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SEARCH FOR SOME PATHOGENIC BACTERIA IN COMMERCIAL HENS AND DUCKS' EGGS SOLD IN ASSIUT GOVERNORATE

(With 5 Tables)

By
M.S. SABREEN

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البحث عن بعض البكتريا الممرضة في بيض الدجاج والبط بمحافظة أسيوط

محمد سعد صابرين

جمعت ٢٢٥ بيضة ممثلة لـ ٤٥ مجموعة بطريقة عشوائية من مختلف الأسواق ومحلات البقالة والسوبر ماركت ومزارع الدواجن المعدة للاستهلاك الأدمي وكذلك من منازل الفلاحين في محافظة أسيوط. وقد شملت عينات البيض ثلاثة أنواع وهي: بيض الدجاج التجاري بنوعيه من السلالات البلدية ومزارع الدواجن وكذلك بيض البط. وقد تم فحص العينات لمعرفة مدى تلوثها ببعض البكتريا الممرضة المحتمل وجودها على قشر وفي محتوى البيض الداخلي. وأظهرت النتائج أن ميكروب الليستريامونوسيتوجينس كان موجوداً بنسبتي ٦,٧% ، ١٣,٣% على قشر بيض مزارع الدواجن والبط على التوالي. أما ميكروب الليستريا أنكوا فقد تم عزله بنسبتي ١٣,٣% ، ٦,٧% من قشر بيض الدجاج بنوعيه على الترتيب. وكذلك تم عزل ٦,٧% من ميكروب الليستريامبورا من على قشر بيض الدجاج البلدي والبط بنفس النسبة (٦,٧%) . وفيما يختص بمحتوى البيض الداخلي فلقد كانت عينة واحدة فقط (٦,٧%) من بيض الدجاج البلدي ملوثة بميكروب الليستريانكوا . أما بالنسبة للميكروب المكور العنقودي الذهبي فقد تم عزله من قشر بيض الدجاج بنوعيه وكذلك قشر بيض البط بالنسب المئوية التالية على التوالي : ٢٦,٧، ٢٦,٧، ٢٠% . وكذلك تم عزل هذا الميكروب من محتوى البيض الداخلي لبيض الدجاج البلدي بنسبة ١٣,٣% . وفيما يتعلق بالبكتريا المعوية – فقد تم عزلها وتصنيفها من عينات البيض المفحوصة ، فقد بينت النتائج أن ميكروب السالمونيلا تيفي ميوريم كان موجودا بنسبتي ١٣,٣% ، ٦,٧% على قشر بيض مزارع الدواجن ومحتوي بيض الدجاج البلدي على الترتيب. أما ميكروب الشيجيلا ، فقد تم عزله بنسبة ٦,٧% من قشر بيض الدجاج التجاري بنوعيه. وكذلك أظهرت النتائج أن النسب المئوية الآتية : ٤٦,٧ ، ١٣,٣ ، ٣٣,٣ ، ٦,٧ ، ٦,٧ ، ٢٠% لقشر ومحتويات عينات البيض المفحوصة كانوا ملوثين بميكروب الأيشيريشيا كولاي على التوالي. كما أسفرت النتائج عن عزل الميكروبات التالية : الأنتيروباكتر، الستروباكتر ، الكلبسيلا، البروفيدنشيا، البروتيس ريتجرى، البروتيس فولجارز، الأريزونا، الألكاجينز دسبار، السيراتيا، السيدوموناس صايوجينس من على قشر ومحتويات العينات المفحوصة لبيض الدجاج بنوعيه

وكذلك بيض البط بنسب مئوية مختلفة . هذا وقد اتضح من هذه الدراسة أن قشر البيض أكثر تلوثا ببعض البكتريا الممرضة عن نظيره من محتوى البيض. وقد نوقشت الطرق الواجب إتباعها لمنع تلوث البيض بهذه البكتريا الممرضة والتي لها أثر خطير على صحة الإنسان.

