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ASSESSMENT OF THE MICROBIAL QUALITY AND AFLATOXINS CONTENT IN POULTRY FARMS EGGS SOLD IN QENA CITY- UPPER EGYPT

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ABSTRACT

Contamination of eggs with microorganisms that possibly affect eggs quality and transmit pathogens or intoxication to consumers, causing public health hazards. A total of 135 fresh poultry farms eggs were collected randomly from different groceries and markets in Qena city, Upper Egypt. Every three eggs from each market were represented as one egg pooled sample (n =45 pools) Each egg shell and content was examined for their microbiological contents in terms of aerobic plate, *enterococci*, total *coliforms*, *faecal coliforms* and yeast and mold counts /ml, presence of salmonella, Escherichia coli, Listeria, and the presence of total aflatoxins (AFs) levels by enzyme linked immune-sorbent assay (ELISA). The results showed microbial growth on 100%of each (45 shell and content pools) of the examined samples and of all, total aflatoxin contamination was determined to trace amounts in three egg samples (6.7 %) (ranging from 0 .7 to 1.15 ppb).Other samples tested were found to be free from any detectable level of aflatoxins. Among poultry farms hen eggs average values of aerobic plate, *enteroccocci* and total yeast and mold counts / ml of egg shells samples were 6.16X 10^3, 1.6 x10^2 and 2.50 x 102 cells/ ml, respectively. However in egg contents, the corresponding counts were lower than that of egg shells samples 1.14 X103, - and 5.14 x10 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 15 (33.3%) and 9(20 %) in shells and contents samples, respectively. Also one egg shell sample was contaminated by *Listeria monocytogene* and salmonella negative. It is concluded that eggs sold in Qena city were of good quality, although occurrence of some pathogenic microorganisms. Therefore, it is recommended that poultry farm hen eggs should not be consumed raw.

Key words: *Microbial quality, Aflatoxins, Poultry farms, Eggs, Upper Egypt.*

INTRODUCTION

Today, eggs remain a staple 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 (Osei-Somuah *et al.,* 2003).

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). Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells (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 egg shells and contents.. Among them, *Listeria monocytogenes*, Yersinia enterocolitica, Escherichia coli, *Salmonella* and *Campylobacter* were detected (Adesiyun *et al.,* 2005).

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 consumers demands worldwide to eggs periodical assessment is required to offer safe and good quality eggs for consumption. The present investigation was therefore, planned to assess and interior quality of consumed eggs at retail levels in Qena city Upper Egypt. Microbiological quality, presence of food pathogens and total aflatoxin residues were investigated.

MATERIALS and METHODS

-Samples collection:
One hundred and thirty-five (135) fresh poultry farms hen eggs were collected randomly from different groceries and markets in Qena city, Upper Egypt. Every three eggs from each market were represented as one egg pooled sample. All 45 egg pooled samples were examined for microbial quality, total aflatoxins (AFs) as well as presence of *Staph aureus*, *listeria*. *E.coli* and *Salmonella*. Two sampling methods were utilized.

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

II• 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 content samples. Then duplicate plating of 1 ml aliquots used for enumeration of each analyzed microbial item. Each sample was divided into two parts, one for extraction of aflatoxins and the other for microbial enumeration, detection isolation and identification. 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 coagulas, 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).
- Detection of aflatoxins

MaxSignal® Aflatoxin Total- enzyme linked immuno-sorbent assay (ELISA) Test kit (Bio Scientific corp., Austin, TX, USA) is a competitive enzyme immunoassay and was used for the quantitative detection of AFs in the samples following manufacturer instructions. The micro wells were measured at 450nm by ELISA reader (Elx800, Bio

Tekinc., Highland Park, Winooski, VT, USA). The optical densities of the samples were determined and compared with that of the kit standard. Egg samples were exposed to some pretreatments the egg pooled samples were diluted with 70% methanol (1:9) mixed and blended for one min. The homogenate was filtered and 50µl of the filtrate was used per well for the assay. The test kit detection limit is 0.05ng/g (ppb).

RESULTS

Table 1: Statistical analyticalresults of various microorganisms recovered from the examined poultry farms egg shells / ml.

Types of microorganisms	No. of examined samples	Positive samples		Counts /ml of positive samples		
		No.	%	Min	Max	average
Aerobic plate counts	45	45	100	500	17600	$6.16X\ 10^3$
Enterococci counts	45	2	4.4	100	220	$1.60\ x10^2$
Yeast and mold counts	45	30	66.66	100	500	$2.50\ x\ 10^2$

Table 2: Statistical analytical results of various microorganisms recovered from the examined poultry farms hen egg contents / ml.

Types of microorganisms	No. of examined samples	Positive samples		Counts /ml		
		No.	%	Min	Max	Average of positive samples
Aerobic plate counts	45	45	100	10	4000	$1.14\ X10^3$
Enterococci counts	45	-	-	-	-	-
Yeast and mold counts	45	21	46.6	10	100	5.14x10

Table 3: Frequency distribution of the positive shells and contents Poultry farms egg samples based on their total coliform, Fecal coliform and E. coli counts.

