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MICROBIOLOGICAL PROFILE OF COMMERCIAL HEN'S EGGS IN ASSIUT GOVERNORATE PART I: OCCURRENCE AND SIGNIFICANCE OF *LISTERIA* SPECIES, *YERSINIA ENTEROCOLITICA* AND SOME IMPORTANT MOLDS IN HEN'S EGGS

(With 4 Tables)

By

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الحالة الميكروبيولوجية لبيض الفراخ التجاري في محافظة أسيوط الجزء الأول: أهمية تواجد ميكروبات الليستيريا واليارسينيا انتيروكوليتيكا وبعض الفطريات الهامة في بيض الدجاج

ألفونس فخري بسطاوروس ، أشرف محمد سيد ، عبد الراضى ثابت ،
رزق الشرنوبي ، أحمد بركات

أجريت هذه الدراسة على عدد ١٥٠ بيضة " كل عينة مكونة من خمس بيضات تمثل ثلاثون مجموعة" جمعت عشوائيا في اشهر الصيف والشتاء من أسواق أسيوط ومحلات البقالة والسوبر ماركت ومنازل الفلاحين وذلك لمعرفة مدى تلوث البيض بميكروبات الليستيريا واليارسينيا انتيروكوليتيكا وبعض الفطريات الهامة ولقد امكن عزل ميكروبات الليستيريا مونوسيتوجين , الليستيريا ايفانوفا , الليستيريا اينوكيا والليستيريا ولشميرى من ٤ (١٣.٣٣%) , ٣ (١٠%) , ٢ (٦.٦٧%) , ١ (٣.٣٣%) عينات من قشر البيض على التوالى اما في حالة محتويات البيض فقد امكن عزل ميكروبات الليستيريا اينوكيا والليستيريا سليجرى من ٤ (١٣.٣٣%) و ٢ (٦.٦٧%) عينات من محتويات البيض ولم تسجل اى نتائج للانواع الاخرى من ميكروبات الليستيريا . وتمت دراسة مدى ضراوة ميكروب الليستيريا مونوسيتوجين المعزول على الفئران البيضاء. أما بخصوص ميكروب اليارسينيا انتيروكوليتيكا فقد اسفرت النتائج عن تلوث قشر البيض بهذا الميكروب بنسبة ١٦.٦٧% ولم يستدل على الميكروب في محتويات البيض المفحوص وعند اختبار ضرواة الميكروب اتضح ان ٦٠% من المعزولات كانت ضارية. اثبت الفحص الميكولوجى باستخدام الوسط الغذائى

Malt extract agar والتحضين عند درجة حرارة ٢٨°م ان متوسط العدد الكلى للفطريـــات كان ٣٢٠/ قشرة , ٧٠/مللى لكل قشرة وكل مللى من محتويات البيض على التوالى وقد تـم عزل ٣٥ نوعا فطريا بالاضافة إلى صنف واحد تنتمى إلى ١٨ جنسا فطريا وكـانت اكـثر الاجناس شيوعا وانتشارا هما جنس اسبرجلليس وبنسليوم. و قد تمت مناقشة الاهمية الصحية والاقتصادية للميكروبات المعزولة وكذلك اقتراح السبل الكفيلة للمحافظة على البيـــض مـن التلوث بهذه الميكروبات.

SUMMARY

150 eggs, representing 30 groups, were collected in summer and winter months from Assiut City markets, groceries, supermarkets and farmers'houses. The samples were examined for the presence of *Listeria* species, *Yesinia enterocolitica* and some important molds. Out of 30 examined egg shell samples, 4 (13.33%), 3 (10%), 2 (6.67%) and 1 (3.33%) contained *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria innocua* and *Listeria welshimerii* respectively. While in case of examined egg contents, *Listeria innocua* and *Listeria seeligeri* could be isolated from 4 (13.33%) and 2 (6.67%) out of 30 samples examined, other *Listeria* species failed detection. Pathogenecity for *Listeria monocyotgenes* to mice were studied. Concerning, *Yersinia enterocolitica*, the organism was existed in 5 (16.67%) out of 30 egg shells samples, while the organism failed to recover from egg contents samples. The virulence tests were carried out on 5 isolated *Yersinia enterocolitica* recovered in this study. The obtained results proved that 3 (60%) out of 5 strains examined were virulent. The mycological analysis revealed that the average values of filamentous fungi were 320/shell and 70/ml of eggs shells and its contents respectively on malt extract agar medium at 28°C. Thirty five species and one variety belonging to 18 genera were isolated. The most prevalent genera were *Aspergillus* and *Penicillium*. other moulds were also encountered with variable frequencies. The economic and public health importance of isolated microorganisms has been discussed and suggested measures for improving the quality of produced hen eggs are given.