SUMMARY

A total of 225 random eggs, representing 45 groups, were collected from different markets, groceries and supermarkets, as well as, from poultry farms and farmers' houses in Assiut Governorate. They included commercial hens' eggs of both native breeds and poultry farms and ducks' eggs. The samples were examined for the presence of some pathogenic bacteria on shells and in the contents. L. monocytogenes was isolated from 6.7 and 13.3% of egg shells of poultry farms and ducks, respectively. L. innocua was detected on egg shells of commercial hens' eggs of both native breeds and poultry farms with an incidence of 13.3 and 6.7%, respectively. While, L. murrayi could be isolated from one sample (6.7%) each of shells of native breeds hens and ducks' eggs. On the other hand, L. innocua was isolated from one sample (6.7%) of egg content of native breeds hens. Staph. aureus was existed in 26.7, 26.7 and 20% on egg shells of hens of both native breeds and poultry farms and ducks, respectively. However, only two samples (13.3%) of commercial hens' egg contents of the native breeds contained Staph. aureus. Sal. typhimurium could be isolated, respectively, from 13.3 and 6.7% of examined samples of egg shells of poultry farms and egg contents of native breeds hens. Furthermore, each of shells of native breeds hens and poultry farms' eggs were contaminated by Shigella spp. in one sample (6.7%). While, E.coli was isolated from 46.7, 13.3 and 33.3% of the examined egg shell samples of both commercial hens and ducks, respectively, and also this bacterium was detected in 6.7, 6.7 and 20% of the examined egg content samples, respectively. Likewise, Enterobacter spp., Citrobacter spp., Klebsiella spp., Providencia spp., Proteus rettgeri, Proteus vulgaris, Arizona spp., Alcaligenes dispar, Serratia spp. and Pseudomonas cyanogenes were recovered from the examined samples of shells and contents of hens and ducks' eggs at varying percentages. It is apparent that egg shells were more contaminated with some pathogenic organisms than egg contents. The public health hazards of such pathogenic bacteria and the suggestive measures for improving the quality of eggs were discussed.

Key words: *Pathogenic bacteria, Hens, Ducks', Eggs.*

INTRODUCTION

Eggs provide a unique, well balanced source of nutrients for all persons of all ages. Eggs and egg products are used in a wide variety of foods, including whole egg custard, mayonnaise, egg salad, egg nog and all types of bakery products. On the other hand, eggs may constitute, if contaminated, a public health hazard and may lead to losses from economic point of view through spoilage. Sources of eggs contamination are numerous such as the egg may become infected before it is laid, when the ovary was infected with bacterial pathogens, also outside eggs are not sterile and the shells soon become contaminated by faecal matter from the bird, by the lining of the nest, by wash water if the eggs are to be washed, by handling and perhaps by the material in which the eggs are packed (Gordon and Tuker, 1965; Dhillon et al., 1974; Matthes, 1984; Foegeding and Leasor, 1989; Board and Fuller, 1994 and Cox et al., 2000). The rate of penetration of these organisms from the intact shell contaminating the egg contents influenced by humidity and storage temperature at which the eggs are produced and stored (Board and Fuller, 1994 and Cox et al., 2000).

Some pathogenic bacteria such as Listeria (especially, L. monocytogenes), Staphylococcus aureus, Salmonella, Shigella, Escherichia coli and other Enterobacteriaceae could be isolated from the shells and contents of marketable eggs at variable percentages by different investigators (Garrad, 1946; Board and Board, 1968; Board, 1977; Moats, 1980; Moursy et al., 1982; Ahmed et al., 1985 & 1987; El-Prince, 1988; Foegeding and Leasor, 1989; Saad and El-Prince, 1995; Bastawrows et al., 1997 and Ring and Atanassova, 2001). Furthermore, numerous cases of food-poisoning outbreaks were traced to these pathogenic bacteria (Dack et al., 1930; Mostafa et al., 1948; Taylor, 1969; Tulloch et al., 1973; Anonymous, 1990; Eley, 1992; Wieneke et al., 1993; Henzler et al., 1994; Brooks et al., 1995; Anonymous, 1996 and ICMSF, 1996). It is worth to mention that the presence of such pathogenic bacteria in the eggs caused serious diseases for human-beings during consumption of such contaminated eggs. The main symptoms of these diseases are septicaemia, meningitis, abortion in pregnant women, gastroenteritis, epidemic diarrhoea in infants, sporadic summer diarrhoea in children, urinary tract infections or others (MacCkie and MacCartney, 1962; Kornacki and Marth, 1982; Brooks et al., 1995; Miller et al., 1997 and Arizcun et al., 1998).

Control of foodborne diseases can be accomplished by reducing the number of pathogens or the amount of their toxins to below that required to cause illness, which can be achieved by thorough heat processing and application of food hygienic practices. Although, critical control measures and legislations for the safety of food with regard to the health of the consumer were introduced, serious health hazards outbreaks due to consumption of the eggs were persisted. Therefore, the aim of this work is to investigate the incidence of some pathogenic bacteria which may be found on the shells and in the contents of commercial hens and ducks' eggs sold in Assiut Governorate.

