Original Research Article

Pathological Investigation of Fowl Adenovirus Infections Associated with Respiratory Diseases in Broilers

ABSTRACT

In the present study, broiler mortalities with respiratory symptoms from 12 farms in the Indore region were examined, with a focus on postmortem investigations to detect Fowl Adenovirus (FAdV) infections. Clinically affected broilers exhibited signs of lethargy, huddling, facial oedema, reduced feed and water intake, and severe respiratory distress, including laboured breathing and nasal discharge. Postmortem findings revealed gross lesions in the trachea, lungs, and liver. The gross lesions ranged from mild congestion to severe haemorrhages. Microscopically, deciliation and basophilic intranuclear viral inclusions were observed. Pulmonary lesions included focal emphysema, haemorrhages, and red hepatization, while hepatic lesions showed enlargement, congestion, and necrosis. Microscopically, intranuclear viral inclusions were evident in both the lungs and liver, further indicating adenoviral replication in these organs. Based on symptoms, gross lesions and microscopic lesions with the presence of intranuclear basophilic inclusion bodies in respiratory epithelium, it is concluded that Fowl adenovirus is involved in the respiratory diseases of broilers in Indore and nearby areas. Fowl adenovirus infection in birds causes mild to severe respiratory distress resulting in low to high mortality.

Key Words: *FADv, Fowl adenovirus, Respiratory disease*

1. INTRODUCTION

Poultry holds a special place in the animal husbandry sector. To combat hunger and malnutrition, poultry serves as an effective converter of carbohydrates into less expensive but higher-quality animal protein through meat and eggs. To maximize output in limited space, intensive chicken production is highly encouraged in the current industrial environment. Among the fastest-growing agricultural industries in India is the poultry sector. India ranks third globally in egg production and sixth in poultry meat production. Its transformation over just 40 years from a small backyard activity to a prominent commercial sector is a significant achievement.

Despite the rapid development of the poultry industry, periodic outbreaks of diseases continue to hinder its progress. Viral diseases, in particular, have assumed threatening proportions, and among these, avian adenoviruses form a highly diverse group that causes diseases in both domestic and wild birds. Adenovirus infections are caused by non-enveloped, icosahedral viruses belonging to the genus *Aviadenovirus* in the family *Adenoviridae*, with linear double-stranded DNA ranging in size from 25 to 46 kbp. Adenoviruses isolated from poultry are classified into five *Fowl adenovirus* (FAdV) species, FAdV-A to FAdV-E, comprising 12 serotypes: FAdV-1 to 8a, and FAdV-8b to 11. Adenovirus strains are distributed worldwide, with different types reported in various geographic regions (Lebdah et al., 2022). Fowl adenoviruses are commonly found in healthy birds but can also cause a range of diseases, with pathogenicity varying from 10% to 90%, depending on the virulence of the strain, as demonstrated in poultry flocks worldwide (Schachner et al., 2018). Fowl adenovirus infection is considered an emerging disease, especially in regions where broilers are reared under intensive conditions (Asrani et al., 1997). The first isolation of an avian adenovirus from diseased birds was reported during an outbreak of respiratory disease in bobwhite quail (Bouquet et al., 1982).

Therefore, it becomes necessary to continuously monitor Fowl adenoviruses (FAdVs) linked to respiratory issues in chickens, due to the limited information available on the circulating FAdV strains associated with respiratory diseases. In light of this, the present study was undertaken with the objective of studying the pathology of Fowl adenovirus infections associated with respiratory diseases in broilers in and around Indore.

2. MATERIALS AND METHODS

2.1 Location and Place of Work

The research work was conducted at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow (M.P.). The research work was carried out from August 2023 – July 2024.

2.2 Sample Collection

Mortalities from 12 commercial farms in and around Indore (M.P.) were submitted to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow for Gross post-mortem examination. Affected birds ranged from 3 to 6 weeks of age. Data on flock size, bird age and total mortality were recorded. After recording the gross lesions, tissues from different organs—such as the lungs, trachea, and liver—were collected in 10% neutral buffered formalin and processed using routine dehydration and paraffin embedding techniques.

