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Biosafety Against Fungal Contamination of Hen's Eggs and Mycotoxins Producing Species

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Abstract. In the present work one hundred random hen's eggs were collected from a poultry farm at El-Behera Governorate, Egypt for detection of fungal contamination and mycotoxins production and evaluation of the sanitary conditions of the farm. The obtained results proved that (38%) of the examined eggs were contaminated with moulds species with a count ranging from 11 to 17×10^3 cfu/g and a mean value of 3.4×10^3/g. The isolated moulds species were identified into *Aspergillus* (14%), *Penicillium* (9%), *Fusarium* (1%), *Mucor* (6%), *Rhizopus* (4%) and *Cladosporium* spp. (5%). The genera *Aspergillus*, *Penicillium*, and *Fusarium* spp. were further identified into *Asp. flavus*, *Asp. niger* and *Asp. fumegatus*, *P. oxalicum* and *P. regulosum* and *Fusarium graminarium*. The isolated mould species were screened for mycotoxins production under laboratory circumstances. The obtained results showed that (10.25%) of the previous mould species were able to produce mycotoxins in synthetic media. Aflatoxins B_1, B_2, G_1, G_2 were produced by two strains of *Asp. flavus* in total concentrations of 1.3 and 1.5 µg/g mycelia, respectively. T_2-toxin was produced by one strain of *Penicillium regulosum* with concentration 0.072 µg/g mycelia; while Zearalenon toxin was produced by one strain of *Fusarium graminarium* in a concentration of 0.146 µg/g mycelia. The dangerous role of contaminated hen's eggs by mould species at poultry farm, as well as, the public health hazard and the high economic losses have been discussed. Great attention for the prophylactic and control measures that should be adopted in the poultry farm to overcome these problems and achieve the consumer safety has been recommended.

Introduction

Egg is one of the most nutritious foods, not only because they are considered one of the highest sources of protein, but also they contain almost every essential vitamin and mineral needed by human. In fact, egg protein is of such quality that it is used as a standard to which other proteins are compared. In addition, eggs have long been important contributors to the nutritional quality of human diet as they provide more nutrients than calories to the average human diet. Thus they deserve to be called "nutrient dense' and to be part of everyone's diet. They also provide a unique, well-balanced, easily digestible source of nutrient for persons at all ages.

As an egg is originally designed to create a chick, it has a complete life support system with many natural, built-in barriers to prevent bacterial entrance and growth, protecting the developing embryo. These barriers protect, as well, the egg on its way from the hen to the consumers.

Though it helps, the porous shell is not a full proof barrier due to the presence of bloom or cuticle. Other barriers to prevent contamination include the inner shell membranes. These layers fight bacteria in several ways. The inner shell membrane is believed to be made of protein fibers interwoven without any pores going straight through and contains a high amount of lysozymes.

In spite of these protective barriers, against microbial flora yet contamination of eggs before laying, transovarian and after laying with a variety of organisms from different sources exists. This can occur through the vent, from nesting materials, floor litter, avian fecal matter improper handling, washing, the type of detergent used, temperature and pH of the washing solution, storage under very humid conditions and inadequate sanitizing of equipment (Board and Fuller, 1994).

Contamination of eggs and egg products with microorganisms possibly means injuring of egg quality which may lead to spoilage and consequently economic losses or perhaps transmission of pathogens inducing cases of food borne infection or intoxication to consumers constituting public health hazard (California Egg Commission, 1999).

Aflatoxins are potent toxic, estrogenic, mutagenic, teratogenic and carcinogenic agents produced by *Aspergillus flavus* and *A. parasiticus* on feed. The major aflatoxins of concern are aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2 (Adams and Moss, 2001).

