

Animal Health Research Institute

Assiut Regional Laboratory

**PUBLIC HEALTH HAZARDS OF SOME BACTERIAL PATHOGENS
ASSOCIATED WITH CONSUMPTION OF EGGS AND STUDYING
THE BEST COOKING METHODS FOR THEIR DESTRUCTION**

(With 4 Tables)

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(Received at 5/2/2008)

**المخاطر الصحية لبعض البكتيريا الممرضة المصاحبة لإستهلاك البيض مع
دراسة أفضل طرق الطهي للقضاء عليها**

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يُقبل الكثيرون على تناول البيض باعتباره مصدراً من مصادر البروتين الحيوانى وكذلك لاحتوائه على العناصر الغذائية والفيتامينات الهامة لمرحل العمر المختلفة للحفاظ على صحة الإنسان. لذا هدفت

الدراسة إلى عزل بعض الميكروبات ذات الأهمية الصحية حيث تكون سبباً في تلوث البيض متسببة في إحداث التسمم الغذائي للإنسان مثل المكور العنقودي الذهبي، الميكروب القولوني (الإيشيريشيا كولاي) ، فصائل السالمونيلا والليستيريا والإريموناس وكذلك اليارسينيا إنتيروكوليتيكا والإريثلوبوتركس. لذا تم جمع أربع مائة وخمسين بيضة بطريقة عشوائية من محلات البقالة ومختلف السوبر ماركت ومنازل الفلاحين بمدينة أسبوط بواقع مائة وخمسين عينة لكل من بيض الدجاج التجاري بنوعيه من السلالات البلدية ومزارع الدواجن وكذلك بيض البط وقد تمثلت العينة الواحدة في خمس بيضات. وقد دلت النتائج على أن بيض الدجاج البلدى هو الأقل تلوثاً حيث لم يتم عزل الميكروب القولوني واليارسينيا إنتيروكوليتيكا والإريثلوبوتركس. وقد غُزلت بعض فصائل السالمونيلا والليستيريا والإريموناس بنسب ضئيلة جداً من القشرة الخارجية والقشرة بأغلفتها فقط وقد مثل المكور العنقودي الذهبي أعلى نسبة للعزل من القشرة الخارجية والقشرة بأغلفتها بمعدل ٢٣,٣% ، ١٣,٣% على التوالي مقارنة بنسبة العزل من المحتوى الداخلى (١٠%). أما بالنسبة لبيض مزارع الدواجن فقد كان أكثر تلوثاً من بيض الدجاج البلدى حيث تم عزل المكور العنقودي الذهبي من القشرة الخارجية والمحتوى الداخلى بنسبتين متساويتين وهما : ٢٣,٣% وكذلك تم عزل الميكروب القولوني (٣,٣%) وبعض فصائل السالمونيلا : سالمونيلا تايفي والسالمونيلا المعوية (الإنترتيدز) وكذلك تم عزل بعض فصائل الإريموناس واليارسينيا إنتيروكوليتيكا من أجزاء البيض المختبر بينما لم يتم عزل فصائل الليستيريا وميكروب الإريثلوبوتركس. ومقارنة ببيض البط التجاري فقد كان الأكثر تلوثاً من البيض البلدى وبيض المزارع حيث تم عزل المكور العنقودي الذهبي بنسب عالية من القشرة الخارجية والقشرة بأغلفتها والمحتوى الداخلى : ٣٦,٧ ، ٣٠ ، ٣٣,٣% على التوالي . كما تم عزل الميكروب القولوني من القشرة الخارجية والقشرة بأغلفتها بنسبتي ١٣,٣ ، ١٠% على الترتيب. أيضاً تم عزل بعض فصائل السالمونيلا والليستيريا والإريموناس واليارسينيا إنتيروكوليتيكا بنسب مختلفة من بعض أجزاء البيض المختبر. بينما لم يتم عزل ميكروب الإريثلوبوتركس كما فى حالتى بيض الدجاج البلدى وبيض مزارع الدواجن. ونظراً لتعدد طرق طهى البيض للإستهلاك فقد هدفت الدراسة إلى معرفة تأثير المعاملات الحرارية على بعض الميكروبات ذات الخطورة على صحة الإنسان. لذا تم القيام بحقن البيض بميكروبات المكور العنقودي الذهبي ، الإيشيريشيا كولاي والسالمونيلا إنترتيدز وطهيها بعدة طرق لمدد زمنية مختلفة للتعرف على مدى حيوية وبقاء هذه الميكروبات. وقد أظهرت نتائج تقييم طرق طهى البيض أن أفضل طريقة لإعداد البيض وتناوله أمنأ هي طريقة الأومليت عند ١٦٣°م ولمدة ٢٥ دقيقة حيث لم يتم عزل أى من الميكروبات المستخدمة بالحقن. كما تم الحصول على نفس النتيجة عند استخدام طريقة التحمير بدون غطاء لمدة إثنتى عشر دقيقة. أما فى حالة طهى البيض مسلوقاً فقد تبين عدم جدوى السلق للمدد الزمنية ٣ ، ٥ دقائق للقضاء على أنواع الميكروبات المختبرة بالحقن حتى عند الدقيقة الثانية عشر لم تصلح فى القضاء على ميكروب الإيشيريشيا كولاي بينما تم القضاء على كل من المكور العنقودي الذهبي والسالمونيلا المعوية (عند ١٢ دقيقة). لذا تنصح الدراسة بتناول البيض البلدى المعد بطريقة الأومليت أو التحمير أو بطريقة السلق لمدة تزيد عن إثنتى عشر دقيقة. وقد ناقشت الدراسة الأهمية الصحية والاقتصادية لبعض الميكروبات التى تصيب الإنسان من خلال تناوله لبيض المائدة وكذلك أفضل الطرق لطهيه والسبل الكافية لمنع تلوث البيض والحفاظ عليه أثناء تخزينه.

