Microbiological Scanning of poultry Eggs marketed in EGYPT.

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ABSTRACT

A one-year study was carried out to scan the microbiological quality of poultry eggs distributed in Egyptian market and also collected from farms in order to determine the egg safety from production to consumption. This study was intended to identify the status of egg safety as one component of the public health issue of food safety. Five that produce eggs farms were surveyed to ensure that standard health programs were applied thoroughly in order to prevent diseases and provide good environmental conditions in the laying houses. One thousand and eight hundred eggs representative 120 batchs of field samples were collected from five different farm, supermarkets, shops of selling egg and from open markets. High total plate count was detected in both the egg collected from open markets and farms. However, detection of organisms (total *Coliform*, *Faecal coliform* and *E coli*) were detected in either samples collected from farms or market, which reflects temperature abuse during storage and handling. Data recorded that, the *coliform* was detected in 86.7%, 56.7%, 86.7% and 100% of egg samples collected from farms, supermarkets, egg shops and open markets, respectively. *Faecal coliform* was detected in 53.3%,63.3% of eggs that collected from farms and open markets. E .coli was detected in 43.3% and 26.7% in egg collected from farms and open markets. *Faecal coliform* and *E coli* not detected in the eggs collected from supermarkets and egg selling shops. The highest isolation frequency of bacillus spp. was found in egg collected from open markets (22.7%), while the lowest was in egg sample collected from supermarket (3.3%) . *Staphylococcus* species were isolated at high frequency of 76.7%,66.7% and 33.3% from eggs collected from open market , egg shop and farms, respectively, while the lowest isolation was detected in those eggs collected from supermarket (6.7%). *Salmonella* species were isolated at various frequencies of 76.7%, 66.7%, 56.7% and 10% from egg collected from open markets, egg shops, farms and supermarkets, respectively.

There is a need for new guidelines for the proper production, marketing and handling of eggs to ensure egg safety. Minimization of the incidence of food born illness could be gained through quality-control programs, refrigeration during transport and storage, and food-safety education for home and hotels egg consumers.

INTRODUCTION

Eggs are among the most nutritious foods on earth and can be a part of a healthy diet. However, they are perishable just like raw meat, poultry, and fish. Poultry, meat, and other raw foods also can be carriers of *Salmonella enteritidis* (SE) and egg shell can lead the list (Farran *et al.*, 2001). Unbroken clean and fresh shell eggs may contain SE that can cause food born illness. Nevertheless, the number of eggs affected is quite small,

there have been cases of food borne outbreaks in the last few years. Unlike egg born Salmonellosis of past decades, the current epidemic is due to intact and disinfected grade A eggs (MMWR, 1996). *Salmonella enteritidis* silently infects the ovaries of healthy appearing hens and contaminates the eggs before the shells are formed.

Traditionally, practices such as the use of raw, undercooking and non-refrigerated eggs in human food were not considered unsafe (St -Louis *et al.*, 1988). More recently, however, the potential for internal SE contamination of eggs has been established and egg-handling practices have been reevaluated. Common egg-handling practices now considered to be unsafe which include: temperature abuse (i.e. holding eggs and egg-containing foods at room temperature instead of under refrigeration); inadequate cooking and pooling eggs to prepare a large volume of an egg-containing food that condition is subsequently temperature abused or inadequately cooked. The presence of SE bacteria in a raw egg alone does not guarantee illness upon consumption. However, the likelihood of developing an SE infection increases when the egg is not handled safely by permitting the bacteria to multiply and a greater number of bacteria to be ingested with the food (US-FDA, 1995). Eggs must be properly handled, refrigerated, and cooked to be safe at the consumer level.

The present investigation was designed to:

1. Study the microbiological quality of those eggs marketed in Egypt in order to determine the egg safety from production to consumption.
2. Suggesting guidance for the hygienic production, storage, packaging and transport of whole eggs distributed for human consumption.

MATERIALS and METHODS

One thousand and eight hundred eggs representative 120 batchs of field samples were collected from different layer farms, supermarkets, shops of egg selling and from open markets. 30 batch from each source were collected and each batch contain 15 samples. Tested whole egg was examined microbiologically to determine its hygienic and quality status.

Preparation of tested samples, initial suspension and decimal dilution were done according to ISO 6887-3-(2001). Ten grams of whole eggs weighted into a sterile stomacher bag and 90 ml diluents (buffered peptone water) was added and, blended for 1-2 min. Then decimal dilution to $1.0x10^3$ in buffer peptone water was made to perform enumeration of total plate count for *Coliform*, *Faecal coliform*, *E-coli* , *Bacillus cereus* and *Staph. aureus*.

The total plate count was done by the method of ISO 4833-2002 on (TPC) agar and incubated at 30° c for 72 hrs.

