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Aspergillosis in Poultry a Critical Overview

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Abstract: Aspergillosis is a respiratory ailment predominantly affecting turkeys, chickens, humans, and various mammals, with less frequent occurrences in pigeons, ducks, geese, and other domestic and wild birds. It is caused by a fungus belonging to the Aspergillus genus. On farms, the disease may be endemic among chickens and turkeys, while in wild birds, it appears sporadic, often affecting individual birds. Typically, immature birds aged 7 to 40 days exhibit the disease. Due to the widespread presence of Aspergillus spp., the condition can be found in environments conducive to its growth. Aspergillosis is commonly attributed to Aspergillus fumigatus, although several other mold species, including A. flavus, A. niger, Rhizopus sp., Mucor sp., and Penicillium sp., may also be responsible. Infection usually occurs through the inhalation of spore-filled dust from contaminated dusty areas. The disease manifests in two forms: acute, affecting birds that have ingested large spore quantities, and chronic, affecting birds with suppressed immune systems. Diagnosis in poultry involves various methods, with culturing being essential due to the nonspecific clinical signs. Aspergillosis lacks effective treatment; hence, prevention is crucial. Implementing good management practices, such as maintaining sanitation, avoiding damp litter or soil, steering clear of moldy or dusty feeds, ensuring adequate ventilation, and sterilizing feed and water systems, is essential for disease prevention and control.

Keywords: Avian Aspergillosis, Disease Control, Epidemiology, Diagnosis and Treatment.



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Introduction

Poultry aspergillosis is a major infection with fungus and is a non-contagious disease in birds. The fungus from the genus Aspergillus caused this disease. When the sickness develops it means the birds have an excessive number of spores in their bodies or when the host's immune system is weak [1]. Stress condition is the primary risk factor [2]. In this fungus aspergillosis bird's respiratory system is most affected and also causes acute mortality and morbidity. Commercial and backyard poultry both mainly typically cause poor management [3]. The most common sickness in the lower respiratory system. Embryos are also affected when the aspergillus species are penetrated in the eggshell which may be caused dead or hatch with a lesion fully grown. Hatchery equipment is also contaminated when a large number of spores or emitted [5]. Mostly A. fumigatus Aspergillus shows a large range of clinical symptoms when the birds inhale the asexual spores (conidia) depending on the bird's immune system [6]. There are two different types of aspergillosis acute and chronic. The young birds are affected by the acute form that may cause severe mortality and morbidity [7]. The chronic type of aspergillosis usually causes infection in older birds due to the weak immune system [8,9]. Fungal spore growth increased due the poor sanitation and ventilation all of these increase the risk of infection in the respiratory system in birds [10]. It mostly affects the newborn chicken and also relates to the infection in hatching the sickness known as brooder pneumonia in young poultry. Avian aspergillosis is also described by Pneumomycosis, bronchomycosis, and colloquial [11]. Low frequent symptoms in the eyes, brain, skin, joints, bones, and visceral infection [12]. Avian aspergillosis is caused by a variety of fungus including Aspergillus fumigates, A. niger, A. flavus, A. terreus, and A. glaucus [13]. The aspergillosis infection is mainly caused by the inhalation of so many tiny, hydrophobic fungus spores (conidia). The infectious spores enter the nasal, tracheal, bronchial, and epithelium of the air sac before entering respiratory tissue and developing Granulomas [14].

Mycotoxins, which are toxic secondary metabolites produced by fungi, pose a threat to poultry when they ingest tainted food. Examples of these mycotoxins include ochratoxin and aflatoxins, originating from fungi such as Aspergillus ochraceus, Aspergillus flavus, and Aspergillus parasiticus, leading to detrimental effects on the well-being of poultry [15]. PCR is a very common



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diagnostic method for Aspergillosis in birds including a combination of clinical manifestations, epidemiological factors, and the isolation and also identification of the causative agent [16]. The control of this infection is by good management techniques like sanitation, sufficient ventilation, and disinfection. These control measures also depend on the correct diagnostics of aspergillosis infection and epidemiology [17]. All these are the objectives of this study.

