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MICROBIOLOGICAL PROFILE OF COMMERCIAL HENS' EGGS IN ASSIUT GOVERNORATE
Part 2: *CAMPYLOBACTER JEJUNI* AND *STAPHYLOCOCCUS AUREUS* ORGANISMS IN HENS' EGGS

(With One Table)

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الحالة الميكروبيولوجية لبيض الفراخ التجارى في محافظة أسيوط
الجزء الثاني: ميكروبات الكامبيلوباكتر جيوجيناى والمكور العنقودي الذهبي في بيض الدجاج

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للتعرف على تواجد ميكروبى الكامبيلوباكتر جيوجيناى والمكور العنقودي الذهبي الضار في البيض تم جمع عدد ٣٠٠ بيضة ممثلة في ستون مجموعة كل مجموعة مكونة من خمس بيضات وذلك من أسواق ومحلات البقالة والسوبر ماركت ومنازل الفلاحين بمدينة أسيوط - تم تجهيز البيض المراد فحصه لمحاولة عزل هذه الميكروبات من على السطح الخارجى للقشرة وكذلك من المحتويات الداخلية للبيض ولقد تبين من هذه الدراسة أن ميكروب الكامبيلوباكترجيوجيناى يتواجد بنسبة ٨,٣٣% من على سطح القشرة ولم يستدل على الميكروب في محتويات البيض المفحوص ولقد تم عمل تصنيف بيوكيميائي لعترات ميكروب الكامبيلوباكترجيوجيناى المعزولة ولقد تبين من التصنيف أن أربعة عترات تقع تحت التصنيف (I) وعترة واحدة تحت التصنيف(II) . أما بخصوص ميكروب المكور العنقودي الذهبي قد تبين من الدراسة أن ٢٨,٣٣%، ٣٦,٦٧% من قشر البيض و١٨,٣٣%، ٢٣,٣٣% من محتويات البيض تحتوى على ميكروب المكور العنقودي الذهبي على الأوساط الغذائية شابمان مانيتول سولت اجار والبيرد باركر أجار على الترتيب وتم مناقشة الأهمية الصحية والسبل الكفيلة للمحافظة على البيض من التلوث بهذه الميكروبات.

SUMMARY

300 of fresh commercial hens' eggs representing 60 groups were collected at random in summer and winter months from Assiut markets, groceries, supermarkets and farmer's houses. Both shells and contents of the eggs were examined bacteriologically for the presence of *Campylobacter jejuni.* (*C.jejuni*) and *Staphylococcus aureus* (*S. aureus*). *C jejuni* was isolated from 5 (8.33%) shell surfaces but not from egg contents. Biotyping of *C.jejuni* isolates revealed that 4 isolates were biotype 1 and one strain was biotype 2. Concerning, *S.aureus*, the organism was existed in 17 (28.33%) and 22 (36.67%) out of 60 shell surfaces using Chapman "mannitol salt" agar and Baird parker agar media, respectively. On the other hand, out of 60 egg content samples examined, 11 (18.33%) and 14 (23.33%) were contaminated by *S.aureus* using Chapman "Mannitol salt" agar and Baird Parker agar media, respectively. The Significance of *C.jejuni* and *S.aureus* as foodborne pathogens was discussed and suggested measures for improving the quality of produced hens' eggs are given.

Key words: *Campylobacter jejuni: Staphylococcus* - hens' eggs

INTRODUCTION

There is an increasing evidence that *Campylobacter jejuni* can cause gastroenteritis in human through consumption of contaminated water and foods including eggs (Acuff *et al.*, 1982 and Finch and Blake, 1985).

Unlike salmonella, campylobacters do not multiply in food so they seldom cause explosive outbreaks of food poisoning. Some 48 foodborne outbreaks have been reported worldwide. Two outbreaks of campylobacter enteritis have arisen from the consumption of raw frozen or inadequately treated eggs (Skirrow, 1998). *C.jejuni* could be isolated occasionally from the inner shell and membranes of refrigerated eggs suggesting that the organism may contaminate liquid eggs which may contain shell membranes after the breaking procedures (Doyle, 1984). Moreover, Hanninen *et al.* (1984) studied the behaviour of *C.jejuni* in liquid egg, at 37°C and found that the organism grew

slowly in either egg yolk or homogenized whole egg, while at 20°C *C.jejuni* died slowly in these materials.

