Detection of Aflatoxin and antibacterial residues in different types of table eggs with studying of the effect of heat treatment.

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A B S T R A C T

Ninety egg samples, represented as (30 each of brown, white farm eggs and balady eggs) were randomly collected from different markets in Cairo and Giza cities. Collected samples were analyzed for detection of the antibacterial and aflatoxins (B_1, B_2, G_1 and G_2) residues in addition to study the effect of heat treatment (boiling and frying) on antibacterial and aflatoxins residues in positive samples. The egg samples were analyzed for antibacterial residues using a modified four plate test using *Bacillus subtilis*. The current results revealed that the incidence of antibacterial residues was 6.6%, 20% and 13.3% in balady, brown farm eggs and white farm eggs respectively. The different heat treatment completely degraded the antibacterial residues in balady eggs. However, some traces of antibacterial residues were existed after boiling and frying of farm eggs. On the other hand, the egg samples were analyzed for total aflatoxins (B_1, B_2, G_1 and G_2) residues using competitive direct enzyme linked immune sorbent assay (CD- ELISA). The current results revealed that total aflatoxins residues in balady eggs, brown farm eggs and white farm eggs were 30%, 16.6% and 20% respectively. The different heat treatment revealed that Aflatoxin residues was almost stable in naturally contaminated egg for up to 15minutes of boiling and frying for 5 minutes , so avoiding aflatoxin transmission into egg appears to be the only practical way to ensure their safety for human consumption. Therefore, the presence of such residues in eggs should be taken in consideration for public health hazard.

Keywords: Aflatoxin, antibacterial residues, table eggs.

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1. INTRODUCTION

Today, eggs remain a stable food within the human diet, consumed by people throughout the world. They are consumed worldwide in various dishes and considered very nutritious and a cheap source of protein (Papadopoulou*et al.*, 1997). Moreover, eggs provide a unique well balanced nutrient for persons of all ages. Their high nutrient content, low caloric value and ease of digestibility make eggs available in many nutritive diets for adults. (Heranzet *al.*, 2007 and Ebubekir*et al.*, 2008). Antimicrobials are used by the poultry industry to enhance growth and feed efficiency and to reduce bacterial disease (Donoghue 2003). In laying hens, antimicrobials are used only to treat and to prevent bacterial infections. Antimicrobial classes used to treat poultry are similar to those used in human medicine and include

aminoglycosides, tetracyclines, beta-lactams, quinolones, macrolides, polypeptides, amphenicols and sulphonamides (Stolker & Brinkman 2005). The therapeutic uses of antibacterial agents in laying hens possess a particular problem because it may result in drug residues in eggs laid during and directly after treatment (Loliger, 1978; Terplan et al., 1979; Siegman and Neuman, 1984). Residues of furazolidone, chloramphenicol, sulpha-quinoxaline, nitrofurazone, tetracyclines and other antimicrobial agents were detected in eggs of treated chickens (Roudautet *al.*, 1989; Tropilo and Stepien , 1989). Antibiotics and sulphonamides residues may be retained in eggs after veterinary medication of laying hens, which may cause allergic reaction, toxicity and skin rashes in human (Rivere and Spoo, 1995). Although, the majority of freshly laid eggs are sterile inside, the shells soon become contaminated with litter, droppings, dust and prevailing environment giving the chance for food borne threat of great public health concern. The presence of fungi and their toxic metabolites (mycotoxins) in poultry ration, on the other hand, is virtually inevitable particularly in tropic areas. Mycotoxins are unavoidable because they are naturally occurring compounds. They contaminate crops before harvesting or invade feedstuffs of laying hen during processing, transport or storage (Liau*et al.,* 2007; Yaling*et al.,* 2008). Aflatoxins are group of polypeptide- derived furanocoumarins, with at least 16 structurally related toxins that have been characterized. These toxins are produced by a number of different Aspergillus species (Cast, 1989; Goto*et al.*, 1996; klick et al., 2000 ; Ito *et al.,* 2001 and Peterson *et al.* 2001). Aflatoxins (AFs) are secondary metabolites of the fungi *Apergillus flavus* and *Aspergillus parasiticus*. These moulds are common contaminants of foodstuff, particularly in the tropical regions (Gourama and Bullerman, 1995) .Aflatoxin contaminated feed may effect on growth and health of poultry and the possible transmission of such toxic residues to edible eggs resulting in potential hazards to human health (Martin *et al.,* 1998). The presence of aflatoxins in egg is a potential threat to the health of the consumer. Growing children are more sensitive than adults are, as egg is one of their main sources of nutrients. Aflatoxin is known to be human carcinogens based on sufficient evidence of carcinogenicity in humans (IARC, 1987, 1993 and Yaling *et al.,* 2008). Effects of aflatoxins are dose - time dependent, and two distinct forms of aflatoxicosis, namelyacute and chronic, can be distinguished depending on the dose and length of time of exposure (Leeson *et al.,* 1995). Many studies have linked aflatoxin contamination of food with some toxic effects such as liver cancer and immune suppression in various animals and humans (Williams *et al.,* 2004 and Jianet *al.,* 2005). The most common analytical methods employed for AFs determination are thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). Among them, ELISA is often used for routine screening due to its several advantages, such as rapidity, simplicity and cost-effective (Rosi*et al.,* 2007; Kursat*et al.,* 2011). Therefore, the present study was conducted to determine the incidence of Aflatoxin and antibacterial residues in chicken eggs in Cairo and Giza markets as well as the effect of heat treatment on such residues.

