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Original Article

Grading of Anatomopathological Disparity in the Cases of Invasive Pulmonary Aspergillosis in wild avian species as recorded in Pigeons (*Columba livia*), Peafowls (*Pavo cristatus*), and Griffon Vultures (*Gyps fulvus*)

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Abstract

Aspergillosis which is caused by *Aspergillus fumigatus*, a fungal pathogen, can vary from a localized infection to severe life-threatening invasive or disseminated systemic diseases in birds. The present study aimed to evaluate and grade the anatomopathological disparity in the cases of invasive pulmonary aspergillosis (IPA) in *Columba livia* (pigeons), *Pavo cristatus* (peafowls), and *Gyps fulvus* (Griffon vultures). Necropsy gross lesions varied from mere congestion of lungs in *P. cristatus*, congestion and large necrotizing masses surrounded by a zone of hyperemia (10 mm dia) in lungs of *C. livia*, and typically disseminated granuloma in the lungs, air sacs, and organs of other serous membranes in *G. fulvus*. Histopathology varied from extensive parenchymal necrosis amidst exuberant fungal invasion in *P. cristatus*, multifocal to focally extensive tissue necrosis with colonies of fungal hyphae surrounded by heterophils and lymphocytes in *C. livia*, as well as typical mycotic granuloma embedded in the lungs, air sacs, and thoracoabdominal serous membranes with angio-invasion in *G. fulvus*. Based on gross and histopathological findings, we diagnosed the cases as Acute Invasive Pulmonary Aspergillosis (AIPA) in peafowls and pigeons, as well as Chronic Invasive Pulmonary Aspergillosis (CIPA) in Griffon vultures. There is a paucity of case reports on aspergillosis in wild avian species, and this report strived to document the cases of IPA in peafowls, pigeons, and vultures. This is the first report of its kind which evaluated anatomopathological disparity of IPA in pigeons, peafowls, and vultures with a proposed anatomopathological grading system which would help to understand and investigate the nature of aspergillosis in different avian hosts.

Keywords: Aspergillosis, Anatomopathological grading, Invasive, Histopathology, Peafowl, Pigeon, Vulture

1. Introduction

Aspergillus species are filamentous saprophytic fungi that are commonly found in seeds soil, decaying vegetation, as well as on and grains, with an opportunistic potential to infect a wide variety of living hosts (1-7). In animals, aspergillosis is primarily a respiratory infection that may be localized or generalized; nonetheless, tissue predilection is highly variable among the species of the affected host (1). The term aspergillosis was coined by Hinson, Moon, and

Plummer in 1952 to cover a wide variety of conditions ranging from a localized infection to fatal disseminated diseases in humans and different animal species (1, 5-7).

Aspergillosis in animals is often caused by *Aspergillus fumigatus* and rarely other *Aspergillus* species (2). In aves, it is a major cause of severe respiratory disease and mortality in birds, affecting all kinds of birds, including domestic, as well as free-living birds, such as wild birds that are kept in captivity, imposing significant economic losses on the poultry industry (4, 8-11). The genus *Aspergillus* has been subdivided into 22 distinct sections, out of which the sections Candidi, Circumdati, Flavipedes, Fumigati, Nidulantes, Ornati, Restricti, Terrei, Usti, Versicolores, and Warcupi contain clinically relevant species (12).

There are more than 200 species of *Aspergillus*; however, only a few species are associated with infections in humans and animals (13). The first species was *Aspergillus fumigatus* which was discovered by Fresenius in 1863 from the bronchi and alveoli of a great bustard (Otis tarda) (14). *A. fumigatus* has become a recognized opportunistic pulmonary pathogen in humans, animals, and birds (4, 9, 11, 15). It is reported that *A. fumigatus* accounts for over 95% of avian infection cases (16). Other *Aspergillus* species, such as *A. glaucus, A. niger, A. flavus, A. terreus*, and *A. nidulans*, may also be isolated from the cases of avian aspergillosis but much less frequently than *A. fumigatus* (4, 17, 18).

In the past, *A. fumigatus* was involved in significant mortality of free-ranging wild birds, including the American crow (19, 20). It was also reported in Tundra swans (21), Mallards (22), or Canada geese (23), and occasionally in waterfowl, gulls, and corvids, following the dumping of moldy seeds in areas where birds regularly feed (24). Infection by *A. fumigatus* has been reported in birds held in captivity, such as vultures, geese, ducks, swans, gulls, penguins, pigeons, and parrots, and exceptions are guinea-fowls and peafowls (4, 15, 25, 26).

The frequency of aspergillosis occurrence is relatively higher in avian species since the anatomy and physiology of the avian lung-air sac system are significantly different from the bronchoalveolar lung of mammals. The air sacs in birds function as bellows to move air through the gas exchange surface of the lungs, and during this process, they become prone to contamination by extraneous particles since they are submitted to an airflow that favors particle deposition. Moreover, birds have few resident macrophages to remove inhaled particles and possess an epithelial surface nearly devoid of a mucociliary transport mechanism which favors precipitation of disease.

