PATHOLOGY OF AVIAN METAPNEUMOVIRUS AND ASSOCIATED PATHOGENS IN CHICKENS

T H E S I S

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In partial fulfillment of requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

VETERINARY PATHOLOGY

BY

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TABLE OF CONTENTS

CHAPTER		PAGE
I)	INTRODUCTION … … … … …	**1-4**
II)	REVIEW OF LITERATURE … … …	**5-36**
III)	MATERIALS AND METHODS … … …	**37-52**
IV)	RESULTS AND DISCUSSION … … …	**53-68**
V)	SUMMARY AND CONCLUSIONS … … …	**69-72**
A)	BIBLIOGRAPHY … … … … …	**I-XVIII**
B)	VITA … … … … … … …	**XIX**
C)	THESIS ABSTRACT … … … …	

LIST OF TABLES

TABLE NO.	PARTICULARS	PAGE NO.
3.1	Equipment and instruments used in experiments	**38**
3.2	avian Metapneumovirus oligonucleotide primers used in this study	**40**
3.3	List of primers used for amplification of different genes of associated viral and bacterial pathogens.	**41**
3.4	Details of the samples collected from different poultry farms in Akola district.	**43-44**
3.5	Component of RNA and Primer mixture	**46**
3.6	Component of Reverse transcription mixture	**46**
3.7	Composition of the PCR reaction mixture	**48**
3.8	Thermal cycling conditions for aMPV	**48**
3.9	Composition of the PCR reaction mixture	**49**
3.10	Thermal cycling conditions for associated viral pathogens	**49**
3.11	Thermal cycling conditions for associated bacterial pathogens	**50**
4.1	Incidence of aMPV and associated pathogens with concurrent infection	**57**

LIST OF PLATES

PLATE NO.	PARTICULARS	AFTER PAGE
4.1	Agarose gel electrophoresis stained with ethidium bromide showing lane 1, 2, 3 and 4 positive sample for aMPV (115 bp, *N gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.2	Agarose gel electrophoresis stained with ethidium bromide showing lane 1, 2, 3, and 4 positive sample for MG (300bp, *Mgc2gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.3	Agarose gel electrophoresis stained with ethidium bromide showing lane 1 and 2 positive sample for ORT (784bp, *16S rRNA*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.4	Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for IC (500bp, *HPG-2 gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.5	Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for IBV (402bp, *N gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.6	Agarose gel electrophoresis stained with ethidium bromide showing lane 1 and 2 positive sample for APEC (500bp, *ecp gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.7	Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for ILTV (588bp, *p32gene*) along with lane P- positive control, N-negative control and L-ladder 100bp	**56**
4.8	Chicken showing whitish purulent nasal discharge upon pressing the nostrils	**60**
4.9	Cross section of nasal cavity showing marked congestion and edema	**60**
4.10	Bird showing severe conjunctivitis along with erythema	**60**
4.11	Bird showing presence of yellowish cheesy caseous exudate in the tracheal lumen	**60**

PLATE NO.	PARTICULARS	AFTER PAGE
4.12	Lung showing dark reddish consolidation,congestion and edema with focal areas of hemorrhages	**60**
4.13	Presence of pale yellowish fibrinous covering over heart and liver indicating fibrinous perihepatitis and pericarditis along with airsacculitis	**60**
4.14	Microphotograph of the middle nasal chamber showing fibrino-necrotic exudate filling the lumen, along with disruption of the respiratory epithelium and infiltration of mononuclear cells in the lamina propria and submucosa (H&E, 100x)	**60**
4.15	Microphotograph of the trachea showing mucosal epithelial necrosis and sloughing, loss of mucous glands, and prominent submucosal edema (H&E, 400x)	**60**
4.16	Microphotograph of the lung showing marked infiltration of inflammatory cells, perivascular edema, and vascular thrombi (H&E, 100x)	**60**
4.17	Microphotograph of the lung showing fibrinocellular exudate filling the parabronchial lumen, along with marked capillary congestion (H&E, 100x)	**60**
4.18	Microphotograph of heart showing focal necrosis and leukocytic infiltration in between the muscle fibers (H&E,100x)	**60**
4.19	Microphotograph of spleen showing congestion, depletion of lymphocytes and multifocal necrosis (H&E,100x)	**60**
4.20	Bird showing marked periorbital swelling with presence of cheesy exudates	**62**
4.21	Cross section of nasal cavity showing marked congested and cheesy flakes in the lumen	**62**
4.22	Trachea showing mucoid exudates in the lumen and congestion of tracheal mucosa	**62**
4.23	Lung showing fibrinous airsacculitis with multiple areas of consolidation and severe congestion	**62**

