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Fungal Contamination of Table Eggs Sold in Khartoum State, Sudan

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Authors' contributions

This work was carried out in collaboration between both authors. Author EAS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NAS managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The current study was conducted to assess fungal contamination of table eggs sold in Khartoum State, Sudan.
Study Design: This descriptive evaluation study was conducted at Mycology Department, Central Veterinary Research Laboratory, Khartoum State, Sudan over 6-month duration.
Methodology: Egg samples (shell and egg contents) were examined directly using lacto phenol cotton blue slide mount technique. The culture of samples in Sabouraud Dextrose Agar (SDA) supplemented with antibiotic was carried out. Identification of the isolates was based on their macro and microscopic morphological characteristics.
Results: Fungal colony was obviously seen on egg membrane and revealed as *Aspergillus flavus* on direct microscopic examination. Whereas *A. flavus* and *A. niger* hyphae were detected on culture from both egg shell and contents.

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Conclusion: Isolation of *A. flavus* and *A. niger* from both egg shell and content by conventional method. The obtained results report the occurrence of *A. flavus* in table eggs sold in Khartoum state, Sudan.

Keywords: Egg shell; egg contents; Aspergillus flavus; conventional methods.

1. INTRODUCTION

Avian eggs provide a well-balanced diet to people of all ages [1,2]. Malnourished and anaemic patients used raw eggs to improve their health [3]. Newly laid eggs are free of microbial contamination. However, on exposure to environmental sources such as soil, dust and dirty nesting materials contamination with microorganisms may develop. In addition to nutrient substances in eggs that favour bacterial growth [4]. Moreover, bad storage with high humidity will support the growth of these microorganisms [5]. Limited data are available on contamination of egg shell and egg contents with fungi [6,7]. However, contamination with *Aspergillus* species is more serious as these fungi produce toxic secondary metabolites known as aflatoxins (AF) produced by several species of the *Aspergillus* mainly *Aspergillus flavus* and *Aspergillus parasiticus* [8] on a variety of food products [9,10]. The potential health problems of AF has been evaluated as being potent hepatotoxic [11] mutagenic and immune-suppressive agents [12,13]. Moreover, mortality and lower productivity of farm animals were reviewed [14]. Contaminated food and animal products such as meat and eggs [15] or exposure to in or outdoor fungi may predispose to aflatoxicosis [16]. The residue of AF in edible eggs may induce health hazards to human [15]. However, residue of AF in poultry meat has been reviewed [17].

2. MATERIALS AND METHODS

2.1 Sample Collection

Total of 120 table eggs was obtained from three different supermarkets located at Soba district and Kuku area. GPS coordinates for both locations are: N 15º51' 0" E 32º67' 0"; N 15º62' 0" E 32º59' 1" respectively. Eggs were kept at a refrigerator for 2-3 weeks while being used for consumption.

2.2 Macroscopic Examination of Eggs

Eggs were examined externally for deformity and any unusual appearance or texture.

2.3 Laboratory Investigation

Egg samples were brought to the Central Veterinary Research Laboratory (CVRL) located at Soba district (Mycology Department) for investigation. Washing of external egg shell with sterile distilled water was done. Later, the inner membranes were exposed using sterile scalpel blade. Some portions were collected in sterile containers for culture.

2.4 Culture of Samples

Egg shell and egg contents were inoculated into two sets of SDA plates supplemented with Streptomycin sulphate (0.05 mg/ml), Benzyl penicillin (0.05 mg/ml) and Gentamicin (0.04 mg/ml). Aspiration of the contents was done according to method described by Baily and Scott (1998). Cultures were then incubated at 27ºC and 37ºC for two weeks and examined at weekly interval for fungal growth.

2.5 Identification of the Isolates

Identification of the isolate was done using conventional methods. When good growth was observed, small portion of the developed colony was taken, mounted in lacto phenol cotton blue and examined directly under compound microscope. Later, sub cultured on corn meal agar and Aspergillus differentiation media base (ASDMB) were done. Slide culture from pure colony was carried out. Identification of the isolate was done on the basis of gross and microscopic morphology according to the described procedure [18].

3. RESULTS

3.1 Macroscopic Examination of Egg

Macroscopic examination of eggs revealed thinness of egg shell and deformity at the pointed end of some eggs. No cracks were seen. On breaking eggs for cooking, some eggs from different racks showed watery contents as a yellowish liquid in addition to greenish colony on the inner shell membrane. Direct examination of

inner shell membrane revealed greenish colony as shown below (Plate. 1). Both egg shell and content culture showed growth of fungi. Out of 120 egg samples 10 were found contaminated with *Aspergillus* species. Three were *A. niger* and 7 were *A. flavus* with a prevalence of 2.5% and 5.8% respectively.