MATERIAL and METHODS

Collection of samples:

225 eggs were collected, at random, from different markets, groceries, supermarkets, poultry farms and farmers' houses in Assiut Governorate. They included commercial hens' eggs of both native breeds and poultry farms and ducks' eggs (75 eggs each). Every 5 eggs (one group) were placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay.

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).

Experimental techniques:

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

1) Isolation and identification of Listeria species were carried out according to Lovett et al. (1987) and Hitchins (1995).
2) Isolation and identification of Staph. aureus were performed according to the recommended methods of Finegold and Martin (1982).
3) Isolation and identification of Salmonella, Shigella, E.coli and other Enterobacteriaceae organisms were carried out according to the methods recommended by Speck (1976). The serological typing of isolates presumed to be Salmonella were carried out at the Department of Bacteriology, Faculty of Medicine, Assiut University.

RESULTS

The obtained results were recorded in Tables 1 - 5.

DISCUSSION

The obtained results recorded in Table 1, show that L. monocytogenes was not detected in the examined commercial hens' egg shell samples of the native breeds. While, the bacterium was isolated from 1(6.7%) and 2(13.3%) of the examined samples of commercial hens' egg shells of poultry farms and ducks' egg shells, respectively. On the other hand, L. innocua was detected in 2(13.3%) and 1(6.7%) of the examined hens' egg shells of native breeds and poultry farms, respectively. However, L. murrayi could be isolated from one sample (6.7%) of each of shells of native breeds hens and ducks' eggs. These results are very low compared with those reported by Saad and El-Prince (1995) who found that hens' egg shells were contaminated by some Listeria species. They isolated L. monocytogenes, L. innocua, L. seeligeri and L.ivanovii from the examined egg shell samples in percentages of 17.8%, 35.6%, 11.1% and 4.4%, respectively. The difference in incidence of Listeria may be attributed to differences in geographic distribution of Listeria; variation in bird husbandry practices or variation in methods of isolation (WHO, 1988 and ICMSF, 1996). In recent years, L. monocytogenes has become of considerable importance to food industry. Moreover, the presence of L. monocytogenes in eggs most likely is due to contamination from the shells during the breaking process or from the processing environment (Foegeding and Leasor, 1989).

Results given in Table 2 point out that L.innocua could be isolated from only one of commercial hens' egg content samples of native breeds with an incidence percentage of 6.7%. Other species of Listeria could not be detected in the examined samples of commercial hens and ducks' egg contents. These findings are in good agreement with those recorded by Saad and El-Prince (1995), while Foegeding and Leasor (1989) could isolate L.monocytogenes from 2 samples of raw, broken, liquid whole egg with a prevalence percentage of 4.8%. Generally, the contents of newly laid egg from healthy birds are usually sterile, yet the shell soon becomes contaminated with microorganisms which can grow and penetrate through its intact shell contaminating the egg contents. It is worth to mention that the use of Listeria species other than L.monocytogenes as indicators of presence of such microorganisms has been proposed (Johnson et al., 1990). From the public health point of view, foodborne listeriosis nowadays is represented a serious public

health problem in many countries since the case fatality rate is high (ICMSF, 1996).

The findings reported in Table 3 declare that 26.7, 26.7 and 20% of the examined samples of commercial hens' egg shells of native breeds, commercial hens' egg shells of poultry farms and ducks' egg shells were contaminated with Staph. aureus, respectively. On the other hand, from the examined 15 egg content samples of native hens' breeds, only two samples (13.3%) contained Staph. aureus, while this organism failed detection in the other types of egg content samples tested. Lower incidence of Staph. aureus on egg shells were recorded by Moats (1980) and El-Prince (1988), but higher results were reported by Ahmed et al. (1985). However, the results obtained from egg content samples agree to some extent with those recorded by Ahmed et al. (1987) and El-Prince (1988). The comparatively low incidence of Staph. aureus in the examined samples of egg contents may be due to the presence of lysozyme in the inner shell membrane which acts as an effective agent against Gram-positive organisms, thus the chance of Staph. aureus entrance into a shell egg is very remote (Baker, 1974). Although, Staph. aureus presence in shell eggs is very rare, yet birds' eggs may be contaminated accidentally from shell, as it has been originated from ova during egg formation (Matthes, 1984), dust, and from the surface of inanimate objects, in addition to, different sources of contamination from skin, nose and throat of poultry workers. Furthermore, considerable number of food-poisoning outbreaks had been attributed to Staph. aureus (Dack et al., 1930; Elcy, 1992 and Wieneke et al., 1993).