PLATE NO.	PARTICULARS	AFTER PAGE
4.24	Microphotograph of the nasal turbinate demonstrating epithelial necrosis, marked vascular congestion, and a dense mononuclear cells infiltrate in the lamina propria, consistent with chronic lymphoplasmacytic rhinitis (H&E, 100x)	**62**
4.25	Microphotograph of the trachea showing marked necrosis with submucosal edema and inflammatory cells infiltration (H&E, 100x)	**62**
4.26	Microphotograph of the lung showing lymphoid hyperplasia in the peribronchiolar area (H&E, 100x)	**62**
4.27	Microphotograph of lung showing fibrinocellular exudate in parabronchial lumen with mononuclear cells infiltration and fibrous connective tissue proliferation (H&E, 100x)	**62**
4.28	Microphotograph of lung showing parabronchiolar necrosis and mononuclear cells infiltration in the lamina propria and peribronchiolar area (H&E, 100x)	**62**
4.29	Microphotograph of liver showing marked periportal mononuclear cell infiltration and areas of focal necrosis (H&E, 100x)	**62**
4.30	Chicken showing severe unilateral periocular swelling with marked erythema and severe conjunctivitis	**62**
4.31	Trachea showing fibrinous exudates in the lumen with mild congestion of mucosa	**62**
4.32	Lung with marked congestion, edema and focal areas of consolidation	**62**
4.33	Enlarged and congested spleen with mottled appearance	**62**
4.34	Microphotograph of the nasal turbinate (concha) from the middle nasal chamber, demonstrating extensive necrosis of the epithelial cells and mucous glands, accompanied by infiltration of mononuclear cells (H&E, 100x)	**64**

PLATE NO.	PARTICULARS	AFTER PAGE
4.35	Microphotograph of the middle nasal chamber showing necrotic exudate within the lumen, accompanied by extensive necrosis and loss of mucous glands and the nasal conchal mucosa (H&E, 40x)	**64**
4.36	Microphotograph of the trachea showing necrosis of the mucosal epithelium, infiltration of mononuclear cells, and edematous changes in the lamina propria (H&E, 400x)	**64**
4.37	Microphotograph of lung showing presence of fibrino cellular exudate in the bronchiolar and parabronchial lumen predominated with mononuclear cells (H&E, 100x)	**64**
4.38	Microphotograph of the spleen showing a thickened capsule with multifocal areas of necrosis and infiltration of heterophils (H&E, 100x).	**64**
4.39	Microphotograph of the liver showing mild vascular congestion, focal areas of hepatocellular necrosis, and infiltration of heterophils and lymphocytes (H&E, 100x)	**64**
4.40	Bird showing severe focally extensive unilateral periorbital swelling, erythema and presence of cheesy exudate in the lumen.	**64**
4.41	Trachea: tracheal lumen showing mucinous exudate and mild mucosal hyperemia adjacent to the cartilaginous rings.	**64**
4.42	Lung showing severe, multifocal coalescing consolidation with firm consistency, necrotizing foci, and fibrinous exudation.	**64**
4.43	Fibrinous pericarditis: presence of yellowish fibrinous layer over pericardium.	**64**
4.44	Microphotograph of the middle nasal chamber of a chicken showing fibrinocellular exudate occluding the lumen, along with necrosis and mononuclear cell infiltration in the lamina propria (H&E, 100x)	**66**
4.45	Microphotograph of infraorbital sinus obliterated by the presence of fibrinous necrotic debris (H&E, 40x)	**66**
4.46	Microphotograph of the trachea showing extensive mucosal and submucosal necrosis with inflammatory cell infiltration (H&E, 400x)	**66**