Key words: *Listeria – Yersinia enterocolitica – Molds – Hen's eggs.*

INTRODUCTION

Several pathogenic microorganisms have been isolated from the surface of chicken egg shells among them are *Listeria monocytogenes*

(Moore and Madden, 1993), *Yersinia enterocolitica* (Chiesa et al., 1989) and some important molds (Fajardo et al., 1995).

L.monocytogenes is a food-borne pathogen which has lately been a cause of great concern not only for the food industry but for regulatory agencies as well as a food-borne pathogen (Farber et al., 1989), *L.monocytogenes* has several characteristics which make it of special concern with respect to food processing and storage. The microorganism is ubiquitous, psychrotrophic and moderately heat resistant (Foegeding and Leasor, 1990).

L.monocytogenes is widely distributed in foods and has been isolated from commercially broken, raw liquid whole egg. Its presence in egg most likely is due to contamination from the processing environment (Foegeding and Stanley, 1990). Contaminated eggs may pose a health threat to humans in some lightly cooked or uncooked egg-based products (Foegeding and Leasor, 1990).

Y.enterocolitica as an emergent food-borne pathogen is widely distributed in nature and in animal also in aquatic reservoirs, can produce enterocolitis, mesenteric lymphadenitis and terminal ileitis with or without pseudoappendicitis symptoms or other extra-intestinal manifestation (Boltone, 1997). The presence of *Y. enterocolitica* on the surface of egg shells has been demonstrated, although its association with human infection has not be observed (Favier et al., 2000a).

Molds as undesirable microorganisms are widely distributed in nature. Unfortunately eggs are susceptible to fungal contamination at different stages till consumption (Fajardo et al., 1995). Certain molds are capable of producing toxic and carcinogenic metabolites or mycotoxins which could be regarded as potential health hazard (Cole and Cox, 1981).

The purpose of this study was to survey commercial hen's eggs for the presence of *Listeria* species, *Y. enterocolitica*, some important molds and to study the pathogenecity and virulence of *L.monocytogenes and Y.enterocolitica*.

MATERIAL and METHODS

A) Collection of samples:

150 samples of normal fresh market eggs 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 a sterile bag and dispatched to the laboratory with a minimum of delay.

B) Sample preparation:

(i) Egg shells.

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

(ii) Egg contents.

The egg samples were handled and prepared to evacuate the contents according to Speck (1976).

(iii)Dilutions:

Ten-fold dilutions up to 10^{-6} were aseptically prepared from the rinse solution as well as from the homogenized egg contents using sterile 0.1% peptone water.

C) Experimental techniques:

I- Isolation of *Listeria spp.*

The warm and cold enrichment procedures were carried out using an enrichment broth consisting of 0.6% Yeast extract, 5g/L. Lithium chloride, 40 mg/L Nalidixic acid, 15 mg/L Acriflavin chloride and 50 mg/L Cycloheximide (McClain and Lee, 1988). The Listeria selective agar (Oxford medium) was used as a selective isolating medium. The remainder of the method was identical to that of McClain and Lee, (1988) and Varadaraj, (1993). Identification of the suspected isolates and species differentiation was done according to Jonson et al. (1990) and the criteria of Rocourt et al. (1986) Table (2). All isolates confirmed as *Listeria monocytogenes* were subjected to mice pathogenecity test as described by Seeliger and Jones (1986).

II- *Yersinia enterocolitica.*

i) Isolation and identification of *Yersinia enterocolitica.*

Enrichment procedures described by Favier et al. (2000b) were used. Then loopfuls from the inoculated enrichment broths were streaked onto Cefsulodin Irgasan Novobiocin (CIN) agar as described by Schiemann (1979). Presumptive colonies "dark red colonies 1.5 mm diameter surrounded by a transparent border " bulls eye like" were randomly selected and identified by Gram stain and classical biochemical tests (Bercovier and Mollaret, 1984).

ii) Detection of virulence of *Yersinia enterocolitica* including the ability to penetrate Guinea pigs conjunctiva, mouse diarrhoea, and autoagglutination as described by Schiemann, (1981).

