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ISOLATION OF YERSINIA ENTEROCOLITICA AND PSEUDOMONAS AERUGINOSA FROM COMMERCIAL HENS AND DUCKS 'EGGS

(With 2 Tables)

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عزل اليرسينيا انتيروكوليتكا والسيدوموناس ايروجينوزا من بيض الدجاج والبط التجارى

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لما كان للبيض من اهمية كبيرة من حيث قيمته الغذائية الا انة قد يصبح مصدرا لا يستهان به لنقل العديد من الامراض التى تهدد صحة الانسان وذلك لأنة عرضة للتلوث بانواع مختلفة من الميكروبات الممرضة والمفسدة والتى تصل اليه من مصادر متعددة منذ انتاجه واثناء توزيعه حتى يصل الى المستهلك. لذلك قمنا بجمع عدد ٢٢٥ عينة من بيض الفراخ البلدى وبيض فراخ المزارع وكذلك بيض البط ممثلة فى ٤٥ مجموعة (١٥ لكل نوع) جمعت عشوائيا من اسواق مدينة اسيوط ومحلات البقالة المختلفة. وقد تم فحص البيض بكتريولوجيا لمعرفة مدى تلوث قشر البيض من الخارج وكذلك محتوياته بميكروبى اليرسينيا انتيروكوليتكا والسيدوموناس ايروجينوزا. ولقد تبين من الفحص ان ميكروب اليرسينيا انتيروكوليتكا تواجد فى عدد ٣ عينات من محتويات بيض الفراخ البلدى بنسبة ٢٠% وامكن عزلة من عينة واحدة من على قشر ذلك البيض بنسبة ٦,٧% بينما لم يتم عزل الميكروب من بيض فراح المزارع سواءمن المحتويات او من على القشر. بينما فى بيض البط التجارى امكن عزله من على القشرة بنسبة ٦,٧% (عينة واحدة) وكذلك من المحتويات بنسبة ٢٠% (٣ عينات). اما با لنسبة لميكروب السيدوموناس ايروجينوزا فقد تم عزل الميكروب من عينة واحدة من قشر بيض فراخ المزارع بنسبة ٦,٧% وكذلك من عينة واحدة من المحتويات بنفس النسبة ، بينما لم يتم عزل الميكروب من محتويات أو من على قشر بيض الفراخ البلدى. وبفحص قشر بيض البط تواجد الميكروب فىعينة واحدة(٦,٧%) وتواجد فى عدد ٢ عينة(١٣,٣%) من محتوياته. ولقد ناقش البحث اسباب التلوث بهذين الميكروبين وخطورة تلوث منتجات البيض اثناء التصنيع والخطر الذى قد يشكله هذا على صحة الانسان ، كذلك الطرق الواجب اتباعها لمنع تلوث البيض بهما.

SUMMARY

Commercial Hens and ducks' eggs (225 eggs) representing 45 groups (5 eggs each as a sample) were collected from Assiut city markets and different groceries. Hens' eggs represent farm hens and native breeds. The collected samples were examined for incidence of Yersinia enterocolitica and Pseudomonas aeruginosa on egg shells and in contents Y. enterocolitica could not be isolated from shells ad contents of farm hens' eggs (shell and content), and the organism recovered from one sample (6.7%) of shells and 3 samples (20%) of the contents of native breeds eggs. Ducks' eggs were contaminated by the same ratio of native breeds hens' eggs. Pseudomonas aeruginosa could not recover from any of native breed eggs (shells and contents) but could be isolated from 6.7% of shells and contents of farm eggs (one sample each). Ducks' eggs proved to contain the organism in the content (2 samples) and on its shell (one sample). The public Health hazard of the isolated organisms and suggestive measures were discussed.