PLATE NO.	PARTICULARS	AFTER PAGE
4.47	Microphotograph of the lung showing bronchiolar necrosis, infiltration of mononuclear cells and presence of necrotic exudate in the lumen (H&E,100x)	**66**
4.48	Microphotograph of the of the heart showing fibrinous pericarditis and venous congestion (H&E,100x)	**66**
4.49	Microphotograph of the liver showing thickened hepatic capsule due to presence fibrino-cellular exudates (H&E,400x)	**66**
4.50	Bird showing purulent exudate oozing from nostrils	**66**
4.51	Cross section of nasal cavity exhibiting caseous cheesy exudates	**66**
4.52	Trachea: tracheal lumen containing fibrinous exudates and congestion of mucosa around tracheal rings	**66**
4.53	Bird showing yellowish fibrinous layer over the surface of heart and liver and airsacculitis	**66**
4.54	Microphotograph of nasal concha from middle nasal chamber showing marked mucosal architecture effacement with luminal sloughing of necrotic cellular debris, submucosal vascular congestion, and inflammatory infiltrate (H&E, 40x)	**66**
4.55	Microphotograph of trachea showing mononuclear cell infiltration within the subepithelial tissue, accompanied by epithelial hyperplasia and degeneration, indicative of a chronic inflammatory response (H & E 400x)	**66**
4.56	Microphotograph of trachea showing disruption of ciliated epithelial cells with necrosis, mild hyperplasia and presence of necrotic exudate in the lumen (H&E 400x)	**66**
4.57	Microphotograph of the lung showing marked parabronchial necrosis and loss of pulmonary architecture due to infiltration of inflammatory exudate admixed of mononuclear cells and heterophils (H&E, 100x)	**66**
4.58	Microphotograph of the heart showing thickened pericardium due to presence of fibrinocellular exudate (H&E, 100x)	**66**

PLATE NO.	PARTICULARS	AFTER PAGE
4.59	Microphotograph of liver showing marked necrosis, inflammatory cells infiltration and haemorrhages (H&E, 100x)	**66**
4.60	Bird showing severe unilateral periocular swelling due to accumulations of fibrinous and caseous exudate within the infraorbital sinus.	**68**
4.61	Tracheal mucosa showing reddish mucoid exudates and marked congestion	**68**
4.62	Lung showing dark reddish consolidation at lateral portion of right lung lobe and congestion	**68**
4.63	Fibrinous pericarditis : Presence of pale whitish layer over the pericardium.	**68**
4.64	Microphotograph of the middle nasal chamber showing extensive destruction of the respiratory epithelium with epithelial sloughing, ciliary disruption, and marked mononuclear cell infiltration in the lamina propria. Additionally, fibrinous necrotic exudate is observed within the middle nasal canal (H&E, 100x)	**68**
4.65	Microphotograph of the nasal turbinate from middle nasal chamber showing mucous gland hyperplasia, epithelial desquamation with luminal sloughing, and fibrinosuppurative exudate within the airway lumen (H&E 100x)	**68**
4.66	Microphotograph of infraorbital sinus showing eosinophilic fibrino-necrotic material distending the lumen and mucosal necrosis, and dense mononuclear inflammatory infiltrates expanding the mucosa and submucosa, consistent with necrotizing sinusitis with abscess formation (H&E, 40x)	**68**
4.67	Microphotograph of the trachea exhibiting marked necrosis of the respiratory epithelium with sloughing of cellular debris, accompanied by submucosal vascular congestion and edema, consistent with acute necrotizing tracheitis (H&E, 100x)	**68**
4.68	Microphotograph of the lung showing marked pulmonary congestion, alveolar capillary engorgement, and infiltration of heterophils (H&E, 100x)	**68**
4.69	Microphotograph of heart showing varying degrees of mononuclear cells infiltration and degeneration of cardiac muscle fibers (H&E, 400x)	**68**

LIST OF ABBREVIATIONS

Abbreviation	Full form
µg	- Microgram
µl	- Microliter
µm	- Micrometre
^{0}C	- Degree Celsius
aMPV	- avian Metapneumovirus
NDV	- Newcastle Disease Virus
IBV	- Infectious Bronchitis Virus
ORT	- *Ornithobacterium rhinotracheale*
ILTV	- Infectious Laryngotracheitis
MG	- *Mycoplasma gallisepticum*
IC	- Infectious Coryza
APEC	- Avian Pathogenic *E. coli*
Fig	- Figure
RT-PCR	- Reverse transcription – polymerase chain reaction
DDW	- Double Distilled Water
DEPC	- Diethylpyrocarbonate
DNA	- Deoxyribonucleic acid
DPX	- Dibutylphthalate Polystyrene Xylene
EDTA	- Ethylenediamine tetra acetic acid
et al.	- Et alia (and others)
etc.	- Et cetera
gm	- Gram
hrs	- Hours
i.e.	- That is
Kb	- Kilo base
Kg	- Kilogram
Kv	- Kilovolts
mg	- Milligram
Ml	- Milli liter
Min.	- Minute
mPCR	- Multiplex PCR
MT	- Million tons
NBF	- Neutral buffered formalin
Cm	- Centimeter
PCR	- Polymerase chain reaction
Pg	- Pico gram
bp	- Base pairs