SUMMARY

representing A total of 450 commercial eggs (Balady of farm hens and ducks) 150 eggs for each were collected randomly from Assiut city markets, different groceries, supermarkets and farmer's houses. Every 5 eggs represent one sample. Shell surfaces, shell surfaces mixed with shell membranes and egg contents were examined for the isolation of some pathogens of public health importance including *Staph. aureus*, *E. coli*, *Salmonella* spp., *Listeria* spp., *Aeromonas* spp., *Yersinia enterocolitica* and *Erysipelothrix* spp. An experimental part was applied to evaluate the best method used for cooking of eggs at different temperatures for different times to determine the safety of eggs for consumption. The obtained results of isolation revealed that *Staph. aureus* recorded the highest percentage of contamination among all the isolated pathogens specially from shell surfaces of all types of eggs. Commercial Balady hen eggs were the best type and advised to be consumed. *Staph. aureus* recovered from 23.3 , 13.3 and 10% of shell surfaces, shell mixed with shell membranes and egg contents, respectively while, *E. coli*, *S. paratyphi*, *S. enteritidis*, *Y. enterocolitica* and *Erysipelothrix* organisms were failed to be detected in the examined Balady hen egg samples. Commercial farm hen eggs came secondary to Balady hen eggs. *Staph. aureus* isolated from both shell surfaces and egg contents with percentages of 23.3% and 13.3% from the shell mixed with shell membranes. *S. enteritidis* recorded high rate of isolation from egg parts 16.7, 10 and 10%, respectively. *E. coli*, some of *Aeromonas* spp. and *Y. enterocolitica* could be isolated from some egg parts examined. *S. paratyphi*, *S. gallinarium*, *Listeria* spp. and *Erysipelothrix* spp. failed to be detected from farm hen egg samples examined. Highest rate of contamination was observed in commercial duck eggs. *Staph. aureus* was recoverd from shell surfaces, shell mixed with shell membranes and egg contents in 36.7, 30 and 33.3%, respectively. *E. coli* also recorded in high percentages of infection in shell and shell mixed with shell membranes (13.3 and 10%, respectively). Moreover, varying percentages of contamination by *Salmonella*, *Listeria* and *Aeromonas* spp. were recorded in different parts of duck egg samples examined, in addition to *Y. enterocolitica* which could be

isolated from shell, shell mixed with shell membranes and egg contents in 10, 3.3 and 6.7 %, respectively. On the other hand, *Erysipelothrix* spp. failed to be detected in all examined duck egg samples. Concerning the experimental part, results showed that cooking of eggs by Omelet method at 163°C for 25 minutes is the best since non of the test organisms used could be detected. Secondary, was the open frying method where *S. enteritidis* destroyed after 1 minute, and complete destruction of *Staph. aureus* and *E. coli* after 12 minutes. Boiling procedure for 7 and 12 minutes were adequate to destroy *Staph. aureus* and *S. enteritidis*, respectively, while, *E. coli* still be alive. The economic and public health importance of some pathogens that affect the human health through consumption of eggs were discussed. Likewise, suggestive measures for improving the quality of produced eggs and the suitable procedure to cook eggs are given.

Key words: Eggs, *Staph. aureus*, *E. coli*, *Salmonella*, *Listeria*,
Yersinia, *Cooking*.

INTRODUCTION

Eggs are one of the few foods that are used among the popular dishes consumed by people at home, restaurants and convenience stores in their natural states with no artificial additives.

Eggs are considered a unique, well balanced source of nutrients and essential food elements for growth and maintenance of health in the human body of all persons of all ages. Beside, the high nutrient contents of eggs, their low caloric values and ease of digestibility make them also valuable in many therapeutic diets for adults. Eggs and egg products are used in a wide variety of foods including whole egg custard, mayonnaise, egg salad, eggnog and all types of bakery products. Also, there are many food uses of eggs as pet foods

for domestic animals, soil fertilization, culture media, artificial insemination and industrial uses include, leather, cosmetics, shampoos and adhesives.

Most freshly laid eggs are sterile, at least from inside in case of good flock management and absence of vertical transmission also by the presence of cuticle, shell membranes and the antimicrobial properties of eggs (Yadava and Vadehra, 1977). But, eggs may constitute, if contaminated, a public health hazard as laying hens can sequester different types of microorganisms in their eggs.

Eggs are liable to contamination either before laying (congenitally) or after laying when the microorganisms reach the egg contents through penetration the shell and cause low egg quality, low shelf life and safety inducing public health hazards (Board and Fuller, 1994), in addition, fecal matter, improper washing, using of contaminated water and bad handling are the common sources of contamination. With attention to duck's eggs, they are highly contaminated than hen's eggs as they are laid near the damp places and due to the rapid deterioration of the antibacterial activity of albumen by the unfavorable surroundings. Bahout (2001) studied the public health importance resulting from consumption of duck's and hen's eggs.