III- Mycological examination:

i) Molds count was determined by using Malt extract agar according to Harrigan and McCance (1976).

ii) Identification of the isolated strains:

Suspected mold strains were identified on Malt extract agar medium according to Moubasher (1993).

RESULTS

The results are tabulated in Tables 1, 2, 3 and 4

DISCUSSION

Foodborne listeriosis nowadays represented a serious public health problem in many countries since the case fatality rate is high (Anon, 1996). Results given in Table (1) point out that *Listeria* spp. could be isolated from 10 of 30 marketable egg shells with an incidence percentage of 33.33%. Samples containing *Listeria* spp. were obtained from locations scattered throughout Assiut City, thus the following discussion generally will not differentiate the source and type of eggs. Our results were low as compared with those reported by Nagah and Enas, (1995) who recorded incidence of 68.89%. On the other hand, Asmaa, (1997) reported lower figure 11.67%. Such variations may be attributed either to variations in media used for isolation of *Listeria* spp. or the degree of contamination of shells with litter and droppings, or the hygienic measures and to what extent they are implemented in poultry farms.

Listeria monocytogenes was detected in 4 of 30 (13.33%) egg shells, while *Listeria innocua, L.ivanovii* and *L.welshimerii* were isolated in percentages of 6.67%, 10% and 3.33% respectively (Table 1). In Assiut, Egypt, *L.monocytogenes* and other *Listeria* species were isolated from egg shells by some investigators (Nagah& Enas, 1995 and Asmaa, 1997). *Listeria* species were isolated from 6 of 30 (20%) egg content samples (Table 1). The most frequently isolated species was *L.innocua*, which was present in each sample contained *Listeria* spp. On

the other hand, *Listetria seeligeri* was found in two of 30 (6.67%) egg content samples, no other *Listeria* species were recovered. Lower figure (6.566%) was recorded by Nagah and Enas (1995). While Leasor and Foegeding (1989) reported higher incidence (30.95%). The higher incidence (13.33%) of *Listeria innocua* in egg contents (Table 1) is in accordance with those reported by Petran and Swanson (1993) as they stated that *Listeria innocua* was found more frequently than *Listeria monocytogenes* in a variety of foods. This may be attributed to the competition between organisms.

Information derived from the results reported in Table (2) revealed that *Listeria monocytogenes* could not be isolated from any samples of egg contents. This may be attributed to two reasons, firstly, presence of significant levels of *L.innocua* in egg contents (Petran and Swanson, 1993). Secondly, the antilisterial effects observed in the raw albumen due to the presence of protein fractions which hydrolyzes bacterial cell wall polysaccharide, causing cell lysis, as well as, the pH of raw egg albumin is not optimal for growth of *Listeria monocytogenes* (Sionkowski and Shelef, 1990). Our results simulates those reported by Nagah& Enas (1995) and Asmaa (1997), who failed to isolate *Listeria monocytogenes* from 45 and 60 samples of egg contents respectively. A contradictory finding was given by Leasor and Foegeding (1989), who could isolate *Listeria monocytogenes* from raw broken liquid whole eggs at a rate of 5%. *Listeria monocytogenes* may gain access to the egg contents through the affection of the oviduct (Gray and Killinger, 1966) or contamination from the shell during the breaking process (Leasor and Foegeding, 1989).

Our results revealed that both warm and cold enrichment methods were useful for isolation of *Listeria* species, resulting in 16 and 12 positive samples respectively.

Although cold enrichment often is the optimum method for isolation of Listeria from foods (Varadaraj, 1993). Our results indicated that the cold enrichment and plating resulted in the detection of low number of *Listeria spp.* in egg shells and contents, and therefore additional studies are needed to further evaluation of the cold enrichment procedure for isolating *Listeria* from eggs. Most likely, additional selective agents are needed in the cold enrichment procedure due to the diverse background microflora in eggs (Leasor and Foegeding, 1989).