Plate 1. Colony of *A. flavus* in the inner eggshell (white arrow)

3.2 Identification of the Isolates

Identification of the developed colonies on SDA revealed greenish granular colony (Plate 2) whereas on ASDMB an orange colony on the reverse side was observed (Plate 3). In addition to black colony of *A. niger*. Moreover, mounting of the sporulating cultures in LPCB stain and slide culture image revealed hyphae of *A. flavus* as well as *A. niger* (Plate.4).

The prevalence of isolated *Aspergillus* was shown below (Plate 5).

4. DISCUSSION

Avian egg has three physical barriers, the cuticle which overlies eggshell and the pore mouth; the outer waxy shell membrane, and the inner shell membrane. They are known to protect eggs from invading microorganism [19]. But contamination of intact eggs before laying transovarian (vertical transmission) will occur before eggs are covered by shell, if infection to reproductive organs developed or after laying (longitudinal transmission) with a variety of organisms from different sources [20]. In the present study contamination of eggshell with *A. flavus* and *A. niger* was observed. This finding is similar to that previously obtained in Egypt [21] where many species of fungi were isolated among which was *Aspergillus species* with high prevalence. This might be due to similarity in climatic conditions where high temperature and humidity favor the growth of fungi. Moreover, culture of internal contents revealed contamination with *A. flavus*. This result coincided with that reported in Nigeria

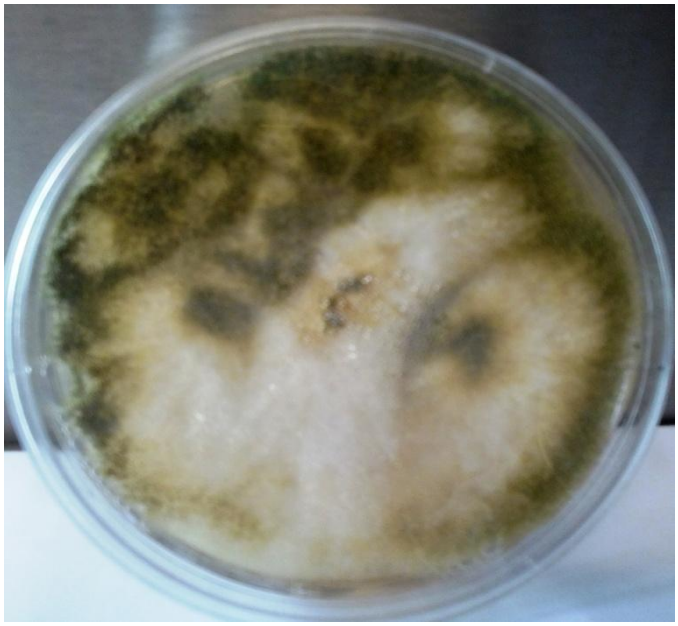

Plate 2. Greenish colony of *A. flavus* on SDA at 27ºC

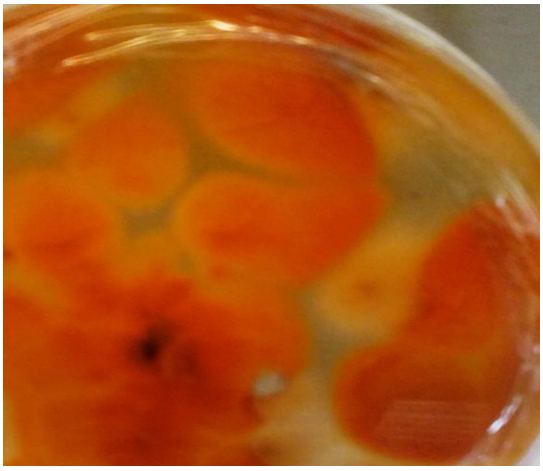

Plate 3. An orange reverse colony of *A. flavus* on Aspergillus differentiation base media