2. MATERIALS AND METHODS

2.1. collection of samples:

A total of ninety egg samples, represented as (30 each of balady eggs, brown and white farm eggs) were randomly collected from different markets in Cairo and Giza cities and subject to analysis for detection of the antibacterial and aflatoxins residues in addition to study the effect of heat (boiling and frying) on antibacterial and aflatoxins residues in positive samples.

2.2. Detection of Antibacterial residues:-

2.2.1. Preparation of samples:

The egg shells were cleaned using cotton swabs moistened with sterile water and wiped with surgical spirit. After thoroughly disinfection, insert the needle of syringe into the egg and take 2ml from the content then dropped into a sterilized beaker. According to (AOAC, 2000), the egg sample was homogenized. The homogenous content of each sample were mixed with 20 ml of phosphate buffer solvent (Freres and Vatdeboaze, 1969). After 10 min. centrifugation at 3000 rpm, the supernatants were tested for antibacterial residues using *Bacillus subtilis* as test organism.

2.2.2. Four plate tests:

They were applied according to (Heitzman, 1994). The presence of antibiotic residues were detected qualitatively by a modified four plate test using *Bacillus subtilis*. The detection of an antibacterial substance in eggs is determined positive when suitable duplicate zones of inhibition of at least 2mm in width (inhibition zones $\geq 2mm$) after incubation period of 18-24 hr. at 30^{o}C.

2.3.Aflatoxins analysis:-

Detection of total aflatoxins (B_1, B_2, G_1 and G_2) was carried out by competitive direct enzyme linked immune sorbent assay (CD-ELISA) according to Salem *et al.*, (2014). The method makes it possible to analyses a large number of samples and does not require time- consuming procedures and sophisticated equipment (Thirumala- Devi *et al.*, 2012).

2.4.Heat treatment:

The positive samples for antibacterial and aflatoxins were subjected to heat treatments as follows.

2.4.1.1.Boiling:

The positive raw egg samples were immersed in boiling water for 15 minutes according to (Antown and Hassan, 2002). Then cooled and examined again.

2.4.1.2.Frying:

3. The content of positive raw egg samples were poured into a sterile frying pan containing a small amount of corn oil (negative for aflatoxin residues) and cooking stir for 5 minutes (Normal cook for frying). (Antown and Hassan, 2002) .Then cooled and examined again.

3. RESULTS AND DISCUSSION

Antibacterial residues in eggs may be produced by administration of antibacterial to laying hens via food or drinking water used by veterinarians for therapy, prophylaxis and growth promotion in laying hens (Roudant and Moretain, 1990). From the results obtained in (Table. 1), it was revealed that the number of positive egg samples for antibacterial residues in balady , brown farm eggs and white farm eggs were 6.6 % ,20% and 13.3 %; respectively .Nearly similar results were

Table (1): Incidence of antibacterial residues in different egg samples (n= 30 for each sample type)

Types of tested egg samples	Number of examined samples	Positive samples		Negative samples	
		No.	%	No.	%
Balady	30	2	6.6	28	93.4
Brown farm eggs	30	6	20	24	80
white farm eggs	30	4	13.3	26	86.7

Table (2): Effect of different heat treatment on antibacterial residues in positive tested egg samples:

Types of tested egg samples	No. of Positive samples	Inhibition zones (mm)		
		Raw	Boiled	Fried
Balady	2	2	0	
		3		0
Brown farm eggs	6	2	1	
		3.5	1	
		3	0	
		2		0
		3		0
		2		0
white farm eggs	4	2	0	
		3.5	1	
		2		0
		2		0

Table (3): statistical analysis of the Aflatoxin residue levels in different tested egg samples (ppb)* (n= 30 for each sample type):

*ppb= part per billion = µg/Kg.