Abbreviation	Full form
Pmol	- Picomoles
rRNA	- Ribosomal Ribo nucleic acid
rpm	- Revolutions per minute
RT	- Room temperature
S/Sec	- Second
sp./ ssp	- Species
TAE	- Tris-acetate-edta
U/L	- Unit per litre
UV	- Ultra violet
V	- Volt
viz.	- Namely
W/v	- Weight per volume

Chapter - I

Introduction

CHAPTER – I
INTRODUCTION

The poultry industry is a vital component of the livestock sector, contributing significantly to farmer's economic growth and creating employment opportunities in both rural and urban areas (Vetrivel and Chandrakumarmangalam, 2013). This sector not only supports livelihoods but also strengthens the agricultural economy (Negassa *et al.*, 2014). Over the past four decades, the poultry industry has undergone substantial development, with each decade focusing on different aspects. The 1970s witnessed a surge in egg production, the 1980s experienced rapid growth in broiler production, and the 1990s saw advancements in integration, automation, and feed production. The current decade aims to capitalize on value-added products and expand international trade opportunities (Kour *et al.*, 2024). Despite the rapid expansion of India's poultry sector, infectious diseases continue to cause significant economic losses. The intensification of poultry farming has increased the prevalence and severity of respiratory diseases in commercial chicken flocks, enabling the rapid spread of multiple pathogens. These diseases result in high mortality rates, directly affecting the profitability of commercial poultry operations (Kalaria *et al.*, 2021).

A decades ago, the major diseases of poultry which were recognized, were due to bacterial, parasitic, and viral agents (Batista *et al*., 2020, Mo and Mo, 2025). In industrialized agricultural nations, the majority of bacterial and parasitic diseases effectively have been managed or even completely eradicated, while more and new viral infections have emerged (Adene, 2004). Viral diseases are less responsive to typical treatment and other management approaches. The poultry industry is currently facing challenges from respiratory viral infections, such as Avian influenza (AI), Newcastle disease (ND), Infectious bronchitis (IB), Infectious laryngotracheitis (ILT), and avian Metapneumovirus (aMPV) infection (Batista *et al*., 2020). Advancements in poultry health management technologies in developed countries have continued to benefit the poultry industry in developing countries. However, some of the so-called emerging poultry diseases are indeed exotic to

India. Findings showed that many native chickens and guinea fowls, have little or no contact with imported chickens and still show antibodies to most of the viral diseases (Batista *et al*., 2020). Therefore, a lack of facilities to monitor and control diseases is the reason why many diseases in poultry flocks go unnoticed and continue to ruin the poultry sector (Liebhart *et al*., 2023).

Avian Metapneumovirus is an emerging poultry pathogen that impacts the poultry industry globally (Jardine *et al*., 2018). It is a worldwide pathogen that affects the upper respiratory system of chickens and turkeys, resulting in clinical symptoms and significant economic losses (Catelli *et al*., 2010), especially in association with secondary bacterial infections (Munir, 2016). Clinically, the disease is thought to resemble *Bordetella avium* (Seal, 2000), However, in experimental conditions, aMPV is mostly linked to *Mycoplasma gallisepticum* and *Escherichia coli* infections (Jirjis *et al*., 2002), and field findings have also observed that other respiratory viruses such as IB virus and ND virus (Villareal *et al*., 2007) can contribute to the disease. Additionally, aMPV impacts the lower respiratory tract, lowers egg production, egg quality, and is closely associated with the clinical manifestations of IB (Nagy *et al*., 2018). When an aMPV infection suddenly develops and spreads quickly among flocks, chicken and turkey exhibit clinical symptoms that are strikingly similar to those of ND (Catelli *et al*., 2010). It was first identified in South Africa in 1978 as "Turkey rhinotracheitis virus" (TRT), and aMPV has since spread globally (Buys *et al*., 1989; Jones, 2010). By 1984, it was detected in chickens in France and the United Kingdom, where it caused "swollen head syndrome" (SHS) (Morley and Thomson, 1984).

