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Mycological and pathological study of broiler mortalities associated with clinically apparent respiratory diseases

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Abstract

In this study, 1136 tissue samples from mortalities of 230 suspected broiler farms were cultured and subjected to isolation, laboratory and histopathology diagnosis of Fungi species. Four fungi species were isolated from respiratory system of necropsied broilers; they were Aspergillus fumigatus, Mucor spp, Aspergillus niger and Aspergillus flavus. At necropsy, extensive yellowish white caseous miliary nodules in the lung and air sacs were seen. Histopathologic examination of tissue sections revealed granulomas in lungs and thickened abdominal air sac membranes. The results of this study indicated that fungal species are present in birds with apparent respiratory diseases. It is very important to be aware of the high prevalence of these organisms, the sources and points at which the chicks become infected with the organisms as well as the diseases they can cause and possibly put them under surveillance as important pathogens of poultry.

Key words: Fungal, Broiler, Isolation, Histopathology, Mortality

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Introduction

Avian fungal diseases occur mostly sporadically, but at times they may take the form of outbreaks. Of all mycotic diseases, the Aspergillosis is more important (Chauhan, 1996). Aspergillosis is the most common opportunistic mycotic infection of the respiratory tract in birds causing high morbidity and mortality (Eswaran et al, 2011). This disease usually involves the air sacs and lungs but may become systemic (Richard et al, 1984). Chicks below 3 days of age are highly susceptible. High moisture content (above 14 per cent) of the feed provides a suitable environment for the growth of the fungi. In addition, if the litter contains sugar cane bagasse it can also support the growth of *Aspergillus* spp. *Aspergillus* is not very effective or invasive pathogen but when it is inhaled in excess the disease becomes clinically apparent. Lowering the body defense mechanism or excessive use of antibiotics makes the birds more susceptible to aspergillosis. Embryos may also get infected through contaminated egg shell. Immunosuppression probably contributes to outbreaks of aspergillosis, because normal chicks are resistant to inhalation of large number of spores (Chauhan, 1996). *Mucor* and *Rhizopus* are all known pathogens of both man, animals and birds either directly as an infection or as a toxicosis (Saidu et al., 1999). These organisms have also been reported to cause disease in animals as well as contaminate the feed where they produce toxins which when ingested by the birds can result in mycotoxicosis.

Morbidity and mortality rate of the poultry varies from flock to flock, age to age and also depends upon the immune status and the degree of exposure of birds. Clinical history, signs, gross morbid lesions, histopathological features of the disease, laboratory fungal culture and isolation and the therapeutic responses could be effective attempts to diagnose the fungal diseases (Fulleringer et al., 2006). The study was carried out to determine the prevalence and distribution of Fungi species among broiler mortalities with apparent respiratory disease.

MATERIALS AND METHODS

Based on history, clinical symptoms and postmortem findings (congested liver, trachea and lungs, thickened air sacs); any suspected mortalities were selected from each farm and were subjected to postmortem examination and subsequent laboratory diagnosis. Totally, 1,136 samples from mortalities of 230 suspected broiler farms were cultured and subjected to isolation and laboratory diagnosis of Fungi species.

The isolation of fungi was carried out from morbid samples (lungs, air sacs and liver) from affected birds. These samples were directly streaked on sabouraud agar plates for culturing and were incubated for 7 days at 37 °C. All samples that had no fungal growth were discarded after seven days (Eswaran et al, 2011).

The growth were observed for the colony morphology i.e. size, color, topography and aerial growth, and these characteristics were used to identify the genus and species (Sajid et al, 2006). Preparations from growths were made for microscopic observation by staining them on glass slides using lactophenol cotton blue stain. The stained slides were observed under using a light microscope (Olympus, Japan). Characteristics of fungi such as hyphae, conidial heads and the arrangement of the conidia were observed. All yeast- like growths were Gram stained and photographs of colonies and microscopic morphology were taken.

The viscera including the lung, kidney, heart, liver and air sacs were harvested for histopathological studies. Direct microscopic examination of caseous nodules, using KOH/DMSO, were performed for detecting the branching hyphae in tissue samples.