Staphylococcus aureus is an important foodborne pathogen and the thermostable enterotoxins -(the usual level found in foods is about 0.5-10μg/100g. food)- elaborated by this organism is of serious concern to public health aspects (Wyah, 1992). Although staphylococci may be found growing in large numbers in foods and animal products (e.g. milk, cheese, eggs and meat, etc), it is generally believed that the primary sources are human handlers or animals (Kloos, 1980). There have been many research works that dealt with *S.aureus* in and on hen's eggs. (Moats, 1980; El-Prince, 1988 and Sabreen, 2001). The purpose of such work was to assess the incidence of *C.jejuni* and *S.aureus* on the shells and in the contents of commercial hens' eggs sold in Assiut city (Egypt), Biotyping of *C.jejuni* isolates, in addition to the comparison of the sensitivity and specificity of two media used for isolation of *S.aureus*.

MATERIAL and METHODS

(I) Collection of samples:

300 of fresh commercial hens' eggs of both native breeds and poultry farms. The samples were collected at random in summer and winter seasons from Assiut city markets, different groceries, supermarkets and farmer's houses. Every 5 eggs (one group) were placed in sterile bag and dispatched to the laboratory with a minimum of delay.

(II) Preparation of Samples:

(A) Egg Shells:

Egg shells were tested by a surface rinse method as described by Moats (1979).

(B) Egg Contents:

The egg was prepared for evacuation of its content according to Speck (1976).

(III) Experimental techniques:

The rinse solution of egg shells, as well as, the homogenous egg contents were subjected to the following examination.

A) Isolation and identification of *Campylobacter jejuni* were carried out according to Doyle (1984) and Barret *et al.* (1988), using preston's media (Bolton and Robertson, 1982).

B) Isolation and identification of *Staphylococcus aureus* were carried out according to the methods recommended by Finegold and Martin (1982), using Chapman medium (Mannitol salt agar) "B30, Difco" and Baird Parker medium "C. 1116-B0293, Biolife S.r.L".

RESULTS

The results are recorded in Table 1.

Table 1: Frequency of isolation of *Campylobacter jejuni* and *staphylococcus aureus* from examined samples.

Type of Egg Samples	No. of examined group samples	Positive samples							
		Type of microorganisms							
		C. jejuni		Total		*S.aureus*			
		C. jejuni Biotype I	*C. jejuni* Biotype II			Chapman Mannitol medium		Baird Parker medium	
		No.	No.	No.	%	No.	%	No.	%
Shells	60	4	1	5	8.33	17	28.33	22	36.67
Contents	60	-	-	-	-	11	18.33	14	23.33

DISCUSSION

In recent years, there has been a significant increase in the incidence of food poisoning outbreaks caused by *Campylobacter jejuni*. It is viewed as pathogena of "emerging public health significance" (Blaser, 1982 and Patel and Sannabhadti, 1991).

Results of shell egg screening studies indicate that *C.jejuni* was detected in 5 (8.33%) out of 60 hens' egg shells. Moreover, 4 out of the 5 isolates were identified as being *C.jejuni* biotype (I) and the remaining one was belonging to *C.jejuni* biotype 2 (Table, 1) Our results support the conclusion that *C.jejuni* biotype 1 was more prevalent than biotype (II) (Wieliczko, 1995 and Sayed, 2000).

All positive shell surface of hens'eggs samples recovered in this study were collected in winter (4 samples) and one in the summer months as the prevaling climitic condition is very hot in upper Egypt in summer months where *C.jejuni* will not grow but rather dies off relatively rapidly at this temperature. This conclusion substantiate those reported by Doyle and Roman (1981) and Doyle and Roman (1982) who concluded that eggs stored at room temperature (25°C) or warmer for more than 48h are not likely to contain detectable cells of

C.jejuni. Therefore, one can assume that the incidence of *C.jejuni* among egg samples denoted the season occurrence of the organism. Although limited sample numbers and seasonally odd sampling times made firm conclusions impossible.

Within the limits of the experimental procedure and number of samples examined the data of the present study suggest that *C.jejuni* was present on the shell of 8.33% of the eggs (Table, 1). This figure was higher than that obtained by Doyle (1984); Moustafa (1993); and Zaki and Reda (1995) who isolated the organism from the same material at incidence rate of 0.88%, 2% and 1.8%, respectively. *C.jejuni* is commonly associated with poultry either broilers or layers (Zaki and Reda, 1995), and there is thus the possibility that egg shells can become contaminated as a result of intestinal carriage of this important pathogen. However, Doyle (1984) suggested that the presence of the organism in the intestinal tract of the hens does not adversely affect their egg laying productivity.

