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Brooder Pneumonia

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Abstract: Brooder pneumonia is an acute form of aspergillosis caused most frequently by *Aspergillusfumigatus* in birds. It causes significant economic loses to the poultry industry with high mortality and morbidity rate in poultry chicks. Chicks become infected when they hatch from eggs in incubators during brooding age and they inhale fungal spores from their surrounding environment. Besides infection in poultry *Aspergillusfumigatus* is considered as major respiratory etiological agent of various birds e.g quails, ducks, malards, turkeys, penguins etc. It also causes infection in other animals and humans. Antimycotics in water and feed are used to treat aspergillosis. Prevention of brooder pneumonia includes sanitation of hatchery and regular fumigation of eggs. advances in diagnosis, treatment, prevention and control of fungal diseases in poultry has not taken much attention. Recently, molecular biological tools have been explored for rapid and accurate diagnosis of important fungal infections. Effective prevention and control measures include: appropriate hygiene, sanitation and disinfection, strict biosecurity programme and regular surveillance/monitoring of fungal infections as well as following judicious use of anti-fungal drugs.
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1. Introduction

Fungal diseases cause huge economic loses to the poultry industry either because of their infectious properties or by release of mycotoxins which are fungal secondary metabolite produced in the poultry feed (Dhama *et al.,*2013; Katole *et al.,* 2013; Patil *et al.,* 2017a, b; Sharma *et al.,* 2019a, b; Singh *et al.,* 2019a, b, c; Singh and Singh, 2020). The Pathogen which is considered as major respiratory pathogen in the birds is *Aspergillusfumigatus*. *A. fumigatus*is filamentous fungi. Other species of *Aspergillus* are *Aspergillusflavus, Aspergillusniger, Aspergillusnidulans* and *Aspergillusterreus* are less commonly involved in avian Aspergillosis as compared to *Aspergillusfumigatu* (Arné *et al.,* 2011; Patil *et al.,* 2014). Aspergillosis in young birds occurs predominantly in acute form whereas chronic form occurs mostly in older age birds (Vanderheyden, 1993). Drugs used for treatment of aspergillosis in birds include fluconazole, itraconazoleketoconazole and also polyene antifungal drugs (Leishangthem *et al.,* 2015). *Aspergillusfumigatus*is a saprophyte and opportunistic fungal pathogen. It is wide spread in the nature, decaying matter and soil. It is one of the species of genus *Aspergillus*. In 1863 this organism was described by a physician named Fresnius. It also plays an important role in the nitrogen and carbon recycling (O'Gorman *et al.,* 2009). *Aspergillusfumigatus* is capable to grow at 37°C. This mold can with stand temperatures more than 50°C with its conidia surviving at temperature of 70 °C (Abad *et al.,* 2010). *A. fumigatus* can also produce cytotoxic and genotoxicmycotoxins (Nieminen *et al.,* 2002). *A. fumigatus* can grow and survive on organic debris. Soil is its natural place to live. It is the most existing fungus in nature with airborne conidia (Mullins *et al.,* 1976). Microscopic features include conidiophore, vesicle, head and conidia. Conidiophore of *A. fumigatus*is 5 to8 µm in width and up to 300 µm in length. Diameter of vesicle is 20 to 30 µm long in length. Head is columnar and conidia have size of 2 to 3.5 µm in diameter (Chakrabarti *et al.,* 2011). *Aspergillusfumigatus* has single layer of phialides (Warris and Verweij, 2005). Its hyphae are septate, unbranched conidiophores that form at right angles from foot cells of hyphae. Pigmented conidia are produced from phialides present on vesicles (Quinn *et al.,* 2011).

Aspergillosis (brooder's pneumonia):

Aspergillosis, commonly known as brooder's pneumonia, is caused mainly by *Aspergillusfumigatus*, most pathogenic fungi affecting poultry (Arne *et al*.,

2011) but *A. flavus* has also been the culprit associated with many cases, Respiratory infection by *Aspergillus* spp. has been reported in almost all types of poultry birds viz., layer cockerels (Steinlage *et al*., 2003), broilers (Martin *et al*., 2007), growers (Zafra *et al*., 2008) and turkey poults (Olias *et al*., 2010). Turkeys are having higher susceptibility to aspergillosis when compared to chickens. *A. fumigatus* infection occurs more frequently in poultry as the spores of this pathogen species are smaller than those of other *Aspergillus* spp. (Richard and Thurston, 1983; Arne *et al*., 2011). Other *Aspergillus* spp. that may affect birds adversely are *A. terreus, A. glaucus, A. nidulans* and *A. niger* (Beernaert *et al*., 2010; Dhama *et al*., 2012). *Aspergilli* can be isolated from environmental samples and are worldwide in distribution. Spores of this fungal pathogen are resistant in nature. Poultry birds coming in contact with the spores through contaminated feed or litter gets affected after inhaling the spores. The predisposing factors for flaring spore generation and dissemination in the air/environment include warm environment, humidity, poor ventilation and sanitation along with long term storage of feed (Tell, 2005; Khosravi *et al*., 2008). The disease develops in brooder stages in chicks as well as passerine birds, especially below three days of age (Pokras, 1988; Chauhan and Roy, 2008; McMillan and Petrak, 1988). Exposure generally occurs by inhalation of spores, which often originate from infected eggs that are opened. Chicks may get infection in the hatcheries itself as by the release of large number of spores in the environment and contaminate hatch mates (Oglesbee, 1997). Aspergillosis is a necrotizing and granulomatous cavitory disease of the lungs with hematogenous spread (Ganguly *et al.,* 2011). High humidity and moderate temperature conditions contributes significantly towards the occurrence and spread of aspergillosis (Dhama *et al*., 2008), thereby facilitating seasonal occurrence of the disease in waterfowls with higher incidences in spring and autumn. Particularly, crippled and malnourished captive birds suffer individually. Contaminant like lead acts as a precipitating factor, especially in geese (Wobeser, 1997; Kapetanov *et al.,* 2011). Brooder pneumonia is respiratory disease of poultry caused by inhaling spore of *Aspergillusfumigatus*. Other species of genus which are less frequently involved to cause brooder pneumonia are *A. flavus, A. niger, A. glaucans* and *A. nidulans*. This disease leads to economic losses of poultry sector. Bothimmunocompetent and immunosuppressed birds are affected by aspergillosis (Spanamberg *et al.,* 2013). Brooder pneumonia is an acute form of aspergillosis that occurs during brooding age of young birds when they are hatched in incubators and the environment is humid. Acute form results in high mortality in chicks whereas chronic form occurs in older age birds having compromised immune system due to poor husbandry conditions at farms of birds. Domestic birds like ducks, poultry, quails and even wild birds are prone toward aspergillosis. Transmission is through inhalation of spores and conidia from feces, soil and feed contamination. Spores are deposited in respiratory tract of bird. Both physiological and anatomical factors of bird respiratory system predisposes to infection (Nardoni *et al.,* 2006). Brooder pneumonia affected chicks show gasping, dyspnea, convulsions and lesions in air sacs and lungs. Other organs that may be affected are eyes, mucosa and trachea. Fungal lesions and nodules are formed with in air sacs and lungs. Lungs show lesions that are characterized by infrequent sudden milliary yellow nodules. Every nodule is enclosed by dark infiltrated area and other parts of lungs are normal. Air sacs are frequently thickened and contain little white yellowish plaque like lesions (Cacciuttolo *et al.,* 2009).