The aMPV is presently categorized as a constituent of the *Mononagavirales* order, the *Pneumoviridae* family, and the Metapneumovirus genus (Rima *et al*., 2017). The single-strand negative sense RNA genome of aMPV is roughly 14 kilobases in size (Munir, 2016). The virus comprises eight genes encoding viral polypeptides, including two glycosylated proteins and three associated with unstructured viruses. The G gene, known for its high variability, encodes a conjugated protein that serves as a major immunogen of aMPV and is a

key target for epidemiological studies and subtype differentiation (Abd El-Ghany, 2023). Viral polypeptides include small hydrophobic protein (SH), viral RNA-dependent RNA polymerase (L), phosphoprotein (P), nucleoprotein (N), fusion protein (F), matrix protein (M), surface glycoprotein (G), and second matrix protein (M2) (Umar *et al*., 2016). The virus nucleoprotein (N) envelopes the viral genome and protects it against nuclease action. The F-protein mediates the fusion between the host cell membrane and the viral envelope, while the G protein facilitates binding to the target cell (Cecchinato *et al*., 2010; Munir, 2016). Furthermore, the primary membrane-associated structural proteins of aMPV, which are the fusion protein (F) and the glycoprotein (G), play essential roles in viral pathogenicity and immunogenicity (Yu *et al*., 2010). It mainly transmits through the aerosols from infected birds to others. However, direct contact with wild birds is also possible route of transmission (Abd El-Ghany, 2023). The aMPV infections are globally distributed in poultry-producing areas with adverse economic losses (Goraichuk *et al.,* 2024). To protect flocks from infection vaccination of aMPV has been implemented in certain parts of the world (Abd El-Ghany, 2023).

The clinical manifestations of aMPV infection in poultry include torticollis, coughing, nasal discharge, and watery eyes, which are commonly observed in broiler flocks and their breeding stock (Hartmann *et al.,* 2015). These symptoms are often accompanied by swelling of the periorbital sinuses, a hallmark of Swollen Head Syndrome (Aung *et al.,* 2008). In laying birds, the infection also leads to a decline in egg production (Sun *et al.,* 2014).

aMPV infection typically exhibits a high morbidity rate ranging from 40% to 100%, while mortality remains relatively low at 1% to 5% (Sun *et al.*, 2014). The severity of the disease is influenced by various factors, such as the age of the birds, concurrent infections, and the overall health status of the affected flock (Umar *et al.,* 2019).

Post-mortem examination of aMPV-infected birds revealed excessive exudates in the respiratory tract, accompanied by head edema, rhinitis, conjunctivitis, sinusitis, laryngitis, and tracheitis. In cases of concurrent bacterial

infection, lower respiratory conditions such as airsacculitis, pneumonia, pericarditis, and perihepatitis were also observed (Zuo *et al.*, 2018, Jirjis *et al.*, 2002). The histopathological analysis of aMPV-infected tissues showed necrosis and destruction of the upper respiratory mucosa, ciliary loss, extensive infiltration of inflammatory or lymphoid cells in the submucosa, and pronounced tracheitis (Cha *et al.*, 2007).

The severity of aMPV infections is influenced by environmental conditions and concurrent bacterial and viral infections (Abd El-Ghany, 2023). Co-infections with pathogens such as *Escherichia coli, Bordetella avium, Ornithobacterium rhinotracheale, Riemerella anatipestifer, Mycoplasma gallisepticum, Avibacterium paragallinarum, Chlamydophila psittaci*, as well as NDV, IBV, and ILTV, can significantly worsen disease outcomes (Sid *et al.*, 2015). The spread of aMPV within farms is determined by biosecurity measures, hygiene standards, flock age, breed, and population density. The virus is highly transmissible through aerosols or contact with contaminated materials (Ali *et al.*, 2019).

Given the significant impact of aMPV on poultry health and production, surveillance for aMPV and associated pathogens is essential to establish effective control measures. Notably, aMPV has not been widely reported within the poultry population in India, and thus, this study was undertaken with the following **Objectives:**

1. To study the incidence of avian Metapneumovirus and associated pathogens in chickens.
2. To study the pathology of avian Metapneumovirus and associated pathogen in chickens.