The pathogenecity of isolated strains of *Listeria monocytogenes* in this work revealed that all isolates induced 100% mortality among

inoculated mice. The early deaths were observed 48 hours after inoculation and become more prominent after 96 hours. On post-mortem the early macroscopic lesions appeared in the form of haemorrhagic foci in the lung, liver and brain and late lesions showed necrosis which started after the fourth day of inoculation. The organism was reisolated from spleen, liver and brain of all dead mice. This result is compatible with those reported by Marco et al. (1992).

The results recorded in Table (1) reveal that 5 (16.67%) out of 30 egg shell samples were contaminated with *Yersinia enterocolitica*. *Yersinia enterocolitica* can also be found in chicken faeces. The egg shell can be contaminated with faeces in the nest or during subsequent manipulation (Berrang et al., 1999). As far as we know little available literatures were dealing with the incidence of *Yersinia enterocolitica* in hen's eggs, but generally Favier et al. (2000b) demonstrated the presence of *Yersinia enterocolitica* on the surface of egg shells, although its association with human infection has not been observed (Favier et al., 2000a).

The summarized data in Table, 1 point out that *Yersinia enterocolitica* could not be isolated from the examined samples of egg contents and this could be attributed to several factors, including the enrichment procedures fail to recover low levels of clinical strains from foods, some strains are sensitive to selective agents commonly used to isolate *Yersinis enterocolitica,* as well as, some of the selective media used don't recover all clinically important serotypes (Chester and Stotzky 1976), besides the compitition of other contaminants. Favier et al. (2000b) failed to isolate *Yersinia enterocolitica* from 88 samples of egg contents. Furthermore, the absence of potentially pathogenic *Yersina enterocolitica* strains has been isolated from other foods (Odumeru et al., 1997) and should also be a norm for fresh eggs.

The virulence tests were carried out on *Yersinia enterocolitica* (5 strains) isolated from shells of hen's eggs. The obtained results proved that 3 (60%) of 5 strains were virulent causing Guinea pig conjunctivitis, mouse diarrhoea model and autoagglutination tests at 22°C and 35°C (Table, 3). There are two factors required for virulence in *Yersinia enterocolitica* , (I) the ability to penetrate the mucosal surface of the intestine, (ii) the presence of virulence factors measured by the autoagglutination test (Pai and Destephano, 1980).

There appeared to be no seasonal differences in the incidence of *Listeria* species and *Yersinia enterocolitica* , although limited sample

numbers and seasonally odd sampling times made firm conclusions impossible.

Considerably more research is needed to be done to determine the role of eggs may play in transmission of foodborne Listeriosis and Yersiniosis. Effective measures to contol the two organisms must be developed, together with better and more efficient isolation procedures.

III- Mycological examination:

A total of 35 species and one variety belonging to 18 genera of fungi (34 species and one variety, 17 genera) from egg shells and (14 species, 7 genera) from egg contents were isolated from 150 (30 groups) of hen's eggs collected from Assiut Governorate (Table 4).

The filamentous fungal average count was 320/shell and 70/ml egg content respectively . In this study low mould counts were recorded, in comparison moderately high counts ($7.4X\ 10^4$/ shell and 9.8X10/ml egg content) were registered by El-Prince (1988). While El-Prince and Hemida (1997) recorded higher average counts ($5X10^5$/shell and $4X10^2$/ml content. Such variations are expected and may be attributed to the variations in the sanitary measures adopted in poultry farms, handling and storage of eggs.

It is apparent that the the number of species and genera recovered from shell samples are higher than those from contents as the shell is more liable to be contaminated.

The most common mould genera isolated from hen's eggs were *Aspergillus* and *Penicillium* which were recovered from 100%, 76.6% of egg shells and 63% and 36.6% of egg contents matching 54.5%, 18.75% and 64.3%, 28.8% of total fungi recovered from egg shells and its contents respectively. They included a wide range of species representing by 10 species of *Aspergillus* of which *A.flavus* was the most prevalent, while 7 species for *Penicillium* were identified of which *P.chrysogenum* was the most recovery. (Table, 4). Concerning *Aspergillus*, our results were substantiate those reported by El-Prince and Hemida (1997). On the other hand, they recovered a lower incidence (9.09% and 3.43% of *Penicillium* from egg shells and contents.