2.3 Histopathology

Different tissue samples were collected in 10% neutral buffered formalin (NBF) and kept for 24–48 hours for fixation. After fixation in 10% NBF, the tissue samples were cut into 2 to 3 mm thick pieces and washed overnight under running tap water. The tissues were then processed by the routine method of dehydration in graded alcohol, cleared in xylene, and embedded in paraffin wax. To remove water, the

tissues were dehydrated in a graded series of acetone: 50%, 70%, 90%, and 100%—for 30 minutes in each concentration. Clearing was done with two changes of benzene for 1 hour each. The tissues were embedded in paraffin wax through four changes of 1 hour each, and wax blocks were moulded using L-molds. Tissue sections were cut at a thickness of 4–5 microns using a semiautomatic microtome and mounted on slides precoated with egg albumin-glycerol mixture (in a 1:1 ratio) (Lillie and Fullmer, 1976).

2.4 Staining procedure

Before staining, the slides were slightly warmed in a hot air oven and placed in a jar containing xylene, which was replaced with fresh xylene after 10–15 minutes to remove paraffin from the tissue sections. After deparaffinization, the slides were passed through a descending series of alcohol: first through two changes of absolute alcohol, followed by 90%, 70%, and 50% alcohol for 2 minutes in each concentration.

Following this, the slides were washed under running tap water. They were stained with haematoxylin for 10–15 minutes, with gentle shaking 2–3 times to ensure even staining. The tissue sections were given 3–4 dips in ammonia water, washed again in running tap water, and then counterstained with 2% aqueous eosin for 2–5 minutes. After eosin staining, the slides were dehydrated through an ascending series of concentrated ethanol, cleared in xylene, and mounted with a coverslip using DPX. Finally, the slides were cleaned, labelled, and examined for microscopic lesions (Lillie and Fullmer, 1976).

3. RESULTS AND DISCUSSION

Affected broilers typically appeared depressed and huddled together, with ruffled feathers and drooping wings. The birds showed reluctance to move, reduced feed and water intake, uneven growth, and occasional facial edema. Most of them exhibited watery white diarrhoea. Severe respiratory distress was prominent, characterized by rapid open-mouth breathing, audible wheezing, and copious mucopurulent nasal discharge. These clinical signs are consistent with previously reported descriptions of IBH outbreaks and respiratory FAdV infections. The clinical signs and symptoms recorded in the present study were similar to the findings of Zadravec *et al.* (2011); Gowthaman *et al.* (2012); Sun *et al.* (2019); Bisht *et al.* (2019); El-Deeb and Mandour, (2019); Levkutova *et al.* (2023) and Hussein *et al.* (2023).

Commented [u1]: These signs are also similar to Newcastle disease in birds. A rapid diagnostic test kit for NCDv (NDV) could also have been done to confirm mixed infections.

Gross pathological examination of the trachea revealed mild to severe congestion of the tracheal rings. In several cases, the tracheal lumen contained a pale, moist, mucopurulent exudate, which was occasionally hemorrhagic in nature. Microscopically, mild cases exhibited an intact columnar epithelium with submucosal congestion and mild infiltration of inflammatory cells. In more severe cases, the tracheal tissue showed extensive hemorrhages in the mucosa, karyomegaly, deciliation of the columnar epithelium, degenerative changes, submucosal edema and hemorrhages, along with infiltration of mononuclear cells (Fig 1).

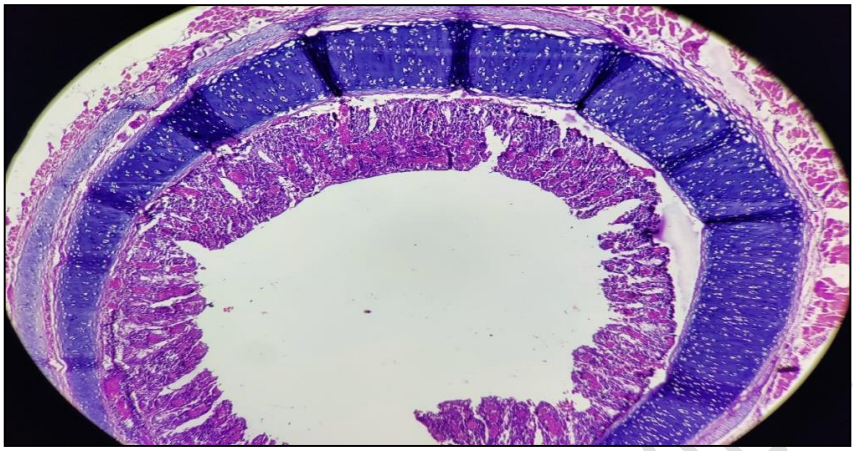

Fig 1: Microphotograph of trachea showing haemorrhages in mucosa, deciliation of columnar epithelium with degenerative changes, oedema, submucosal haemorrhages and infiltration of mononuclear cells in a FAdV positive 37 days old broiler (H & E 10x)

