



## RESEARCH ARTICLE

### ***Aspergillus* species associated with dead-in-shell chick embryo in some hatcheries in Northwest Nigeria**

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#### Özet

**Kwanashie NC, Kazeem HM, Umoh JU, Abdu PA.** Kuzeybatı Nijerya'da bazı kuluçkahanelerde kabuk altı embriyo ölümünün *Aspergillus* türleri ile ilişkisi. **Eurasian J Vet Sci, 2014, 30, 1, 11-13**

**Amaç:** Bu çalışma, kabuk altı embriyo ölümü tespit edilen yumurtalardaki *Aspergillus* türlerinin yaygınlığını belirlemek amacıyla gerçekleştirildi.

**Gereç ve Yöntem:** Dört kuluçkahaneden 6 aylık bir sürede toplanan 3 bin adet kabuk altı ölüm tespit edilen tavuk yumurtası materyal olarak kullanıldı. On adet yumurtanın içeriği, yumurta yüzeyinin dekontamine edilmesinden sonra toplandı ve bunlardan hazırlanan sürüntü materyali, yatay konumdaki Sabouraud Dekstroz Agar (SDA) ve mısır unlu agar (CMA) vasatı yüzeyine tamamen yayılarak kültüre edildi.

**Bulgular:** Toplanan 300 yumurtadan 122'sinde (%40.67) *Aspergillus* genuslarına ait 4 türden *A. fumigatus*, *A. niger*, *A. flavus* ve *A. terreus* mantarları üretildi. İzole edilen 122 *Aspergillus* türünün %48.36'ı (59 adet) *A. fumigatus*, %22.13'u (27 adet) *A. niger*, %18.03'u (22 adet) *A. flavus* ve %11.48'i (14 adet) *A. terreus*'tur.

**Öneri:** İncelenen örneklerden izole edilen *Aspergillus* türleri kabuk altı embriyo ölümlerinde primer veya sekonder etken olarak rol oynayabilir. Kuluçkahanelerin düzenli aralıklarla belirlenen mantarlara karşı dezenfekte edilmesi önerilir.

**Anahtar kelimeler:** *Aspergillus*, yumurta, tavuk.

#### Abstract

**Kwanashie NC, Kazeem HM, Umoh JU, Abdu PA.** *Aspergillus* species associated with dead-in-shell chick embryo in some hatcheries in Northwest Nigeria. **Eurasian J Vet Sci, 2014, 30, 1, 11-13**

**Aim:** The present work was carried out to determine the prevalence of *Aspergillus* species in dead-in-shell chick embryos.

**Materials and Methods:** A total of three thousand dead-in-shell embryonated chicken eggs were collected from the four hatcheries over a period of six months. The content of 10 eggs were pooled after decontamination of the egg surface and swab of pooled contents inoculated onto the entire surface of Sabourauds Dextrose Agar (SDA) and Corn Meal Agar (CMA) slants and growths.

**Results:** Out of the 300 groups of pooled eggs a total of 122 (40.67%) isolates of fungi belonging to 4 species of the Genera *Aspergillus* viz *A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus* made up 48.40% (59), 22.10% (27), 22.30% (26) and 5.70% (14) of the 122 *Aspergillus* respectively were isolated.

**Conclusions:** The presence of these *Aspergillus* species indicates that they may have been primary or secondary contributors to the embryonic mortality. Decontamination of hatcheries at regular intervals is recommended for control of these fungi.

**Keywords:** *Aspergillus*, hatcheries, chicken.



## Introduction

The survival of the poultry industry is dependent on the continual supply of day-old-chicks by commercial hatcheries. The fertility and hatchability of eggs produced by the breeder flock influences this needed continual supply of day-old-chicks. Therefore, factors that may result in low fertility and hatchability are a source of concern to the management of the hatcheries (Peters et al 2008, Wondmeneh et al 2011). Dead-in-shell chick embryo constitutes one of the several factors that account for lower hatchability of incubated eggs (Orajaka and Mohan 1985) and bacteria and fungi have been reported as one of the causes of dead-in-shell chick embryo. These microorganisms gain access to the embryo by the transovarial route or by penetration of the tiny pores located on the egg shell. Condition of the hatcheries such as its humidity and temperature makes it a conducive environment for growth and rapid colonization by fungi especially *Aspergillus* species which are highly ubiquitous and a known cause of embryo mortality in poultry (Hashempouret al 2011). Embryonic mortality may result from fungi (*Aspergillus* sp. and *Penicillium* sp.) contamination during the incubation, and hatchery houses are ideal environments for fungi development - high temperature, high relative humidity and high level of organic material (Gigli et al 2009). These embryonic mortality (dead-in-shell) results in great economic loss for the producers of day-old chicks as well as the farmers (as a result of scarcity of day-old chicks which results in high cost of these birds).