Furthermore, among the pathogenic food poisoning organisms that affect the public health of humans due to consumption of eggs is *Staph. aureus* which is of serious concern to public health (Wyah, 1992). Its thermostable enterotoxins elaborated in large numbers of foods and animal products including eggs causing rapid onset of nausea, vomiting and diarrhea within 6 hours of ingestion of food. Several outbreaks of *Staph. aureus* food poisoning have been recorded, involving large number of individuals throughout the world (Ko and Chang, 1995). Also, there have been many research works that dealt with *Staph. aureus* in and on hen's and duck's eggs (Sabreen, 2001 and Bastawrows *et al.*, 2002).

E. coli constitutes a major economic menace to poultry industry and consequently is of public health importance for human causing profuse watery diarrhea which is varying in its severity and persistence due to inflammation of

the intestinal mucosa (Schiavoni and Vergora, 2000). The organism is taken as index of recent fecal contamination. Quiroga *et al.*, (2000) stated that diarrhoeogenic *E. coli* is the major agent involved in diarrhoeal disease in developing countries. Keshimaki *et al.* (2001) could isolate *E. coli* from 35% of diarrhoeal and 26% of non diarrhoeal cases.

E. coli can multiply in egg content and cause infection when the number of the organism reaches $10^5 - 10^7$ organisms/g (Eley, 1996). Numerous cases of food poisoning outbreaks were traced to the members of the family *Enterobacteriaceae* (Brooks *et al.*, 1995). Likewise, several investigators screened eggs for members of the family *Enterobacteriaceae* in shell, shell membranes and whole egg contents (Lambiri *et al.*, 1995; Bastawrows *et al.*, 2001 and 2002).

Moreover, *Salmonella* spp. remains a potential threat to human health, as well as, broiler chickens. The public health importance of avian salmonellosis has for long been appreciated, particularly the association of gastroenteritis in man resulting from consumption of infected hen's and duck's eggs (Abouzeed *et al.*, 2000). Different *Salmonella* spp. could be isolated from human diarrhoeal swaps by several authers (Urio *et al.*, 2001 and Biendo *et al.*, 2003).

Cold tolerant pathogens like *Listeria*, *Aeromonas* and *Yersinia* have assumed increased importance recently in shell eggs and egg products. While, there have been no documented outbreaks of listeriosis associated with eggs, *L. monocytogenes* is of concern because of its ability to grow in refrigerated whole egg and egg yolk (Sionkowski and Shelef, 1990). Moreover, it was isolated from commercial raw liquid whole eggs (Moore and Madden, 1993).

A. hydrophila can provoke extra intestinal and gastrointestinal disease in humans. It has been recovered as the primary or single gastrointestinal pathogen in children (Janda *et al.*, 1984). Isolation of *Aeromonas* from eggs has been reported by Varnam and Evans (1991), as well as, Kumar *et al.* (2000).

Production of a heat stable enterotoxin is a feature of both virulent and non virulent strains of *Y. enterocolitica*, and other non pathogenic yersinias. The ability of some strains of *Y. enterocolitica* to produce toxin has been demonstrated at 25°C, but not at refrigerated temperature (Walker, 1986).

Erysipelothrix infection is most commonly associated with diseases of animals and infrequently in humans (Hassanein *et al.*, 2001).

Because, there is considerable demand for precooked egg products, it is important to determine the safety of these products with respect to *Staph. aureus*, *E. coli* and *S. enteritidis*. Survival behaviors of heat shocked cells of these pathogens by applying different temperatures were studied by several investigators (Baker *et al.*, 1983; Bradshaw *et al.*, 1985).

The present study aims to:

Isolation of some pathogenic microorganisms of public health importance from shell surfaces, shell mixed with shell membranes and egg contents of different types of consumed eggs.

Study growth and survival behavior of some pathogens by applying different temperatures during its preparation for consumption.

MATERIALS and METHODS

A- Collection of samples:

300 of fresh commercial hen eggs of native breeds (Balady) and poultry farms and 150 of commercial duck eggs bought from farmer's houses, different groceries and Assiut city markets. Every 5 eggs constitute one group.

B- Preparation of samples:

Egg shells were tested by two methods a surface rinse for shell surfaces and by blending method for shell and shell membranes as described by Moats (1980). Egg contents were prepared and evacuated according to Speck (1976).

C- Isolation of some pathogens of public health importance:

- *Staph. aureus*: Using sodium chloride broth and Baird-Parker agar (Biolife) (Finegold and Martin, 1982).
- *E. coli*: Using MaConkey's broth and agar. The technique recommended by Quinn *et al.* (1994) was used for identification.
- *Salmonella* spp.: By using selenite F broth and SS agar and identification was done according to Quinn *et al.* (1994).
- *Listeria* spp.: were isolated on Oxford agar plates (Curtis *et al.*, 1989) after enriched in *Listeria* enrichment broth and identification was performed according to Hitchins (1995).
- *Aeromonas* spp.: Using trypticase soy broth with Ampicillin and trypticase soy Ampicilline agar (FAO, 1979) and identification was applied according to Popoff and Veron (1976).
- *Y. enterocolitica*: Isolated on agar plates of Cefsulodin Irgasan Novobiocin by Oxoid antibiotic (CR log) (Schiemann, 1979) after enrichment in phosphate buffer saline and identified according to Speck (1984).
- *Erysipelothrix* spp.: Isolation of *Erysipelothrix* spp. were performed according to selective method (Hassanein *et al.*, 2001) using brain heart infusion (BHI) broth containing 0.1% Tween 80, 5% horse serum, 50 µg/ml gentamicin, 0.1% sodium azide, and 0.001% crystal violet and BHI agar containing 0.1% Tween 80, 50 µg/ml gentamicin, and 0.1% sodium azide.