The enumeration of *Coliform* group was done by pour plate method according to ISO 4832-2004 on crystal violet neutral red bile lactose (VRBL) and incubated at 37°C for 48 hrs.

Counts of *Faecal* (Thermo tolerant) *coli* form enumeration were done by poor plate method according to NMKL No 125 – 1996 on crystal violet neutral red bile lactose (VRBL) and incubated at 44.5°C for 24 hr.

Escherichia coli enumeration was done by surface plate method according to NMKL No 125 – (1996) modified on VRB-Mug incubated at 44.5°C for 24 hr. *Bacillus cereus* count was done using MYP agar (30° C/48 hrs), (ISO 7932,1993).*Staph. aureus* was isolated on Baird Parker agar (37° C /24 hrs) (ISO 6888, 1998).

Salmonella were tested according to ISO 6579,(2001), Pre–enrichment. 25g sample was performed in 225 ml buffer peptone water and incubated at 37°C for 16 – 20 hrs, then 1 ml and 0.1 ml were transfered into 10 ml in two selective enrichment broth selenite cystine broth and Rappart – vassiliadis broth (RV) and incubated at 37°C and 41.5°C for 24 hr, respectively. One lop from each selective enrichment was streaked on Hektone enteric agar, XLD and phenol red Brilliant green agar at 37°C for 24 hrs. Suspected colony were subjected to biochemical identification on lysine decarboxylase, triple sugar iron agar and urea agar at 37°C for 24 hrs. Positive colony subjected to conformation by using API 20 E system and serology antibody reaction.

Quality control :

General guidelines on quality assurance for the preparation of culture media in the laboratory were followed according to ISO 11133-1 (2000) and ISO 11133-2 (2002).

RESULTS AND DISCUSSION

As far as the microbial quality of whole egg samples is concerned ,there is difference between the four sample sources (farms, supermarkets, egg selling shops and openmarket). All samples were found to contain heterotrophic microbial populations. The highest total plate count (a mean of 2000 cfu/g ranged from 1400cfu/g to 2500 cfu/g) was found in samples collected from open market . The lowest mean count of 830 cfu/g(range of 500 - 1100 cfu/g) was detected in sample collected from supermarket(table 1). The results showed a difference in total plate count for different samples of eggs. These counts suggest the need for treatment such as the use of ultraviolet light (UV) radiation to lower the total microbial count in egg shell .The effects of 254nm ultraviolet light (UV) radiation on aerobic plate count (APC) of egg shells were investigated by Chavez *et al.*,

2002 who stated that a significant reduction (1 to 2 log) in colony forming units per egg was detected which ensure that UV light treatment at high intensities and low time intervals has the potential to reduce aerobic plate counts of egg shells.

However ,detection of indicator organisms (total *Coliform,Faecal coliform* and *E. coli*) were found either in samples collected from production source or at the end source consumers of (Market) , which reflects temperature abuse during storage and handling. Data recorded in Table 1 revealed that *Coliform* was detected in 86.7%, 56.7%, 86.7% and 100% of egg sample from farm, supermarket, egg selling shop and open market with a range of (100- 1100), (30- 300), (50- 500) and (50- 900), respectively. *Faecal coliform* was detected in 53.3%,63.3% egg which is collected from farm and open market, respectively while, *E coli* was detected in 43.3% and 26.7% in egg collected from l farm and open market. The results showed that there was a difference in both the total *Coliform* counts and *Fecal coliforms* for different egg samples . The highest count of *Coliform*, *Faecal coliform* and *E coli* were detected in egg collected from open market and farm which indicates that production defects together with mishandling contributed to quality deterioration . *F. coliform* and *E coli* were not detected in those eggs collected from supermarkets and egg selling shops.

At oviposition period, shell egg is either sterile or harbor very few microorganisms. Contamination takes place afterward from nesting material, floor litter, and avian fecal matter that allow penetration and multiplication of Gram-positive microorganisms, as well as Gram-negative group which are mainly a part of the normal flora (Hale-Boothe and Arnold, 2002).In this study , the incidence of three microbial genera was studied to detect contamination impact (Table 2).The highest isolation frequency of *Bacillus* spp. was found in egg collected from open market (22.7%), while the lowest was in egg sample collected from supermarkets (3.3%) . *Staphylococcus* species were isolated at high frequency of 76.7%,66.7% and 33.3% from egg collected from open markets , egg selling shop and farm respectively . The lowest isolation was recorded from egg collected from supermarkets (6.7%) .This reflect improper washing or sanitation of the shell surface followed by excessive handling of sweated eggs.