Count/ml	Poultry farms egg shells						Poultry farms egg contents					
	Total coliform		*Fecal coliform*		*E.coli*		Total coliform		*Fecal coliform*		E.coli	
	No./21	%	No/15	%	No/9	%	No/12	%	N0/9	%	No/	%
3 -	8	38.7	5	33.3	6	66.6	6	50	9	100	-	-
10^{1-}	4	19	10	66.7	3	33.3						
10^{2-}	9	42.9					3	25				
10^{3-}							3	25				
Total	21	100	15	100	9	100	12	100	9	100	-	

Table 4: Incidence of *Staph aureus* and *Sal.* Spp. Isolated from the examined poultry farms egg samples.

Egg samples	*Staph. spp.*				*Sal. spp.*	
	Staph. aureus		CNS			
	No./45	%	No./ 45	%	No/45	%
Shells	15	33.3	7	15.55	0	0
Contents	9	20	4	8.9	0	0
Total	24	53.3	11	24.4	0	0

CNS= coagulase negative Staph. aureus

Table 5: Incidence of. Listeria species recovered from the examined poultry farms egg samples.

Listeria species	Egg shells		Egg contents	
	Positive samples		Positive samples	
	No/45	%	No./45	%
L. monocytogene	1	2.2	0	
L. innocua	0	0	0	
L.seeligri	0	0	0	
L.gray	4	8.9	0	

Table 6: Incidence of fungi recovered from the examined poultry farms eggs samples.

Fungal species	Egg shells		Egg contents	
	No/45	%	No/45	%
Aspergillusflavus	6	13.3	4	8.9
Aspergillusniger	2	4.4	1	2.2
Aspergillusfumigatus	5	11.11	2	4.4
Penicillium spp.	2	4.4	1	2.2
Cladosporium spp.	8	17.8	2	4.4
Mucor spp.	4	8.9	0	0
Total	27	60	10	22.2

Table 7: Results of AFs content residues in the examined poultry farms egg samples.

Examined samples	Total No. of samples	No. of positive	%	AFs concentration (ug /g or ppb of egg content)
Poultry farms eggs	45	3	6.7	(0.71, 0.81, 1.15)

DISCUSSION

Results presented in Table 1, declared that the average counts of aerobic plate, *enterococci*, and yeast and mold /ml of the rinsing solution of the examined poultry farms hen eggs shell samples were $6.16x\ 10^3$, $1.60x10^2$, and $2.50x10^2$, respectively.

The average aerobic plate counts were $6.16x10^3$ and $1.14x10^3$ for shell and content of poultry farms hen eggs less than accepted $10x10^5$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, *enterococci, total coliforms*, *faecal coliforms*, yeast and mold detection of specific pathogens and their toxins is recorded by A.P.H.A (1992) as the microbial quality reflected the care with which poultry farms hen 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 poultry farms hen eggs shell. 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 (Table1-2) pointed out that the average value of *enterococc*i was $1.60x10^2$ in examined poultry farms hen eggs shell which failed to be detected in hen egg contents these result lower with those staled with El- Prince *et al.* (2003). Moreover, *E. coli* filed to detect in contents these

results agree to a certain extant with those obtained by Seleim 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 recovered from the eggs shell in a percentage of 20% however *E.coli* could not be detected in eggs content. In contrast, the contamination of egg shell and content with *E. coli* was previously investigated by Abdel -Hady and Emara (1997) and Moustafa *et al.* (2001), 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 is 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 total coliform in poultry farms hen eggs shell were 42.9% lied in the range of 10^2- < 10^3 cfu/ ml while in contents were 50% lied in the range of 3->10 cfu/ml. The rest of the positive samples of *total coliforms* in shells were distributed as 38% and 19% lied between 3-> 10 and 10^1> 10^2 cfu /ml. Otherwise the highest frequency distribution of positive samples of fecal coliforms in shells were 66.6% lied in the range of 10^1->10^2 cfu/ml and in contents were 100% lied in range of 3-<10 while the highest frequency distribution of positive samples of E. coli in shells were 66.6 lied between 3-<10 and in contents failed to be detected.

A health issue associated with poultry farms hen eggs is their contamination by pathogenic bacteria in this study Table 4, Staph. aureuswas recovered from 15 (33.3%)and 9(20%) of the total examined shells and contents of poultry farms hen eggs, respectively. As well the *Staph.aureus* scored lower percentage of contamination than that recorded by Korashy *et al.* (2008) and El –Malt (2013). 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 shell surfaces, 7(5.55%) and 4 egg contents (8.9%) samples contamination.