The tissue samples of these organs were fixed in 10% buffered formaldehyde solution, dehydrated in graded ethanol, embedded in paraffin, sectioned at 6 micrometer and stained with hematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) for histopathology (Luna, 1968).

RESULTS

At necropsy, extensive yellowish white caseous miliary nodules with different sizes in the lungs and air sacs were observed but in some chickens with poor body condition caseous plaques covered by green-blue colonies were observed on the air sacs especially in abdominal region.

Histopathologic examination of tissue sections, stained with H&E and PAS, revealed granulomas with a caseous core, containing branching and dichotomous hyphae, surrounded by a granulomatous reaction and giant cells in lungs (Figures 1, 2, 3 and 4). Abdominal air sac membrane was thick and infiltrated by polymorphonuclear inflammatory cells covered by the characteristic conidial head (figures 2 and 5).

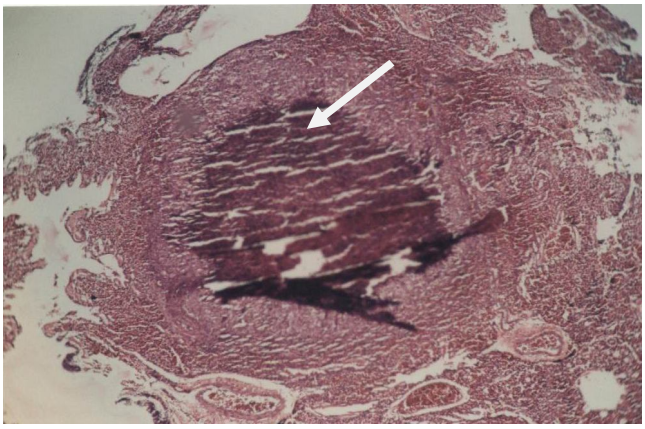

Figure 1. Microscopic lesion of acutely fatal aspergillosis in broiler chicken: a granuloma with caseous center and peripheral granulomatous reaction is observed (H&E, 40×)

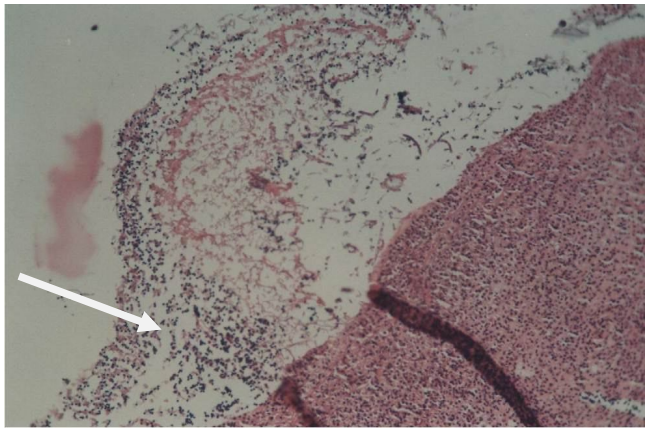

Figure 2. Acutely fatal aspergillosis in a broiler chicken: a thickened abdominal air sac is infiltrated by polymorphonuclear inflammatory cells and characteristic conidiophores (*Aspergillus fumigatus*) (H&E, 100×)