Origin

A fungus is associated with the genus Aspergillus, Family: Trichocomaceae, Order: Eurotiales Class: Eurotiomycetes, Division: Ascomycota, and includes the Kingdom. That is why Fungi causes aspergillosis in chicken. More than 250 species in the genus Aspergillus, however, a few well-known species were taken into consideration constituting significant opportunities for diseases in invertebrates and vertebrates [18]. Belonging A. niger, Aspergillus fumigatus, A. terreus, A. flavus, and A. glaucus among other species. A. fumigatus that fungus causes the most sickness. These fungi are grown in warm and humid environments at room temperature, and also on organic material, broken eggs in hatcheries [19]. In poultry A. fumigatus is a most important agent that causes aspergillosis due to its very small spores that are willingly inhaled. When the environments are heavily contaminated mixed infections are possible in case of aspergillosis like A niger, A. nidulans, A. terreus, and A. amstelodami they may also be isolated in this case [20]. Aspergillus species are sometimes understood as asexual reproduction because these fungi reproduce by ascospores. And they grow and start hyphae and also come together and produce the mycelia [21]. Conidiophores are reproduced by superficial hyphae of mycelia and are made by vesicles and foot cells. Conidia or spores can reproduce in one line chain in this restricted growth. Conidia are single-celled and sometimes one or more called nuclei [22]. The spreading of fungus can be functionally by the conidia. So many different enzymes can be reproduced by it. Aspergillosis is used as a food in so many different products. For fungus growth, the favorable condition is two in which organic matter or moisture is available. The fungus can produce any material [23]. Conidia exhibit variations in shape, appearing either spherical, elliptical, or oval, and their surfaces may range from smooth to slightly textured. The hue of conidia impacts the coloration of the conidial head, thereby influencing the overall colors of the colony [24].



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Epidemiology

A large number of aspergillus species have evolved both parasitic and saprophytic lifestyles as a host in this world. Illness triggered by certain Aspergillus species predominantly impacts the respiratory systems of humans and other creatures, with birds and mammals encountering it more regularly than mammals [25]. Wild birds and domestic birds both can contract the fungus. A common death of zoo birds is Aspergillus. Where the environmental conditions are good, this fungus Aspergillus found there's. This Aspergillus reproduces in warm 25°C and humid conditions and also where organic materials are found in places. In hatcheries in contaminated eggs [17]. In tropical nations, this disease is most common. In the first two weeks, chicken died up to 15%. The outbreak is related to the hatcheries and also the seasonal distribution of aspergillosis [26].

Transmission

Animals, humans and all birds are also affected by aspergillosis. Wild and domestic, both birds, including chickens, ducks, and quails, have been affected by this fungus Aspergillus. Respiratory exposure to numerous minuscule, water-repellent fungal spores (conidia) is responsible for Aspergillosis. Inhaling conidia or spores present in polluted feed, excrement, ground, and ova can lead to infection in the developing embryo [27]. Upon infiltrating the nasal passages, trachea, bronchi, and air sac epithelium, infectious spores enter respiratory tissue, where they proliferate by transforming into tubular hyphae, forming granulomas. Subsequently, they are disseminated through the bloodstream to various tissues such as the kidney, pericardium, brain, and bone marrow. Lesions develop as a consequence of the invasion, accompanied by the infiltration of heterophils, lymphocytes, monocytes, and specific large cells, inducing an inflammatory condition and response [28, 29].

Clinical signs

The onset of acute aspergillosis is marked by a diverse array of general clinical indications, including loss of appetite, fatigue, disheveled feathers, breathing difficulties, excessive thirst, increased urination, stunted growth, or sudden mortality. Referred to as "brooder pneumonia," this



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condition results in severe respiratory hardship among chicks infected either within the egg or posthatching and poses a particularly high fatality risk during the initial ten days of life [30]. Mortality rates are (5-50%) in poultry farms. Increase in a few days and reduce to the previous level. Hyperpnoea with panting, wheezing, cyanosis, nonproductive coughing, Dyspnea, gasping, and occasionally nasal discharge are respiratory symptoms [31]. Emaciation, ataxia, dyspnea, sadness, dehydration, tremor, and opisthotonos are the symptoms of the chronic type. seizures, convulsions, lateral recumbence, torticollis, and hind limb paresis may be involved are the nervous system [31].

Macroscopic lesions

The primary sites affected are the lungs, air sacs, and other organs. Involvement of the respiratory system is usually evident before any clinical symptoms manifest. Typical lesions include white to yellowish granulomas, varying in size from miliary (2 cm), and affecting the serosae and parenchyma of one or more organs. Sliced surfaces may reveal one or several necrotic regions. The lung parenchyma displays either localized granulomas of diverse sizes or consolidation [11]. Cheesy caseous plaques form on thicker membranes as granulomas consolidate within air sacs, where the presence of grey-greenish velvet serves as an indication of potential fungal sporulation. In the cerebrum and cerebellum of broiler breeders, confined abscesses manifest as whitish to grayish patches. Mycotic pod dermatitis is observed in footpads, characterized by swollen and adherent eyelids with a turbid discharge, clouded cornea, and the presence of cheesy yellow exudates within the conjunctival sac.

Additionally, signs include keratinized epidermal disruption, encrustations, and acute inflammation. [27].