D- Evaluation of different methods used for egg cooking to destruct some pathogens (Baker *et al.*, 1983; Chantarapanont *et al.*, 2000):

- Test organisms used: *Staph. aureus*, *E. coli* and *S. enteritidis* were grown in brain heart infusion broth at 37°C.
- Preparation of an egg for inoculation by the test organisms:
 - A small hole was made at the blunt end of an egg with a sterile drill.
 - Aseptically injection of an inoculum (0.5 ml) containing 1×10^8 c.f.u of the tested organism into the egg yolk by a sterile needle. Then the

hole is covered after injection by Ducocement. The egg was ready for cooking by one of the following methods:

- 1- **Boiling:** The inoculated eggs with test organisms were immersed in boiling water bath for 3, 5, 7 and 12 minutes then cooled and examined.
- 2- **Frying:** The contents of the inoculated eggs were poured into a sterile frying pan containing a small amount of oil and cooking still for 1, 4 and 12 minutes. Then cooled and examined.
- 3- **Omelets:** In a sterile greased aluminum pan the contents of inoculated eggs were baked in an oven at 163°C for 25 minutes then samples were taken for detection of the test organisms.

RESULTS

The results were shown in Tables 1-4

Table 1: Incidence of some pathogens of public health significance isolated from commercial Balady hen eggs.

Egg samples	Staph. aureus		Salmonella spp.				Listeria spp.								Aeromonas spp.	
			S. typhi		S. gallinarum		L. innocua		L. ivanovii		L. murrayi		L. grayi		A. hydrophila	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Shell	7	23.3	1	3.3	-	-	1	3.3	1	3.3	1	3.3	-	-	1	3.3
Shell mixed with membranes	4	13.3	-	-	1	3.3	1	3.3	1	3.3	1	3.3	2	6.7	-	-
Content	3	10.0	1	3.3	-	-	-	-	-	-	-	-	-	-	-	-

Table 2: Incidence of some pathogens of public health significance isolated from commercial farm hen eggs.

Egg samples	Staph. aureus		E. coli		Salmonella spp.				Aeromonas spp.						Yersinia enterocolitica	
					S. typhi		S. enteritidis		A. hydrophila		A. sobria		A. caviae			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Shell	7	23.3	1	3.3	1	3.3	5	16.7	2	6.7	1	3.3	1	3.3	1	3.3
Shell mixed with	4	13.3	1	3.3	-	-	3	10.0	1	3.3	-	-	-	-	1	3.3

Shell mixed with membranes	9	300	3	100	1	33	1	33	4	133	-	-	-	-	4	133	2	67	1	33	1	33
Content	103	-	-	-	-	1	33	1	33	-	-	-	-	12	400	3	100	3	100	2	67	

Table 4: Temperature - time relationship for destruction of some pathogens in eggs cooked by different methods.

Method of cooking	Time of cooking / minutes	Survival of some pathogens after cooking		
		<i>Staph. aureus</i>	<i>E. coli</i>	<i>S. enteritidis</i>
<i>Boiling</i>	3	+	+	+
	5	+	+	+
	7	-	+	+
	12	-	+	-
<i>Frying</i>	1	+	+	-
	4	+	+	-
	12	-	-	-
<i>Omelet (163 °C)</i>	25	-	-	-

DISCUSSION

Human infection due to consumption of infected eggs has now been reported in numerous countries all over the world. There has been steady increase in the recorded incidence of infection and the number of countries which reported this infection (Wieneke *et al.*, 1993; Ko and Chang, 1995). Worthily to mention that a single contaminated egg may result in the infection of a large number of people (Varnam and Evans, 1991).

The present study showed that *Staph. aureus* scored highest percentages of contamination among all pathogenic organisms isolated from the shell surfaces and shell mixed with shell membranes of all type of eggs examined (Tables 1, 2 and 3).

In case of commercial Balady hen eggs, *Staph. aureus* recorded 23.3 and 13.3% in the shell surfaces and shell mixed with shell membranes, respectively (Table 1). The obtained results were higher than data were recorded by Alaboudi *et al.* (1988) and El – Essawy *et al.* (1989). While, they were lower than those observed by Ahmed *et al.* (1985) and Bastawrows *et al.* (2002). The variability in these results may be referred to the health status of hens as the transovarian transmission of *Staph. aureus* to eggs which recorded by Mathes and Hanske (1977) or accidental transmission from shell (Mathes, 1984). Additional evidence in Table 1 indicating that low rate of contamination by *Staph. aureus* in egg contents of Balady eggs (10%) and this may related to the presence of lyzosome in the inner shell membranes which acts as an effective agent against Gram-positive bacteria minimizing the chance of interance of the organism to the egg content which constitutes a great threat to the human health specially children (Baker, 1974). It is apparent from Table 1, failed detection of *E. coli*, *S. paratyphi*, *S. enteritidis*, *Y. enterocolitica* and *Erysipelothrix* spp. from parts of commercial Balady hen eggs. Consequently, Balady eggs proved to be the best for consumption if compared by farm hen and duck eggs. Even the other *Salmonellae*, *Listeria* spp. and *A. hydrophila* could be isolated in low percentages, one sample for each (3.3%).