Types of tested egg samples	Positive samples		Minimum	Maximum	Mean ± SE**
	Number	%			
Balady	9	30	0.9	14.3	6.7 ± 1.7
Brown farm eggs	5	16.6	0.34	7.3	3.2 ± 1.2
White farm eggs	6	20	0.75	9.1	4.34 ± 1.1

**Mean ± SE = mean ±standard error for positive tested egg samples only.

Table (4): Effect of different heat treatment on Aflatoxin residues in positive egg samples (ppb):

Types of tested egg samples	positive samples	Heat treatment			
		Raw	Boiled	Raw	Fried
Balady (9+ve samples) (4 samples boiled and 5 samples fried)	1	1.4	1.4	0.9	0.9
	2	9.8	9.73	11.55	11.5
	3	7.5	7.4	6.95	6.9
	4	8.76	8.7	9.2	9.15
	5			3.9	3.9
	Mean	6.86	6.8	6.5	6.36
	Reduction %	-	0.9%	-	2.2%
Brown farm eggs (5 +ve samples) (3samples boiled and 2 samples fried)	1	0.34	0.3	1.6	1.5
	2	7.3	7.25	4.6	4.56
	3	2.4	2.4		
	Mean	3.35	3.3	3.1	3.03
	Reduction %	-	1.5%	-	2.3%
White farm eggs (6 +ves amples) (3samples boiled and 3 samples fried)	1	0.75	0.75	0.9	0.9
	2	9.1	9	7.3	7.1
	3	2.5	2.4	5.46	5.4
	Mean	4.12	4.05	4.55	4.46
	Reduction %	-	1.2%	-	2%

reported by Antown and Hassan, (2002) and Fath EL- Bab, (2012). Whilelower results were obtained by (salem*et al.,* 2009). Table (2) showed that effect of different heat treatment (boiling & frying) on antibacterial residues in positive egg samples (the eggs proved to be positive to the presence of antibiotics residues (inhibition zones ≥ 2mm) detected by measurement of inhibition zone(mm). The present results revealed that boiling for 15 minutes or frying for 5 minutes of balady, brown farm eggs and white farm eggs were degraded the antibacterial residues, which resulted in disappearance of inhibition zones exceptof some traces of antibacterial residues were recorded in brown farm eggs and white farm eggs. These results may be attributed to the sensitivity of the antibacterial agents used in such chickens to heat treatment.Nearly similar results were reported by Antown and Hassan (2002) .The present results do not indicate that allantibacterial are thermo labile. For example Rose *et al.*, (1996) stated that the oxytetracyclin was unstable in water at 100^{o}C with a half – life of about 2 min., but more stable in oil at 180^{o}C where the half – life of about 8 min. However, Kuhne *et al.*, (2001) for

tetracycline. The author mentioned that there was a significant decrease of tetracycline by about 50 % after heat treatment. While, some antibacterial are heat stable such as chloramphenicol (Hamman *et al.,* 1978). Moreover, The mean remaining activity of enrofloxacin residue reduced to 68 % after cooking (Van Egmond*et al* ., 2000) and Fath EL- Bab (2012)stated that the concentration of tetracycline and enrofloxacin residues in the examined egg samples before boiling were 380 and 693 ppb while after boiling were 201.4 and 332.64 ppb with a reduction rate 47% and 52%; respectively. Moreover, the concentration of tetracycline and enrofloxacin residues in the examined egg samples before frying were 380 & 693 ppb and after frying were 159.6 & 214.83 ppb with a reduction rate 58 % and 69%; respectively. Existence of antibacterial residues in food stuff can pose hazards to human health. Among them are sensitivity to antibacterial, allergy reactions and imbalance of intestinal microflora, bacterial resistance to antibiotics in microorganisms and losses in the food industry (Cunha, 2001; Kirbis, 2006 and Lolo *et al* ., 2006) . Also, antibacterial residues in food produce some pathological effects as immune pathological effects, autoimmunity, carcinogencity, mutagencity, nepho-toxicity, hepatotoxicity, reproductive disorder, bone marrow toxicity and allergy (Nisha, 2008). Table (3):it was revealed thatnumber of positive tested egg samples for total aflatoxins (B_1, B_2, G_1&G_2) residues in balady, brown farm eggs and white farm eggs were 30%, 16.6%and 20%; respectively. The mean values of aflatoxins residues in balady was 6.7 ± 1.7 ppb with the minimum and maximum values were 0.9 ppb and 14.3 ppb; respectively. The mean values of aflatoxins residues in brown farm eggs were 3.2 ± 1.2 ppb, with the minimum and maximum values were 0.34 ppb and 7.3 ppb; respectively and the mean values of aflatoxins residues in white farm eggs was 4.34 ± 1.1 ppb with the minimum and maximum values were 0.75 ppb and 9.1 ppb; respectively. This result were higher than that previously reported by Khafaga *et al.*, (2010) , salem *et al.,(*2009), but lower than those reported by Hassan (1995) who detected AFG2 residue contamination (80µg/kg) in baldy egg samples. Most of positive examined samples especially in baldy eggs contain aflatoxins residues more than the permissible limits of aflatoxin recommended by FAO (1997) which is 5 ppb for all aflatoxins residues. The higher level in the current study indirectly reflects the higher degree of exposure of poultry to aflatoxins in their ration. Table (4) showed high stability of aflatoxins residues in contaminated eggs after boiling for 15 minutes and fried for 5 minutes, with a negligible mean reduction %, ranged from 0.9% -1.5% in boiled eggs for 15 minutes and from 2% - 2.3% in fried eggs for 5 minutes. Nearly similar findings were reported with Samarajewa, *et al.*, (1990) Rustom, (1997) and Soliman, (2002). So avoiding aflatoxin transmission into egg appears to be the only practical way to ensure their safety for human consumption.

