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Isolation and Characterization of Yeasts Associated With Hatchery Dead - In - Shell Embryos, In Zaria

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Abstract: *This study was conducted to isolate yeast from dead-in-shell embryos of poultry as a potential cause of in-viability in poultry eggs. A total of two thousand dead-in-shell poultry eggs were sampled over a period of five months, on a weekly basis from a reputable hatchery in Zaria, Kaduna State, Nigeria. The eggs were disinfected in accordance with the standard protocol using sodium hypochlorite and 70 % alcohol. Ten eggs were pooled into sterile beaker as sample processed per week with a total of 200 pooled samples in five months and inoculated on Sabauraud`s Dextrose Agar (SDA) and Corn Meal Agar (CMA) in accordance with standard microbiological procedures. Out of the 200 pooled samples, Fifteen (15) yielded yeast isolates, on microbiologic examination. Further biochemical analysis using urease, citrate and triple sugar ion test confirmed nineteen (19) of the pooled samples as Aspergillus Species. One hundred, and forty-four (144) pooled samples were negative for fungi and twenty-two (22) had no growth on both Corn Meal Agar and Sabauraud`s Dextrose Agar. These findings revealed that Yeasts and Aspergillus species can be associated with dead-in-shell embryo of poultry eggs which can thus be incriminated as potential cause of embryo death in poultry eggs. Hence, the need to create awareness on biosecurity measures amongst poultry farmers, hatcheries and other relevant stakeholders within the poultry industry.*

Keywords: *Isolation, Yeast, Dead-in-shell, Embryo, Hatchery.*

I. Introduction

Yeast is a unicellular fungus, and budding yeast are true fungi of the Phyllum Ascomycetes, class Hemiascomycetes (Haley, 1971; Loftus, 2005), belonging to the order Saccharomycetales (Pfaff *et al*., 1978; Alvarez et al., 2009). Yeasts are a heterogenous group of fungi that superficially appear to be homogenous as majority of unrecognized ascomycetes isolated in the laboratory are heterothallic (Haley, 1971; Loftus, 2005).

Yeast are found in a wide dispersion of natural habitats especially on plants, leaves, flowers, soil and salt water (Haley, 1971), as well as skin surfaces, mucous membranes and intestinal tract of warm- blooded animals, as symbionts and/or as parasites (Pfaff *et al*., 1978). Pathogenic yeasts are: *Histoplasma capsulatum, Blastomyces dermatitides, Cryptococcus neoformans, Coccidiodes immitis* and the yeast –like fungus, *Candida albicans* (Chester *et al.*, 1970),

The major characteristic of yeast is their ability to ferment sugars for the production of ethanol and physiological characteristics which can be used to identify species (Campbell *et al.*, 1988), this identification and characterization of yeast species have been based on morphological, physiological and biochemical properties (Covadonga *et al*., 2002). Yeasts like *Candida species* have been reported to infect birds (Cynthia *et al*., 2005). And the adverse effect of yeast causes food spoilage and diseases cumulatively, leading to economic loss and decreased production (Haley, 1971). Although, they have beneficial effects as rich source of B-vitamins, niacin, and folic acid (Ismail *et al*., 2000). So therefore, maximizing their benefits while minimizing their detrimental effects, requires a thorough understanding of their complex characteristics (Campbell *et al*., l988; Beokhout *et al*., 2003).

Embryo death of poultry eggs is of great economic loss in the poultry industry in Kaduna State, Nigeria (Kwanashie, 2009), as dead-in shell loss of poultry eggs lead to decrease productivity on hatch-day. Consequently, depriving farmers and consumers who depend on hatcheries for supply of day–old chicks for restocking and research purposes (Wain *et al*., 1976 ; Kwanashie, 2009).

Yeast has been isolated from processed poultry products as far back as 1976 (Wain *et al*., 1976), and from dead – in - shell embryonated chicken eggs from hatcheries in Zaria, Kaduna state (Kwanashie, 2009). Also, *C. albicans* has been reported to invade the chick chorioallantoic membrane (CAM) of poultry eggs (Gow *et al*., 2003), with dead-in-shell chicken embryo constituting one of the several factors that accounts for low hatchability of incubated eggs (Kwanashie, 2009), and leads to great productive losses and hence, financial losses in the poultry industries.