Data reported in Table 1 revealed failed detection of *L. monocytogenes* from the egg shells of all samples examined and this contrasted the results of Saad and El–Prince (1995) and Bastawrows *et al.* (2001) while, absence of *L. monocytogenes* from egg contents came in line with the same last investigators and contradictory to Leasor and Foegeding (1989). The obtained results may attribute to the unsuitability of pH of raw egg albumen for growth of *L. monocytogenes*. Furthermore, the presences of the antibacterial properties of the eggs which hydrolyse the polysaccharide bacterial cell wall causing cell lysis (Yadava and Vadehra, 1977). In addition, presence of *L. innocua* with significant level may competate isolation of *L. monocytogenes* (Pateran and Swanson, 1993).

L. innocua revealed 3.3 and 3.3% from egg shells and shell mixed with membranes (Table 1). It was more present than *L. monocytogenes* and other species of *Listeria* as reported by Pateran and Swanson (1993). Presence of *Listeria* spp. in eggs most likely is due to contamination from shells during the breaking process or from the environment (Foegeding and Leasor, 1989).

In case of commercial farm hen eggs, Table 2 showed that *Staph. aureus* could be isolated from shell surfaces and egg contents with incidence of 23.3% for each, while shell mixed with shell membranes revealed 13.3%. The present study clearly demonstrated that highly contaminated egg contents of farm eggs than Balady ones. Table 2 revealed the percentage of 3.3% contamination by *E. coli* in each of shells, shells mixed with shell membranes and egg contents of farm hen eggs. The obtained results were lower than that recorded by El–Essawy *et al.*, (1989). *E. coli* is a normal inhabitant of the intestinal tract of both man and animals and may contaminate

manure and this explains its presence in egg content and shell surface since the organism can grow and penetrate the shells contaminating the contents (Mayes and Takeballi, 1983) so, the organism is taken as index of recent fecal contamination (Garrad, 1946).

Certain strains of *E. coli* are responsible for egg deterioration and fishy flavors (Frazier and Westhoff, 1986). Likewise, high rate of contamination by *S. enteritidis* was recorded in commercial farm hen egg samples in shell surfaces, shell mixed with shell membranes and egg contents in percentages of 16.7, 10 and 10%, respectively. These results were in agreement with those reported by Shirota *et al.* (2001) who suggested that *S. enteritidis* was more associated with human food borne disease outbreaks than other *Salmonella* serotypes particularly those associated with eggs and egg products. Contamination of eggs by the organism occurs before the egg is laid before the formation of the shell (Humphrey, 1999). 90% of food borne Salmonellosis caused by *S. enteritidis* is through the shell of eggs (Schroeder *et al.*, 2005). *A. hydrophila* and *Y. enterocolitica* revealed high incidence (10%) from egg contents of farm eggs (Table 2). *Y. enterocolitica* could not be detected from egg contents by Favier *et al.* (2000) and Bastawrows (2002) while 3.3% could be isolated from shells and shells mixed with shell membranes in the present study. However, high incidence of *Y. enterocolitica* was recorded by Favier *et al.* (2000) and Bastawrows *et al.* (2001) from shell surfaces of eggs.

Y. enterocolitica is found in the chicken faeces and the shell can be contaminated with faeces in the nest or during subsequent manipulation (Berrang *et al.*, 1999). The variation in results could be attributed to the enrichment procedures and the selective media used which fail to recover low levels of clinical strains (Chester and Stotzky, 1976), beside the competition of other contaminants. *Listeria* spp. and *Erysipelothrix* spp. could not be isolated from all examined parts of commercial farm hen egg samples. From the public health point of view, farm hen eggs came secondary to Balady hen eggs for consumption.

The summarized results in Table 3 proved that commercial duck eggs were the worst for consumption. *A. hydrophila* and *Staph. aureus* were the predominant pathogens isolated from shell surfaces and egg contents of duck eggs in percentages of 43.3 and 40%, 36.7 and 33.3%, respectively. The obtained results were in accordance to Kumar *et al.* (2000) who detected that *A. hydrophila* was the predominant species isolated from poultry eggs in 51.52%, followed by *A. sobria* (39.39%) and *A. caviae*.

Shell surfaces can be contaminated congenitally from the carrier ducks which disseminate the organisms to widely distributed areas which receive the infected eggs for hatchability. Duck shell surfaces can be contaminated also from the excreta of farm animals live with ducks in the same place as a bad habit of the Egyptian farmer. Dhillon *et al.* (1974) stated that contaminated water, environment and intestinal tract are the main sources of shell contamination. Shell mixed with shell membranes of commercial duck eggs also recorded high incidence of infection by *Staph. aureus* in incidence of 30%, *E. coli* in 10% and 13.3% by *S. enteritidis* and *A. hydrophila* (for each). *S. typhi*, *S. paratyphi*, *A. caviae* and *Y. enterocolitica* recorded 3.3% for each (Table 3). Bad habits of ducks as laying eggs near dirty and damp places, in addition to the rapid deterioration of the antibacterial activity of albumen on storage give the chance to raise the rate of contamination in duck eggs.

Eggs are one among the major animals foods mostly marketed raw and frequently consumed raw, semi-raw in many dishes and form an important part of meals contain raw eggs as an essential ingredient (home made ice cream, mayonnaise, eggnog etc.). These dishes are not heated up to the (FAO, 1979) recommended temperatures, 155F° for at least 15 seconds (Mermelstein, 2001) and this is not enough to render an egg free from pathogenic organisms as yolk is high nutritive medium permits multiplication of the organisms. Several methods of microbial destruction were discussed by Serrano *et al.* (1997) and Brackett *et al.* (2001). Table 4

clarified that boiling procedure used for destruction of the inoculated test organisms is not enough at 3 and 5 minutes. While, boiling for 7 minutes was enough to destroy *Staph. aureus* only. By elongation the time of cooking for 12 minutes, destruction of *S. enteritidis* was obvious, while *E. coli* still be alive.