The specific pathogenicity of *A. fumigatus* in invasive pulmonary aspergillosis (IPA) is conditioned by its ability to sporulate abundantly, with each conidial head producing thousands of conidia of about 2-3 µm diameters. Therefore, they allow its easy penetration into small bronchioles, which leads to subsequent colonization and tissue invasion (8, 9, 27-29). Timely disease investigation, explorative pathology, and pathogenesis, employing rapid and reliable diagnostic methods, as well as appropriate antifungal therapies, could be of great help in controlling this important disease of avian species (4, 9, 11, 30-32). Nevertheless, the timely confirmatory detection of aspergillosis is difficult owing to the absence of ideal diagnostics, which delays the diagnosis, therapy protocol implementation, and prognosis of this disease in birds (32).

Furthermore, owing to the low efficacy of treatment regimens, poultry experts and avian practitioners should check the conditions favoring the emergence of Aspergillosis so as to advise appropriate preventive and control strategies (8, 33). In the recent past, we have recorded sporadic mortality due to acute and chronic forms of aspergillosis in several captive and free-ranging pigeons, peafowls, and Griffon vultures, particularly during the winter months. Despite the potential significance of *Aspergillus* infection and associated mortality, only one case report of IPA in Himalayan vulture has been reported from India (25).

To the author's best knowledge, the present study is the first recorded evidence in India that describes anatomopathological features of acute and chronic invasive pulmonary aspergillosis (AIPA and CIPA) in

Pavo cristatus (peafowls), *Columba livia* (pigeons), and *Gyps fulvus* (Griffon vultures) with an attempt to grade the lesions based on gross and histopathology. We strongly emphasize that aspergillosis is a serious cause of respiratory disease in birds; therefore, an investigation of sporadic mortality in captive or wild birds should include aspergillosis in the list of diseases to be screened.

2. Materials and Methods

2.1. Study Area and Case Description

The study area, Bareilly, is located at 28˚10′N, 78°23′E in Western Uttar Pradesh, India, with a semi-tropical climate. In summer, the temperature usually ranges between 25°C and 44°C, and in winter, it ranges between 4°C and 24°C, with an average rainfall of 1087 mm. The cases of aspergillosis recorded in this study were investigated between November and January, the peak winter period in northern parts of India, during which the temperature in Western Uttar Pradesh ranges from 4°C to 24°C. Two pigeon carcasses from a flock maintained under captivity and one free-ranging pigeon carcass, as well as two peafowl carcasses and two Griffon vulture carcasses which were found dead in the forest premises, were submitted to the Avian Diseases Section, Division of Pathology for disease investigation. The clinical signs, among the pigeons under captivity, were inappetence, abnormal respiratory rales, and watery droppings. The peafowl carcass exhibited marked debility with soiled vent, and the vulture carcasses were very weak and emaciated.

2.2. Necropsy Examination

The carcasses were grossly evaluated for physical status, external injury, and ectoparasites if any. Swabs from choana and cloaca were collected in phosphate-buffered saline to screen important avian viral pathogens, including Newcastle disease virus and Influenza-A virus. A systematic necropsy was performed for each carcass, and the lesions in each organ system were recorded. Representative tissue samples from the affected organ systems were collected and processed for laboratory investigation procedures, including lactophenol cotton blue staining, cultural isolation, and identification, as well as histopathology.

2.3. Culture, Isolation, and Identification

Sterile swabs and tissue triturates from the affected lungs of all the carcasses of pigeons, vultures, and peafowl were streaked onto Sabouraud Dextrose Agar (SDA) and incubated at 37°C. The culture plates were examined after 24-36 h for fungal growth, and the obtained fungal culture was stained with lactophenol cotton blue and examined by direct light microscopy to identify the *Aspergillus* species based on morphological features.

2.4. Histopathology

Representative tissue samples from lungs, air sacs, heart, liver, spleen, and kidneys were preserved in 10% neutral buffered formalin and processed for routine paraffin embedding. Tissue sections were cut at 5 µ thickness and stained with hematoxylin and eosin (H&E). The tissue sections were examined for histopathological tissue alterations; thereafter, the lesions were recorded and photographed.

2.5. Anatomopathological Grading

Based on gross and histopathological features described for different forms of avian aspergillosis and on the basis of anatomopathological disparity of aspergillosis recoded in different avian hosts, it was attempted to grade the lesions of aspergillosis in avian hosts. We have graded the lesions based on the parameters related to gross and histopathology which would help to differentiate mere infection, peracute, acute local, as well as acute invasive and chronic invasive or disseminated forms of aspergillosis in avian species. The anatomopathological grading proposed in this study consisted of Arabic numerical grades ranging from 0-4, and the lesions suggestive of the peracute nature of disease were graded as Grade X. The detailed parameters for different grades are presented in table 1.