The microbiological safety of foods is discussed in relation to significance and occurrence of pathogenic micro-organisms and yeasts have been incriminated in the context of food safety (Lund *et al*., 2000;

Doyle et al., 2001; Hocking, 2003; Kwanashie, 2009), when compared with other microbial groups, yeasts are not seen as aggressive pathogens, but they are capable of causing disease in humans as opportunistic organisms (Barnett and Cofrancesco, 2000; Georgiev, 2003; Hazen and Howell, 2003). Yeasts like *Candida albicans* (Calderone, 2002) and Cryptococcus neofprmans (Schaars *et al*., 2006) have been incriminated in mucocutaneous, cutaneous, respiratory, central nervous and systemic infections (Georgiev, 2003). The development of allergic and adverse reactions in animals and humans (Graham and Roostita, 2006), due to consumption of foods supplemented with viable and nonviable yeasts to enhance the growth of domesticated animals, poultry and also as probiotic organisms in food have been documented (Klaenhammer, 2001; Dawson, 2002; Metcalfe *et al*., 2003; Van der Aa Kable *et al.*, 2005). This study therefore, seeks to isolate potential yeast associated with dead-in-shell embryos and determine if it's a potential cause of embryo death.

II. Materials And Methods

Study Area: The study was carried out in Zaria, Kaduna State, Nigeria. Zaria is located between latitude $11^{o}04$N and longitude $7^{o}42$E, covering an area of 300 km^2. The vegetation is Northern Guinea Savannah, with rainfall ranging from 0.0 to 816.0 mm/month and temperature of 17 ^{0}C to 33 ^{0}C (Mortimore, 1970). The study area is characterized by three climatic seasons which consists of the cold dry season (November – February), hot-dry season (March – April) and the wet/rainy season (May – October) (Ayo *et al.*, 1999). The monthly mean temperature records show a range from 13.8 to 36.7°C and an annual rainfall of 1092.8 mm (Agbogu *et al.*, 2006). The town has a population of about 408,198 - 547,000 and a population growth rate of 3.5% per annum (MED, 1996). Approximately 40- 75% of the population's livelihood is from agriculture (ABU, 2000).

Sample Collection And Sampling Method: A total of two thousand dead-in-shell poultry eggs, were collected from a reputable hatchery in Zaria from January 2006 to May 2006, and transported to the Veterinary Mycology Laboratory of Ahmadu Bello University, Zaria. The eggs were collected and processed using a pooled sample method with 10 eggs per beaker, making a total of 200 pooled samples.

Sample Processing Method: The egg shells were first disinfected using sterile cotton wool soaked in 70 % alcohol or sodium hypochlorite to remove dirt and possible contaminants. The tip of a spatula for cracking the egg was flamed before use. The site for cracking on each egg was cleaned again with alcohol before cracking. The allantoic fluid of 10 eggs were emptied into a sterile beaker and stirred using a sterile glass rod to mix the allantoic fluid properly.

Media Preparation: The SDA (Oxoid, UK.) and CMA (Oxoid, UK), were used following the manufacturer`s instructions. 0.05 mg/ml of chloramphenicol (Austwick, 1974) was added before been poured into plates and bottles.

Innoculation Of Media: A sterile swab was then used to inoculate the pooled sample unto a labeled sterile universal bottle/petri dishes containing SDA and CMA impregnated with 250mg of chroramphenicol antibiotics. Another sterile swab was used to inoculate the pooled samples into sterile labeled plates/universal bottles containing the prepared sterile media with antibiotics, which were all incubated aerobically at room temperature (25 ^{o}C) for 3-5 days, and observed daily for growth. All culture negative growth plates were discarded after 2-3 weeks.

Colonial Identification: The resulting colonies were stained using lactophenol cotton blue stain and Gram`s stain following standard procedure, as described by Hughes *et al*. (2004) and viewed under the microscope at low power objective magnification (x4 and x10) and oil immersion (x100), respectively.

Biochemical Characterization: Biochemical analysis using urease, citrate and triple sugar ion (TSI) test for confirmation of yeast were carried out according to standard keys, as described by Louvois *et al*. (1979).

III. Result

A total of one hundred and seventy-eight (178) micro-organisms were isolated, 15 (7.5%) were yeast isolates, 19 (9.5%) were *Aspergillus spp*, 144 (72%) were bacterial organism (Table I) and this was based on their colonial morphology on media and microscopic appearance on slide (Table II).

Table I: Frequency of Isolation of organisms from dead – in – shell embryos.

