قسم المراقبة الصحية على الأغذية
كلية الطب البيطري - جامعة أسيوط
رئيس القسم : أ٠د/ توفيق البسيونى

التلوث الميكروبى لبيض الفراخ

أحمد عبدالحميد ، نجاح سعد ، مصطفى خليل

يحتل البيض مكانة مرموقة كقيمة غذائية عاليه الا أنه قد يكون ناقلا ومسببا لكثير من الأمراض التى تصيب الانسان أو يتعرض للفساد نتيجة لتلوثه مما يسبب خسارة اقتصادية ٠ لذلك تم جمع ١٧٥ عينة تمثل ٣٥ مجموعة من البيض المعروض في أسواق أسيوط للاستهلاك ٠ وتم فحص هذه العينات ميكروبيولوجيا ووجد أن متوسط العدد الكلي للميكروبات والخمائر والفطريات وكذلك الميكروبات السبحية المعوية كانت على التوالي ٩٣×١٠^{٤} ، ١×١٠^{٢} ، ٣١×١٠^{٢} ٠ كذلك تم عزل عديد من الميكروبات المختلفة الممرضة والغير ممرضة والتي قد تؤدي الى فساد البيض وتؤثر على صحة المستهلك ٠

ولقد امكن عزل ميكروب السالمونيلا من ٤ عينات من البيض المفحوص (٤٣ر١١٪) ، كذلك تم الكشف على بقايا المواد المثبطة كبقايا الأدوية فى هذه العينات ووجد أن ٢٨ر١٤٪ من العينات المفحوصة تحتوي على هذه المواد المثبطة ٠

وقد تم مناقشة الأهمية الصحية لوجود هذه الميكروبات على الصحة العامة من ناحية وعلى فساد البيض والخسارة الاقتصادية الناتجة من ناحية أخرى ٠ كذلك نوقشت خطورة وجود بقايا المضادات الحيوية والأدوية المستخدمة في البيض المعروض للاستهلاك الآدمى٠

وتناول البحث الاشتراطات الواجب اتخاذها لمنع تلوث البيض في المزارع وأثناء تخزينه أو توزيعه ٠

Dept. of Food Hygiene,
Faculty of Vet. Med., Assiut University,
Head of Dept. Prof. Dr. T.A. El-Bassiony.

MICROBIAL CONTAMINATION OF MARKET HEN EGGS

(With 4 Tables)

By
A.A-H. AHMED; NAGAH M. SAAD and M.K. MOUSTAFA

(Received at 8/1/1987)

SUMMARY

175 eggs (35 groups) were collected in summer months from Assiut city markets and examined microbiologically. The obtained results revealed that the mean values of aerobic plate, yeast & mould, S.aureus and Enterococci counts/ml were 93×10^4, 1×10^2, 10 and 31×10^2, respectively. Coliforms, faecal coliforms and E.coli were detected in 7(20%), 5(14.28%) and 4(11.4%) of the examined samples respectively. S.aureus, Staph. epidermidis, Micrococci, Enterococci, E.coli, Enterobacter aerogenes, Providencia spp., Edwardsiella spp., Serratia spp., Proteus spp. and Arizona spp. could be isolated from the examined samples. Also, Salmonellae were detected in 4(11.43%) of the examined sampoles. the seriological typing revealed that they are Sal. typhimurium, Sal. newport, Sal. ohio, and Sal. wanyatt. On the other hand, inhibitory substances were detected in 14.28% of the examined samples.

INTRODUCTION

It is generally accepted that milk and eggs are nature's most perfect food. The egg is a remarkable natural package. The contents are as rapidly perishable as milk, yet the fragile shell, if undamaged and dry will usually keep the egg edible for many months. The microbial contamination and subsequent results of this contamination of eggs intended for human consumption have attracted the attention of several workers (HARRY, 1963; BOARD, 1966 & 1968; SAUTER and PETERSEN, 1974; BOARD, 1977 and FURUTA and MURNYAMA, 1981).