Histopathology

Microscopic observations reveal perivascular edema and congestion in pulmonary, peri-alveolar, and parietal alveolar blood vessels. The typical structural organization of the lungs and air sacs is replaced by disseminated pyo-granulomatous foci. The center of these granulomatous foci contains



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caseous necrosis and necrotic cellular debris, surrounded by inflammatory cells such as heterophils, lymphocytes, macrophages, and multinucleated giant cells. Nodules exhibit coagulative necrotic centers. On the pleura and underlying pulmonary lobules, there are concentrated, more severe densification and inflammatory lesions [33].

Diagnosis

Although symptoms of aspergillosis are vague and challenging to pinpoint, the most common approach to diagnosing it relies on clinical manifestations and observable lesions. Diagnosis typically hinges on assembling information from various sources such as medical history, clinical presentation, postmortem observations, hematology, biochemistry, serology, radiological changes, endoscopy, and fungal culture. It's important to note that no single test can guarantee a conclusive diagnosis [34].

Isolation and Identification of Causative Agent

Mold presence can be identified through microscopic examination of tissues from the infected individual. Various methods can be employed to obtain tissue samples for testing, including extracting a portion of the affected tissue, swabbing the lesion, or detaching one of the plaques. It is crucial to handle the diagnostic samples with care, employing aseptic techniques. A small segment of the nodule immersed in 20% potassium hydroxide (KOH), separated, and placed on a microscope slide can be studied under a microscope. The exudate can be examined for hyphae by heating the slide over a flame. Typically, samples are placed on Sabouraud dextrose agar, and incubated for 24 hours at 37°C, and the pathogenic organism can be isolated by observing distinctive conidial heads and colonies [35]. Special fungal stain analysis during histopathological examination reveals granulomas containing mycelium in tissue samples, encompassing organs like the lungs, trachea, throat, and thoracic air sacs. The samples are treated before being embedded in paraffin pieces and undergo staining using the hematoxylin and eosin (HE) procedure. Utilizing other specialized stains such as Periodic acid-Schiff (PAS), Bauer and Gridley's, Groote's, and Gomori Methanamine Silver stains simplifies the identification of fungal hyphae and mycelia [9].



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Immunity and serologic tests

On farms, serological assays are not currently employed to investigate aspergillosis outbreaks and have not been validated in poultry. Due to the generic nature of fungal antigens, serologic testing has limited applicability. Although enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion measurements of the antibody response were inconsistent [36]. The early detection of circulating galactomannan using sandwich ELISA is associated with both high sensitivity and specificity [37].

Immunohistochemistry, utilizing either monoclonal or polyclonal antibodies, is a robust and accurate approach to ascertain the presence of A. fumigatus within lesions. To enhance the sensitivity of serodiagnostic tests for Aspergillosis, protein electrophoresis can be employed. This process involves elevations in globulins alongside reductions in albumin, increased levels of alpha 2 globulins, and elevated concentrations of serum amyloid [38].

Diagnostic imaging (Radiography and Endoscopy)

Radiographic assessment serves as a rapid and efficient means to initially evaluate underlying diseases in non-critical avian cases. When examining the avian lungs and air sacs for signs of inflammation or granulomas and to assess the overall well-being of the lower respiratory tract, employing non-invasive imaging techniques such as lateral and dorso-ventral radiography proves beneficial. Despite the invasiveness of endoscopy, it provides a visual diagnosis of fungal development and organs with abnormal growths in the bird's trachea and coelomic cavity are sampled using biopsy, fine-needle aspiration, and culture methods [39].

Molecular diagnosis

Polymerase chain reaction (PCR) has been utilized in identifying and detecting fungal isolates in various avian species, including ostriches, penguins, falcons, turkeys, and white storks. However, further research is necessary before PCR assays can be routinely incorporated into the diagnostic practices of avian practitioners. In experimental settings, PCR assays have been employed in conjunction with other indicators, particularly in turkey models and various bodily fluids.



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Although PCR is highly sensitive in analyzing fungal DNA from bodily fluids or serum, it can yield false-positive results [40]. Molecular biological approaches have been applied to assess fungal contamination in poultry flocks, particularly in identifying the source of fungal isolates. Aspergillus DNA detection holds value, especially when examining frozen and fixed organ samples or in cases where culture fails to identify the infectious agent [41].