The results came in line with Baker *et al.*, (1983); Schuman *et al.*, (1997) and Soliman and El-Tabiy (2007). The present study demonstrated a combined relationship between destruction of the test organism and the time-temperature used during the method of eggs cooking. Following up Table 4 it clarified that by applying the open frying on one side all the test organisms destroyed at 12 minutes. *Staph. aureus* and *E. coli* could be isolated when fried for 1 and 4 minutes. However, *S. enteritidis* was completely destroyed and could not be isolated. The results were in contrast to Baker *et al.* (1983).

Table 4 illustrated that all the test organisms were completely destroyed in the omelets at 163C° for 25 minutes. This evidence due to high temperature and long time during omelet cooking procedure. The same results were recorded by Baker *et al.* (1983). The obtained results recommended that omelet procedure is the best for consuming safe eggs while, frying and boiling methods were inadequately to destroy some of the test organisms. Boiling must be adopted for more than 12 minutes to ensure complete destruction of pathogens may contaminate eggs. Heat treatment – time temperature conditions aims to achieve a decrease in the number of viable organisms (Stadelman *et al.*, 1996 and Schuman *et al.*, 1997).

There is a considerable demand for using high temperatures during cooking of eggs to destroy the present pathogens. Gossett and Baker (1981) studied the textural problems and greenish discoloration which affected eggs due to high temperatures used and suggested the addition of citric acid which gives favorable effects due to thermal destruction of microorganisms.

So, in order to remove or reduce the risk of some of pathogenic organisms of public health importance contaminate eggs, there are several points must be adopted. Chosen of healthy mother's hens are necessary to obtain eggs of free pathogens. Hygienic measures applied in the farms during handling and storage. Using of hot soapy water with those come in contact with eggs and egg containing foods in work areas. Eggs must be held at low temperature (5 °C) to prevent proliferation of the pathogens. Cleaning with sanitizer minimizes the contamination of the shells, beside pasteurization of egg products as statutory requirements in many countries. Educational programs for consumers informed the risks resulted from eating under cooked eggs particularly the elderly and immune-compromised persons who are more susceptible to infection.

REFERENCES

- Abouzeed, Y.M.; Hariharan, H.; Poppe, C. and Kibenge, F.S. (2000): Characterization of Salmonella isolates from beef cattle, broiler chickens and human sources on Prince Edward Island. *Comp. Immunol. Microbiol. Infect. Dis.*, 23 (4): 253–266.
- Ahmed, A.H.; Moustafa, M.K.; Aboul – Khier, F. and El – Bassiony, T. A. (1985): Bacterial contamination of egg shells. *Assiut Vet. Med. J.* 14 (27): 122–127.
- Alaboudi, A.R.; Hamed, D.A. and Ali, D.S. (1988): Microbial content of market eggs. *Indian J. Anim. Sci.*, 58 (7): 768–770.

- Bahout, A.A. (2001): Influence of shell quality on bacterial infection of the commercial hen's Eggs. Ph. D. Thesis, Fac. Vet. Med., Zagazig Univ., Egypt.
- Baker, R.C. (1974): Microbiology of eggs. J. Milk Food Technol. 37: 265–268.
- Baker, R.C.; Hogartv, W.P. and Vadehra, D.V. (1983): Survival of *Salmonella typhimurium* and *Staphylococcus aureus* in Eggs cooked by different methods. Poultry Science. 62: 1211–1216.
- Bastawrows, A.F.; Sayed, A.M.; Thabet, A.R.; El Sharnouby, R. and Barakat, A. (2001): Microbiological profile of commercial hen's eggs in Assiut governorate. Part 1: Occurrence and significance of *Listeria* species, *Yersinia enterocolitica* and some important molds in hen's eggs. Assiut Vet. Med. J. 45 (89).
- Bastawrows, A.F.; Sayed, A.M.; Makar, N.H. and Thabet, A.R. (2002): Microbiological profile of commercial hen's eggs in Assiut governorate. Part 2: *Campylobacter jejuni* and *S. aureus* organisms in hen's eggs. Assiut Vet. Med. J. 45 (89).
- Berrang, M.E.; Frank, J.F.; Buhr, R.J.; Bailey, J.S. and Cox, N.A. (1999): Egg shell membrane structure and penetration by *Salmonella typhimurium*. J. Food Prot. 62: 73–76.
- Biendo, M.; Laurans, G.; Thomas, D.; Dechepy, O.; Hamdad – Daoudi, F.; Canarelli, B. and Fb, F. (2003): Regional dissemination of *Salmonella enterica* serovar enteritidis in season dependent. Clin. Microbiol. Infect. 9 (5): 360–369.
- Board, R.G. and Fuller, R.C. (1994): Microbiology of the Avian Egg. 1st. Ed. Chapman and Hall. PP. 94–128.
- Brackett, R.E.; Schman, J.D.; Ball, H.R. and Scouten, A.J. (2001): Thermal inactivation Kinetics of *Salmonella* spp. Within intact egg heated using humidity controlled air. J. Food Prot., 64 (7): 934–938.
- Bradshaw, J.G.; Peeler, J.T.; Corwin, J.J.; Hunt, J.M.; Tierney, J.T.; Larkin, E.P. and Twedt, R.M. (1985): Thermal resistance of *Listeria monocytogenes* and *E. coli* O157:H7. J. Food Prot. 48: 743–745.
- Brooks, G.F.; Butel, J.S.; Nicholas, O.L.; Jawetz, E.; Melnick, J.L. and Adelberg, E.A. (1995): Medical Microbiology. 20th Ed. Prentice. Hall international Inc. PP. 206–217.
- Chantarapanont, W.; Slutsker, L.; Tauxe, V. and Beuchat, L.R. (2000): Factors Influencing inactivation of *Salmonella enteritidis* in hard cooked eggs. J. Food Prot. 1: 36–43.
- Chester, B. and Stotzky, G. (1976): Temperature dependent cultural and biochemical characteristics of rhamnose positive *Yersinia enterocolitica*. J. Clin. Microbiol. 3: 119–127.
- Curtis, G.D.; Mitchell, R.G.; King, A.F. and Griffin, E.J. (1989): A selective differential medium for isolation of *Listeria monocytogenes*. Lett. Appl. Microbiol. 8: 95–98.
- Dhillon, A.S.; Maurer, A.J.; Deibel, R.H. and Haller, R.W. (1974): Feeding of different levels of Salmonellae to chickens. Indian J. Poult. Sci. 9: 103–107.
- El- Essawy, H.A.; Saudi, A.M. and Sallam, S.S. (1989): Microbiological studies on market hen eggs. Alex. J. Vet. Sci. 5 (2): 219–225.
- Eley, A.R. (1996): Microbiol Food Poisoning. 1st Ed. Chapman and Hall publisher, London, New York.