Key words: *Y. enterocolitica, P. aeruginosa, eggs*

INTRODUCTION

It has been recognized that eggs are important and popular food in all countries and almost for all ages. Eggs provide a unique well balanced source of nutrients, including high quality protein, vitamins, useful amount of minerals and easily digested lipids, However, the nutrients that make eggs a high quality food for humans are also a good growth medium for bacteria. In the rare event that egg contains bacteria, as the inside of an egg was once considered almost sterile (Brooks and Taylor, 1955 and Frazier and Westhoff, 1978). But, over the recent years, different types of microorganisms have been found inside eggs. As, if the ovary is infected with bacterial pathogens, the egg may become infected before it is laid. Besides, other types of microorganisms could be deposited along with dirt on the outside of an egg and from fecal matters, from hens, by lining of the nest, by wash if the eggs are to be washed, by handling and perhaps by materials in which eggs are packed (Board and Fuller, 1994 and Cox *et al.*, 2000).

The risk of getting a food borne illness from eggs is very low, in spite of the good growth medium for bacteria. Unfortunately, some food

poisoning outbreaks due to consumption of eggs had been reported (Philbrook *et al.*, 1960, Adler, 1965 and Bowmer, 1965). Different types of bacteria including Psudomonas have been isolated from eggs. (Longree, 1980). Also, it has been proved that egg shells are pervious to microorganisms such as Salmonella typhi, E.coli, Serratia marcescens, Pseudomonas aeruginosa, Pseudomonas fluorescens and Alkaligenes bookeri (Haienes and Moran, 1940; Garibaldi and Stokes, 1958 and lifshitz *et al.*, 1964).

Yersinia enterocolitica is a food borne pathogen that is widely distributed in nature, animal and aquatic reservoir. They are able to grow at low temperature (Stern *et al.* 1980) and can produce toxins in foods (Boyce *et al.*, 1979; Francis *et al.*, 1980). These enterotoxins may be able to resist the temperature used in food processing and storage (Boyce *et al.*, 1979). Y.enterocolitica could lead to enterocolitis, mesenteric lymphadenitis and terminal ileitis. Also it has been reported that Y.enterocolitica invades the epithelium cells of gastrointestinal tract to produce intestinal diseases in animals and humans. There has been interest in the recovery of Y.enterocolitica from foods since the implication of Yersinia entercolitica in food poisoning occurred in New York due to consumption of chocolate milk (Black *et al.*, 1978). The reports on Y.enterocolitica prevalence in eggs and egg products are sketchy, however, it has been shown that Y.enterocolitica can be found on the surface of egg shells (Favir *et al.*, 2000).

Pseudomonas aesuginosa, has been implicated in various human infections, including enteritis, various respiratory diseases, urinary tract infection, infections of bones, joints, nails, skin and wounds (Clement and Millard, 1953; Winso, 1957; Burlina, 1962 and Chernosky and Duckes, 1963). As reported by Haines and Moran (1940), Pseudomonas aeruginosa can grow and then penetrate through intact shell to the egg contents. This phenomenon was previously assured by Garibaldi and Stokes (1958), that egg shells were pervious to Pseudomonas aeruginosa. Ahmed *et al* (1985) could isolate the organism from the shells of examined hens' eggs. In a study conducted by Das *et al.* (1996), Pseudomonas aeruginosa was one of the causative organisms of nosocomial diarrhea occurred in Calcutta, the eggs were among the source of infection. Therefore, this work aims to investigate the incidence of Yersinia enterocolitica and Pseudomonas aeruginosa on shells and in contents of commercial hens and ducks' eggs.

MATERIALS and METHODS

Collection of samples:

A total of 225 eggs representing 45 groups of commercial hens and ducks' eggs, were collected randomly from Assiut city markets and different groceries. Hens' eggs included farms and native breed hens. Each group (5 eggs) represent one sample, was placed in a sterile plastic bag and dispatched to the laboratory with a minimum of delay. The eggs were prepared and examined for the presence of Yersinia enterocolitica and Pseudomonas aeruginosa

Preparation of samples:

(A) Egg shells were tested by surface 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 was subjected to the following examination:

1- Isolation and identification of Yersinia entrocolitica

(a) - Enrichment procedure: 1 ml of rinse solution of eggshells, as well as, from the homogenous egg contents was placed aseptically into enrichment broth (Trypticase soy broth) and incubated at 37°Cfor 24 hours (Greenwood and Hooper, 1989).

(b) Isolation technique: A loopful of the incubated broth was streaked directly onto Cefsuldin Irgasan Novobiocin (CIN) as described by Schiemann (1979), and then incubated at 37c for 24h. The presumptive colonies were identified according to Schiemann and Devenish (1982).