This study was conducted to determine the prevalence of fungi especially *Aspergillus* species and yeast in dead-in-shell in some major hatcheries in Kaduna State.

## Materials and Methods

### Study area

This study was carried out at in Kaduna State, Nigeria, located at between latitudes 11°32' and 9°20'N and longitude

8°50' and 6°51'E. The State is positioned in the Northern Guinea Savannah zone of Nigeria. The area is characterized by a cold dry season (November-February), hot-dry season (March-April) (both dry seasons) and the wet/rainy season (May-October) (Adenkola et al 2010). The annual rainfall peaks in the month of August with the average of 146 mm. The average humidity is highest in August with 75.6 mm/Hg and lowest at the months of December- January with 38.2 mm/Hg. The mean temperatures for the zone are 10.7°C and 38.75°C minimum and maximum respectively (Kwanashie et al 2012). The study was conducted throughout the year (July- December).

### Collection of samples

Four hatcheries (two located in Kaduna metropolis (1 and 2) and two located in Zaria (4 and 5) were used for the study. These were the major hatcheries located in the area of study. A total of three thousand Dead-in-shell embryonated chicken eggs were collected from the hatcheries over a period of six months. The hatcheries located in Zaria, hatched once a week, each with a capacity to hatch about 10.000 chicks at a time, while the two hatcheries studied in Kaduna metropolis hatched once a month and had the capacity to hatch about 5.000 chicks at a time. The environment around hatcheries number 2 and 4 were not kept clean. The dead-in-shell eggs were transported to the Microbiology Unit of the Faculty Veterinary Medicine of the Ahmadu Bello University Zaria for processing. Time of collection of samples was at the end of incubation i.e. on the day the chicks hatched.

### Laboratory isolation

The eggs were decontaminated using sodium hypochlorite/savlon and wiped with 75% alcohol. Then using a sterile scissors each egg was punctured at the blunt end and any fluid and unabsorbed yolk was drained into a sterile beaker. The samples taken from 10 eggs were pooled in a sterile 80ml beaker. Isolation and identification of the fungi was carried out as described previously by Darise (1987). Briefly,

Table 1: *Aspergillus* species isolated from four hatcheries in Zaria and Kaduna.

Hatchery	Species and number (%) of isolated <i>Aspergillus</i>				Total
	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>	
1	12 (40)	8 (26.7)	8 (26.7)	2 (6.7)	30 (24.59)
2	30 (60)	8 (16.0)	7 (14)	5 (10)	50 (40.98)
3	9 (37.5)	7 (29.2)	3 (1.3)	5 (2.1)	24 (19.67)
4	8 (47.2)	4 (22.2)	4 (22.2)	2 (2.8)	18 (14.75)
Total	59 (48.36)	27 (22.13)	22 (18.03)	14 (11.48)	122(100)





the swabs of the content of individual pools were directly streaked on the entire surface of Sabouraud Dextrose Agar (SDA) and Corn Meal Agar (CMA) slants for culturing and incubated for 7 days at room temperature (25-27°C). *Aspergillus* species were identified based on colony characteristics, slides were also prepared for identification of mycelium and hyphae arrangement with lactophenol cotton blue staining method.

### Results

Out of the 300 groups of pooled eggs a total of 122 (40.67%) isolates of fungi belonging to 4 species of the Genera *Aspergillus* viz *A. fumigatus*, *A. niger*, *A. flavus* and *A. terreus* made up 48.40% (59), 22.13% (27), 18.03% (22) and 11.48% (14) of the 122 *Aspergillus* species isolated (Table 1). *Aspergillus fuminatus* represented the major isolate while hatchery 2 had the highest occurrence of *Aspergillus* species.

### Discussion

The findings of this study does not only indicate a high contamination of the hatcheries by *Aspergillus* it also indicate different levels of contamination between the hatcheries. The different management practices in the different hatcheries could have accounted for the differences in the levels of contamination with *Aspergillus* spp. Hatchery number 2 was not only the most contaminated with *Aspergillus* but also had the highest level of *A. terreus* contamination. *Aspergillus* species have been reported to be pathogenic in poultry and also implicated in dead-in-shell embryo (Sajidet al 2006, Hashempouret al 2011). *Aspergillus* causes mycoses or mycotoxicosis which may likely be the mechanism that resulted in the death of these embryos. The source of the *Aspergillus* may be from the environment or eggs contaminated by faeces which represent a good substrate for *Aspergillus* (Rediget al 1980, Fatunmbi and Bankole 1984). Thus eggs predispose to penetration by the *Aspergillus* species (Wilson 1991).

### Conclusions

*Aspergillus* may have been partly or wholly responsible for the dead-in-shell embryonated chicks observed in the hatcheries. There should be a proper decontamination of hatcheries and hatchery equipment after each hatching. This will help reduce the level of contamination of the hatcheries by *Aspergillus* species and other fungi and thus reduce the likelihood of dead-in-shell embryos caused fungi.

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