Microscopic lesions were further characterized by epithelial destruction, prominent degenerative changes, mucosal hemorrhages, and infiltration by a mixed population of inflammatory cells. Notably, basophilic intranuclear inclusion bodies were observed in both intact and sloughed tracheal epithelial cells, occupying most of the nuclear space (Fig 2). These inclusions are indicative of adenoviral replication.

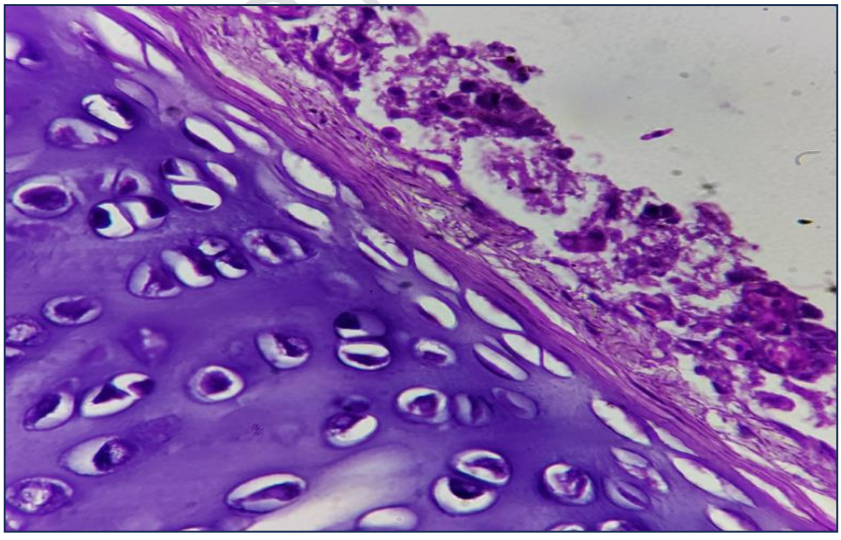

Fig 2: Microphotograph of trachea showing basophilic intranuclear inclusion bodies in the tracheal epithelial cells (H & E 100x) (arrows)

The gross lesions observed in this study are consistent with those previously reported by Meulemans et al. (2001) and in the experimental findings of Dhillon and Kibenge (1987). Similarly, the microscopic features align with the descriptions provided by De Herdt et al. (2013). The detection of characteristic basophilic intranuclear inclusion bodies in the tracheal epithelium, suggesting active replication of Fowl adenovirus, corroborates the findings of Zadravec et al. (2011). However, these observations contrast with those of Bisht et al. (2019), who reported eosinophilic intranuclear inclusion bodies in the laryngeal and tracheal epithelium.

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Gross examination of the lungs revealed rounding of the lobar borders and marked congestion, along with focal areas of emphysema (Fig 3). Microscopically, lungs from mildly affected birds displayed congestion and hemorrhages primarily in the parabronchial regions. In more severe cases, the lungs exhibited multifocal areas of extensive hemorrhage, with alveolar spaces engorged with red blood cells, indicative of red hepatization (Fig 4). Additionally, sections revealed coagulative necrosis of the lung parenchyma (Fig 5). Basophilic intranuclear inclusion bodies were frequently observed within the alveolar epithelial cells (Fig 6), signifying active viral replication.