From the results recorded in Tables 4 & 5, it is evident that egg shells of poultry farms and egg contents of native breeds were found to have Sal. typhimurium, with incidence percentages of 13.3 and 6.7%, respectively. While, no other species of Salmonella could be detected in the other examined samples of egg shells and contents. The findings of examined samples are nearly similar to those obtained by Ahmed et al. (1985 & 1987); El-Prince (1988) and Bastawrows et al. (1997). Lower occurrence were reported by El-Agroudy and Awad (1966) and Ring and Atanassova (2001). However, Yang et al. (2001) studied the influence of holding temperature on the growth and survival of Salmonella spp. and Staph. aureus and the production of staphylococcal enterotoxin in egg products, and they revealed that Salmonella spp. and Staph. aureus in the egg products multiply best at 37°C, followed closely by 22 and 18°C, and neither pathogen showed growth in the egg products held at 5°C. On the other hand, the vertical transmission theory states that Salmonellae

come from an infected hen. The horizontal transmission theory states that Salmonellae invade the egg through the shell after the egg is laid. In truth, both routes are probably involved (Cox et al., 2000). Also, contaminated water may act as an effective route of transmission (Dhillon et al., 1974), where Salmonella find their way from alimentary tract via blood to ovaries (Gordon and Tuker, 1965). Board and Fuller (1994) reported that when eggs are broken, Salmonella present on egg shells may contaminate the contents and grow rapidly if storage is at ambient temperature. Moreover, much of the evidence of a link between Salmonella and eggs comes from the table egg industry. Numerous Salmonella infections and outbreaks have been recorded, usually involving the consumption of raw or undercooked eggs (Taylor, 1969; Anonymous, 1990; Henzler et al., 1994 and Anonymous, 1996). On the other hand, Shigella spp. were detected in one (6.7%) sample from each of native breeds and poultry farms' egg shells (Table 4), but could not be assured due to lack of antiserum. Nearly similar percentages were reported by Ahmed et al. (1985) and El-Prince (1988).

E.coli was detected in 7(46.7%), 2(13.3%) and 5(33.3%) out of 15 samples of both commercial hens (native breeds and poultry farms) and ducks' egg shells, respectively (Table 4). The obtained results agree to a certain extent with those reported by Ahmed et al. (1985); El-Prince (1988) and Bastawrows et al. (1997). While, E.coli was isolated, respectively, from 6.7, 6.7 and 20% of examined egg content samples of both commercial hens and ducks (Table 5). A higher incidence of E.coli (42.2%) was obtained by Bastawrows et al. (1997). E. coli is taken as an index of recent faecal contamination, as it is a normal inhabitant of the intestinal tract of both man and animals, and always present in manure (Garrad, 1946). This organism can grow and penetrate the shell contaminating the egg contents (Mayes and Takeballi, 1983). Likewise, E.coli is considered one of the main contaminants of deteriorated eggs (Moursy et al., 1982). Furthermore, some strains of E.coli are now considered as common causes of traveller's diarrhoea and/or infantile illness, gastroenteritis and sporadic summer diarrhoea in children, as well as many cases of food-poisoning (MacCkie and MacCartney, 1962; Tulloch et al., 1973; Kornacki and Marth, 1982 and Brooks et al., 1995). Therefore, the isolation of E.coli from the examined egg samples in this study constitutes a great threat to the health of humans especially children and also of unproperly consumed, some individuals, especially athletics drink freshly beaten eggs.