Differential diagnosis

The specific clinical manifestations of avian aspergillosis vary depending on the affected organs and systems. Early mortality in broiler chicks should be carefully examined for potential Aspergillosis, as it can be triggered by mycotoxicosis, acute bacterial septicemia, or carbon monoxide poisoning. Diseases such as infectious laryngotracheitis, infectious bronchitis (characterized by coughing, gasping, and neck extension during inspiration), and Newcastle disease all lead to dyspnea and watery, greenish diarrhea. Differential diagnoses include mycobacteriosis, colibacillosis, dactyl-aria infection (manifesting as nervous signs), other mycoses like ochronosis and zygomycosis, as well as nutritional encephalomalacia [4]. During necropsy, the presence of granulomatous lesions is typically used to differentiate pulmonary aspergillosis from other avian respiratory illnesses. However, Staphylococcus aureus pneumonia in newborn chicks can resemble it. Additionally, pneumonia and exudative fibrinous or fibrinoheterophilic air vasculitis are frequently observed in mycoplasmosis, colibacillosis, poultry cholera, and chlamydophilosis patients. When granulomas predominate, considerations must also include mycobacteriosis and other mycoses. Aspergillus ocular edema resembles infectious coryza or vitamin A deficiency in chicks [42].

Treatment

The treatment of aspergillosis is challenging due to the ineffectiveness of medications, as the fungus becomes encapsulated by the bird's inflammatory response, isolating it from the bloodstream. In cases of widespread tissue infection where only systemic medications are employed, the prognosis for the disease is typically unfavorable. Optimal outcomes are achieved



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by desiccating the granulomatous lesions and applying topical medication alongside organized therapy. Treating aspergillosis necessitates the use of one or more systemic antifungal medications. Commonly utilized medicines include itraconazole, ketoconazole, clotrimazole, miconazole, and fluconazole [31]. While amphotericin B has traditionally been the preferred medication for aspergillosis treatment, it comes with serious adverse effects and a high mortality rate. For improved results, itraconazole and voriconazole can be administered after amphotericin B. Additionally, less toxic lipid formulations of amphotericin have proven to be efficient [43].

Prevention and Control

A cure for aspergillosis is currently unavailable, and vaccination is not a commercially viable method for prevention. Spontaneous recovery may occur if re-exposure to the mold is avoided. Key control measures include:

- Reducing exposure to the fungus and related risk factors.
- Removing birds from contaminated areas.
- Ensuring hatchery hygiene in young chickens by meticulously cleaning and disinfecting hatching equipment.
- Eliminating contaminated materials to prevent additional exposure, such as removing old litter and moldy feed.
- Refraining from using moldy litter or feed to prevent an outbreak of aspergillosis.
- Minimizing the disturbance of infected materials to reduce further spore aerosolization.
- Implementing higher air exchange rates or improved ventilation to potentially mitigate the severity of the epidemic.

Avoid the incubation of severely infected or fractured eggs, as they can stimulate fungal growth and may rupture, releasing spores into the hatching machine [5, 44].

Public Health Significance

Aspergillosis poses a significant occupational and zoonotic threat, primarily impacting individuals and workers who come into contact with infected birds. The respiratory symptoms of aspergillosis



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in humans can be particularly severe, especially in individuals with chronic conditions such as diabetes, cancer, and tuberculosis, and those with compromised immune systems undergoing longterm antibiotic, antimetabolite, and corticosteroid treatments. The recent outbreak of aspergillosis in chickens has caused alarm and concern within the community that consumes poultry [45]. Human contraction of aspergillosis can occur through handling infected birds, inhaling spores from contaminated feed and litter, neglecting sanitation and hygiene standards in the environment, and consuming improperly cooked contaminated poultry. It is challenging to salvage food that has molded because most of the mycotoxins produced by Aspergillus are not broken down by cooking temperatures. Individuals regularly exposed to materials like grain, hay, cotton, wool, and other items contaminated with fungus spores are at a higher risk. Healthy children may experience fever and dyspnea when exposed to numerous conidia. Allergic bronchopulmonary aspergillosis (ABPA) is a condition affecting individuals with pre-existing asthma, eosinophilia, and intermittent bronchial obstruction [46].

Conclusion and Recommendations

In young chickens or turkeys, the most prevalent manifestation of aspergillosis is a respiratory illness, although it can also manifest as an ophthalmic or neurologic condition. A diagnosis is typically established based on the identification of characteristic gross lesions, which may be further supported by histopathology, the isolation of the infective agent, and culture. Successful cure is not attainable; therefore, effective control revolves around minimizing exposure to contaminated materials. To mitigate aspergillosis-related economic losses in poultry farms, the following measures are recommended: Thoroughly clean and disinfect feeding and watering equipment. Prevent overcrowding in the poultry house, ensure proper ventilation, and refrain from using dusty and moldy feed. Maintain cleanliness in the hatching apparatus, and use an antifungal substance to disinfect both the chicken house and the litter. In suspected outbreaks, infected birds should be promptly removed, and feed should be treated with mold inhibitors to impede the spread of the disease.



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