Chapter - II

Review of Literature

CHAPTER – II
REVIEW OF LITERATURE

Respiratory diseases are a major cause of high mortality rates in avian production worldwide due to their complex and multifactorial nature (Sid *et al*., 2015). Respiratory diseases have various causes, including viral pathogens (Umar *et al.*, 2016). Viruses like Infectious Laryngotracheitis (ILT), Infectious Bronchitis (IB), Avian Metapneumovirus (aMPV), Avian Influenza (AI), and Virulent Newcastle Disease Virus (vNDV) are major contributors to respiratory diseases that cause high mortality in chickens (Hicham *et al.,* 2015). Various pathogens contribute to the complexity of poultry respiratory diseases, including avian metapneumovirus, Newcastle disease virus (NDV), avian influenza virus, infectious bronchitis virus, and several *Mycoplasma* species (*Mycoplasma iowae, Mycoplasma meleagridis, Mycoplasma gallisepticum*, *Mycoplasma synoviae*). Additionally, bacterial pathogens such as *Escherichia coli, Avibacterium paragallinarum, Bordetella avium, Pasteurella multocida*, *Ornithobacterium rhinotracheale,* and *Chlamydophila psittaci* contribute to respiratory infections, leading to significant economic losses in the poultry industry (Van de Zande *et al*., 2001). Among these avian Metapneumovirus (aMPV) is a globally distributed virus that targets the upper respiratory tract of chickens and turkeys, leading to noticeable clinical signs and substantial economic losses (Catelli *et al*., 2010), particularly when linked to secondary bacterial infections (Cecchinato *et al.,* 2012). The disease is reported to have clinical similarities to *Bordetella avium* infection (Seal, 2000), However, under experimental conditions, aMPV infection is primarily linked to co-infection with Escherichia coli and *Mycoplasma gallisepticum* (Jirjis *et al.,* 2002), Field observations have also shown an association with other respiratory viruses, including infectious bronchitis virus and Newcastle disease virus (Villareal *et al.,* 2007). aMPV also impacts the lower respiratory tract, leading to decreased egg production, reduced egg quality (Nagy *et al.,* 2018).

2.1 Etiology of avian Metapneumovirus:

The aMPV is presently categorized as a constituent of the Mononagavirales order, the Pneumoviridae family, and the Metapneumovirus genus (Rima *et al.*, 2017). The family is divided into two genera: Metapneumovirus, which include human metapneumovirus [hMPV] and avian Metapneumovirus [aMPV]), and mammalian respiratory syncytial viruses (Lamb *et al.*, 2009; Amarasinghe *et al.*, 2019). aMPV is a respiratory virus that infects chickens, turkeys, and other avian species. In turkeys, it leads to turkey rhinotracheitis (TRT), while in chickens, it causes swollen head syndrome (SHS) (Mernizi *et al*., 2023).

2.2 Structure of Avian Metapneumovirus:

The virus genome is unsegmented and consist of single stranded, negative-sense RNA, enveloped, linear, non-segmented, and pleomorphic (80 to 200 nm) or spherical of approximately 14 kilobases with a helical symmetry (Al-Hially *et al.*, 2023). The chromosome contains eight genes that encode viral polypeptides, including two glycosylated proteins and three proteins associated with unstructured viruses. The highly variable G-attachment gene, which encodes a conjugated protein and serves as a major immunogen of aMPV, is a key target for epidemiological studies and subtype differentiation (Chacon *et al.*, 2011). viral polypeptides include small hydrophobic protein (SH), viral RNA-dependent RNA polymerase (L), phosphoprotein (P), nucleoprotein (N), fusion protein (F), matrix protein (M), surface glycoprotein (G), and second matrix protein (M2) (Ling and Pringle,1988). The avian metapneumovirus (aMPV) virion is enclosed in a phospholipid sheath derived from the host cell's cytoplasm, with fusion (F), attachment (G) glycoprotein, and small hydrophobic (SH) proteins embedded within it (Wei *et al*., 2013). The viral gene order is 3′-leader-N-P-M F-M2-SH-G-L-trailer-5′ (Ling and Pringle, 1988). Moreover, the aMPV characteristics differ from mammalian pneumoviruses at the molecular level (Dal *et al.*, 2020). Since aMPV lacks hemagglutination and neuraminidase activity in its G-attachment protein, it can be differentiated from other paramyxoviruses (Abd El-Ghany, 2023). For instance, the glycoprotein G is responsible for attaching to the host cell and