2- Isolation and identification of Pseudomonas aeruginosa.

(a) Enrichment procedure. 1 ml of shell rinse solution, as well as, from homogenous egg contents was inoculated into Citrimide broth and incubated at 42 C° for 48 hours (Shriniwas, 1975).

(b) Isolation technique: Loopfuls from incubated broth tubes were streaked onto Citrimide agar plates and incubated at 42 C° for 24-48 hours (Shrininwas, 1975). Loopfuls from suspected colonies were picked up into agar slants and incubated at 42 C° for 24 hours for further identification according to Finegold and Martin (1982).

RESULTS

The obtained results were recorded in Tables 1 and 2

Table 1: Incidence of Y. entrocolitica in the examined samples of Hens and ducks' eggs.

Examined samples	No. of samples examined	Positive samples			
		Egg shell		Egg content	
A. Hens' eggs		No	%	No	%
Farms	15	-	-	-	-
Native breeds	15	1	6.7	3	20
B. Ducks' eggs	15	1	6.7	3	20

Tables 2: Incidence of pseudomonas aeruginosa in the examined samples of Hens and ducks' eggs.

Examined samples	No. of samples examined	Positive samples			
		Egg shells		Egg contents	
A. Hens eggs		No	%	No	%
Farms	15	1	6.7	1	6.7
Native breeds	15	-	-	-	-
B. Ducks' eggs	15	1	6.7	2	13.3

DISCUSSION

As recorded in Table 1, Y. enterocolitica could not be detected on egg shells or in contents samples of the examined hens' eggs collected from poultry farms. The data proved that 1/15 (6.7°C) of eggshells and 3/15 (20%) of egg contents samples of native breed hens were positive for the organism. Duck's eggs samples were found to be contaminated by Y. enterocolitica on 1/15(6.7%) of their shells and in 3/15(20%) of their contents. No data available to compare with except the research newsletter published by Favir *et al.* (2000) that Y. enterocolitica could not be detected among the natural flora on any of the egg tested.

Presence of Y. enterocolitica on eggshells and in egg contents samples could be attributed to the fecal matter soiled the eggshells of native breed hens and ducks' eggs. Furthermore, previous isolation of

Coilforms, E.coli, Salmonella, Enterococci, Alkaligenes and pseudomonas organisms from eggshells and contents by Alford et al. (1950); Ahmed *et al.* (1985 &1987); El-prince (1988) and Bastawrows *et al.* (1997), assures the possibility of contamination by Y. enterocolitica. The study of Henning. (1939) stated that eggshells are frequently contaminated by infected fecal matter and under favorable conditions, the organisms penetrate the shell into the egg contents. Also, Matthes (1984) proved that storage temperature and types of packing materials for transportation influence the penetration through eggshells into the contents.

The summarized data in Table 2 revealed that the hens' eggs of poultry farms were contaminated by Pseudomonas aeruginosa on 1/15(6.7%) of their shells samples and by the same ratio in their contents. In case of egg samples of native bread hens, P.aeruginosa failed to recover from any of their shells or contents. While, 2/5 (13.3%) of ducks' eggs content were positive for P.aeruginosa, and 1/15(6.7%) of their shells carried the organism (Table 2). P. aeruginosa was previously isolated from egg shells of commercial hens' eggs by Garibaldi and Stokes (1958); Lifshitz *et al.* (1964 &1965) and Ahmed *et al* (1985) in different percentages. The investigation carried by Moursy *et al* (1982) pointed out that P. aeruginosa could be detected in 18.18% of contents of unsold aged hens' eggs. However, in a previous study conducted by Miller and Crawford (1953), P. aeruginosa could be isolated from 9% of examined egg content samples of commercial hens' eggs. It was noticed that P. aeruginosa can grow and then penetrate through the intact shell to the egg content and the rate of penetration depends on the temperature of storage and Humidity (Haines and Moran, 1940). Furthermore, Garibaldi and stokes (1958) had proved that eggshells are pervious to P. aeruginosa. The public health hazard of P. aeruginosa has been stated, since its implication in nosocomial diarrhea occurred in Calcutta, where the eggs were among the source of infection (Das *et al.*, 1996).