Fresh eggs usually contain less than 10 microorganisms per gram and seldom 100/g. (SPECK, 1976). Many workers have suggested that the egg can be easily contaminated within a short period after laying (LORENZ & STARR, 1952). It has been reported that the shell is pervious to microorganisms such as E.coli, Salmonella paratyphi, Serratia marcescens and Pseudomonas aerogenosa (HAIENS and MORAN, 1940 and GARIBALDI and STOKES, 1958). While, TANNER (1944) proved that E.coli and Proteus group were among the frequent contaminants found in egg yolk. Furthermore, SAVOV (1966) could isolate 60 strains of E.coli from yolk of 226 eggs (26.5%). On the otherhand, S.aureus and Micrococcus roseus could be isolated from spoiled eggs. While, Salmonella, E.coli, Proteus, Enterobacter, Strept. faecalis, and different types of fungi, were recovered from deteriorated eggs examined by AHMED, et al. (1974) and MOURSY, et al. (1982).

Besides, the public health hazard of different types of microorganisms which could contaminate egg content, it is of great concern to mention that the growing use of antibiotics and sulpha drugs in poultry farms, creats new problems from the public health point of view. In 1978, EL-RASHEDY could detect antibiotics residues in 14% of examined egg samples while, EL-BASSIONY, et al. (1985) detected inhibitory substances in 6.67% of examined egg samples. These drugs may exist in eggs as residues and may cause allergic reaction, toxicity, skin rashes in infants or even bacterial resistance (O'BRINE, 1974).

This investigation was planned to secure the microbial contamination of market hen eggs and to ascertain different types of microorganisms, as well as, the ihibitory substances which could be present in market eggs.

MATERIAL and METHODS

I. Collection of samples:

175 eggs were collected in summer months at random from Assiut city markets, different groceries and supermarkets. Every 5 eggs (one group) were placed in a sterile plastic bag and dispatched to the laboratory.

II. Preparation of samples:

Egg samples were handled and prepared to evacuate the content according to SPECK (1976). The contents of each group were received into a sterile container and were mixed using a sterile mixer until the sample becomes homogenous. Serial dilutions were prepared using sterile 0.1% peptone water.

1- Total bacterial count Standard plate count was determined as described in Standard methods (APHA, 1972).

2- Coliform group (MPN), Faecal coliforms and E.coli count were determined according to the recommended methods described by SPECK (1976).

3- Enterococci count by using ESD agar of EFTHYMIOU, et al. (1974).

4- Yeast and mould count by using Malt extract agar of HARRIGON and McCANCE (1976).

5- Isolation and enumeration of S.aureus were determined by using Baird-Parker agar plates (BAIRD-PARKER, 1962) while, the isolation and identification were carried out according to FINEGOLD and MARTIN (1982).

6- Isolation and identification of other Staphylococci and Micrococci were the same as described by FINEGOLD and MARTIN (1982).

7- Isolation and identification of Enterobacteriacae were performed according to the recommended methods of SPECK (1976). The seriological typing of isolates presumed to be Salmonella were carried out at the Dept. of Bacteriology, Faculty of Medicine, Assiut University.

8- Detection of inhibitory substances:
2 ml of homogenous content of each samples were mixed with 20 ml of the solvent (FRERES and VATDEBOAZE, 1969). After 10 min. centribugation at 3000 r.p.m., the supernatent was tested for residues as described by GUDDING (1976). Bacillus subtilis (ATC 6633) was the test organism, its maintenance and preparation of the test plates were done according to APHA (1972).

RESULTS

The obtained results from the examined samples are recorded in Tables 1-4.

DISCUSSION

The results recorded in Table 1. show the min., max. and average values of total bacteria and Yeast and Mould counts. A lower count of total bacteria was recorded from eggs, and a range of 10^4-10^5 bacteria/g. of fresh liquid eggs were obtained by SPECK (1976). Yeast and mould were established as a cause of spiolage and could be isolated from rotton eggs (AHMED, et al. 1974, FRAZIER & WESTTHOFF, 1978 and MOURSY, et al. 1982). Fungal and bacterial penetration through egg shell has been stated by ROMANOFF and ROMANOFF (1949) while, WESTON and HALNAN (1927) proved that the hyphae of mould facilitate th entry of bacteria. Staph. aureus (/ 10/g.), Staph. epidermidis and Micrococcus were isolated from the examined egg samples (Table 1&3), and this may be attributed to transovarian transmission (MATHES and HANSCHKE, 1977). Besides, micrococci were found to be common contaminant of oviduct from cloacal region (HARRY, 1963). On the otherhand, PAVARINOV (1929) could isolate S.aureus and micrococci from spoiled eggs.