S/N	TYPE OF ORGANISM	NO. OF ISOLATES	PERCENTAGE OF ISOLATES (%)
1	Yeast	15	7.5
2	*Aspergillus spp.*	19	9.5
3	Bacteria	144	72
4	Negative cultures	22	11
	TOTAL	200	100%

Table II: Cultural and Microscopic characteristics of Yeasts from dead – in – shell embryos.

AGAR	NO. POSITIVE	COLONIAL MORPHOLOGY	REMARKS
CMA	14	Whitish/cream/yellow, tiny/large, raised/flat, smooth, round, moist, domed, swampy colonies.	Yeast
SDA	15	White/cream/brown/grey, tiny/small/large flat/raised, smooth, moist, swampy colonies	Yeast

KEY: CMA = Corn meal agar. SDA = Sabouraud`s Dextrose agar.

TABLE III: Biochemical characteristics of yeast isolates from dead – in – shell embryos.

S/NO	POOLED SAMPLE NO	TRIPPLE SUGAR ION	CITRATE TEST	UREASE TEST
1	71	Alkaline/Acid + Gas	Negative	Negative
2	72	Alkaline/Acid	Negative	Negative
3	73	Acid/Acid + Gas	Positive	Positive
4	76	Acid/Acid + Gas	Positive	Negative
5	77	Alkaline/Acid + Gas	Negative	Negative
6	78	Alkaline/Acid + Gas	Negative	Negative
7	79	Acid/Acid + Gas	Positive	Negative
8	80	Acid/Acid + Gas	Negative	Negative
9	125	Alkaline/Acid + Gas	Negative	Negative
10	128	Alkaline/Acid + Gas	Positive	Negative
11	134	Acid/Acid + Gas	Positive	Negative
12	135	Acid/Acid + Gas	Negative	Negative
13	144	Acid/Acid + Gas	Positive	Positive
14	147	Acid/Acid + Gas	Positive	Positive
15	155	Alkaline/Acid	Negative	Negative

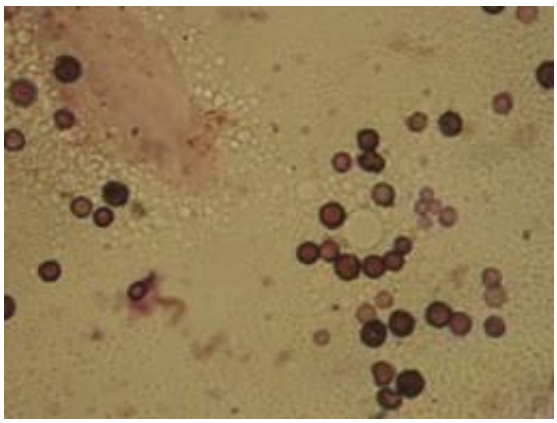

Figure I: Microscopic appearance of yeast cells grown on corn meal agar.