Aflatoxins are potent hepatotoxins and carcinogens. Their effects vary with dose, duration of exposure and nutritional status. The clinical signs of acute aflatoxicosis is represented by lack of appetite, weight loss, jaundice, neurological abnormalities, ascitis and oedema of the lower extremities. Mortality is high and death occurs suddenly as a result of massive gastrointestinal hemorrhage**.** Affected human beings with chronic aflatoxicosis may be predisposed to secondary infectious diseases because of the immunosuppressive effects of aflatoxins that may affect cell mediated immunity, extend whole blood clotting time, prothrombin time, and recalcification time (Hendrickse, 1997).

Freshly laid eggs are generally devoid of organisms. However, following the exposure to environmental conditions, eggs become contaminated with different types of microorganisms from various sources including soil, dust and dirty nesting materials. Consequently the bad storage of eggs under very humid conditions could support the multiplication of these contaminating microorganisms present on eggshell. Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells. After laying, freshly laid eggs cool and the contents contract leading to drawing of water and microorganisms through the shell pores. Some factors influence the bacterial penetration such as environmental temperature and humidity enhancing the infection and spoilage (Frazier and Westhoff, 1987). Thus, the rate of egg contamination depends mainly on hygienic measures applied in the farm during transportation, handling and storage.

Owing to the continuous consumers' demand for fresh eggs, and egg products, it is extremely necessary, not only to increase egg production, but also to safeguard consumers against health hazards. This study is a trial to throw light on the importance for the prophylactic and control measures that should be adopted in the poultry farm to overcome these problems and achieve the consumer safety.

Materials and Methods

Collection of Samples, Isolation and Identification of Mould

One hundred random samples of hen's eggs collected from a poultry farm at El-Behera Governorate were examined for determination of total mould count, as well as for detection of mycotoxins produced by some isolated moulds. The surface of each egg was aseptically immersed in 70% ethyl alcohol and left to drain. A hole was made in the blunt end of the shell and the egg content was aseptically evacuated into sterile blender and mixed well. Serial dilutions were done from each egg content for detection of the total mould count/g of egg content according to Bailey and Scott (1998).

The isolated organisms were picked up, purified and identified according to Ajello and Georg (1964), Booth (1977) and Bailey and Scott (1998).

Detection of Mycotoxins Producing Strains of Mould Isolates

Hyphal tip colony of pure strains of isolated *Aspergillus*, *Penicillium*, and *Fusarium* spp. was prepared for toxin production according to Mashaly, *et al*. (1982) and El-Deeb (1988).

The stored spores of each fungus were activated on potato dextrose agar at 28ºC for 7 days. The different strains of each fungus were incorporated separately onto tube containing 10 ml of synthetic media (Difco, 1984) for determination of the dry weight of the mycelia.

The inoculum was 0.1 ml activated fungal spores suspension containing 10^7 spores/ml. The tubes were exposed to steam at 100ºC for 5 minutes by autoclaving to kill the living fungi, and then incubated at 28ºC for 7 days. The mycelial dry weight of the growth in each one was estimated by filtering the tube content in a Whatmann filter paper No. 41. The filter paper containing the fungal hyphae and spores was placed in vacuum oven at 40ºC until complete drying.

Mycotoxins and Aflatoxin Determination

The aflatoxins produced by different strains of isolated fungi were determined in both dry fungus material and previous filtrate of the media

as described by El-Deeb (1988). The mycotoxins produced by each strain of isolated mould were determined according to Scott (1983).

Aflatoxin and mycotoxins standards obtained from sigma chemical company, USA were used for comparison (Double Wave Length TLC Scanner Model CS-910 made by Shimatzu Co., Japan).

Results and Discussion

Data of Table 1 reveal that moulds were recovered from 38% of the examined egg magma with a mean value of $3.4 \times 10^3 \pm 7.6 \times 10^2$/ml. The highest frequency percentage (60.53) lies within the range of $10\text{-}10^2$ (Table 2). These findings substantiate what have been reported by Ahmed, *et al*. (1987) and Amer (1990).

Table 1. Total mould count/g of examined egg content.