- FAO. (1979): Manuals of Food Quality Control (4-microbiological analysis) Rome.
- Favier, G.I.; Escudero, M.E.; Velazquez, L. and Guzman, A.M. (2000): Reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria in egg shell by washing with disinfectants and their effect on the shell microstructure. *Food Microbiol.* 17: 73–81.
- Finegold, S.H. and Martin, W.J. (1982): Bailly and Scott. *Diagnostic Microbiology* 6th Ed. Mosby Co. St., Louis, Toronto, London.
- Foegeding, P.M. and Leasor, S.R. (1989): Heat resistance and growth of *Listeria monocytogenes* in liquid whole egg. *J. Food Prot.* 56 (7): 616–618.
- Frazier, W.C. and Westhoff, D.C. (1986): *Food Microbiology*. 6th reprint Tata McCraw Hill Publishing Co. Ltd. New Delhi.
- Garrad, E.H. (1946): Coliform contamination of eggs. *Cand. J. Research.* 24 (C): 121–125.
- Gossett, P.W. and Baker, R.C. (1981): Prevention of green – gray discoloration in cooked liquid whole eggs. *J. Food Sci.* 46: 328–331.
- Hassanein, R.; Sawada, T.; Kataoka, Y.; Itoh, K. and Suzuki, Y. (2001): Serovars of *Erysipelothrix* species isolated from the tonsils of healthy cattle in Japan. *Vet. Microbiol.* 82, 97-100.
- Hitchins, A.D. (1995): *Listeria monocytogenes*. In 8th Ed. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC International Pub. Co., Gaithersburg, MD, USA.
- Humphrey, T.J. (1999): Contamination of eggs and poultry meat with M. E. Potter, and P. G. Wall (ed.), *Salmonella enterica* serovar enteritidis in humans and animals. *Epidemiology, pathogenesis and control*, Iowa State University Press, Ames, IA, PP. 183–192.
- Janda, J.M.; Brenden, R. and Botton, E.J. (1984): Differential susceptibility to human serum by *Aeromonas* Spp. *Curr. Microbiol.* 11: 325–328.
- Keshimaki, M.; Eklund, M.; Pesonen, H.T. and Siitonen, A. (2001): EPEC, EAEC and STEC in stool specimens: Prevalence and molecular epidemiology of isolates. *Diagn. Microbiol. Infect.* 40 (4): 151–156.
- Ko, H.C. and Chang, T.Y. (1995): Using the reversed passive latex agglutination method to detect enterotoxigenic *Staphylococcus aureus* and enterotoxin in foods. *J. Food & Drug Analysis.* 3: 57–63.
- Kumar, A.; Bachhil, V.N.; Bhilegaonakar, K.N. and Agarwal, R.K. (2000): Occurrence of entero-toxigenic *Aeromonas* species in foods. *J. Commun. Dis.* 32: 169–174.
- Lambiri, M.; Mavridou, A.; Richardson, S.C. and Papadakis, J.A. (1995): Isolation of *Salmonella* from animal feeds and the environment during 1985–1990 in Greece. *Acta Microbiologica Hellensca.* 40 (4): 297–302.
- Leasor, S.B. and Foegeding, P.M. (1989): *Listeria* Spp. In commercially broken raw liquid whole egg. *J. Food Prot.* 52: 777–780.
- Mathes, S. (1984): Diminution of egg quality caused by avian diseases and microbiological contamination. *J. World's poul. Sci.* 40 (81).