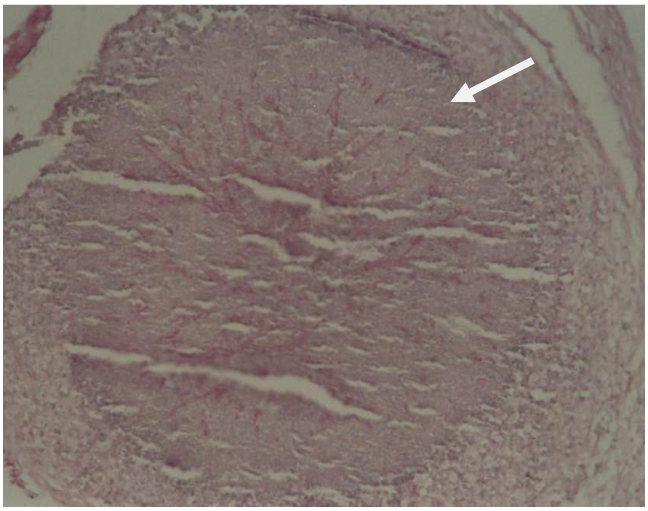

Figure 3. Pneumonia in a broiler chicken due to *Aspergillus fumigatus*, a single granuloma contains numerous, dichotomously branched, septate hyphae radiating from the center of a mass of necrotic granulocytes (H&E, 100×)

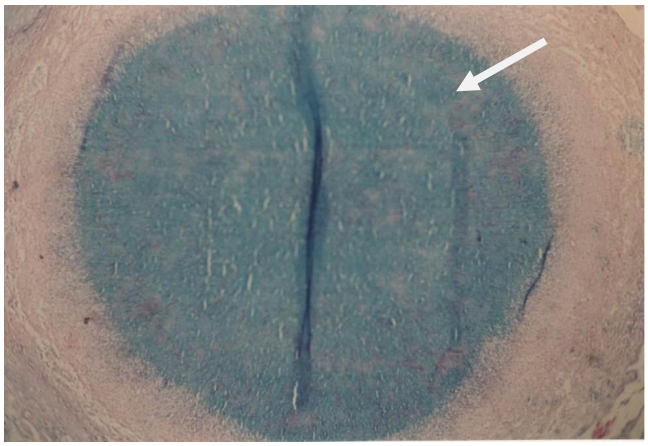

Figure 4. Pneumonia in a broiler chicken due to *Aspergillus fumigatus*, a single granuloma contains numerous, dichotomously branched, septate hyphae radiating from the center of a mass of necrotic granulocytes (PAS, 100×)

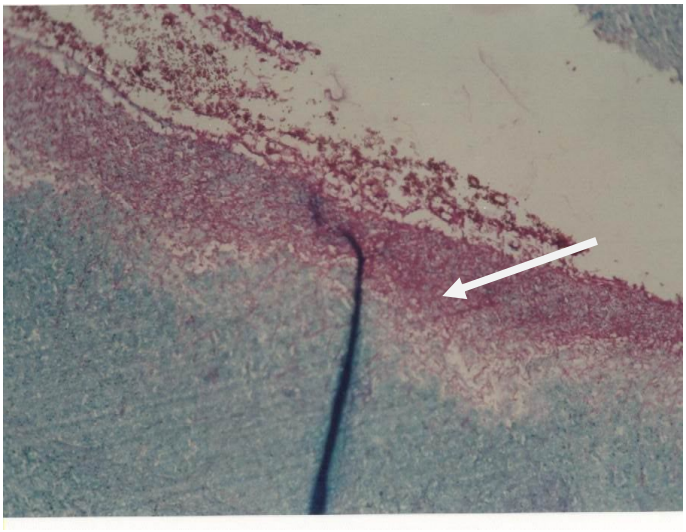

Figure 5. Acutely fatal aspergillosis in a broiler chicken: a thickened abdominal air sac is infiltrated by polymorphonuclear inflammatory cells and characteristic conidiophores (*Aspergillus fumigatus*) is seen on the air sac (PAS, 100×)

Four fungi species were isolated from respiratory system of necropsied broilers; they were *Aspergillus fumigatus*, *Mucor* spp, *Aspergillus niger* and *Aspergillus flavus. Aspergillus niger* and *Mucor* spp were the most frequently isolated. Incidence of *A. niger* isolation was 10.91 % where as the isolation rate of *Mucor* and *A. fumigatus* were 8.18 and 3.78 % respectively. *A. flavus* isolated from 2.37% samples and *Rhizopus* spp was not isolated from of examined samples (table 1).