Enterobacter spp., Citrobacter spp., Klebsiella spp., Providencia spp., Proteus rettgeri, Proteus vulgaris, Arizona spp., Alcaligenes dispar, Serratia spp. and Pseudomonas cyanogenes could be isolated at varying percentages from the examined samples of each of shells and contents of commercial hens' eggs of both native breeds and poultry farms and ducks' eggs (Tables 4 & 5). Some of these organisms were previously isolated from egg samples by several investigators (Garrad, 1946; Board and Board, 1968; Board , 1977; Mackenzie and Skerman, 1982; Moursy et al., 1982; Becirevic, 1983; Ahmed et al., 1985; Ibeh and Izuagbe, 1986; Ahmed et al., 1987; Alaboudi et al., 1988; El-Prince, 1988; El-Essawy et al., 1989 and Bastawrows et al., 1997). Moreover, some of isolated strains were claimed to be incriminated in food-poisoning and urinary tract infections and occasionally other infections and were often resistant to antimicrobial therapy (Wyah, 1992 and Brooks et al., 1995). From the economic point of view, Enterobacter spp., Klebsiella spp., Serratia spp. and others were implicated in different types of objectionable changes, rendering the eggs unfit for human consumption (Board and Board, 1968).

It is well established that the risk of some pathogenic bacteria recovered from the examined egg samples. Therefore, recommendations for prevention of this risk are, proper hygienic measures adopted in the farm, handling and storage of eggs are necessary for obtaining eggs of good quality. Likewise, the practice of cleaning eggs by washing sanitizers is common in egg industry nowadays. As well as, effective inspection of drainage system, public education programmes and thorough heat processing. At the end, pasteurization of egg products has been introduced as a statutory requirement in many countries.

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Table 1: Incidence of Listeria species recovered from the examined commercial hens and ducks' egg shells.

Listeria species	Commercial hens' eggs				Ducks' eggs	
	Native breeds		Poultry farms			
	Positive samples				Positive samples	
	No./15	%	No./15	%	No./15	%
L. monocytogenes	0	0	1	6.7	2	13.3
L. innocua	2	13.3	1	6.7	0	0
L. murrayi	1	6.7	0	0	1	6.7
Total	3	20.0	2	13.3	3	20.0

Table 2: Incidence of Listeria species recovered from the examined commercial hens and ducks' egg contents.

Listeria species	Commercial hens' eggs				Ducks' eggs	
	Native breeds		Poultry farms			
	Positive samples				Positive samples	
	No./15	%	No./15	%	No./15	%
L. monocytogenes	0	0	0	0	0	0
L. innocua	1	6.7	0	0	0	0
L. murrayi	0	0	0	0	0	0
Total	1	6.7	0	0	0	0

Table 3: Incidence of Staph. aureus recovered from the examined Commercial hens and ducks' eggs.

Category	Commercial hens' eggs				Ducks' eggs	
	Native breeds		Poultry farms			
	Positive samples				Positive samples	
	No./15	%	No./15	%	No./15	%
Egg shells	4	26.7	4	26.7	3	20.0
Egg contents	2	13.3	0	0	0	0

Table 4: Incidence of Salmonella, Shigella, E.coli and other Enterobacteriaceae organisms recovered from the examined commercial hens and ducks' egg shells.

Organisms	Commercial hens' eggs				Ducks' eggs	
	Native breeds		Poultry farms			
	Positive samples				Positive samples	
	No./15	%	No./15	%	No./15	%
Sal. Typhimurium	0	0	2	13.3	0	0
Shigella spp.	1	6.7	1	6.7	0	0
E.coli	7	46.7	2	13.3	5	33.3
Enterobacter spp.	1	6.7	2	13.3	3	20.0
Citrobacter spp.	2	13.3	1	6.7	0	0
Klebsiella spp.	0	0	0	0	1	6.7
Providencia spp.	3	20.0	0	0	1	6.7
Proteus rettgeri	1	6.7	2	13.3	1	6.7
Arizona spp.	0	0	1	6.7	0	0
Alcaligenes dispar	1	6.7	1	6.7	0	0

Table 5: Incidence of Salmonella, Shigella, E.coli and other Enterobacteriaceae organsisms recovered from the examined commercial hens and ducks' egg contents.

Organisms	Commercial hens' eggs				Ducks' eggs	
	Native breeds		Poultry farms			
	Positive samples				Positive samples	
	No./15	%	No./15	%	No./15	%
Sal. typhimurium	1	6.7	0	0	0	0
E.coli	1	6.7	1	6.7	3	20.0
Enterobacter spp.	1	6.7	0	0	0	0
Citrobacter spp.	0	0	0	0	3	20.0
Providencia spp.	1	6.7	0	0	1	6.7
Proteus rettgeri	1	6.7	0	0	0	0
Proteus vulgaris	0	0	0	0	1	6.7
Arizona spp.	1	6.7	0	0	0	0
Serratia spp.	2	13.3	0	0	0	0
Pseudomonas cyanogenes	0	0	0	0	1	6.7