Table 1. Anatomopathological grading of avian aspergillosis

Grade	Gross lesions	Microscopic lesions
Grade 0	No obvious pathological changes were observed in the trachea, bronchi, lungs, and air sacs.	Normal histoarchitecture was maintained in the trachea, bronchi, lungs, and air sacs. No fungal elements in routine histopathology or special staining
Grade 1	Focal miliary plaques or amorphous necrotic lesions involving <10% of lung portion in one or both lung lobes, Airsacs appeared normal.	Focal areas of caseous necrosis with or without intralesional fungal elements, surrounded by marked congestion of air capillaries and parabronchial vessels. Fungal elements may not be detectable by routine histopathology; however, the conidial wall can be demonstrated by special staining for melanin.
Grade 2	Multifocal raised soft to firm nodular lesions involving up to 30% of the lung parenchyma, while other portions of the lung exhibited only congestion. The clavicular, thoracic, or abdominal air sacs may become cloudy and show plaque-like lesions.	Multifocal areas of extensive tissue necrosis with intralesional colonies of fungal elements were predominantly surrounded by heterophils and fewer lymphocytes with restricted tissue invasion; nonetheless, no granulomatous reaction was usually observed. Fungal hyphae or conidiophores may be found in aerated air sacs.
Grade 3	Multifocal to coalescing pleomorphic firm to hard nodules in lungs involved up to 30%-60% of parenchyma. Plaques and firm greyish to yellowish nodules in air sacs	Multiple foci of caseation necrosis were surrounded by typical granulomatous reaction characterized by a large number of macrophages, multinucleated giant cells, and lymphocytes in lungs, air sacs, and other communicating organs or multiple areas of pyogranulomatous reaction with fungal hyphae and conidia. Tissue destruction and fungal invasion were widespread.
Grade 4	Random or diffuse pleomorphic mixed type (soft/firm/hard) of granulomatous or pyogranulomatous nodules in lungs involving up to 60%-80% of parenchyma, observed on air sacs, peritoneum, and serous membrane of other visceral organs, including blood vessels wall.	Typical mycotic granulomas with or without fibro-vascular reaction in the respiratory system, as well as other organs. The fungal hyphae may be found invading the blood vessels wall and into the lumen (angioinvasive). There may be infection and inflammation on distant non-communicating organs.
Grade X	Peracute form: severely congested and edematous lungs, No distinct nodules	Lung parenchyma revealed diffuse congestion of blood vessels, leaky or collapsed air capillaries, and exuberant invasion by fungal hyphae and conidia. No granulomatous lesions were detected in the lungs.

3. Results

3.1. Culture, Isolation, and Identification

Microscopic examination of lactophenol blue-stained smears prepared from cultures isolates revealed uniseriate and columnar conidial head with chains of globose to subglobose conidia. The conidiophores were short, smooth, and terminating in flask-shaped vesicles, with a single row of phialides characteristic of *Aspergillus fumigatus*.

3.2. Necropsy and Histopathological Findings

The pigeon carcasses were presented with good body conditions and displayed no evidence of ectoparasites, traumatic injury, or any debilitating conditions. On necropsy, the lung parenchyma revealed a large necrotic mass in the ventral surface of the left lung (Figure 1A), which was found extended to the dorsal surface as a circular brownish mass with hyperemic borders (Figure 1B). Histopathological examination of the lung sections from pigeons revealed multifocal to focally extensive areas of tissue necrosis with colonies of fungal hyphae, conidia amidst infiltration by heterophils, and lymphocytes (Figure 2A and 2B).

Acutely branching septate fungal hyphae and conidia were found at the periphery of necrotic tissue. No granulomatous lesions were detected in any of the pigeon carcasses. The presented peafowl carcasses were very weak and debilitated. Upon gentle pressure on the nasal sinuses, catarrhal to mucopurulent exudate

was observed. Necropsy revealed diffuse congestion in one male peacock and mild consolidation of lungs in a peahen. No lesions were found in air sacs or other visceral organs. Histopathology revealed the areas of parenchymal necrosis accompanied by exuberant colonies of fungal hyphae, conidiophores, conidia amidst diffuse heterophilic, and lymphocytic infiltration (Figure 2C and 2D). No distinct granulomatous reaction was observed, and the Griffon carcasses were severely emaciated.

On necropsy examination, we observed variably thickened and rough air sacs with varying sizes of greyish to yellowish hard nodules (Figure 1C). The clavicular, thoracic, and abdominal air sacs were consistently involved in both vulture carcasses. The lungs were severely consolidated, and the parenchyma had firm to hard discrete nodules which were distributed equally in both lungs (Figure 1D). The epicardium and serosa of major blood vessels also demonstrated similar nodules (Figure 1E). The spleen was markedly enlarged and displayed greyish nodules on the surface (Figure 1F). Aspergillus nodules were found embedded in other abdominal visceral organs as well.