As shown in Tables (2&3), Coliforms, faecal coliforms, and E.coli were recovered from the examined samples. The count of Coliforms was 1000/ml. in 5 samples and from 1-10&100-1000/ml. in the other two samples. SPECK (1976) stated that Coliforms count generally ranged from 10^3-10^5/g. in fresh liquid egg. Moreover, Coliforms have been listed as one of the organisms causing rott in Australian eggs (SCOTT, et al. 1950-1951). A higher incidence of E.coli (26.5%) was obtained by SAVOV (1966). Furthermore, Enterobacter aerogenes, Providencia, Edwardsiella, and Serratia spp. were recovered from the examined egg samples. some of these organisms were found among the common contaminant of rotton eggs (BOARD, 1965 and 1966).

Enterococci (Strept. faecalis, Strept. faecium and Intermediate) as shown in Tables (1&3) were recovered from 31.43% of the examined samples. Strept. faecalis was previously isolated from rotton eggs by AHMED, et al. (1974). The occurence of Enterococci in eggs could be attributed to the fact that they are among the heaviest contaminants of oviduct (PETTGER, 1913). On the otherhand, it has been found that Proteus contaminate egg yolk frequently (TANNER, 1944) and this findings go parallel with our results (Table 3) where, Proteus spp. (prot rettergi. and prot. vulgaris) was isolates from 11.43% of the examined egg samples. The organisms were isolated from deterurated eggs by BOARD and BOARD (1968); AHMED, et al. (1974) and MOURSY, et al. (1982).

Occurence of Salmonella as shown in Table 3, was 11.43% of the examined egg samples. The seriological typing reveal that the isolated strains were Sal.typhimurium, Sal. newport, Sal. wanyat. and Sal. ohio. while one strain was classified as arizona spp. and revovered from one sample. Salmonella was previously isolated from fresh liquid eggs and from frozen and dried eggs (SPECK, 1976 and FRAZIER and WESTHOFF, 1978) while, MOURSY, et al. (1982) could isolate Sal. typhimurium, Sal. newport and other types of Salmonellae from rotton eggs. The incidence of salmonella is quite understood, as the organism may contaminate the content from infected matters soiled the shell, which is pervious to salmonella and can penetrate into the egg (LORENZ & STAAR, 1952 and GARIBALDI and STOKES, 1958). Moreover, contaminated water may act as an effective route of transmission (DHILLON, et al. 1974) where salmonellae find theri way from alimentary tract via blood to ovaries (GORDON and TUCKER, 1965). Many

of these microorganisms which could be isolated from the examined egg samples have been implicated in human infection. as well as food poisoning outbreaks (SMITH and CONANT, 1960; MACKIE and MAcCARTHEY, 1962; FRAZIER, 1967 and TAYLOR, 1969).

Inhibitory substances residues (Table 4) were detected in 14.28% of the examined egg samples. Similar results were reported by EL-RASHEDY, et al. (1978) while, lower finding (6.67%) was obtained by EL-BASSIONY, et al. (1985). The presence of these residues in eggs may constitute a public health hazard (O'BRINE, 1974).

In conclusion, fresh eggs are liable to be contaminated by different types of bacteria during formation or after laying from the infected matters soiled the shell. These bacteria under bad storage and misshandling of eggs may lead to economic losses through spoilage of eggs, as well as, constiute a public health hazard. Educational program should be imposed for egg producer and handlers.

REFERENCES

Ahmed, A.A.; Abu-Gabal, M.; Enab, S.A. and Moustafa, T.H. (1974): Investigation of microbial spoilage of hen eggs. Assiut Vet. Med. J. Vol. 1, No. 1&2: 77-84.

A.P.H.A. (1972): Standard methods for examination of dairy product, 12th Ed. American Public Health Association, Inc., New York.