Fig 3: Photograph of congested lungs showing focal areas of emphysema in FAdV positive broiler

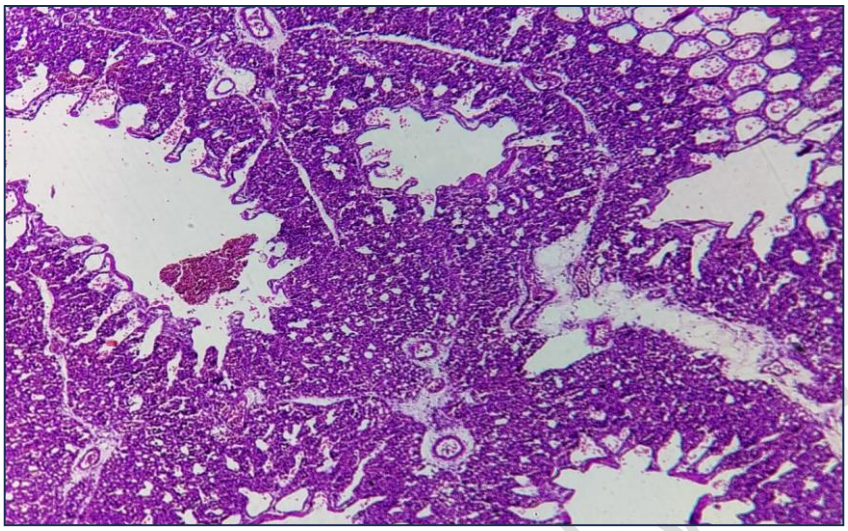

Fig 4: Microphotograph of lungs showing multifocal areas of severe haemorrhages, alveoli filled with RBCs indicating red hepatization of lungs and haemorrhages in the parabronchial lumen (H & E 10x)

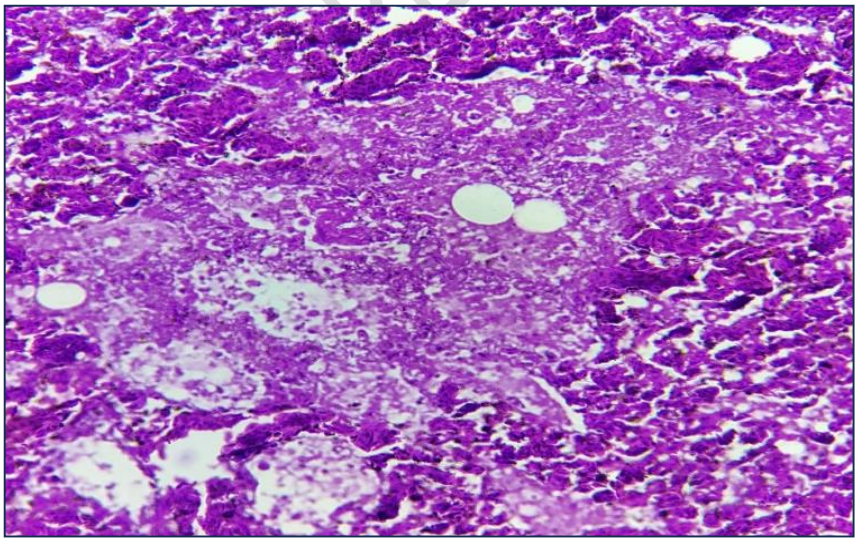

Fig 5: Microphotograph of lungs showing coagulative necrosis in the lung parenchyma (H & E 40x)

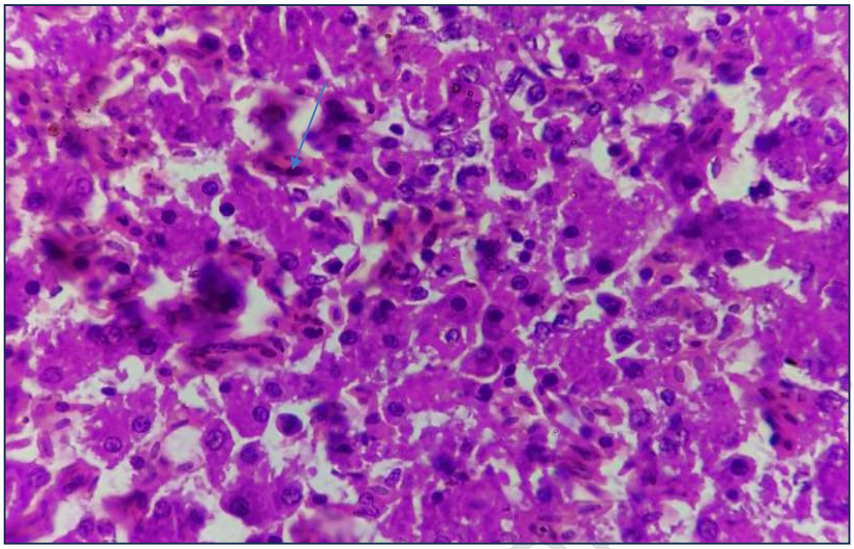

Fig 6: Microphotograph of lungs showing basophilic intranuclear inclusion bodies in alveolar epithelium (H & E 100x) (arrows)

The gross and histopathological pulmonary lesions documented in the present study are in agreement with the findings of Radwan et al. (2019) and Lebdah et al. (2022), further supporting the pathogenic role of Fowl adenovirus in inducing severe respiratory pathology in broilers.