In this study isolated *L. monocytogenes* from the egg shells (1of 45 samples) but not from egg contents. While L. gray was detected in examined eggs with low ratio 4 samples (8.9%) of 135 poultry eggs (n=45 samples) in their shells but not in the contents Table 5, El-Malt, and Abdel-Hameed (2009) examined table eggs obtained L. gray 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* species 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 Leasor and Foegeding (1989) from the outer surface of the egg shells Likewise, Farber *et al.* (1992). Sayed*etal.* (2009) found that egg shells were contaminated with 7% of *Listeria spp.* while no contamination was found in egg contents. On the other hands, Moore and Madden (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 spp. from poultry farms 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 *Enterobacteriacea*e 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 2.50 x 10^2cfu /ml in eggs shells while averaged 5.14x10cfu/ 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 were 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 Bahobail *et al.* (2012), the fungal load found averaged 1.3 $\log_{10}$cfu/ml.

It is apparent from results outlined in Table 6, that 27 (60%) and 10 (22.2%) fungi species belong to 4

genera were presented in samples of shells and contents, respectively. *Aspergillus*was the most prevalent genus constituting 13(28.9%) and 7(15.55%) in eggs *shell and content, respectively. It* was represented by 3 species, Aspergillusflavus 6(13.3%) and 4(8.9*), Aspergillusniger* 2(4.4%) and 1(2.2), and *Aspergillus fumigates5 (11.1%)* and 2(4.4) in egg shells and contents, respectively. However the genus *Cladosporium r*anked second in percentage of isolation constituting 8 (17.8) and 2(4.4) in shells and contents poultry farms samples, respectively. Furthermore, *Penicillium sp*p. 2(4.4) and 1(2.2) in egg shells and contents, respectively. And *Mucorisolated from* examined shells only. Mycological examination in the current work revealed four genera, which agrees with published reports where Aspergillus spp., Penicillum spp., cladosporum spp. And Mucorspp. have been recovered from washed eggs or their contents (Salem *et al.,* 2009; bahobail (2012).

In Table 7, The AFs contamination was detected to trace amounts only in three (6.7 %) of egg samples ranged 0.7 to 1.15 ppb. The positive samples did not exceed the maximum residual limits of aflatoxins recommended by WHO (2005) which is 5 ppb. Similar findings were reported by Ahmed *et al.* (2002), Aly and Anwer (2009), Salem *et al.* (2009), bahobail (2012) were aflatoxin contamination of egg was minimal comparing with the limit recommended by WHO (2005).

In CONCLUSION, the results showed that eggs of Qena city market are generally in a good quality, however because of the presence of minimal pathogenic microorganisms in some samples, we recommended that poultry farms eggs should not be consumed raw.

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تعيين الجودة الميكروبيولوجية ووجود الأفلاتوكسين في بيض مزارع الدجاج في مدينة قنا – مصر العليا

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نظرا لتلوث البيض بالميكروبات التي تؤثر على سلامته وكذلك بالميكروبات المسببة للتسمم الغذائي للمستهلك وتسبب مخاطر صحية ، تم جمع مائة خمسة وثلاثون بيضة بطريقةعشوائية من بيض مزارع إنتاج الدجاج وقد تمثلت المجموعة الواحدة بثلاث بيضات (٤٥ مجموعة) من محلات البقالة والاسواق المختلفة المنتشرة بمدينة قنا (مصر العليا). في كل عينة تم فحص القشرة ومحتويات البيض ميكروبيولوجيا وكذلك تم تعيين مستوى السموم الأفلاتوكسينفي بيض الدجاج باستخدام اختبار الاليزا. وقد أوضح الفحص الميكروبيولوجي لعينات قشرة البيض أن المتوسط الكلي للميكروبات الهوائية، والمكور القولوني، والخمائر والفطريات على الترتيب : ٦,١٦×١٠^٣، ١,٦٠×١٠^٢، ٢,٥٠ ×١٠^٢ / مللي. وبفحص عينات محتويات بيض الدجاج وجد أن المتوسطات الكلية للميكروبات السابقة أقل من نظيرتها في قشر البيض ١,١٤×١٠^٣، - ، ٥,١٤×١٠ على الترتيب. وكذلك تم عزل ميكروب الايشريشيا كولاي من عينات قشرة البيض ولم تعزل من عينات محتويات بيض الدجاج. ونسبة التلوث بميكروب المكور العنقودي الذهبي كانتا ١٥(٣٣,٣٪)، ٩(٢٠٪) لكل من القشرة ومحتويات البيض على التوالي. وقد تم عزل الليستيريا من عينة من قشرة البيض. وقد دلت النتائج على عدم وجود لميكروب السالمونيلا. وقد تم تعيين ثلاث عينات ملوثة بالسموم الأفلاتوكسين بنسبة (٦,٧ %) وكانت الكمية تتراوح ما بين (٧، ppb١,١٥:) وكانت باقي العينات خالية. وقد نوقشت الطرق الواجب إتباعها لمنع تلوث البيض بتلك الميكروبات وإتباع الاشتراطات الصحية لحماية صحة المستهلك.