Some food born diseases are well recognized, but are considered emerging because they have recently become more common. For example, outbreaks of Salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence on many continents and is largely related to consumption of poultry or eggs (MMWR,

1990). Until recently, *Salmonella enterica* serotype *Enteritidis* has remained sensitive to most antibiotics. However, national surveillance data from Denmark show that quinolone resistance in SE has increased from 0.8% in 1995 to 8.5% in 2000, which exert a public risk (Molback *et al.*, 2002). In this study,*Salmonella* species were isolated at various frequencies (Table 2) (76.7%, 66.7%, 56.7% and 10% from egg collected from open market, egg shop, layer farm and subermarket, respectively).

The major source of *Salmonella* is shell cracks that allow penetration of the micro-organisms to the interior contents. Improper washing plays a vital role especially when the temperature of the washing solution is less than that of the egg resulting in water drawn to the egg. Also when the water with high iron levels is used, excessive iron overcomes the ability of conalbumin to inhibit microbial invasion. Conalbumin is one of the natural barriers of the egg white that binds iron and other metals retarding the growth of microorganisms (Farran *et al.*, 2001). The possible routes of *Salmonella* from the laying hens to eggs are; transovarial, translocation from peritoneum to yolk sac or oviduct, and penetration of the shell as the egg pass through the cloaca (Mason and Ebel, 1992).

Egg safety has been identified, as one component of the public health issue of food safety. There is a need for new guideline for hygienic production, storage, packaging and transport of whole eggs intended for human consumption. Concerning table eggs production, bird recycling by induced molting can be an effective management tool, enabling matching of egg production with demand and reduce bird cost per dozen eggs (Holts and Gast, 2002). Through an induced molt, the productive life of a flock can be extended to an age of 105 weeks instead of 72 weeks. Generally, most studies reported that molting improves the post-molt performance of the laying hens when compared to the pre-molt performance. This improvement includes egg size, shell quality, internal egg quality and rate of egg production. Egg temperature (initial and throughout processing and storage), washing water pH, and enviromental temperature play key roles in reducing microbial growth in shell eggs (Mason and Ebel, 1992). Modern technology also may aid towards reducing *Salmonella enteritidis* in shell eggs. For example, ionizing radiation, cryogenic cooling using gaseous nitrogen (GN), liquid nitrogen (LN), and gaseous carbon dioxide (GC) (Jones *et al.*, 2002), and reducing *Salmonella* colonization in the chicks' intestines by spraying newly hatched chicks with a mixture that contains 29 bacteria (Fulton *et al.*, 2002). The feasibility of in-shell pasteurization for destruction of *Salmonella enteritidis* was investigated by Holt *et al.*(1996),

and found that this method gave 5-7 log reduction of (SE) with an acceptable overall functionality of internal egg components. Furthermore, vaccination of hens with *Salmonella enteritidis* (SE) has become an important industry management tool to reduce both the incidence of SE in flocks and the production of SE-contaminated eggs (Holt *et al.*, 1996). Also, electrostatic charging of particles in enclosed spaces has been shown to be an effective means of reducing airborn dust (Mitchell *et al.*, 2002). The guideline also requires retail establishments to refrigerate shell eggs promptly when they are received and to store the eggs at 45° F (7.2° C) or cooler. To prevent food born illness at the consumer level, a safe handling instruction should be included in the label (US-FDA, 1995). The statement must appear on the label prominently, conspicuously, as follows:

"To prevents illness from bacteria: keep eggs refrigerated, cook eggs until yolks are firm, and cook foods containing eggs thoroughly".

In general, the recommendations for preventing egg-associated food born illness could be concluded as follows :

- Persons should avoid consumption of raw or undercooked eggs.
- In hospitals, nursing homes, and commercial kitchens, pooled eggs or raw or undercooked eggs should be substituted with pasteurized egg products.
- Eggs should be cooked at > 145 F (> 63° C) for > 5 min (until the white is Completely set and the yolk begins to thicken) and eaten promptly after cooking.
- Nationwide Consistent Standards should be followed to improve confidence in the egg supply.
- Flock-based egg-quality-assurance programs that meet national standards and include microbiological testing should be adopted by industry nationwide.
- Standards must be risk-based and must achieve intended purpose.
- Flexibility for implementation to recognize the different configurations of egg layinghouses and ranches to allow the technological advanced methods.
- Inspector Training is a prerequisite for a successful program and consistent enforcement and inspection are needed.
- Education, at all levels, is essential in reducing food born illness.

Table 1 Incidence of different microorganisum (cfu/g) in Eggs collected from different Sources.