From the aforementioned results, it is clearly evident that Y.enterocolitica and P. aeruginosa recovered from the examined egg samples should be considered a public health hazard concern. These pathogens may thrive from shells or contents into other egg products, grow and multiply sufficiently to the risk level of food poisoning.

Recommendation for prevention of their risk include, proper hygienic measure should be adopted in poultry farms side by side with educational program for the employee, and for the public to know how to handle and store egg or egg products. Likewise, the practice of

cleaning eggs by removal of dirt and faecal matter, washing by sanitizing solution is recommended and is common in egg industry. Moreover, pasteurization of egg products must be adopted as a statutory requirement.

REFERENCES

Adler, H.E. (1965): Salmonella in eggs an appraisal. Food Technol.19: 623.

Ahmed, A. A-H.; Moustfa, M.K.; Aboul - Khier, F. and El-Bassiony, T. A. (1985): Bacterial contamination of egg shells. Assiut Vet. Med .J. Vol.14 (27): 123-127.

Ahmed, A-H. Ahmed; Saad, Nagah, M. and Moustafa, M.K. (1987): Microbial contamination of market hens' eggs. Assiut Vet. Med. J. Vol. 1, No. 1: 77 –84.

Alford, L.R.; Holmes, N.E.; Scott, W.J. and Vickery, J.R. (1950): Studies on the preservation of shell eggs. I.Nature of wastage in Australian export eggs. Aust .J.Appl.Sci.1:208 - 214

Bstawrows, A.F.; Khalil, Nawal, Gh.; Sddek, S.R. and Aly, Seham, M. (1997): Bacteriological studies on Enterobacteriaceae isolated from table eggs . Assiut Vet. Med. J. 36:203 –214.

Black, R.E.; Jackson, R.J.; Tsai, T.; Mdvesky, M.; Shayegani, M.; Feeley, J.C.; Macleod, K.I.E. and Wakelee, A.M. (1978): Epidemic Y.nterocolitica infection due to contaminated chocolate milk. New England j. Med. 298(2)76-79.

Board, R.G. and Fuller, R. (1994): Microbiology of the avian eggs .1 st ed. Chapman and Hall P. 112-128.

Bowmer, E.J. (1965): Salmonella in food (A review). J. Milk Food Technol. 28: 74-86.

Boyce, J.M; Evans, D.J. Jr.; Evans, D.G. and Dupont, H.L. (1979): Production of heat – stable, methanol- soluble enterotoxin by Y.enterocolitica. Infect. Immun. 25: 532-537, Enviromental Baeteriology. Vol. 32,No.2

Brooks, J. and Taylor, D. I. (1955): Eggs and egg products. Rep. Fd. Invest. Bd, Lond.no 60 H. M.S.O.

Burlina, A. (1962): Patobgia polmonare del Lattante da Pseudomonas aeruginosa. Riv. Pat. Once. 22: 1095- 1123 (After Lusis and soltys, 1971).

Chernosky, M.E. and Duckes, C.D. (1963): Green nails. Importance of Pseudomonas aeruginosa in onychia Archs Derm. 88: 548- 553 (After Lusis and Soltys, 1971).

Clement, R. and Millard, L. (1953): L' infection a bacilli pyocyanique chez L'enfant. Press med, 61: 661-662. (After Lusis and solty, 1971).

Cox, N.A.; Berrang, M.E. and Cason, J.A. (2000): Salmonellae penetration of egg shells and proliferation in broiler hatching eggs- A review. Poult.Sci 79: 1571-1574.

Das, A.s; Mazumder, D.N.; Pal, D and Chattopadh-Yay, UK. (1996): A study of nosocomial diarrhea in Calcutta. Indian J. Gastroenterol. Jan; 15(1); 12-3.

El-Prince, Enas, M. (1988): Microbiological quality of hens'eggs. M.V.Sc. Thesis. Faculty of Vet. Med. Assiut University.

Francis, D.W.; Spaulding, P.L. and Lovett, J. (1980): Enterotoxin production and thermal resistance of Y, enterocolitica in milk. Applied and Environomental Microbiology, 40: 174-176.