Information derived from the results reported in Table 1 revealed that *C.jejuni* could not be isolated from any of samples of egg contents. This may be attributed to two reasons, firstly, *C. jejuni* does not easily penetrate the egg shell (Doyle, 1984), but when it does, it does not survive for more than 6h in egg content (Neill *et al.*, 1985) this may be due to a bacteriocidal effect of the albumen (Hanninen *et al.*, 1984). Secondly, prophylactic antibiotics may also influence campylobacter infection (Annan-Prah and Jane, 1988 and Watson *et al.*, 1992). Moreover, Compylobacter organisms are markedly labile organisms, being sensitive to environmental conditions such as chilling and drying (Koidis and Doyle, 1983 and Wyah, 1992). In conclusion, results of egg screening indicate that *C.jejuni* is not likely to contaminate the content of sound eggs. This conclusion substantiates what have been reported by Doyle (1984) who failed to isolate the organism from 226 egg content samples. Furthermore, Neill *et al.* (1985) immersed hens'eggs in a suspension of *C.jejuni* and, although the organism was recovered from shell membranes, all yolk and albumen samples were campylobacter negative. On the other hand, a contradictory finding was given by Moustafa, (1993) who could isolate the organism from 4% hens'eggs contents.

C.jejuni is responsible for food poisoning and the symptoms include abdominal pain and severe diarrhoea, often leading to prostration, the illness can last up to 2 weeks. The infective dose has

been shown to be as low as 500 bacterial cells (Wyah, 1992). The pathogenic mechanisms by which campylobacter causes diarrhea in humans seem to be adhesion of the mucous membrane, toxin production, and/or invasion of the epithelial cells, since the clinical effect varies between watery to bloody diarrhea (Lindblom and Kaijser, 1995).

As shown in Table 1, out of the examined samples 17 (28.33%) and 22 (36.67%) of shell surfaces were contaminated with *Staphylococcus aureus* by using Chapman "Mannitol salt agar" and Baird Parker media respectively. Lower incidence (26.7%) was reported by Sabreen (2001), but higher figure (80%) was reported by Ahmed *et al.* (1985) by using the same media. While out of 60 groups of egg content samples, 11(18.33%) and 14 (23.33%) yealding *S.aureus* by using the media mentioned above, respectively. This finding disagreed with that reported by Ahmed *et al.* (1987); Alaboudi *et al.* (1988); El-Essawy *et al.* (1989) and Sabreen (2001) who recorded lower incidences (14.28%, 7.4%, 3.33% and 13.3%) respectively.

One can only speculate as to why such variability exists. The health status of hens, the hygienic measures adopted in the farm or during handling and storage of eggs may influence the existence and spread of *S.aureus* in eggs. Methods used to obtain and transport samples and to isolate the organism may also be important factors.

Although, *S.aureus* presence in shell eggs is very rare, yet hens'eggs get contamination with the organism either as a result of transoverian transmission (Mathes and Hanschke, 1977) or accidentally from shell (Mathes, 1984).

As shown in Table 1, Baird Parker medium showed a high percentage of isolation in comparison to Chapman "Mannitol salt agar" medium. Therefore, Baird Parker is the medium could be used for isolation *S.aureus* from eggs as it permits the detection of very small number of the organism.

It is apparent that the rate of isolation of *S.aureus* recovered from shells are higher than those from egg contents. This may be due to two reasons firstly, the presence of lysozyme in the inner shell membrane which act as an effective agent against gram-positive organisms, thus the chance of *S.aureus* enterance into the contents is very remote (Barker, 1974). Secondly, the shell is more liable to be contaminated.

Several outbreaks of *S.aureus* food poisoning have been recorded, involving large number of individuals through out the world (Eley, 1992; Wieneke *et al.*, 1993 and Ko and Chang, 1995). The heat stable enterotoxin probably acts on the lining of the abdomen and the onset of symptoms is characteristically rapid, normally within 6 hours of ingestion of the food. Nausea, vomiting and diarrhoea are usual, with collapse of the sufferer in severe cases (Wyah, 1992).

The isolation of 14 strains of *S.aureus* from the content of 300 eggs in this study, constitutes a great threat to the health of humans especially children and some individuals, especially athletics who drink freshly beaten eggs. Thus, proper farm hygiene, handling and storage of eggs are necessary for obtaining eggs safe from the potential hazards of this prevalent pathogen.

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