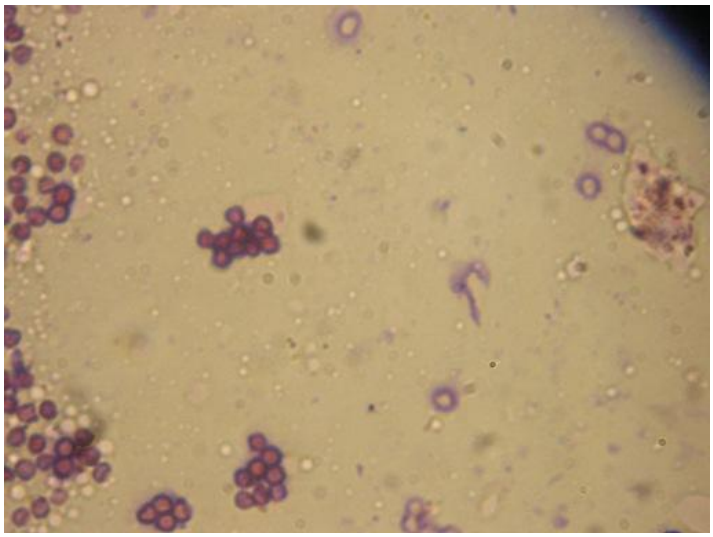

Figure II: Microscopic view of a budding yeast cell

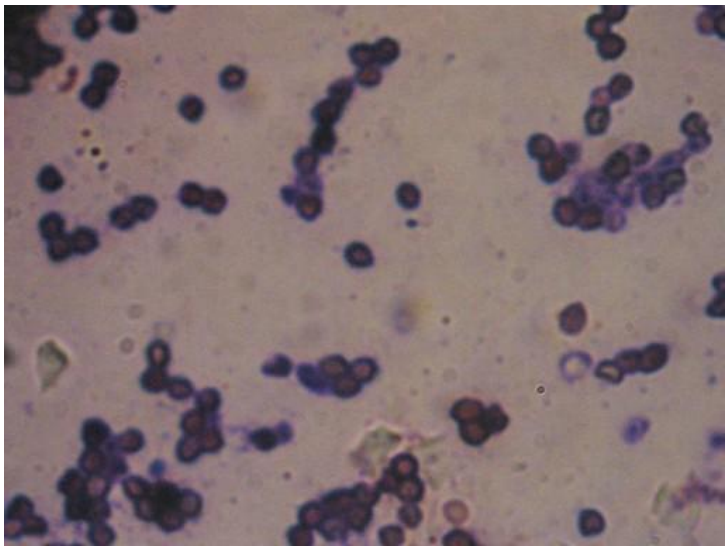

Figure III: microscopic appearance of yeast cells stained with Giemsa`s stain

IV. Discussion

The percentage of yeast isolates observed (7.5%, Table 1), was higher than what was isolated by Cecilla *et al.,* (2004) from fertile poultry eggs. This can be attributed to the fact that their samples were collected at day 19 and 21 pre-hatching. In this study, samples were collected on hatch-day, providing longer budding

time for the yeast consequently, leading to an increased population. Furthermore, the number of samples involved in this study was larger hence, increasing the possibility of higher isolates.

This study, isolated micro-organisms with varying colonial and microscopic morphology (Table I and Table II) and the possibility of isolating organisms from dead-in-shell poultry eggs is in concordance with the work of Gordon and Jordan (1985), as reported, egg contamination does occur at the broiler breeder farm. In this study, it is also more likely that contamination occurred during or after; the fertile eggs have been transported to the hatchery (Papadopoulou *et al*., 1997). It has been reported that pathogens like bacteria, yeast and other fungi (Table I) causes' yolk sac infection, which is a major cause of mortality in the hatchery and post hatching, which leads to increased number of dead-in-shell poultry eggs (Coutts, 1981; Mosqueda *et al*., 1985; Dzoma *et al*., 2001; Walker *et al*., 2002; Cecilla *et al*., 2004).

The large bacteria percentage (Table I) observed in this study agrees with the work of Cecilla *et al*., (2004), where 588 organisms were isolated from fertile eggs with 14 of the organisms being yeasts. This can be attributed to fertile egg contamination at breeder farms, especially in farms with unhygienic farm practices and poor biosecurity mearsures. A total of 72% (Table I), bacterial organism were isolated and this might be due to the relatively simple nutritional requirements of these organisms and their ability to grow in iron chelating agents like ovotransferrin (Seviour *et al*., 1972). This bacterial infection of embryos is a major cause of reduced hatchability, early chick mortality and production losses.

Twenty-two of the plates had no result (Table I) based on the fact that, they showed no growth on both CMA and SDA media after four weeks and were discarded. However, we attributed embryo death of the fertile eggs in these 22 samples to be likely due to unfavourable environmental and incubator conditions at the hatchery, as the eggs were fertile but embryo died after candling at day 18 or would have been discarded (Standard procedure at the hatchery) before hatch-day, when we collected our samples from the hatchery. In this study, 29 (Table II) of the samples showed positive yeast growth on SDA and CMA.

Most of the reports on yeasts in poultry are in relation to spoilage of fresh and processed poultry carcasses (Viljoen *et al*., 1998; Ismail *et al*., 2000). In this study, 15 yeast isolates were isolated, yeast colonies are known to possess proteolytic and lypolytic activity (Ismail *et al*., 2000; Wooley, 2003), which aids their ability to play a role in death of embryo by breaking down the yolk sac constituents and making nutrients more readily available for bacterial growth which explains the large percentage of bacteria isolates observed and hence play a role in yolk sac infection.

V. Conclusion

This study has shown that, Yeasts and *Aspergillus species* and other possible bacterial contaminant can be incriminated in embryonic death of poultry eggs. Most hatching eggs were contaminated with these organisms due to hatchery and breeder farms related problems, especially in hatcheries/farms with very poor or inefficient biosecurity measures. This study recommends the application of sound hygienic practices and other biosecurity measures to hatcheries, poultry farmers and relevant stakeholders.

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