An overview of aspergillosis in poultry: A review

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Abstract
Aspergillosis is an infectious, fungal disease caused by *Aspergillus* species, particularly *Aspergillus fumigatus*. Infection occurs by inhalation of spores and penetration through egg shell. The disease occurs in two forms, acute and chronic. The acute form occurs by ingestion of large amount of spores, whereas, the chronic form affects birds with reduced immunity. Clinical signs include anorexia, emaciation, dusty or moldy feeds, adequate ventilation, and disinfection of litter and affects older birds only.

Etiology
Aspergillosis is infectious and non-contagious fungal disease of poultry. The etiological agent belongs to genus *Aspergillus* which is ubiquitous, saprophytic mould with worldwide distribution. *Aspergillus* is the most common fungal infection of respiratory tract in birds causing high morbidity and mortality. Thus causing a significant economic losses especially in poultry. It occurs due to mis-management problem in back yard and commercial poultry. The disease commonly affects lower respiratory system. Inhalation of *A. fumigatus* asexual spores (conidia) can cause wide range of clinical symptoms depending upon the immune status of the bird. Acute aspergillosis occurs in young birds resulting in high morbidity and mortality. Chronic form is sporadic and causes lesser mortality and affects older birds only, with immunosuppression due to poor husbandry practices. Poor sanitation and poor ventilation in the house as well as food contamination enhances fungal growth and increases the possibility of invasion by air borne spores.

Transmission
Aspergillosis affects not only chickens and other animals but also humans. All the domestic birds like poultry, quails and duck, as well as wild birds are susceptible to aspergillosis infection. Aerosal transmission of fungal spores from contaminated feed, soil, fecal material and contamination of egg in ova, infect the developing embryo. The small and non-expanding lungs and nine air sacs together constitute a primary nidus for infection because the air (or spores) reaches the caudal air sacs prior to which, it pass through the part of the lungs in which the gas exchange takes place.
Pathogenesis
Aspergillosis is caused by inhalation of small, hydrophobic fungal spores (conidia) into the lungs [8]. During inhalation, small spores trapped in nares (two holes in beak leading to respiratory system), trachea and enter into primary bronchi (mesobronchi) and delivers the inhaled air to the posterior thoracic air sac and abdominal air sacs which further reaches epithelial surfaces in the lungs [7]. These spores get engulfed by alveolar macrophages in lung [9] and go through interstitium into haematoagogenous route and lymphatic system further spread into multiple organs [10]. Recent studies suggest that A. fumigatus conidia may be able to resist killing by alveolar macrophages [11]. Two types of tissue reactions have been recognized: 1. granulomatous or deep nodular form 2. infiltrative or superficial diffuse form. In the granulomatous form, neither exudative inflammation nor vascular lesions in the neighboring tissues are seen. This type of encapsulated reaction develops both in non-aerated and aerated organ (lungs and the air sacs) [7, 12, 13]. In non-encapsulated infiltrative type, the fungus frequently invades blood vessels. In aerated organs, the fungus may form aggregates of radiating hyphae containing large numbers of conidiophores and conidia in the absence of a structured granuloma formation [7, 13]. 3. Mixed type composed of both tissue reactions in the same tissue [14, 15].

Clinical signs
Susceptible birds develops polymorphic clinical forms with regard to either localized or disseminated lesions. Aspergillosis occurs in two forms 1. Acute (less than 1 week) and 2. Chronic (weeks or months). Acute aspergillosis occurs by inhaling large number of spores where as chronic is associated with immuno-suppression [16].

1. Acute form
Young birds have acute or peracute infection resulting in high morbidity and mortality. Clinical signs include dyspnoea, anorexia, cyanosis, polydypsia, foul smell diarrhoea and emaciation. Occasionally the birds may die suddenly without showing any clinical signs (Peracute).

2. Chronic form
It is more common in older birds. Clinical signs includes inappetence, emaciation, dyspnoea, gasping, increased thirst, fever, diarrhea and signs of nervous involvement [17]. Ocular changes include ophthalmitis, blepharospasm, photophobia, and Mycotic keratitis (peri orbital and eyelid swelling with cheesy yellow exudates in the conjunctival sac [18, 19]) as well as necrotic granulomatous dermitis [20]. Neurological signs include paralysis, ataxia, tremor, torticollis, lameness, convulsions, opisthotonous condition [21, 22, 23, 24].