The remaining fungal genera and species were isolated in moderate, low and rare frequencies of occurrence (Table 4).

Contaminations of eggs with the objectionable mould will cause off-flavors "musty odors" and tastes spoilage and fungal rotting of eggs rendering it unfit for marketing and consumption. Most of molds

recovered in this work were reported as egg shell polutant and causing spoilage of eggs (Frazier and Westhoff, 1986).

Some species of *Penicillium* were found to be associated with pulmonary and urinary tract infections as well as "yellow rice disease" causing several deaths in man (Banwart, 1980). On the other hand, *Penicillium* species cause yellow, blue or green spots inside the shell. The hyphae of the mold may weaken the yolk membrane enough to cause its rupture, after which the growth of the mold is stimulated greatly by the food relased from the yolk (Frazier and Westhoff, 1986).

The contamination of eggs with the encountered molds is an indicative of bad sanitary measures adopted in poultry farms or during handling and storage of eggs. Thus, proper farm hygiene, handling and storage of eggs are necessary for obtaining eggs of good quality and to safe guard consumers from being infected.

REFERENCES

Anon (1996): Microorgainsims in foods, microbiological specifications of food pathogens. Co-published by James & James Ltd., London.

Asmaa, A.A.H. (1997): Occurrence of *Listeria* species in laying hens. Assiut Vet. Med. J. 38 (75): 97-103.

Banwart, G.J. (1980): Basic food microbiology. A bridged test book. Ed. Avipublishing Co., INC., Vest part, connecticut, USA.

Bercovier, H. and Mollaret, H.H. (1984): Yersinia, P. 498-506. in N. R. Krieg (ed.) Bergey's manual of systemic bacteriology, Vol. I The Williams & Wilkins Co., Baltimore.

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.

Boltone, E.J. (1997): Yersinia enterocolitica : The charisma continues. Clin. Microbiol. rev. 10: 257-267.

Chester, B and Stotzky, G (1976): Temperature dependent cultural and biochemical characteristics of Rhamnose positive *Yersinia enterocolitica.* Journal Clin. Microbiol., 3:119-127.

Chiesa, C.L.; Pacifico, V.; Cianfranco, E.; Nanni, A.; Medici, S.; Monto, P.; Triglione, P.; Mariani, P.; and Midulla, M. (1989): Sources of 2883 strains of *Yesinia enterocolitica* and related species

isolated in Italy: Clinical, ecological and epidemiological features. Microecol. Ther. B: 331-334.

Cole, R.J. and Cox, R.H. (1981): Handbook of toxic fungal metabolites. New York, Academic Press.

El-Prince, Enas (1988): Microbiological quality of hen's eggs. M.V.Sc. Thesis. Fac. Vet. Med., Assiut Univ., Egypt.

El-prince, Enas and Hemida, S.K. (1997): Mycological quality of commercial hen's eggs. Assiut Vet. Med. J. 36 (72): 275-285.

Fajardo, T.A.; Auantheswaran, R.C.; Puri, V.M. and Kanbel, S.J. (1995): Penetration of *Salmonella enteritidis* into eggs subjected to rapid cooling. J. Food Prot., 58 (5): 473-477.

Farber, J.M.; Sanders, G.W. and Johnston, M.A. (1989): A survey of various foods for the presence of *Listeria* species. J. Food. Prot., 52 (7): 456-458.

Favier, G. I.; Escudero, M. E.; Matter, M. A. and Guzman, M. S. (2000a): Survival of *Yersinia enterocolitica* and Mesophilic aerobic bacteria on eggshell after washing with hypochlorite and organic acid solutions. J. Food Prot. 63 (8): 1053-1057.

Favier, G.I.; Escudero, M.E.; Velazquez, L. and Guzman, A.M.S. (2000b): Reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria in eggshell by Washing with surfactants and their effect on the shell microstructure. Food microbiol. 17: 73-81.

Foegeding, P.M. and Stanley, N.W. (1990): Listeria monocytogenes F 5069 thermal death times in liquid whole egg. J. Food Prot. 53 (1): 6-8.

Foegeding, P.M. and Leasor, S.B. (1990): Heat resistance and growth of *Listeria monocytogenes* in liquid whole eggs. J. Food Prot., 53 (11): 9-14.