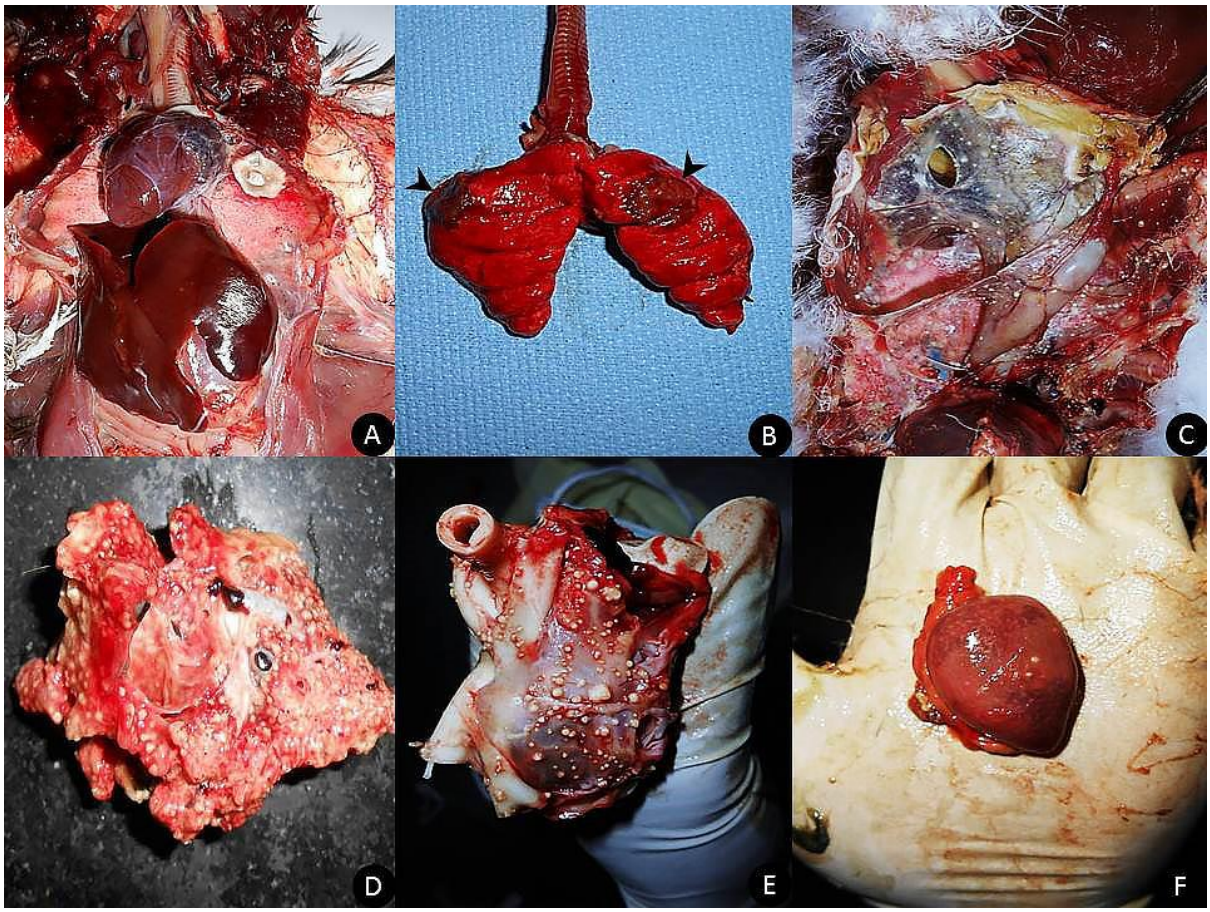

Figure 1. (1A): Pigeon lung– a large white necrotic mass in the cranioventral surface. (1B): Peafowl lung–diffuse congestion and large circular soft necrotic masses with elevated hyperemic edges. (1C): Thoraco-abdominal air sacs from a vulture–discrete greyish pleomorphic miliary nodules. (1D): Lung of a Griffon vulture–multiple greyish hard nodules deeply embedded in the parenchyma. (1E): Heart of a Griffon vulture–pleomorphic nodules in the epicardium and serosa of major blood vessels. (1F): Spleen from a Griffon Vulture–discrete nodules embedded in the spleen.