Gross Lesions
The primary location is lungs, air sacs and other organs. Majorly the respiratory tract involvement can be seen prior to the development of clinical signs. Typical lesions consist of white to yellowish granulomas ranging from milary (2 cm) involving serosa and parenchyma of one [25] or multiple organs. Single or multiple necrotic areaseay may be visible on cut surfaces. Lung parenchyma is either consolidated or has focal granulomas of different size [26, 27, 28]. When coalescing in air sacs, granulomas form cheesy caseous plaques on thickened membranes where fungal sporulation may occur as evidenced by grey-greenish velvet [13, 21, 22, 28, 29].

Circumscribed white to greyish areas were observed in the cerebellum of broiler breeders [31].

Microscopic lesions
The microscopic lesions observed are congestion of pulmonary and peri-alveolar blood vessel and peri-vascular edema. The normal structure of the lung and air sacs were replaced by pyo-granulomatous foci. The center of the granulomatous foci contained caseous necrosis and necrotic cellular debris surrounded by infiltration of inflammatory cells like heterophils, lymphocytes, macrophages and multinucleated giant cells were seen. The nodules consisted of coagulative necrotic center. Focal inflammatory lesions were present on the pleura and the underlying lung lobules [33].

Diagnosis
As the signs of aspergillosis are non-specific making diagnosis is difficult. Diagnosis is based on history, clinical signs, postmortem findings, hematology, biochemistry, serology, radiographic changes, endoscopy, and culture of the fungus. Regular aspergillosis cases in birds are commonly diagnosed based on postmortem findings of white to yellowish caseous nodules in the lungs or air sacs since clinical diagnosis is difficult [32]. The history of the bird reveals a stressful event, environmental factors and immune suppressive condition. The tissue samples (lungs, trachea, pharynx and thoracic air sacs as well as other organs) fixed in 10% neutral buffered formalin are processed and embedded in paraffin blocks and stained with haema toxylin and eosiin (HE) method. Other special stains such as Periodic acid-Schiff (PAS), Bauer’s and Gridley’s stains, Grocott’s and Gomori Methanamine Silver stain easily identify the hyphae and mycelia of fungus. The pathogenic organism can be isolated by culturing on Sabouraud’s glucose agar or antibiotics and incubated at 37°C for 24 hours with characteristic conidial head and colony [33].

Treatment
Treatment for Aspergillosis is not effective because of the reduced bird’s inflammatory response to drugs. Prognosis of disease is poor when there is extensive infection in tissue and only systemic drugs are used. The best treatment is topical application along with systematic therapy. It involves the use of one or more systemic antifungal agents like -itraconazole, ketoconazole, clotrimazole, miconazole, fluconazole and Amphotericin B. But itraconazole is a choice of treatment for this disease [31].

Prevention and control
Aspergillosis has no effective treatment and prevention by vaccination is not commercially practicable. Majorly control depends up on reducing exposure to the fungus and associated risk factors. Aspergillus fumigatus in young chicken can be controlled to some extent by hatchery sanitation. Mouldy litter or feed should be avoided to prevent aspergillosis outbreak. It is better to treat poultry house and litter with antifungal compounds [34]. Removal of mouldy feed, cleaning of bulk feed container, removal of old litter and replaced with new litter in poultry house. All the hatching equipments should be thoroughly cleaned and disinfected properly. Contaminated hatchery should be fumigated with formaldehyde or thiabendazole with dose rate- 120-360 g/m3 and [35].
Recommendations
The following recommendations are advised to prevent economic loss in poultry farms by aspergillosis:
Proper cleaning and disinfection of feed and water utensils, Avoid overcrowding in poultry house, Provide proper ventilation in poultry house, Avoid using of mouldyand dusty feed, Maintain proper sanitation of hatching equipment, Treat the poultry house and disinfect the litter with antifungal compound, Infected birds should be culled to avoid spread of disease, Mould inhibitors are used in the feed for suspected outbreak.

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