4. CONCLUSION AND RECOMMENDATION

The results cleared the occurrence of antibacterial drugs and total aflatoxins (B1, B2, G1 and G2) residues with different percentages and levels in different types of eggs. Therefore, some recommendation must be followed as:

When antibacterial drugs used as a therapeutic agent in the treatment of laying hens a suitable withdrawal time should be elapsed. Inspection should be regularly performed to the farms before marketing to ensure that the farms follow the rules of pre -marketing withdrawal period. Eggs collected during and shortly after antibacterial medication should not be used for human consumption. Cooking time and temperature can play major roles about antibacterial reduction. It needs of quality control measures that compete against possible aflainto-xication in consumed eggs by more care during producing, handling and storing to minimize moulds contamination to safeguard human from being infected. Feed of laying hens should be regularly checked for aflatoxins and strict measures should be carried out to avoid contamination with Aflatoxin. Laboratory quality assurance program, monitoring of analysts and validation of analytical methods. Periodical examination of eggs in local market for presence of antibacterial and aflatoxins residues. The poultry farms must be kept under the veterinary supervision. Application of good hygienic practices (GHPS) during eggs production.

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الكشف عن بقايا الأفلاتوكسين والمضادات البكتيرية فى الأنواع المختلفة من بيض المائدة مع دراسة تأثير المعالجة الحرارية

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الملخص العربي

اجريت هذه الدراسة لبيان مدى تواجد الأفلاتوكسين والمضادات البكتيرية فى الأنواع المختلفة من بيض المائدة. حيث تم اجراء الدراسة على عدد 90 عينة (30 عينة من كل من البيض البلدي وبيض المزارع البنى والابيض) عشوائية تم جمعها من محلات البقالة والسوبر ماركت بمدينتي القاهرة والجيزة وقد تم ا لكشف عن بقايا المضادات البكتيرية وسموم الأفلاتوكسين بالإضافة إلى دراسة تأثير المعالجة الحرارية (الغليان لمدة 15 دقيقة والقلي لمدة 5 دقائق) على بقايا المضادات البكتيرية وسموم الأفلاتوكسين في العينات الإيجابية فقط. وقد وجد ان نسبة بقايا المضادات البكتيرية هي 6.6٪و 20٪ و 13.3٪ في البيض البلدي وبيض المزارع البنى والأبيض على التوالي. كما وجد ان نسبة بقايا سموم الأفلاتوكسين هي 30٪ و16.6٪ و20٪ في البيض البلدي وبيض المزارع البنى والأبيض على التوالي. وبالمعالجة الحرارية لهذى البقايا وجد ان بقايا المضادات البكتيرية قلت بنسبة كبيرة وفى بعض العينات اختفت. كما وجد ان بقايا سموم الأفلاتوكسين ظلت ثابتة ولم تتأثر بالغلي او القلي. وتم مناقشه الأهمية الصحية لوجود هذه البقايا ووضع التوصيات المناسبة للحد من تواجدها في البيض.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2):177–187 , ديسمبر2014)