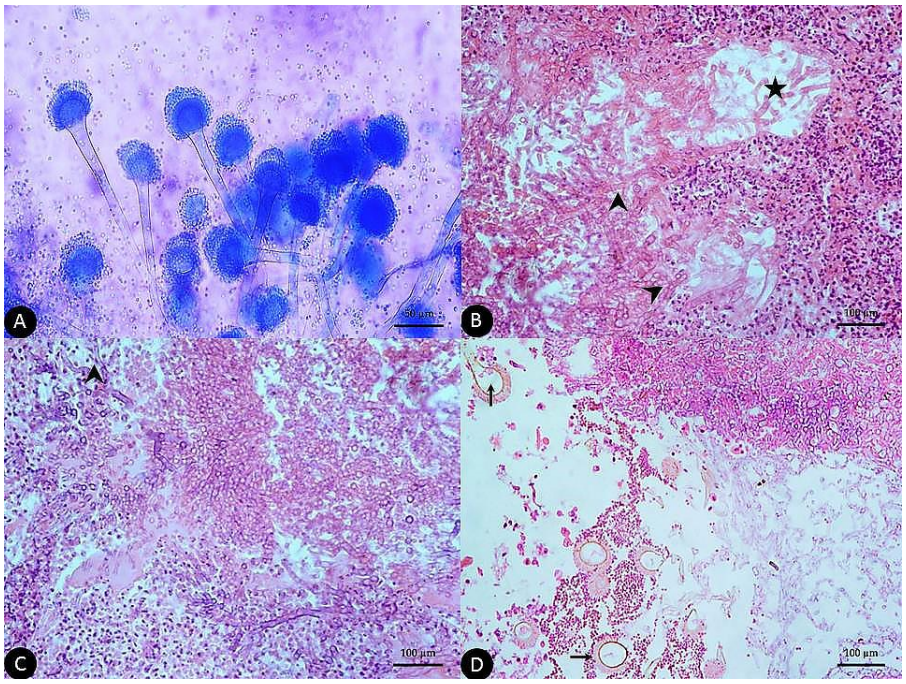

Figure 2. (2A): Smear from culture–uniseriate hyphae with short and smooth conidiophores terminating in flask-shaped vesicles, with a single row of phialides (Lactophenol Cotton Blue X 400). (2B): Pigeon lung–colonization of fungal hyphae and conidia in the parenchyma, along with severe heterophilic infiltration (H&E X 200). (2C): Peafowl lung–severe necrosis, extensive tissue invasion by fungal hyphae and conidia amidst heterophilic and lymphocytic infiltration (H&E X 200). (2D): Peafowl lung–fungal hyphae, conidiophores with flask-shaped vesicles harboring a single row of phialides (H&E X 200). Note: No granulomatous reaction was observed in any of the tissue sections obtained from pigeons and peafowls.

Histopathological examination of lungs and air sacs revealed typical mycotic granulomas (Figure 3A and 3B). They were characterized by the central area of caseous necrosis surrounded by epithelioid cells and multinucleated foreign body giant cells with palisaded nuclei, followed by lymphocytes and plasma cells (Figure 3C and 3D). The fungal hyphae were found invading deep into the lung parenchyma with intense tissue destruction and inflammatory reaction predominated by heterophils and lymphocytes. Many blood vessels in the lungs and air sacs exhibited fungal hyphae within the vascular lumen, invading the vascular wall (Figure 4).

Few blood vessels were thrombosed with typical perivasculitis and parenchymal necrosis. The sections of air sacs revealed abnormal thickening due to excessive inflammatory exudate and increased fibroplasia. The inflammatory exudate in the air sacs consisted mainly of lymphocytes. The outer lining of air sacs exhibited exuberant growth and invasion of fungal hyphae (Figure 4). The tunica serosa and tunica mucosa major blood vessels, including the aorta and pulmonary artery, exhibited mild to moderate heterophilic serositis with several acutely branching fungal hyphae invading the muscular layer (Figure 4).

The histopathological tissue alterations, intralesional demonstration of fungal elements, isolation of *Aspergillus* in pure culture from the infected organs, and identification by its characteristic growth and microscopic features are considered sufficient for the diagnosis of aspergillosis. Therefore, we did not attempt molecular confirmation, although a polymerase chain reaction targeting ITS1-5.8S-ITS2 DNA is routinely used to determine the species identity.

3.3. Anatomopathological Grading

Considering the grading criteria based on gross and histopathological findings, the lesions in acute invasive pulmonary aspergillosis (AIPA) in *Columba livia* and *Pavo* cristatus, as well as chronic invasive pulmonary aspergillosis (CIPA) in *Gyps fulvus*, were graded as Grade 2, Grade X, and Grade IV, respectively.