Frazier, W.C and Westhoff, D.C. (1986): Food Microbiology. 3 rd Ed., 6 reprint. Tata Mc Graw. Hill publishing Co., Ltd. pp. 267-268.

Gray, M.L. and Killinger, A. H. (1966): *Listeria monocytogenes* and listeric infections, Bacteriol. Rev. 30: 309-382.

Harrigan, W. F. and McCance, M.E. (1976): Laboratory methods in food and dairy microbiology. Academic press, London, New York, San Francisco.

Johnson, J.L.; Doyle, M.P. and Cassens, R.G. (1990): Listeria monocytogenes and other *Listeria spp.* in meat products. A review J. Food Prot. 53 (1): 81-91.

Leasor, S.B. and Foegeding, P.M. (1989): *Listeria* species in commercially broken raw liquid whole egg. J. Food Prot. 52 (11): 777-780.

Marco, A.; Domingo, M.; Prots, N.; Altimira, J.; Briones, V.; Blanco, A. and Dominguez, L (1992): Difference in pathogenecity between *L.monocytogenes* and *L.ivanovii* after intraperitoneal and subcutaneous inoculation. The eleventh international symposium on problems of Listeriosis. Eigtveds pakhus Copenhagen 11-14 May.

McClain, D. and Lee, W.H. (1988): Development of USDA. FSIS method for isolation of *Listeria monocytogenes* from raw meat and poultry. J. Assoc. Off. Anal. Chem. 71: 660-664.

Moats, W.A. (1979): The effect of washing eggs under commercial conditions on bacterial loads on egg shells. Poultry sci., 58: 1228-1233.

Moore, J., and Madden, R.H. (1993): Detection and incidence of *Listeria* species in blended raw eggs. J. Food Prot. 56: 652-654.

Moubasher, A.H. (1993): Soil fungi in Qatar and other Arab Countries: The Scientific and Applied Research Centre, Univ. of Qatar, Doha, 1: 566.

Nagah, M.S. and Enas-El-Preince (1995): Prevalence of *Listeria* species in hen's eggs sold in Assiut City. Assiut Vet. Med. J. 33 (65): 127-131.

Odumeru, J.A.; Mitchell, S.J. Alves, D.M.; Lynch, J. A.; Yee, A.J.; Wang, S.L. Styliadis, S.J. and Faber, J.M. (1997): Assessment of the microbiological quality of ready-to-use vegetables for health-care food services. J. Food Prot. 16: 954-960.

Pai, C.H. and Destephano, L.C. (1980): Virulence factors in *Y.enterocolitica.* Abs. Ann. Metting Am. Society for Microbiology. B52, 23.

Petran, R.L. and Swanson, K.M.J. (1993): A research note: Simultaneous growth of *Listeria monocytogenes* and *Listeria innocua.*J. Food Prot. 56 (7): 616-618.

Rocourt, J.; Hof, H.; Schnettenbrunner, A.; Mallnverni, R. and Brille, J. (1986): Meningite purulente aige a *Listeria seeligeri*: Chez un adulte immunocompents. Schweiz. Med. Wochenschr. 116: 248-251.

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

Schiemann, D.A. (1981): An enterotoxin negative strain of *Yersinia enterocolitica* serotype O:3 capable of producing diarrhoea in mice. Infection and Immunity, 32: 571-574.

Seeliger, H.P.R. and Jones, D.E. (1986): Genus listeria . pp. 1235-1245. In: Bergey's manual of systematic bacteriology, Vol. 2 (Sneath, P. H. A., Mair, N. S., Sharpe, M. E. and Holt, J. G. eds, The Williams and Wilkins Co., Baltimore, M.S.

Sionkowski, P.J. and Shelef, L.A. (1990): Viability of *Listeria monocytogenes* strain Bric-1 in the avian egg. J. Food Prot. 53 (1): 15-17.

Speck, M.L., (ed) (1976): Compendium of methods for microbiological examination of food. American public health Association. Washington, D. C.

Varadaraj, M.C. (1993): Methods for detection and enumeration of foodborne bacterial pathogens: A critical evaluation. J. Food Sci. Technol., 30 (1): 1-13.

