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**Original article**

## Isolation of fungi associated with dead-in-shell chick embryo in hatcheries

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### ARTICLE INFO

*Article history:*

Received 11 February 2013

Accepted 12 April 2013

Available online 24 April 2013

*Keywords:*

Dead-in-shell

Hatcheries

Fungi

Yeast

*Candida*

Chicken

### ABSTRACT

The present work was carried out to isolate and identify fungi species present in dead-in-shell chick embryo. A total of three thousand dead-in-shell embryonated chicken eggs pooled into 300 eggs (10 eggs per group) were collected from the four hatcheries over a period of six months. The contents of the pooled eggs were inoculated onto Sabourauds Dextrose Agar (SDA) and Corn Meal Agar (CMA) slants. Out of the 300 groups of pooled eggs a total of 60 (20.00%) fungi isolates of belonging to 5 genera viz *Candida*, *Mucour*, *Rhizopus*, *Curvularia* and *Penicillium* making up 48.33% (29), 18.33% (11), 13.33% (8), 11.67% (7), and 8.33% (5) respectively of the fungi isolate respectively. The presence of these fungi gives an indication of the level of contamination and sanitary conditions of the hatcheries. It also indicates that fungi be a primary or secondary contributor to the embryonic mortality (dead-in-shell).

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## 1. Introduction

Dead-in-shell chicken embryo constitutes one of the several factors that account for lower hatchability of and incubated eggs (Orajaka and Mohan, 1985; Hashempour *et al.*, 2011). Hatchery losses associated with embryonic mortality may result from non specific bacterial infections of incubated eggs, and fungal elements in the environment especially *Aspergillus* species (Wilson 1991). Other causes include nutritional deficiency, prolonged egg storage, high temperature in early incubation, and inadequate ventilation (Ghazikhanian, 1980; Wilson, 1991). Disease producing microorganisms have been known to enter the egg in two ways, either by trans-ovarian egg transmission or by egg shell transmission. The trans-ovarian egg transmission is when the microorganism is already in the egg as it is laid by the hen. Such types of diseases resulting from this mode of transmission are known as the "true egg-transmitted diseases" (Ghazikhanian, 1980). Bacterial organisms in this category are *Mycoplasma gallisepticum* causing sinusitis and air sacculitis, *Mycoplasma meleagridis* which causes air sacculitis, poor poul performance and skeletal problems. *Mycoplasma synoviae* also causes air sacculitis and leg problems in growing turkeys and *Salmonella* group (Ghazikhanian, 1980). Aerobic bacterial flora from dead-in-shell chicken embryos such as *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus* spp, *Pseudomonas* spp and *Proteus* spp have been reported (Orajaka and Mohan, 1985).

The egg shell transmission in contrast to the trans-ovarian route of infection, makes use of the over 11,000 tiny pores of an egg shell through which an organism can penetrate (Ghazikhanian, 1980). Both bacterial and fungal agents are implicated in egg shell transmission and *Aspergillus* and *Candida albicans* are organisms that penetrate the egg shell during incubation, (Gow *et al.*, 2003; Hashempour *et al.*, 2011). Despite the prevalence and reports of fungi as a threat to poultry production, there is paucity of information on their involvement in the production of dead-in-shell chick embryos, thus the need for this study.

## 2. Materials and methods

### 2.1. Collection of samples

Samples of dead-in-shell embryonated chicken eggs were collected from four hatcheries, two in Zaria (Z1 and Z2) and two from Kaduna (K3 and K4). Two of the hatcheries located in Zaria, hatched once a week and had capacity to hatch 10,000 eggs at a time. The other two hatcheries hatched located in Kaduna, only once a month with capacity for 5,000 eggs at a time. The environment around hatcheries number 2 and 4 were not kept clean. The dead-in-shell eggs were collected into a clean bucket and transported to the Microbiology Unit of the Faculty Veterinary Medicine of the Ahmadu Bello University Zaria for processing. A total of three thousand dead-in-shell embryonated chicken eggs from the four hatcheries were collected over a period of six months. Time of collection of samples was at the end of incubation, on the day the chicks are hatched. The hatcheries selected the major ones in the area of study.

### 2.2. Laboratory isolation

A paper towel soaked in sodium hypochlorite/savlon was laid on the working surface. With gloved hands the eggs were picked up and wiped all round with 75% alcohol soaked cotton wool to remove external debris and other contamination. Then using a sterile scissors each egg was punctured at the air sac end and any fluid and unabsorbed yolk was drained into a sterile beaker. The samples were pooled 10 eggs to a sterile beaker. The pooled samples were then stirred with a sterile glass rod, one sterile glass rod for one pooled sample. A sterile cotton swab was then used to take an inoculum from each beaker and inoculated on the entire surface of Sabourauds Dextrose Agar (SDA) and Corn Meal Agar (CMA) slants. The slants were labeled appropriately and covered leaving the cover loose to allow some air in. The inoculated bottles were incubated at room temperature for seven days and observed for growth. Fermentation and assimilation tests were used to identify yeasts that were isolated in the procedure above using sugars. The sugars are lactose, glucose, sucrose, galactose, mannose, mannitol, sorbitol, and trehalose according to Harley (1971).