such a process has not yet been identified clearly for aMPV (Suarez *et al.*, 2020). The glycoprotein G also shows higher genetic heterogeneity compared to other polypeptides. This genetic variation allowed differentiating vaccine strains from field ones, by analyzing the G gene (Catelli *et al.*, 2010; Lupini *et al.*, 2011). In addition, glycoprotein G is relevant in the case of molecular and epidemiological investigations and triggers opportunities for intensive sequencing and characterization of strain (Franzo *et al.*, 2020). The virus nucleoprotein (N) envelopes the viral genome and protects it against nuclease action. The F-protein mediates the fusion between the cell membrane and the viral envelope, while the G protein facilitates binding to the target cell (Cecchinato *et al.*, 2010). Viral RNA dependent RNA polymerase (L) and Phosphoprotein (P) are required for RNA genome replication (Easton *et al.*, 2004; Wei *et al*., 2013). Furthermore, the primary membrane-associated structural proteins of aMPV, which are the Fusion protein (F) and the glycoprotein (G), play essential roles in viral pathogenicity and immunogenicity (Yu *et al*., 2010).

2.3 Transmission of avian metapneumovirus :

Although airborne transmission can introduce viral particles directly to host cells, close contact with infected birds and physical interaction appear to be essential for experimentally reproducing aMPV infection (Mernizi *et al.*, 2023). Viruses have been detected in the reproductive tracts of layer birds, but there is no evidence of vertical transmission (Hassan and Abdul-careem, 2020). However, it is important to note that this mode of transmission has been observed in ducks, indicating that semen may serve as a source of the virus (Mernizi *et al.*, 2023). The role of wild birds in transmission has also been highlighted (Umar *et al.*, 2016). This could explain the rapid global spread of aMPV following the initial outbreak in South Africa (Cook and Cavanagh, 2002). The appearance of aMPV in wild birds is essential for understanding the disease's epizootiology (Felippe *et al.*, 2011). Turkeys are regarded as the primary host species responsible for the spread of aMPV in countries with a long history of large-scale poultry farming. However,

evidence of significant strain exchanges between turkeys and broilers suggests that turkeys may not play a dominant role in aMPV epidemiology (Franzo *et al.*, 2020).

2.4 Pathogenesis of avian Metapneumovirus

The aMPV infects the upper respiratory tract, like the nasal turbinate and trachea, and to a minor extent, the lungs and the air sacs, which are affected particularly when intercurrent infections are involved and exacerbated, prolonging the respiratory disease (Al-Ankari *et al.*, 2001). Besides, the ability of the virus to replicate in the reproductive system has been confirmed. Although the role of aMPV is not clear enough when found in the oviduct, it is currently often assumed, in field investigations, and based on suitable diagnostic tools, that a drop in egg production could be associated with aMPV infection, even without clear evidence (Hess *et al.*, 2004). The G protein enables virus attachment on the cell receptors, while F promotes the fusion of the envelope with the cell membrane; the nucleocapsid is then released in the cytoplasm, where aMPV replication occurs (Easton *et al.*, 2004). aMPV follows the generally accepted replication and transcription models for other Mononegavirales (Fearns *et al.*, 2002). Negative single stranded RNA viruses require the ribonucleic complex both for transcription and replication. This means their genome needs to be encapsidated with N, P and M2 protein together with the viral polymerase to initiate the infectious cycle (Mernizi *et al.*, 2023). Thus, the virus targets epithelial cells of both respiratory and genital tracts (Suarez *et al*., 2020). Twenty-four hours after infection, the virus can be detected in the nasal cavity and the trachea. The maximum viral quantity is obtained between three and five days post-infection. aMPV in turkeys was isolated from the nasal cavity for up to 14 days, and its viral genome was detected for up to 17 days (Mernizi *et al.*, 2023). In broilers, the replication of aMPV is very similar. However, the virus recovered only a few days after experimental inoculation. In that case, the virus presence was limited to nasal and sinus tissues, trachea, and occasionally lungs with small amounts. Hence, this particularity reflected the short-lived and localized damage of aMPV in chickens (Catelli *et al.*, 1998). Because the virus is associated with the deformation of cilia and ciliated epithelial cells of both

nasal turbinates and trachea, as mentioned before, it may result in the loss of cilia (Hartmann *et al.,* 2015) and the increase of susceptibility of the epithelium to secondary pathogens (Suarez *et al.,* 2020). Macrophages may contribute to the spread of aMPV from the respiratory tract to other peripheral tissues (Suarez *et al.*, 2020). Immunosuppressive properties of some aMPV strains, such as the interference with innate immune response in vitro (Hartmann *et al.,* 2015). When birds are infected with aMPV only, antibody production and waning of respiratory signs coincide with the clearance of the virus infection (Suarez *et al.,* 2020).