Diagnosis of Brooder Pneumonia

Histopatholgical examination is done by following standard method: Tissue from affected birds are taken in 10 percent formol solution. Then fixationis done properly followed by preparing 4 to 6 µm of paraffin embedded tissue sections and they are stained by Haematoxylin and Eosin method and then microscopy is done and pathognomonic features of disease are observed (Luna, 1968). Microscopic examination of tissues and other specimens can be done by using routine potassium hydroxid preparations, calcofluor white, periodic acid-shiff (PAS), Grocott's stain and Gomorime then amine silver. Direct microscopic methods are not sensitive and samples are positive only in advanced disease (Chandler and Watts, 1987). Isolation of *Aspergillusfumigatus*is done by directly streaking from infected organie lung, liver, air sacs on saboraud dextrose agar followed by incubation at 25°C for 5 days. Then macroscopic and microscopic features are observed. Macroscopic character includes color, texture of colony, margins and pattern on ridges and microscopic features include conidia, foot cell and hyphae.

Mounting methods include Lactophenol cotton blue stain (Ali *et al.,* 2017). *Aspergillusfumigatus* was detected by polymerase chain reaction by amplifying 26S gene of 401 base pairs by using specific oligonucleotide primers. 93% correlation was found between PCR results and culture results (Spread bury *et al.,* 1993). Molecular methods for confirmation of *Aspergillusfumigatus* involves amplifying various molecular targets in fungi e.g D1-D2 domain, 28S subunit, rod let A, ITS including 5.8S rRNA gene, betatubulin region and gene by PCR (Iwen *et al.,*

2002). There are various serological methods that can be used for diagnosis of brooder pneumonia in poultry. Various serological techniques include enzyme linked immune sorbent assay, agar gel immunodiffusion, counter immune electrophoresis (Cray *et al.,* 2009). Mass spectrometry identification methods such as matrixasisted laser desorption ionization time of flight mass spectrophotometry can identify by using *Aspergillusfumigatus* culture. This method MALDI-TOF MS can identify the organism not only at specie level but also at strain level of organism (Clark *et al.,* 2013).

Treatment of Brooder Pneumonia

Brooder pneumonia is treated by antifungaldrugs in which itraconazole, is administered orally by adding in feed, ketoconazole is administered orally, fluconazole administered orally, fluconazole is given orally, miconazole and clotrimazole are topical. Amphotericin B is given through nebulization. Every drug has different dosage e.g dosage of itraconazole is Dosages of ketoconazole 20-30 mg kg twice for 2-6day (Leishangthem *et al.,* 2015). It is fungistatic and stops germination of spore. 5-fluorocystosin is fungistatic drug and it stops germina3tion of spores inhaled by birds (Ganguly *et al.,* 2011).

Antifungal Drugs Used in Treatment

Azoles are antifungal compounds. Azoles stop cytochrome P 450 dependent enzyme lanosterol 14alpha demethylase that is encoded by cyp51A gene in *Aspergillus* species. Cytochrome P 450 dependent enzyme converts lanosterol to ergosterol in fungal cell membrane consequently it causes cell membrane rupture. Cell membrane function is affected and toxicmethylated sterols begin to accumulate in cell membrane of fungi and ergosterols start depleting in the cell membrane (Manavathu *et al.,* 1998). It raconazole has broad spectrum activity against pathogenic fungi (Diekema *et al.,* 2003). Amphotericin B is polyene. Polyenes binds commonly to ergosterol in cell membrane of fungi and affect function by causing impairment of cell membrane barrier, metabolic upset, leakage of cell components and death (Ernst *et al.* 2002). Flucytosine impairs pyrimidine metabolism and consequently inhibition of DNA, RNA and Protein synthesis occurs (Vanderheyden, 1993). Many vaccines are used to control aspergillosis. Those vaccine contain different parts of fungal elements i.e. germ line cells, mycelia, spores, conidia, whole cell filterate (Ganguly, 2016).

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