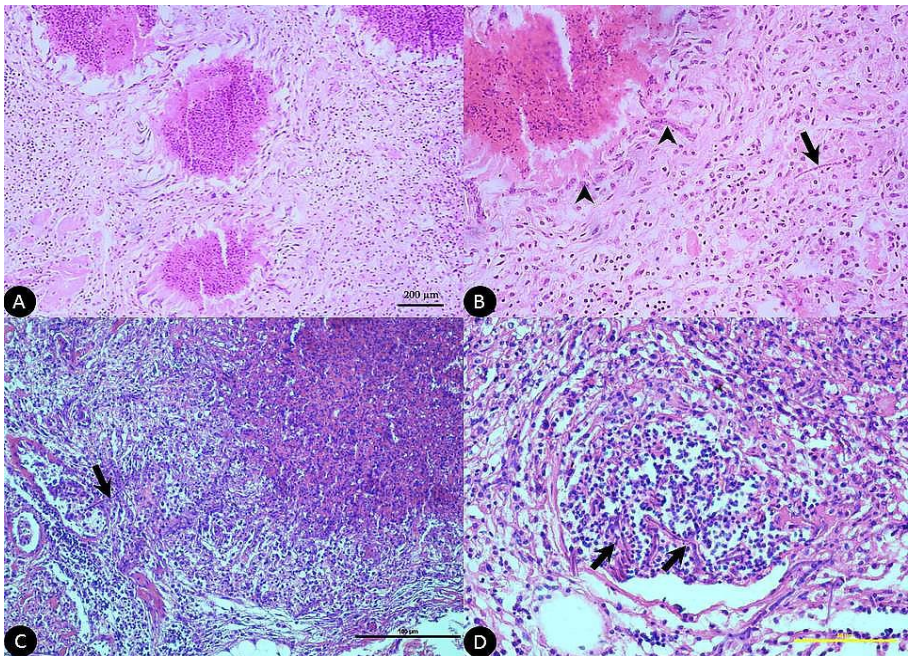

Figure 3. Lung section from a vulture. (3A): – multifocal granulomas with central caseous necrosis amidst infiltration by mononuclear cells (H&E X 100). (3B) Higher magnification of (3A): caseous necrosis surrounded by epithelioid cells, multinucleated foreign body giant cells (arrowheads), fibroblasts, and lymphoid cells with an invading fungal hypha (bold arrow) (H&E X 400). (3C): Extensive tissue necrosis surrounded by heterophilic and lymphocytic infiltration, and the invasion of fungal hyphae in the tissue parenchyma and also into the blood vessels (bold arrow). (3D): A blood vessel within the lung–numerous fungal hyphae amidst lysed erythrocytes and few lymphocytes (bold arrow) (H&E X 400).

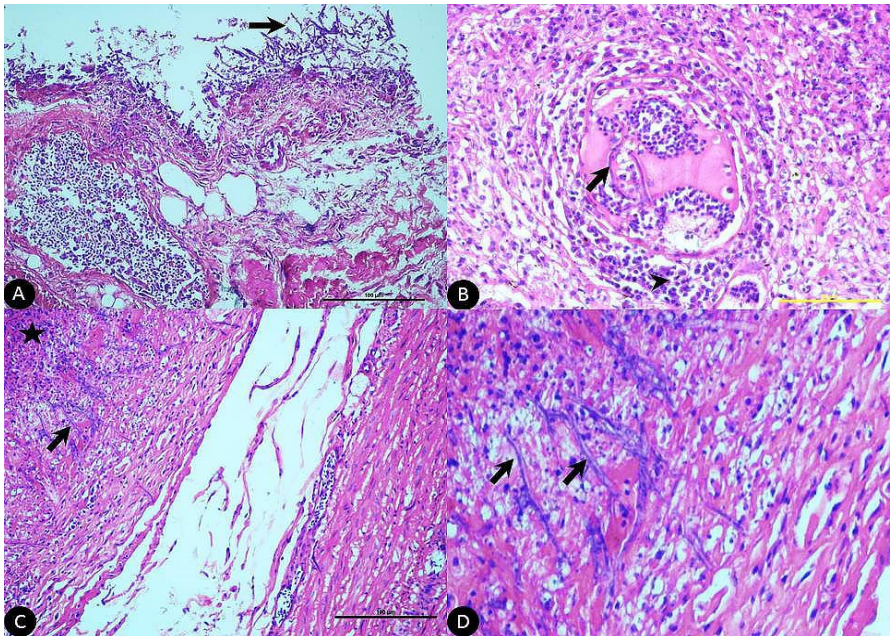

Figure 4 (4A): – section of abdominal air sac shows abnormal thickening, heterophilic infiltration amidst exuberant growth of fungal hyphae on the mesothelial lining (H&E X 100). (4B) Higher magnification of (4A): exuberant growth of fungal hyphae (bold arrow) (H&E X 400). (Figure 4c): a blood vessel within the air sac showing the invasion of fungal hyphae and lymphocytic infiltration (bold arrow) (H&E X 400). Extensive tissue necrosis surrounded by heterophilic and lymphocytic infiltration, and invasion of fungal hyphae in the tissue parenchyma and also into the blood vessels (bold arrow). (4D): Aortic wall illustrating the invasion of fungal hyphae on tunica serosa, as well as into the tunica media (bold arrow) (H&E X 400).