No. of samples	No. of +ve samples	%	Minimum	Maximum	Mean ± SEM
100	38	38	11	17×10^3	$3.4 \times 10^3 \pm 7.6 \times 10^2$

Table 2. Frequency distribution of examined eggs contents based on their total mould count/g.

Interval	Frequency	
	No.	%
0-10	4	10.53
$10\text{-}10^2$	23	60.53
$10^2\text{-}10^3$	11	28.94
Total	38	100

The results obtained in Table 3 show that the most prevalent mould species was *Aspergillus* which represented about (14%) of the total isolates, while *Penicillium*, *Fusarium*, *Mucor*, *Rhizopus*, and *Cladosporium* species were isolated from 9, 1, 6, 4, and 5%, respectively. Most of the isolated genera have been detected by Ahmed, *et al*. (1974), Moursy, *et al*. (1982), Torkey (1982) and Amer (1990).

From the economic point of view the penetration of fungi into eggs leads to spoilage of its content in the market as well as some species were incriminated in public health hazard (Ray, 2001).

Table 3. Incidence of the isolated mould spp. in the examined egg content.

Isolated spp.	No. of isolates	%
Aspergillus flavus	2	2
Asp. niger	5	5
Asp. fumigatus	7	7
Penecillium oxalicum	5	5
P. rugulosum	4	4
Fusarium ograminarium	1	1
Mucor spp.	6	6
Rhzopus spp.	4	4
Cladosprium spp.	5	5

Aflatoxin and Mycotoxins Production

Results in Table 4 reveal that two strains of *Aspergillus flavus* have the ability to produce aflatoxin in a total concentration of 1.3 and 1.5 µg/g mycelia. Another species of *Aspergillus* failed to produce aflatoxin.

Table 4. Aflatoxin produced in synthetic media by pathogenic mould strains isolated from egg contents.

Strain type	Mycelial dry weight (mg)	Aflatoxin concentration (µg/g mycelia + 10 ml media)				
		B1	B2	G1	G2	Total
Asp. flavus	15.1	0.48	0.365	0.396	0.130	1.376
Asp. flavus	15.8	0.628	0.295	0.481	0.194	1.598

The data presented in Table 5 show that one strain of *Penicillium regulosum* produced T2-Toxin while Zearalenone was produced by a strain of *Fusarium graminarium*. Aflatoxin could occur as a natural contaminant of poultry feed (Edds and Bortell, 1983) and the egg may contain aflatoxins due to the chronic exposure of birds to these chemicals via contaminated feed (Jones, *et al*., 1982).

Table 5. Mycotoxins produced in synthetic by pathogenic mould isolated from egg contents.

Strain type	Mycelial dry weight (mg)	Mycotoxin concentration µg/g mycelia + 10 ml media	
		T_2-Toxin	Zearalenone
Asp. flavus	13.5	0.072	–
Asp. flavus	13.5	–	0.146

Some moulds especially *Fusarium* and *Penicillium* species could penetrate into the eggs at different temperatures and produce their toxins which increase with the storage time (Torkey, 1982).

Aflatoxin B1 and M1, as well as, G1 and G2 were detected in both yolk and white of eggs of laying hens by Truckess, *et al*. (1983). Furthermore, the presence of Zearalenone which is the toxic by-product of *Fusarium* species was reported by Mirocha, *et al*. (1981). Concerning health hazards, aflatoxins are considered as acute toxins and produce potential carcinogenicity.

Zearalenone was responsible for estrogenic signs, diarrhea, emesis, and hemorrhage which reduce the fertility rate and lead to economic losses (Wyllie and Rehouse, 1978). Furthermore, the T2-toxin was also detected in many cases of mycotoxicosis and skin lesions (Mirocha, *et al*., 1981).

