitary measures during handling and transportation of eggs. Similar prevalence was found by Nitcheva *et al.* (1990) who isolated L. monocytogenes from the egg shells (1of 71 samples) but not from egg contents. In contrast, *L. monocytogens* was isolated with high frequency from samples of eggs collected at processing plants Leassor and Forgeding (1989) from the outer surface of the egg shells Likewise, Farber *et al.* (1992). Sayed *et al.* (2009) found that egg shells were contaminated with 7% of Listeria spp. while no contamination was found in egg contents. On the other hand, Moore and Maddan (1993) recorded that 72% of raw blended egg samples were positive for *Listeria* spp. which 37.8% were identified as *L. monocytogenes.*

Failure to isolate *Salmonella s*pp. from quail eggs in the current study may due 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 isolated three *Salmonella* strains out of 1100 domestic eggs. Also, Musgrove *et al.* (2005) identified one out of 105 Enterobacteriaceae isolates, recovered from 84 shell surfaces, as *Salmonella*. Poppe *et al.* (1998); De Reu *et al.* (2006) and de Boer and Witt (2000) reported that 0.07 to 0.4% (egg shell and egg content), 0.18% and 0.03% of table eggs, respectively were *Salmonella*-positive. This variability in *Salmonella* occurrence may be due to sample size, timing of sampling, sites of the eggs that were tested, techniques used, and investigations of eggs lay by artificially or naturally infected hens (Humphrey, 1994).

The fungal load found averaged $9x10^3$ cfu /ml in eggs shells while averaged $6x10^3$/ ml in the contents (Tables 1- 2). However, lower fungal count was reported in table eggs Ahmed *et al.* (2002); Suba *et al.* (2005) and Salem *et al.* (2009) which was reported to be > 5 $\log_{10}$ cfu/ ml. Other studies indicated lower count of 1 $\log_{10}$ cfu /ml in egg samples Ahmed *et al.* (1987) and El-Essawy *et al.* (1989). Jones *et al.* (2004) found an average fungal concentration of 1.5 log cfu/ ml egg shell in the day of egg collections while averaged o.1 log cfu/ml in the content, while Bahoball *et al.* (2012), the fungal load found averaged 1.3 $\log_{10}$ cfu/ml.

It is apparent from results outlined in Table 6, that 16 (53%) and 8 (27%) fungi species belong to 6 genera were presented in samples of shells and contents, respectively. Pencillium was the most prevalent genus constituting 6(20%) it was represented by 4 species, *P.crysogenum* and *P. funiculosum* 1(3.3%), *P. paxilli* and P. *cyclopium* 2 (6.7%) in egg shells. However in egg contents, Pencillium was represented by 2 species *P.cryscogenum* and *P. cyclopium* 2 (6.7%). The genus *Cladosporium* ranked second in percentage of isolation constituting (16.7% and 6.7%) in shells and contents samples, respectively. Furthermore, Fusarium spp, Mucor heimalis, Trichoderma viride, and Trichothecium roseum were infrequently recovered from examined shell and content samples.

It is worth to mention that some of fungi species such as Fusarium moniliforme and Trichothecium rosarium are mycotoxin – producing molds which posses potential hazards to food safety and human health Martins *et al.,* (1998). Likewise, Sanchez *et al.* (1980) studied the environmental fungus genera were encountered most frequently, furthermore different types of molds were isolated by several investigators as El-Essawy *et al.* (1989); Obi and Igbokwe (2007); Salam *et al.* (2009) and Bahobail *et al.* (2012). These molds including Penicillum, cladosporium, Aspergillus, Alternaria alternaria, Mucor, Rhizopus genera from examined avian eggs.

CONCLUSION

From the above achieved results, it is noted that quail eggs need more care during handling, also, they were liable to contamination by some pathogenic microorganisms. If such eggs are consumed raw or semi-raw may be responsible for sporadic or epidemic diseases. Moreover, some species of fungi

encountered are known to be mycotoxin producers which threaten human health. Therefore, to safeguard human from being infected, the hygienic measures adapted in the farm during handling and storage are necessary to obtain good quality eggs and fit for human consumption.

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تعيين المخاطر الميكروبيولوجيه فى بيض السمان في محافظة قنا ، مصر العليا

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نظرا لتلوث بيض السمان بالميكروبات التي تؤثر على سلامته وكذلك بالميكروبات المسببة للتسمم الغذائي للمستهلك وتسبب مخاطر صحية ، تم جمع عينات عشوائية من بيض السمان (١٥٠ بيضة) مثلث ٣٠ مجموعة من مزارع السمان المختلفة المنتشرة بمدينة قنا (مصر العليا). كل عينة تم فحص القشرة ومحتويات البيض ميكروبيولوجيا. وقد أوضح الفحص الميكروبيولوجي لعينات قشرة البيض أن المتوسط الكلي للميكروبات الهوائية, والمكور القولوني, الكوليفورم , الفيكال كوليفورم ,الايشيرشيا كولاي والخمائر والفطريات على الترتيب : ١×١٠^{٤}، ٧×١٠^{٢}، ٥×١٠^{٢}، ١×١٠، ٧٨٤,١٠×٠، ٩×١٠^{٣} / مللي. وبفحص عينات محتويات بيض السمان وجد أن المتوسطات الكلية للميكروبات السابقة أقل من نظيرتها في قشر البيض (٧×١٠^{٣}، ١×١٠^{٣}، ٩.١×١٠، < ١٠ ، ٦×١٠^{٣}) على الترتيب. وكذلك تم عزل ميكروب الايشريشيا كولاي من عينات قشرة البيض ولم تعزل من عينات محتويات بيض السمان. ونسبة التلوث بميكروب المكور العنقودي الذهبي كانتا ١٢(٤٠٪) ، ٥(١٧٪) لكل من القشرة ومحتويات البيض على التوالي. وقد تم عزل الليستيريا من ٢ عينة من قشرة البيض. وقد دلت النتائج على عدم وجود ميكروب السالمونيلا. وقد تم تصنيف الفطر المعزول بنسبة ١٦(٥٣٪) ، ٨(٢٧٪) من اصناف الفطر من عينات القشرة ومحتوى البيض على الترتيب. وتم تصنيف ٦ اصناف من الفطر. وقد نوقشت الطرق الواجب اتباعها لمنع تلوث البيض بتلك الميكروبات واتباع الاشتراطات الصحية لحماية صحة المستهلك.