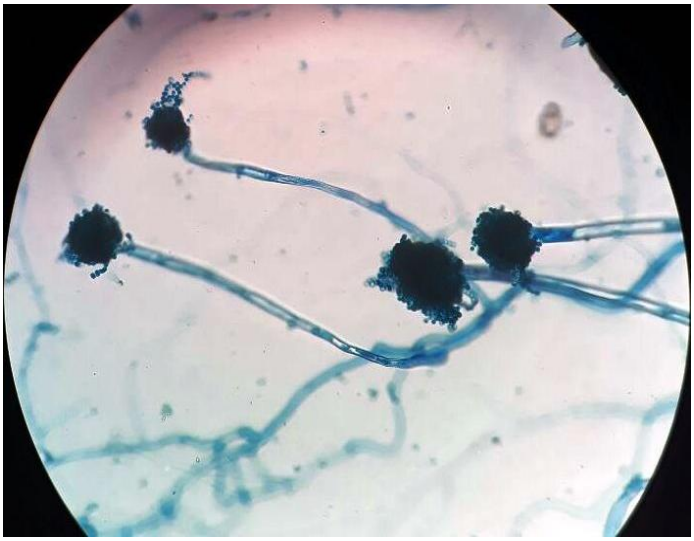

Plate 4. Slide culture showing *A. flavus* hyphae

[7]. Contamination of egg contents might be due to occurrence of fungi in the oviduct of the hen that contaminate chicken droppings or poultry feeds [3,22]. This finding is important as the growth of fungi in food is regarded as an indicator for the presence of mycotoxins leading to food borne mycotoxicosis [23]. In the current study, contamination of eggshell and content might be due to the use of contaminated poultry feeds or poultry feeds raw materials or general low hygienic measures in such farms. However, contamination with *A. flavus* is a threat to health due to the production of aflatoxins that have been found to be carcinogenic, teratogenic and mutagenic in humans and birds. In the present study, isolation of *A. flavus* reflects the importance of such fungi as it was known to cause acute health problems like diarrhoea, nausea, abdominal pain or even death [24] or chronic illness like cancer and other irreversible effects [25]. These pathogenic moulds may found their way to penetrate and contaminate eggs and produce their toxins under favorable conditions. The penetration of such fungi into eggs leads to their spoilage as well as some species were incriminated in public health hazard [26]. Therefore, special care should be taken to reduce egg contamination through application of proper farm hygienic programs, good handling and storage methods, as well as, the periodical examination of eggs and poultry feed [27].

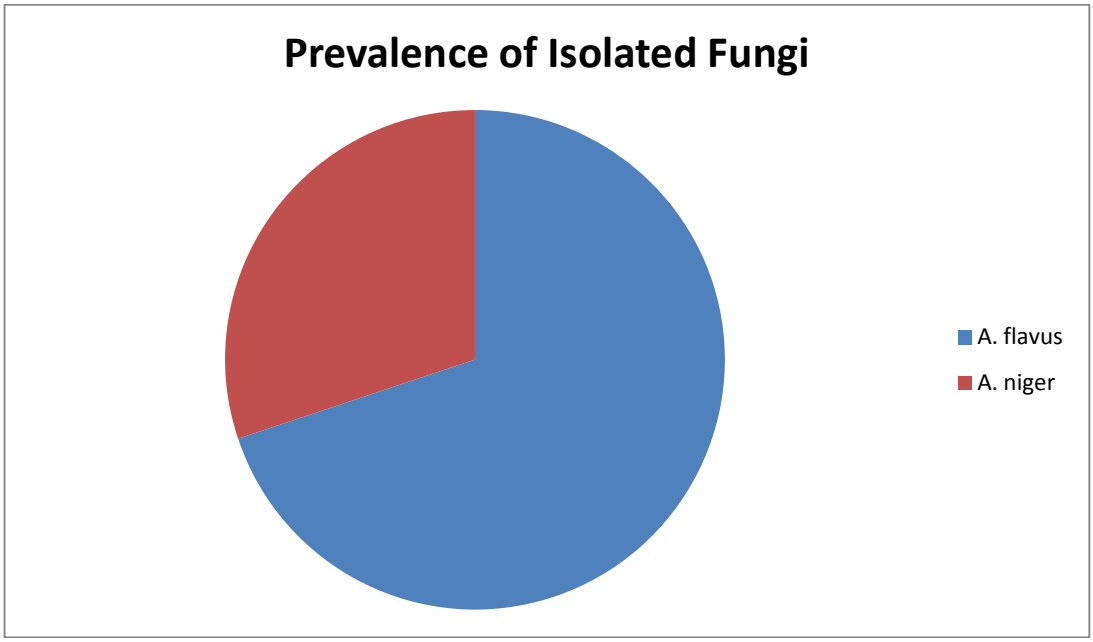

Plate 5. Prevalence of isolated *Aspergillus* species

5. CONCLUSION

Isolation of fungi from table eggs reflects the importance of the study. The present study revealed existence of *A. flavus* and *A. niger* in eggshell and contents using conventional methods. Identification of the isolates was based on their morphological characteristics. Contamination of eggs and egg products with fungi may affect egg quality leading to egg spoilage and predispose to food-borne diseases which represent one of the most severe diseases of poultry, human and animals.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

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