Gross lesions observed in the liver of broilers affected by FAdV included enlarged, swollen, pale and congested liver with multiple haemorrhagic areas (Fig 7 and 8). Microscopically, the liver showed focal necrotic areas surrounded by mononuclear cell infiltration and fatty change represented by large and small vacuoles. Other lesions recorded were multiple areas of haemorrhages, oedema and mild infiltration. Degenerative changes varied and included fatty change (Fig 9). Numerous hepatocytes displayed characteristic basophilic intranuclear inclusion bodies (Fig 10).

Fig 7: Broilers showing congested and swollen liver

Fig 8: 37 days old broiler showing pale and swollen liver with multiple haemorrhages

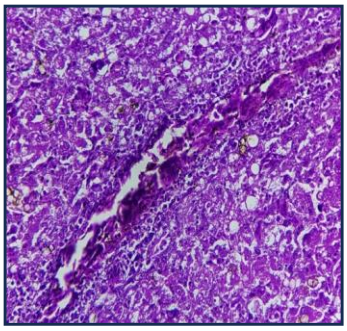

Fig 9: Microphotograph of liver showing fatty change and infiltration of mononuclear cells (H & E 40x)

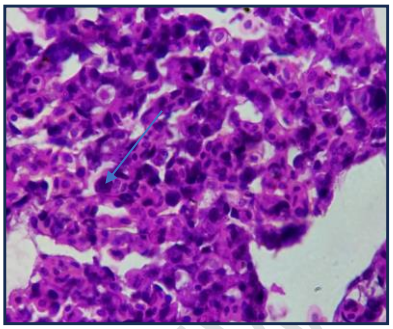

Fig 10: Microphotograph of liver showing basophilic intranuclear inclusion bodies in hepatocytes of 37 days old broiler affected with FAdV (H & E 100x) (arrows)

Gross lesions recorded in the present study were similar to findings recorded in the research done by De Herdt *et al.* (2013); Mariappan *et al.* (2018); El-Deeb and Mandour, (2019); Chitradevi *et al.* (2021) and Sultan *et al.* (2021). Microscopic lesions in the present study were consistent with the findings of De Herdt *et al.* (2013); Shrivastava *et al.* (2019); El-Deeb and Mandour, (2019); Cizmecigil *et al.* (2020); Safwat *et al.* (2022); and Helmy *et al.* (2024). In the present study, characteristic basophilic intranuclear inclusion bodies were observed, contrasting with the findings of Chitradevi *et al.* (2021), who reported eosinophilic intranuclear inclusion bodies. The presence of the intranuclear inclusion bodies in the trachea, lungs and liver, is clear evidence of the replication of adenovirus in these sites (Cook, 1983). No gross lesions were seen in other organs, except for occasional mild congestion.

4. Conclusion

The present study provides strong evidence implicating Fowl adenovirus (FAdV) as a significant etiological agent in the respiratory diseases affecting broiler chickens in Indore and surrounding regions. This conclusion is supported by the clinical signs observed in affected birds, including respiratory distress, reduced feed intake, and depression, along with characteristic gross lesions such as congestion and mucoid exudates in the trachea and lungs. Histopathological examination revealed consistent microscopic changes, including epithelial degeneration, hemorrhages, and most notably, the presence of prominent intranuclear basophilic inclusion bodies in the respiratory epithelium—hallmark features of adenoviral infection. The findings of this study underscore the role of FAdV in inducing a spectrum of respiratory pathology ranging from mild to severe, often leading to varying levels of morbidity and mortality in commercial broiler flocks. Given the economic implications of such infections, early diagnosis, regular surveillance, and appropriate biosecurity measures are essential for effective disease control. Future studies focusing on molecular characterization of circulating

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FAdV strains and their pathogenic potential would further contribute to developing targeted vaccines and management strategies.

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