Baird - Parker (1962): An improved and selective medium for isolating coagulase positivbe staphylococci. J. Appl. Bacteriol. 25, 1: 12-19.

Board, P.A. and R.G. Board (1968): A diagnostic key for identifying organisms from rotton egg. Brit. Poult. Sci. 9, 111-120 (Cited after Moursy, et al. 1982).

Board, R.G. (1965): The porperties and classification of the predominant bacteria occuring in rotton eggs. J. Appl. Bacteriol. 28: 437-453.

Board, R.G. (1966): The course of microbial infection of the hen's egg. J. Appl. Bacteriol. 29: 319-341.

Board, R.G. (1968): Microbiology of egg. A review P. 133-162 in T.C. Carter (ed). Egg quality. A study of hen's egg. Oliver and Boyd, Edinburgh.

Board, R.G. (1977): The microbiology of eggs. P. 49-64. In W.J. Sttadelman and O.J. Cotteril (eds) Science and Technology. AVI Publishing Co., Inc. Westport, Cnnecticut.

Dhillon, A.S.; A.J. Maurer, R.H. Deibel, and R.W. Haller (1974): Feeding of different levels of salmonelae to chickens. Ind. J. Poult. Sci. 9: 103-107.

Efthymiou, C.J.; P. Baccash, V.J. Labombard and D.S. Epistein (1974): Improved isolation and differentiation of enterococcus in cheese. Appl. Microbiol., 28: 417-422.

El-Bassiony, T.A.; M.K. Moustafa; A.A-H. Ahmed and S. Mousa (1985): Studies on the drug residues in eggs. Assiut Med. J. 9, 2: 19-29.

El-Rashedy, A. (1978): Studies on the identification of antibiotic products in food and foodstuffs. Thesis, Dept. of Nutrition, High Institute of Public Health, University of Alexandria.

Finegold, S.M. and W.J. Martin (1982): Baily and Scotts Diagnostic Microbiology. 6th ed. C.V. Mosby, Co. St. Louis. Toronto. London.

Frazier, W.C. (1967): Food Microbiology 2nd ed. TATA McGraw Hill publishing Co. Ltd Bombay, New York.

Frazier, W.C. and D.C. Westthoff (1978): Food Microbiology 3rd ed. TATA McGraw Hill publishing Co. Ltd. New Delhi.

Freres, D. and P. Vatdeboaze (1969): Recherch des residues activite antibiotque dans les tissus animeux. Bult. Acud. Vet. 1:42. (Cited after El-Rashedy, 1978).

Furuta, K. and S. Muruyama (1981): Bacterial contamination of eggs during incubation and hatching and of fluffs of newly- hatched chicks. Brit. Poult. Sci. 22: 247-254.

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

Gordon, R.F. and J.F. Tucker (1965): The epizootology of Salmonella menstom infection of fowls and the effect of feeding poultry food artificially infected with salmonella. Brit. Poult. Sci. 6: 251-264.

Gudding, R. (1939): An improved bacteriological methods for detection of sulfonamide residues in foods. Aceta Vet. Scand., 17, 458-464.

Haiens, R.B. and T. Moran (1940): Porosity of and bacterial invasion through the shell of the hen's egg. J. Hyg. 40: 453-461.

Harrigan, W.F. and M.E. McCance (1976): Laboratory Methods in Food and Dairy Microbiology. Academic Press London, New York, Sann Francisco.

Harry, E.G. (1963): Some observation on the bacterial content of the ovary and oviduct of the fowel. Brit. Poult. Sci. 4: 63-70.

Lorenz, F.W. and P.B. Starr (1952): Spoilage of washed eggs. 1 Effect of sprayed versus static water under different washing temperature. Poult. Sci. 31: 204-214.

Mackie, K.J. and I.E. MoocCartney (1967): Handbook of Bacteriology, 10th ed. E&S Livingston Limited, London.

Mathes, S. and J. Hanschke (1977): Experimentelle Unter- Suchungen Zur Ubertragung von Bakiterien Uber das Huhnerei Berl. Munch Tierarztl Wshr. 90: 200-203. (Cited after Mayes and Takeballi, 1983).