4. Discussion

Pulmonary aspergillosis denotes a spectrum of diseases caused by infection with a fungus of the *Aspergillus* species. Pulmonary aspergillosis includes invasive aspergillosis that occurs from angioinvasion of fungal elements, simple aspergilloma that results from mere colonization of pulmonary cavities, and chronic cavitary pulmonary aspergillosis resulting from the germination of fungi and activation of the host immune system (3, 6, 7, 34). The importance of aspergillosis in humans and different animal species has increased in the recent past (1-4, 6, 7), and pulmonary aspergillosis has remained as a continuous spectrum of disease, with the evolution of disease occurring in association with alterations in the host immune response (11, 35, 36).

These fungi, particularly the *A. fumigatus*, tend to produce several toxins and enzymes which help in the degradation of complex macromolecules to provide nutrients for the germination of fungi and also play a critical role in the pathogenesis of the disease (3, 11, 35, 37-39). The most important toxin is gliotoxin which is reported to be a potent inhibitor of the host mucociliary clearance and induces T cell cytotoxicity, which further suppresses the host immune system. The other fungal metabolites, such as 'conidial inhibitory factor' and 'complement inhibitory factor', can interfere with opsonization and phagocytosis, resulting in substantial immunosuppression, cytotoxicity, and tissue destruction by necrosis (1, 29, 40).

The disease has been detected in a wide variety of domesticated and captive wild birds (4, 8, 33, 41, 42); nonetheless, only a few reports are available on spontaneously occurring aspergillosis in pigeons, peafowl, and vultures across the globe. Although hundreds of *Aspergillus* species have been recognized, *A. fumigatus* is by far the most common pathogenic species in humans and animals due to the easy dispersion of its small-sized and hydrophobic spores or conidia (43). Infection by *Aspergillus* species has been reported in almost all domesticated avian species and production types, including pullets in cages, layer cockerels, broilers, and broiler breeders, growers of chicken or turkey poults, goslings, common duck breeders, greater rheas, ostriches, Japanese quails, and pigeons (8, 15). Respiratory signs reported in avian pulmonary aspergillosis include dyspnoea, gasping, hyperpnoea with panting, nonproductive coughing, wheezing, cyanosis, and sometimes nasal discharge (4, 31, 44-47).

In the present investigation, the pigeon flock held in captivity exhibited dyspnoea, moist rales, and wheezing, while peafowl showed exudation of catarrhal discharges from nares; however, respiratory signs could not be established for vultures. The disease in avian species can be diagnosed based on postmortem findings of white caseous nodules in the lungs or air sacs of the affected birds (26, 32, 37, 48). Nonetheless, culture characteristics, microscopy, and demonstration of a fungal element in tissues by histopathology or immunohistochemistry are standard methods for accurate diagnosis (32, 49, 50).

The severity of initial infection or the dose of inhaled conidia or spores, the degree of disease development, as well as the intensity of host and pathogen interaction, determine both morphology and extend of macroscopic lesions. Typically, the gross lesions consist of white to yellowish nodules ranging from miliary (<1mm in diameter) to large roughly spherical nodules (>2cm) on serosae and in the parenchyma of one or multiple organs. Focal or multifocal necrotic areas are visible on the cut surfaces of affected organs. The primary location of lesions in the air sacs and lungs, although other organs, such as the esophagus, proventriculus, gizzard, small intestine, liver, kidney, spleen, skin, trachea, peritoneum, brain, eye, muscle, or heart, may be also involved (15).

The gross pathological finding recorded in *Gyps fulvus* was typical chronic invasive aspergillus granuloma in both cases; however, in *Columba livia*, *Pavo cristatus*, the gross lesion was suggestive of severe acute necrotizing pneumonitis. Tokarzewski, Ziółkowska (51) has reported similar gross pathology in *Aspergillus fumigatus* infection in a pigeon flock. As per our review of literature, there is no report on

aspergillosis in peafowls. Although we examined only two peafowl carcasses, the gross and histopathological lesions were consistently the same in the lungs of both peafowl carcasses, suggesting a peracute to the acute nature of the disease.

In the present study, the histopathological lesions in pigeons and peafowl were typical acute necrotizing mycotic pneumonitis involving both the lungs. Moreover, in vultures, the lesion was typical mycotic granuloma characterized by central caseous necrosis surrounded by numerous multinucleated giant cells, epithelioid cells, and lymphoid cells, as described earlier (52, 53). The presence of giant cells in aspergillosis has been reported to be highly variable (54). The lungs and air sacs of vulture carcasses revealed the presence of numerous giant cells; nonetheless, no giant cells were detected in pigeons and peafowl. In their study,Tokarzewski, Ziółkowska (51) pointed to the presence of giant cells in the chronic and disseminated form of aspergillosis in a pigeon flock in Poland; however, there was no granulomatous reaction in the lungs of pigeons and peafowls examined in this study.