Favir, G.; caudero, M; Mattar, M. (2000): Survival of Yersinia enterocolitica, and mesophilic aerobic bacteria on egg shells after washing with Hypochlorite and organic acid solutions. J. Food Prot. 63: 1053-1057.

Finegold, S.H. and Martin, W.J. (1982): Baily and Scott Diagnostic Microbiology 6th ed C.V. Mosby Co. St. Louid, Torento, London.

Frazier, W.C. and Westhoff, D.C. (1978): "Food Microbiology" 3rd ed. McGraw Hill, Inc., New York. Contamination, preservation and spoilage of eggs. p. 256-269.

Garibaldi, J.A. and Stokes, J.L. (1958): Protective role of shell membranes in bacterial spoilage of eggs. Food. Res. 23:283-290.

Greenwood, M.H. and Hooper, W.L. (1989): Improved methods for the isolation of Yersinia species from milk and foods. Food Microbiol., 41(3); 99-104.

Grover, S. and Srinivasan, R.A. (1988): Isolation and characterization of Pseudomonas aerugionsa from milk and milk products. Indian J. of Dairy Sci., 41: 3. 326-329.

Haines, R.B. and Moran, T. (1940): Prosity of, and bacterial invasion through the shell of hen's eggs. J.Hyg. Camb. 40: 453-461.

Henning, M.W. (1939): Salmonella infection in birds.Ondrstepoort.J. 13,125.

Lifshitz, A.; Baker, R.C. and Naylor, H.B. (1965): The exterior structures of the egg as nutrients for bacteria. J. food Sci., 30:516-519.

Lifshitz, A.; Baker, R.C. and Nayloro, H.B. (1964): The relativ importance of chicken egg, exterior structures in resisting bacterial penetration. J. Food Sci. 29: 94-99.

Longree, K. (1980): Quantity food Sanitation. I- Food supply of animal origin. P 131-175.3rd Ed. Published by John Wiley & Sons, Inc. NewYork and Tronto.

Lusis, P.I. and Soltys, M.A. (1971): Pseudomonas aeruginosa. Veterinary bulletin, V. 41, No.3: 169-176.

Matthes, S. (1984): Diminution of egg quality caused by avian diseases and microbial contamination. J. World' Poul.Sci. 40:81.

Miller, W.A. and crawford, L.B. (1953): Some factors influencing bacterial penetration of eggs. Poult.Sci. 32(2): 303 –309.

Moats, W.A. (1979): The effect of washing eggs under commercial conditions on bacterial loads on egg shells. Poult. Sci. 58: 1228-123

Moursy, A.W.; Al-Ashmawy, A.M. and Moursy, E.A. (1982): Microbiological studies on deteriorated hen eggs. Assiut Vet. Med. J. 9:91-96.

Philbrook, F.R.; Maccreedy, R.A.; Van Roekel, H.; Andersen, E.S.; Smyser, C.F.; Jr.; Sanen, F.J. and Groton, W.M. (1960): Salmonellosis spread by a dierty suplement of avian source. New Engl. J. Med. 263: 713-718.

Schiemann, D.A. (1979): Synthesis of a selective agar medium for Y. enterocolitica. Canadian J. of Microbiol, 25: 1298- 1304.

Schiemann, D.A and Devenish, J.A. (1982): Relation-ship of Hela cell infectivity to biochemical, serological and virulence characteristics of Y.enteroerocolitica. Infect. Imun., 35: 497-506.

Shriniwas, J. (1975): J. Aims, I, 94. (After Grover and Srinvasan, 1988).

Speck, M.L. (ed). (1976): Compendium of Methods for Microbiological examination of Food. A.P.H.A., Washington, D.C.

Stern, N.J.; Pierson, M.D. and Kotula, A.W. (1980): Effects of pH and sodium chloride on Y. enterocolitica growth at room and refrigeration temperatures. J. Food Sci., 45: 64-67.

Winso, S. (1957): Fall au pseudomonas Senterit hos svin. Medlemsbl-Sver Vet Farb 9, 104. (After Lusis and Soltys, 1971).