Mayes, F.J. and M.A. Takeballi (1983): Microbial contamination of the hen's egg. A review J. Food Protect. 46, 12: 1092-1098.

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

O'Brine, T.E. (1974): Ame. J. Hosp. Pharm. 31, 844-854. Dairy Sci. ABst. 39(7), 391.

Pavarinov, G.L. (1929): Ann. Chim. Applicata. 19: 266 (Cited after Ahmed, et al. 1974).

Rettger, L.F. (1913): The bacteriology of hen's egg, with special reference to its freedom from microbic invasion. Sterrs Agr. Expt. Sta. Bull. 75: 191-213. (Cited after Mayes and takeballi, 1983).

Romanoff, A.L. and A.J. Romanoff (1949): The avian egg. Wiley adn Sons. New York.

Savov, D. (1966): Pathogenic Escherichia coli carriage in fowels. Vet. Med. Nauki, Sof. U. 519. (Cited after Ahmed, et al. 1974).

Sauter, E.A. and C.F. Petersen (1974): The effect of egg shell quality on penetrationby various Salmonellae. Poult. Sci. 53: 2159-2162.

Scott, W.J., et al. (1950-1951): Studies in the preservation of shell eggs. Aust. J. Appl. Sci. 1: 208, 2: 205 (Cited after Ahmed, et al. 1974).

Smith, T.D. and N.T. Conant (1960): Zinsser Microbiology, 12th ed., Appleton Century Croft, Inc. New York. (Cited after Ahmed, et al. 1974).

Speck, M.L. (ed) (1976): Compendium of methods for microbiological examination of food. American Public Health Association, Washington, DC.

Tanner, F.W. (1944): The microbiology of food. 2nd ed., Garrad Press, Illinois, USA.

Yaylor, J. (1969): Salmonellae and Salmonellosis. pp. 25, In: Bacterial food poisoning. London, The Royal Society of Health.

Weston, W.A.R.D. and E.T. Halnan (1927): Black Spot of eggs. Poult. Sci. 6: 251-258.

Table (1)

Counts of some microorganisms recovered from egg content

Types	Positive samples		Count/ml.		
	N/35	%	Min.	Max.	Average
APC	35	100	/ 30	22×10^6	93×10^4
Yeast & mould	35	100	/ 10	1×10^3	1×10^2
S.aureus	5	14.28	*/ 10	*/ 10	*/ 10
Enterococci	11	31.42	*/ 10	15×10^3	31×10^2

APC : Aerobic Plate Count.
*/ : no colonies could be detected onthe plate.

Table (2)

Statistical analytical results of Cliforme, Faecal coliforms and E.coli count/ml (MPN)

Types	Positive samples		Count/ml.			
	No/35	%	1-10	10-100	100-1000	1000
Coliforms	7	20	1	0	1	5
Faecal coliforms	5	14.28	1	3	1	0
E.coli	4	11.43	2	0	1	1

Table (3)
Incidence and frequency distribution of different isolates recovered from egg contents

Isolates	Positive samples		Isolates	
	No/35	%	No/35	%
Staph. aureus	5	14.28	5	7.69
Staph. epidermidis	11	31.42	11	16.92
Micrococci	7	20.00	7	10.77
Enterococci				
Strept. faecalis	10	28.57	10	15.38
Strept. faecium	5	14.28	5	7.69
Intermediate	3	8.57	3	4.62
E.coli	4	11.43	4	6.15
Enterobacter aerogenes	4	11.43	4	6.15
Providencia spp.	5	14.28	5	7.69
Edwardsiella spp.	1	2.86	1	1.54
Serratia spp.	2	5.71	2	3.08
Proteus				
Proteus rettergi	3	8.57	3	4.62
Proteus vulgaris	1	2.86	1	1.54
Arizona spp.	1	2.86	1	1.54
Salmonellae				
Salmonella typhimurium	1	2.86	1	1.54
Salmonella newport	1	2.86	1	1.54
Salmonella wanyatt	1	2.86	1	1.54
Salmonella ohio	1	2.86	1	1.54
			65	100

Table (4)
Incidence of inhibitory substances in eggs

No. of examined samples	Positive samples	
	No/35	%
35	5	14.28