Clinical or antemortem diagnosis of avian aspergillosis has remained a challenge, although few diagnostic tests, such as biochemistry, hematology, radiography, laparoscopy, or endoscopy, are employed in exotic pet birds; nonetheless, they are not feasible in poultry or other free-ranging birds (32, 55). The most commonly used diagnostic methods in veterinary practice include histopathology, immunohistochemistry using monoclonal or polyclonal antibodies, and polymerase chain reaction (PCR) targeting the amplification of specific ITS1-5.8S-ITS2 DNA of *A. fumigatus* (32, 56). Diagnosis based on anatomopathological features is very reliable but highly variable in different avian hosts and often involves multiple factors from the infected host, the ability of the fungus to sporulate, and finally, the precipitating micro-environment (52).

In the current study, we diagnosed the cases of invasive pulmonary aspergillosis (IPA) by the detection of frequently septate and acute branching fungal hyphae, as well as globose to subglobose conidia, in infected and inflamed tissues by histopathology and isolation of *A. fumigatus* in pure culture. It has been reported that the histological diagnosis of invasive aspergillosis is carried out by detecting the presence of invasion by the *Aspergillus* species based on the morphology of hyphae which are 2-4 mm wide, frequently septate, and acutely branch at 45°. Definitive diagnosis requires a culture of the specimen positive for Aspergillus (57, 58); however, birds can host Aspergillus conidia in their respiratory system without any clinical symptoms or macroscopic lesions, leading to a dormant infection (52). We detected highly invasive fungal elements at intralesional sites, as well as in the vicinity of lesions, including angioinvasion in different histological sections and isolated Aspergillus in pure culture from the infected tissues, which are considered sufficient for pathological investigation and confirmation.

In recent years, we have recorded cases of aspergillosis in domestic birds, including poultry, Japanese quails, emu, and turkeys (unpublished), as well as a few wild birds, such as vultures, pigeons, and peafowls (25, 53). Although the fungal species of *A. fumigatus* was consistently isolated from all the birds, the anatomopathological features were distinct among different kinds of the avian host. Therefore, we attempted to analyze the anatomical disparity and grade the lesions based on published literature, as well as our own findings on different birds.

This grading system would help pathologists in systematic investigation and gaining a thorough understanding of disease severity, which in turn would help to formulate suitable therapeutic interventions and control strategies at least in birds held in captivity. Although infection by *A. fumigatus* species mainly affects the respiratory system, dissemination to other

organs, such as the brain, has also been reported (54). Nonetheless, in the present study, no dissemination was observed in pigeons and peafowls; however, in vultures, dissemination of the lesions was detected in organs of thoracic and abdominal cavities.

The clinicopathologic of aspergillosis has been linked to poor weather conditions and climate, particularly high air moisture and very low temperature in winter, as well as high temperature in spring (28). The reported cases of aspergillosis in pigeons, vultures, and peafowls all were recorded during the winter months when the environmental temperature ranges between 4°C and 24°C. The optimum temperature required by the *A. fumigatus* for its growth and metabolism is reported to be about 37°C; however, this fungus can survive in a wide range of temperatures from 12°C to 65°C (43). The normal body temperature of these birds, vultures, peafowls, and pigeons, usually ranges between 40°C and 42°C. In this regard, a slight reduction in the internal core body temperature at the peak of winter, along with the high air moisture, might have provided optimum conditions for the proliferation of *A. fumigatus* and establishment of disease in the succumbed birds.

5. Conclusion

The diagnosis of aspergillosis based on clinical manifestation and simple postmortem examination is imprecise. Moreover, the application of molecular tools and immunohistochemistry in routine disease investigation is costly in many laboratories. Systematic necropsy accompanied by isolation and identification of Aspergillus in pure culture, as well as the demonstration of fungal elements within the lesions by histopathology and histochemistry, are reliable and inexpensive methods in this regard. Mycotic pneumonia caused by *Aspergillus* species is a leading cause of morbidity and mortality in captive and free-ranging birds; however, the magnitude of the disease varies among different avian hosts.

Anatomopathological grading of lesions would help to evaluate the severity and forms of aspergillosis in different avian hosts. Future perspectives may be directed towards understanding the host-pathogen interaction at a molecular level by studying host cell responses to Aspergillus cytotoxins using different avian host models. Molecular pathogenesis mechanisms, including the role of different cytokines, such as Tumor Necrosis Factor–α (TNF-α), Interferon–γ (IFN γ), and Transforming Growth Factor-β (TGF-β), might be elucidated by real-time expression to understand the factors that exacerbate the magnitude of infection at a cellular level in different avian hosts.

Authors' Contribution

Study concept and design: A. K. M., P. M. and K. D.
Acquisition of data: A. K. M., P. M. and K. D.
Analysis and interpretation of data: A. K. M., P. M., K. D. and S. K. L.
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Ethics

All experimental procedures were performed according to the , ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India ethics committee.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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