- Mathes, S. and Hanscke, J. (1977): Experimentelle unter – suchungen Zur Ubertragung Von Bakterien Uber das huhnerei. Bert. Munch Tierar Zti. Wshr. 90: 200–203.
- Mayes, F.J. and Takeballi, M.A. (1983): Microbiol contamination of the hen's eggs. J. Food Prot. 46 (12): 1092–1098.
- Mermelstein, N.H. (2001): Pasteurization of Food of shell eggs food technology. December, 72: 73–79.
- Moats, W.A (1980): Classification of bacteria from commerical egg washers and washed and unwashed eggs. J. Appl. Environ. Microbiol. 4: 710–714.
- Moore, J. and Madden, R.H. (1993): Detection and incidence of *Listeria* species in blended raw eggs. J. Food Prot. 56: 652–654.
- Pateran, R.L. and Swanson, K.M. (1993): Simultaneous growth of *Listeria monocytogenes* and *Listeria innocua*. J. Food Prot. 56: 616–618.
- Popoff, M. and Veron, M. (1976): A taxonomic study of the *Aeromans hydrophila*. *Aeromonas pancctata* group. J. Gen. Microbiol. 94: 11–22.
- Quinn, P.J.; Carter, M.E.; Markery, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology. Wolfe pub. Europ Limited 209–236.
- Quiroga, M.; Oviedo, P.; Chinem, I.; Pegels, E.; Husulak, E.; Binztein, N.; Rivas, M.; Schiavoni, L. and Vergora, M. (2000): A symptomatic infections by diarrheagenic *Escherichia coli* in children from Misiones, Argentina, during the first twenty months of their lives. Rev. Inst. Med. Trop. Sao Paulo, 42 (2): 9–15.
- Saad, Nagah, M. and El – Prince, Enas, M. (1995): Prevalance of *Listeria* species in Hen's Eggs sold in Assiut City. Assiut Vet. Med. J. 33 (65).
- Sabreen, M.S. (2001): Search for some pathogenic bacteria in commercial hens and ducks' eggs sold in Assiut Governorate. Assiut Vet. Med. J. 45 (89): 91–103.
- Schiavoni, L. and Vergora, M. (2000): A symptomatic infections by diarrheagenic *Escherichia coli* in children from Misiones, Argentina, during the first twenty months of their lives. Rev. Inst. Med. Trop. Sao Paulo, 42 (2): 9–15.
- Schiemann, D.A. (1979): Association of *Yersinia enterocolilica* with the manufacture of cheese and occurrence in pasteurized milk. App. Environ. Microbiol. 36, 27 : 4-7.
- Schroeder, C.M.; Alecia, L.N.; Wayne, D.S.; Allan, T.H.; Frederick, J. A.; Jonathon, S.R.; Eric, D.E.; Terry, D.W.; Kristin, G.H. and David, P.G. (2005): Estimate of illness from *Salmonella enteritidis* in eggs, United States, 2000. Emerging Infections Diseases, 11 (1): 113–115.
- Schuman, J.D.; Shelaon, B.W.; Vandepopuliere, J.M. and Ball, H.R. (1997): Immersion heat treatments for inactivation of *Salmonella enteritidis* with intact eggs. J. Appl. Microbiol. 83: 438–444.
- Serrano, L.E.; Murano, E.A.; Shenoy, K. and Olson, D.G. (1997): D values of *Salmonella enteritidis* isolates and quality attributes of shell eggs and liquid whole eggs treated with irradiation. Poult. Sci. 76: 202–205.

- Shirota, K.; Katoh, H.; Murasa, T.; Ho, T. and Otsuki, K. (2001): Monitoring of layer feed and eggs for *Salmonella* in eastern Japan 1993 and 1998. J. Food Prot. 64 (5). 734–737.
- Sionkowski, P.J. and Shelef, L.A. (1990): Viability of *Listeria monocytogenes* strain Brie – 1 in the avian egg. J. Food Prot. 53 (1): 15–17.
- Soliman, Zienab, I. and El – Tabiy, Azza, A. (2007): A study on effect of immersion heat treatment on viability of *Salmonella enteritidis* in table eggs. Assiut Vet. Med. J. 53 (115).
- Speck, M.L. (1976): Compendium of Methods for Microbiological Examination of Food. American Public Health Association, Washington, D.C.
- Speck, M.L. (1984): Compendium Method for Microbiological Examination of Food. American Public Health Association, Washington, D.C.
- Stadelman, W. J., Singh, R. K., Muriana, P. M. and Hou, H. (1996): Pasteurization of eggs in the shell. Poult. Sci. 75: 1122–1125.
- Urio, E.M.; Collison, E.K.; Gashe, B.A.; Sebunya, T.K. and Mpuchane, S. (2001): *Shigella* and *Salmonella* strains isolated from children under 5 years in Gaborone, Botswana and their antibiotic susceptibility patterns. Trop. Med. Int. Health. Jap. 6 (1): 55–59.
- Varnam, A.H. and Evans, M.G. (1991): Foodborne Pathogens, Wolfe Pub. Ltd, Aylesbury, England.
- Walker, S.J. (1986): *Yersinia enterocolitica* and *Yersinia enterocolitica* like bacteria in milk. Doctoral Thesis: Queen's Univ. Belfast.
- Wieneke, A.A.; Reberts, D. and Gilbert, R.J. (1993): Staphylococcal food poisoning in the United Kingdom, 1969–1990. Epidemiol. Infect. 110: 519.
- Wyah, G.M. (1992): Immunoassays for Food Poisoning Bacteria and Bacterial toxins. 1st Ed., Chapman & Hall, PP: 5–13.
- Yadava, N.K. and Vadehra, D.V. (1977): Mechanism of egg white resistance to bacterial growth. J. Food Sci. 42: 97–99.
- Sci. 42: 97–99.