Table 1. Isolation rate of fungi Species

	Mycological culture Absolute frequency/ Relative frequency (%)				
	Positive	**Negative**	**TOTAL**		
Number	*287*	*849*	*1136*		
Percent	*25.26%*	*74.73%*	*100*		
Fungi Species	***A. Fumigatus***	***A. Niger***	***A. flavus***	***Rhizopus*. spp**	***Mucor*. spp**
Incidence of isolation	3.78 %,	10.91%	2.37%	0%	8.18 %

Discussion

The results of this study indicated that fungal species are present in birds with apparent respiratory diseases. As mentioned in histopathological findings, miliary granulomatous pneumonia, granulomatous reactions and infiltration of air sacs by polymorphonuclear inflammatory cells were seen. These findings are considered as indicative of aspergillosis (Randall, 1996; Jones et al 1997), and in our cases, the pathologic lesions of aspergillosis were obvious and confirmed by fungal diagnostic cultures.

In this report, all miliary granulomatous lesions of aspergillosis were limited to lung and air sacs, and there were no lesions in liver, kidney, brain and heart. This is the acute form of aspergillosis. Aspergillosis presents in several forms in birds. The lesions depend on the chronicity of the infection and the number of spores inhaled. Colonization may be limited to the site of primary infection. The spores grow on the mucous membranes of the lungs and in the air sacs. It occurs as an acute aspergillosis, due to an overwhelming dose of fungal spores. In other form, aspergillosis can present as a chronic infection of the air sacs. Occasionally, depending on the host resistance, aspergillosis can be found in localized granulomatous lesions of the sinuses, trachea, internal organs and body cavity. The acute form is usually seen in young birds (Riddell, 1997; Schmidt et al, 2003). Similarly, in our study affected birds were young broiler chickens and the lesions limited to lung and air sacs without any other organs involvement.

Aspergillus species have been reported to be one of the most common fungal infections of poultry (Greenacre et al., 1992). The spores of *Aspergillus* have been reported to be highly thermo tolerant growing at temperatures between 15°C - 53°C. Wood shavings were used as a litter in broiler farms, initially have low moisture content (9.0 % to 26.0 %). It is therefore significant that they could contain a number of species of the *Aspergillus* as a source to contaminate broilers.

Aspergilus fumigatus produces gliotoxin, *A. flavus* produces aflatoxin B1 while *A. niger* produces oxalic acid (Joseph, 2000). Deem has been reported that *A. fumigatus* accounts for about 95% of aspergillus cases in birds followed by *A. flavus* (Deem, 2003). However, as indicated in Table 1, isolation rate of *A. niger* was more than other isolates followed by *Mucor*, *A. fumigates* and *A. flavus*. *A. niger* is pathogen of birds and man (Redig et al., 1980). Some of these fungi species especially those that produce mycotoxins can contaminate the feed of the birds which they may subsequently consume resulting in mycotoxicosis (Kwanashie et al., 2013).

The presence of these fungi may pose a potential cause of increased mortality of the birds and threat to the health of the birds, ultimately resulting in economic loses to the farmer in the event of

an outbreak. Fungal infection in poultry is very difficult and expensive to treat with a guarded prognosis (Joseph, 2000).

Deannis et al (1973) in a study on UK broiler farms isolated *Mucor* and *Rhizopus* from initial and final dust and litter samples. In another study in Malasia, Yudiarti et al (2012) isolated *Aspergillus*, *Mucor* and *Rhizopus* from gastrointestinal system of Indigenous chickens. Stanislaw et al (2007) in a case report of *Aspergillus fumigatus* infection in a pigeon flock recorded dominant presence of *A. fumigatus* and some colonies of *Mucor* spp in bedding litter and feed ingredients (wheat, barley, vetch).

Mucor and *Rhizopus* are all known pathogens of both man, animals and birds either directly as an infection or as a toxicosis (Saidu 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 (Kwanashie et al., 2013).

These fungi species may produce mycoses or mycotoxins and affect the health of the birds. It is therefore very important to be aware of the high prevalence of these organisms, the sources and points at which the chicks become infected with the organisms as well as the diseases they can cause and possibly put them under surveillance as important pathogens of poultry.