Aspergillus species may induce pulmonary aspergillosis, pulmonary allergy, skin infection, nasal infection, as well as, nail and external ear infections (external otitis) while, *Mucor* and *Rhizopus* species are frequent contaminants of foods. These members may involve the rhino facial-cranial area, the lungs, gastrointestinal tract, skin and possibly other organ systems, as well as, they can induce intra-ocular infection, external otomycosis, orbital cellulitis and deep wound infection (Washington, 1981).

Feed is a potent source of aflatoxin-producing *Aspergillus* which constitute a big threat to the poultry industry**.** Aflatoxin is expected to be present in eggs under wide range of conditions at almost allover the year because the genus *Aspergillus* is ubiquitous in nature; in the soil, in the grains and in the air**.** Aflatoxin causes poor growth, decrease of feed utilization, decrease of egg production and decrease of hatchability, egg quality and egg weight (Ray, 2001).

Aflatoxins are potent toxic, estrogenic, mutagenic, teratogenic and carcinogenic agents produced by *Aspergillus flavus* and *A. parasiticus* on feed. The major aflatoxins of concern are aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2 (Adams and Moss, 2001).

In conclusion, the pathogenic moulds found their way to penetrate and contaminate eggs and may produce their toxins under favorable conditions. Therefore, special attention should be directed to safeguard

the eggs against their contamination through application of correct farm hygiene programs, good handling and storage methods, as well as, the periodical examination of eggs and poultry feed.

From the public health point of view, certain strains of moulds were implicated in food poisoning outbreaks due to production of aflatoxins, as well as some moulds, are capable of forming toxins that cause mycotoxicosis in man and neoclassic diseases including leukemia (Ray, 2001).

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السلامة الحيوية للتلوث الفطري لبيض الدجاج والأنواع المنتجة للسموم

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المستخلص. تم جمع عدد ١٠٠ بيضة من إحدى مزارع الدواجن في محافظة البحيرة، من أجل الكشف عن التلوث الفطري وإنتاج السموم الفطرية (الميكوتوكسين)، وذلك بغرض تقييم الظروف الصحية في المزرعة. وأثبتت النتائج أن ٣٨٪ من البيض كان ملوثا بأنواع العفن، تراوح عددها ١١ و١٧ x١٠^{٣}/جرام بقيمة متوسطة ٣,٤ x١٠^{٣}/جرام. وقد تم تحديد أجناس الفطريات الآتية: أسبرجلس (١٤٪)، بنسيليوم (٩٪)، فيوزاريوم (١٪)، ميوكر(٦٪)، ريزوبس (٤٪)، والكلادوسبوريوم (٥٪). أما الأنواع الخاصة بالأجناس المذكورة فكانت أسبرجلس فلافس، أسبرجلس نيجر، أسبرجلس فيوميجاتوس، وبنسليوم أكساليكوم، وبنسليوم ريجولوزم، وفيوزاريوم جرامينياريوم. وقد تم إجراء مسح للأنواع المعزولة للوقوف على قدرتها على إنتاج السموم الفطرية في البيئات الاصطناعية. وكان تركيز الأفلاتوكسين B_1 وB_2 وG_1 وG_2 الناتج من سلالتي الأسبرجلس فلافس ١,٣ و١,٥ ميكروجرام/جرام ميسليوم على التوالي. أما التوكسين T_2فتم إنتاجه من سلالة واحدة

من البنسليوم ريجولوزم بتركيز ٠,٠٧٢ ميكروجرام/جرام ميسليوم، أما الزيارالينون فقد تم إنتاجه من سلالة واحدة من الفيوزاريوم جراميناريوم بتركيز ٠,١٤٦ ميكروجرام/جرام ميسليوم. وقد تم مناقشة الدور الخطير لبيض الدجاج الملوث بأنواع الفطريات المختلفة في الإضرار بالصحة العامة، والخسائر الاقتصادية المترتبة على ذلك، مع التركيز على طرق التحكم والوقاية التي يمكن تطبيقها في مزارع الدواجن للتغلب على هذه المشاكل ولضمان سلامة المستهلك.