## 3. Results

Out of the 300 group of pooled eggs a total of 60 fungi comprising of 5 genera (*Candida*, *Mucor*, *Rhizopus*, *Curvularia* and *penicillium*) were isolated. *Candida* species made up 48.33% (29) of the total fungi isolated. The dead-in-shell chick embryos evaluated from the Zaria hatcheries has the highest level of fungal contamination, 36.66% and 40.00% compared to 10.00% and 13.33% observed in the dead-in-shell chick embryos evaluated in the hatcheries in Kaduna metropolis (Table 1).

The 29 *Candida* isolates were comprised of 6 species *C. albicans*, *C. pseudotropicalis*, *C. tropicalis*, *C. guillamondi*, *C. kruzai* and *Geotrichum candidum* with *Candida albicans* making up 79.31% (13) of the *Candida* isolates (Table 2).

**Table 1**

Fungi isolated from dead-in-shell chick embryo in four hatcheries in Zaria and Kaduna.

Hatcheries	Total No. of pooled eggs	No of fungi isolated (% positive)					Total No. positive
		<i>Candida</i> spp	<i>Mucor</i> spp	<i>Rhizopus</i> spp	<i>Curvularia</i> spp	<i>Penicillium</i> spp	
Z1	80	10 (45.45)	6 (27.27)	2 (9.09)	2 (9.09)	2 (9.09)	22 (36.66)
Z2	120	15 (62.50)	3 (12.50)	4 (16.67)	1 (4.17)	1 (4.17)	24 (40.00)
K3	70	1 (16.67)	1 (16.67)	1 (16.67)	2 (33.33)	1 (16.67)	6 (10.00)
K4	30	3 (37.50)	1 (12.50)	1 (12.50)	2 (25.00)	1 (12.50)	8 (13.33)
Total	300	29 (48.33)	11 (18.33)	8 (13.33)	7 (11.67)	5 (8.33)	60 (20.00)

**Table 2***Candida* species isolated from dead-in-shell chick embryo in four hatcheries in Zaria and Kaduna.

Hatcheries	<i>Candida</i> species isolated (%)						Total isolated
	<i>C. albicans</i>	<i>C. pseudotropicalis</i>	<i>C. tropicalis</i>	<i>C. guillamondi</i>	<i>C. kruzai</i>	<i>Geotrichum candidum</i>	
Z1	5	1	1	2	1	1	10
Z2	7	2	3	1	1	1	15
K3	1	0	0	0	0	0	1
K4	1	1	0	0	1	0	3
Total	13 (79.31)	5 (17.24)	4 (13.79)	3 (10.34)	2 (6.90)	2 (6.90)	29

#### 4. Discussion

The findings of this study showed a colonization of the chicks by molds of the genera *Candida*, *Penicillium*, *Curvularia*, *Mucor*. *Candida albicans* have been reported to penetrate the egg shell during incubation, (Gow et al., 2003). *Mucor*, *Rhizopus*, *Curvularia* and *Penicillium* are all known pathogens of both man, animals and birds either directly as an infection or as a toxicosis (Sai'du et al., 1999). These organisms have also been reported to cause disease in the animals as well as contaminate the feed where they produce toxins which when ingested by the birds can result in mycotoxicosis. The presence of these fungi is an indication that they may have contributed to the death of these bird likely by the production of toxins. These toxins are known to be necrotic and/or immunosuppressive resulting in tissue destruction and predisposition to other deadly agents (such as bacteria) respectively and finally death. These fungi may also contaminate the hatchery and thus the apparently healthy birds which are hatched resulting in transportation of these fungi to the farms and subsequent colonization of such farm with resultant loss in production or death of birds.

Humidity control is sometimes implicated in embryo death and humidity is also a factor in the proliferation of fungi. A constant incubation temperature of 36.5<sup>0</sup> C which majority of these hatcheries use is also good for *Candida* proliferation (Wilson, 1991) which showed a high isolation rate in this study.

It is therefore very important to be aware of the high prevalence of these organisms, the sources and points at which the embryonated eggs become infected with the organisms as well as the diseases they can cause and possibly put them under surveillance as important pathogens of poultry. Sanitization of hatchery is very important therefore it is recommended that only clean eggs should be set for hatching. The use of antifungal spray to decontaminate the hatchery in between setting as well as cleaning of equipments after each hatch is also advocated.

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