Table (1): Incidence of *Listeria species* and *Yersinia enterocolitica* recovered from commercial hen's eggs.

Samples Hen's egg groups (30)	positive samples													
	Listeria species												*Yersinia enterocolitica*	
	Listeria monocytogenes		Other *Listeria* species								Total			
			L.ivanovii		*L.innocua*		*L.seeligeri*		*L.welshimerii*					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hen's egg shell	4	13.33	3	10	2	6.67	-	0.00	1	3.33	10	33.33	5	16.67
Hen's egg contents	-	0.00	-	0.00	4	13.33	2	6.67	-	0.00	6	20	-	0.00
Total	4	13.33	3	10	6	20	2	6.67	1	3.33	16	53.33	5	16.67

Table (2): Differentiation of *Listeria* species according to the criteria of Rocourt, (1986)

Species	Beta-haemolysis	CAMP test with *S.aureus*	Fermentation of			pathogenicity for mice
			Mannitol	L.Rhamnose	D.xylose	
L.monocytogenes	+	+	-	+	-	+
L.ivanovii	++	-	-	-	+	+
L.seeligeri	+	+	-	-	+	-
L.innocua	-	-	-	V	-	-
L.welshimeri	-	-	-	V	+	-

V = Variable reaction

Table (3): Virulence of *Yersinia enterocolitica* strains isolated from examined samples

Type of samples	No. of strains examined	tests for detection of virulence												Virulence strains		Avirulence strains	
		Guinea pig conjunctivitis				Mouse diarrhoea model				Autoagglutination Test							
		Grade of reaction				Grade of reaction				Grade of reaction							
		+++	++	+	-	+++	++	+	-	+++	++	+	-	No.	%	No	%
Hen's egg shells	5	2	1	-	2	1	1	1	2	1	2	-	2	3	60	2	40

Table (4): Average total counts and percentage frequency of fungal genera and species isolated from 150 hen's eggs (representing, 30 groups)using Malt extract agar at 28°C.

Genera & species	Egg shell surfaces			Egg contents		
	ATC	NCI & OR		TC	NCI & OR	
Acremonium strictum	22	11	M			
Alternaria	4	4	L			
A.alternata	2	2	R			
A.chlamydospora	2	2	R			
Aspergillus	174	30	H	45	19	H
A.fisherii	2	2	R			
A.flavus	52	20	H	30	17	H
A.flavus var. columnaris	13	9	M			
A.fumigatus	11	7	L	3	3	R
A.niger	80	24	H	4	4	L
A.ochraceus	1	1	R			
A.oryzae	1	1	R	2	2	R
A.sydowii	5	5	R	1	1	R
A.tamarii				2	2	R
A.terreus	7	6	L	3	3	R
A.versicolor	2	2	R			
Cladosporium	19	10	M	2	2	R
C.cladosporioides	16	10	M	2	2	R
C.sphaerospermum	3	2	R			
Emericella nidulans				1	1	R

Table (4):Continued

Genera & species	Egg shell surface			Egg contents		
	ATC	NC& OR		TC	NCI & OR	
Eurotium amstelodami	4	2	R			
Fusarium oxysporum	10	7	L			
Gibberella	10	8	M	1	1	R
G.fujikuroi	8	6	L	1	1	R
G.intricans	2	2	R			
Mucor hiemalis	1	1	R			
Nectria haematococca	3	3	R	1	1	R
Nigrospora sphaerica	2	2	R			
Paecilomyces variotii	2	2	R			
Penicillium	59	23	R	20	11	M
P.aurantiogriseum	4	4	L			
P.chrysogenum	36	19	H	18	9	M
P.citrinum	3	3	R			
P.duclauxii	2	2	R			
P.funiculosum	4	4	L	1	1	R
P.jensenii	1	1	R			
P.oxalieum	9	7	L	1	1	R
Pleospora herbarum	1	1	R			
Scopulariopsis brevicaulis	2	2	R			
Stachybotrys chartarum	1	1	R			
Trimatostroma betulinum	5	5	L			
Ulocladium botrytis	1	1	L			
Average gross total count	320	30	H	70	22	H
No. of genera	17			6		
No. of species	34 +1			14		

Abbreviations:

ATC = Average total counts

NCI = Number of cases of isolation

OR = Occurrence remark's