References

Chauhan, H. V. S., & Roy, S. 1996. *Poultry diseases, diagnosis and treatment*. New Age International.

Dennis, C., & GEE, J. M. 1973. The Microviral Flora of Broiler-house Litter and Dust. *Journal of General Microbiology*, *78*(1), 101-107.

Deem, S.L., 2003. Fungal disease of birds of prey. *Veterinary Clinics of North America: Exotic Animal Practice*., 6, 363-376

Eswaran, M. A., Sukumar, K., Malmarugan, S., Poomathi, K., & Rajeswar, J. J. 2011. Pulmonary aspergillosis in emu (*Dromaius novaehollandiae*). *Journal of Veterinary and animal Sciences*. 7 (5): 254-255

Fulleringer, S. L., Seguin, D., Warin, S., Bezille, A., Desterque, C., Arne, P. & Guillot, J. 2006. Evolution of the environmental contamination by thermophilic fungi in a turkey confinement house in France. *Poultry science*, *85*(11), 1875-1880.

Greenacre C.B., Latimer K.S., Ritchie B.W. 1992. Leg paresis in a black palm cockatoo (Probosciger ater-rimus) caused by aspergillosis. *Journal of Zoo and Wildlife Medicine*, 23, 122–126.

Joseph, V. 2000. Aspergillosis in raptors. *Seminars in Avian and Exotic Pet Medicine*, 9: 66–74.

Kwanashie, C. N., Kazeem, H. M., Abdu, P. A., & Umoh, J. U. 2013. Distribution of Aspergillus species among apparently healthy birds in poultry farms in Kaduna state, Nigeria. *Scientific Journal of Microbiology*, *2*(3), 61-64.

Luna, L.G. 1968. Manual of Histologic staining methods of the Armed Forces Institute of pathology. 3rd edition, McGraw-Hill Book Company, New York, USA

Mihaylov, G., Petrov, V., Marutsov, P., Simeonov, R., & Simeonova, G. 2008. A Case of Aspergillosis in A Bearded Vulture (GYPAETOS BARBATUS). *Trakia Journal of Sciences*, 6(1), 144-146.

Randall, C. J. and Reece, R. L. 1996. A color atlas of avian histopathology, Mosby-Wolfe, London, UK

Redig, P.T., Fuller, M.R., Evans, D.L. 1980. Prevalence of Aspergillus fumigatus in free-living goshawks (Accipiter gentilis atricapillus). *Journal of Wildlife Diseases*. 16, 169-174.

Richard, J. L., Thurston, J. R., Peden, W. M., & Pinello, C. 1984. Recent studies on aspergillosis in turkey poults. *Mycopathologia*, 87(1-2), 3-11.

Riddell, C. 1987. "*Avian histopathology*. American Association of Avian Pathologists."Lawrence: Allen Press Inc.

Saidu, L., Abdu, P.A., Oyelola, K., Raji, M.A., 1999) Suspected new castle disease and mucormycosis in flock of seven day old ostrich. *Zariya Veterinarian*, 6(1), 154- 157.

Sajid, M. A., Khan, I. A., & Rauf, U. 2006. *Aspergillus fumigatus* in commercial poultry flocks, a serious threat to poultry industry in Pakistan. *Journal of Animal and Plant Sciences*, 16(3-4): 79-81.

Schmidt, R.E., Reavill, D.R., and Phalen, D. N. 2003, Pathology of pet and aviary birds, Iowa state press,

Tokarzewski, S. T. A. N. I. S. Ł. A. W., Ziolkowska, G., Lopuszynski, W., & Nozdryn-Plotnicki, Z. 2007. Aspergillus fumigatus infection in a pigeon flock. *Bulletin-veterinary institute in Pulawy*, *51*(4): 563-567

Yudiarti, T., BI, V. Y., Murwani, R., & Kusdiyantini, E. 2012. Isolation of fungi from the gastrointestinal tract of indigenous Chicken. *Journal of the Indonesian Tropical Animal Agriculture*, *37*(2), 115-120.