Sources	T.P.C (cfu/g)			*Coliform* (cfu/g)			*F.coliform* (cfu/g)			*E. coli* (cfu/g)		
	N.D	Mean	Range	N.D	Mean	Range	ND	Mean	Range	N.D	Mean	Range
Farms *		1100 (100%) #	(600-1600)	4 ** (13.3%)	490 (86.7%)	(100-1100)	14 ** (46.7%)	17 (53.3%)	(15-100)	17 ** (56.7%)	21 (43.3%)	(15- 55)
Supermarkets *	-	830 (100%) #	(500 - 1100)	13 ** (43.3%)	60 (56.7%)	(30-300)	30 ** (100%)	-	-	30 ** (100%)	-	-
Egg selling shops *	-	1400 (100%) #	(900-2100)	4 ** (13.3%)	230 (86.7%)	(50-550)	30 ** (100%)	-	-	30 ** (100%)	-	-
Open Markets *	-	2000 (100%) #	(1400-2500)	-	430 (100%)	(50-900)	11 ** (36.7%)	70 (63.3%)	(30-350)	22 ** (73.3%)	26 (26.7%)	(20 – 150)

T.P.C : Total plate count ,Cfu : Colony forming unit and N.D : not detected < 10 cfu/g

* Thirtybatches of each source werecollected.

** Number of samples out of 30 batches.

\# Percentage of the detected samplesfor the different examined microorganisums.

Table 2 Prevalence of pathogenic microorganisms in Egg collected from and different egg sources.

Microorganisms / source	Farm	Supermarkets	Egg selling shops	Open markets
Salmonella	17 (56.7%)*	3 (10%)	20 (66.7%)	23 (76.7%)
Staph.aureus	10 (33.3%)	2 (6.7%)	18 (60%)	21 (70%)
Bacillus cereus	4 (13.3%)	1 (3.3%)	7 (23.3%)	8 (26.7%)

* Number of contaminated samples and its percentage based on the total No.of the detected batches (30).

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الملخص العربي

الكشف الميكروبى لبيض الدجاج فى السوق المصريه

فؤاد الطحان

مركز البحوث الزراعية -المعمل المركزىلتحليل متبقيات المبيدات والعناصر الثقيلة فى الاغذية- دقى - جيزة

اجريت دراسه لمدة عام لفحص بيض الدجاج من الناحيه الميكروبيه فى السوق المصريه ومزارع دواجن البيض للتعرف على سلامتها للاستهلاك الادمى

وتمت هذه الدراسه على خمسه مزارع للدجاج البياض للتاكد من البرامج الصحيه القياسيه التى طبقت بدقه لكى تحمى من الأمراض والتى تعطى ظروف بيئيه جيده

واستخدمت فى هذة الدراسه الف وثمانىمائه بيضه موزعه على مائه وعشرون قسم من العبنات الميدانيه التى جمعت من مزارع البياض والسوبر ماركت ومحلات بيع البيض ومن الاسواق المفتوحه مقسمه إلى 30 قسم من كل سوق على التوالى .

واظهرت النتائج أرتفاع العدد الكلى للبكتريا الكليه من كل البيض المجموع من الأسواق المفتوحه ومزارع البيض على الرغم من أن الكشف عن الميكروبات الممرضه مثل بكتريا مجموعه القولون وجدت اما فى مزارع دجاج البيض او الأسواق ويرجع ذلك إلى درجه الحرارة أثناء التخزين والتعامل مع البيض

وقد شملت نتائج مجموعه بكتريا القولون بنسبه(86.7% و 56.7% و86.7% و100%) فى عينات البيض المجموعه من المزارع والسوبر ماركت ومحلات البيض والأسواق المفتوحه على التوالى

وقد أظهرت نتائج مجموعه البكتريا البرازيه بنسبه(53.3% و63.3%) للبيض المجموع من مزارع البيض والأسواق المفتوحه بينما (E.coli) قد وجدت بنسبه(43.3% و26.7%) فى البيض المجموع من مزارع البيض والأسواق المفتوحه .

ولم تظهر البكتريا البرازيه و (E.coli) فى البيض المجموع من السوبر ماركت ومحلات البيض .

وكانت اعلى نسبه تواجد للميكروبات الهوائيه المتجرثمه فى البيض المجموع من الأسواق المفتوحه(22.7%) بينما الاقل وحدة فى البيض المجموع من السوبر ماركت(3.3%)

واظهرت النتائج الكشف عن البكتريا العنقوديه ان النسبه الاعلى(76.7% و 66.7% و33.3%) فى البيض المجموع من الأسواق المفتوحه ومحلات البيض ومزارع البيض على التوالى بينما وجدت النسبه الاقل فى البيض المجموع من السوبر ماركت (6.7%) وكما اظهرت النتائج عن تواجد ميكروب السالمونيلا (salmonella) بنسب مختلفه (76.7% 66.7% 56.7% 10%) فى البيض المجموع من الأسواق المفتوحه ومحلات البيض ومزارع البيض والسوبر ماركت على التوالى

هناك حاجة إلى ارشادات لحصول على إنتاج جيد والتسويق والتسويق للبيض للحصول على بيض صحى