2.5 Diagnosis of avian Metapneumovirus

Avian metapneumovirus (aMPV) has been detected in various organs of infected poultry, including the lungs, trachea, ovary, uterus, and other visceral tissues (Ali *et al*., 2019). However, some sample types are considered more effective for virus detection. Research suggests that choanal swabs, ocular and nasal secretions, and tissue samples from the turbinates and sinuses yield higher detection rates (Aung *et al.,* 2008). Since aMPV remains viable in the sinuses and turbinates for only 6–7 days, it is essential to collect samples immediately after infection to maximize detection (Cook, 2000; Aung *et al.,* 2008). The isolation of aMPV from birds with severe clinical symptoms is rare, as these symptoms are often exacerbated by secondary bacterial infections. This challenge in virus recovery is particularly evident in chickens affected by swollen head syndrome (Cook and Cavanagh, 2002). Given the virus's fragile nature, samples intended for isolation should be transported on ice to the laboratory without delay. If immediate transport is not possible, samples should be stored at –50°C to –70°C or preserved on dry ice. Furthermore, FTA cards have demonstrated their efficiency in collecting and transporting aMPV isolates, including respiratory tissue homogenate supernatants and smears, for molecular RNA characterization (Awad *et al.,* 2015).

2.6 Incidence Of aMPV

Nakamura *et al.* (1997) studied the pathology, microbiology and biochemistry of swollen head syndrome in broiler chicken in japan. The virus isolation was attempted from tracheal samples by several methods including embryonating eggs, chicken kidney cells and tracheal organ culture. The tracheal suspension was inoculated into the embryonating specific pathogen free chicken egg via the allantoic cavity of 10 day old embryo and also onto the chorioallantoic membrane of 11 day old embryos. The suspension was inoculated onto chicken kidney cell culture prepared from SPF chickens, and onto tracheal organ cultures of 20-day-old SPF chick embryos. This results, no viral agents were isolated from tracheal samples by any of the isolation methods.

Cavanagh *et al.* (1999) conducted a comprehensive study to monitor the prevalence of avian metapneumovirus (aMPV) and infectious bronchitis virus (IBV) in commercial broiler flocks in eastern and northern England, as well as Scotland. They employed molecular methods, specifically reverse transcription polymerase chain reaction (RT-PCR), to detect aMPV subtypes A and B, and multiplex RT-PCR for IBV detection. The study revealed a high incidence of IBV, with 11 out of 13 flocks (85%) testing positive. In contrast, field infection with aMPV subtype B was only detected in the final week of the study in flocks that had not received an aMPV-A vaccine or had only received an aMPV-A vaccine. Notably, no field aMPV-B infections were found in flocks vaccinated with an aMPV-B vaccine.An additional 30 flocks, mostly unvaccinated against aMPV, were also monitored, and the results showed a significant incidence of aMPV-B (50%) compared to aMPV-A (3%).

Maherchandani *et al*. (2004) utilized an enzyme-linked immunosorbent assay (ELISA) to detect avian metapneumovirus (aMPV) in field samples. A total of 900 samples, collected from 45 flocks (20 samples per flock), were tested individually and then pooled in groups of 3, 4, 5, and 7 samples for comparison. At a theoretical prevalence of 20%, the probability of detecting an infected flock was 0.93 for a pool size of 2 and 0.86 for a pool size of 7. This study demonstrates the

effectiveness of pooling strategies in optimizing aMPV detection while maintaining high diagnostic sensitivity.

Banet-Noach *et al*. (2005) screened five broiler flocks, two breeder flocks, and three layer flocks with respiratory symptoms for avian metapneumovirus strains. Results showed all three layer flocks were RT-PCR negative, while two breeder flocks and one broiler flock tested positive.

Gharaibeh and Algharaibeh (2007) conducted a serological and molecular study on avian pneumovirus (aMPV) in chickens exhibiting respiratory disease in Jordan. A total of 38 chicken flocks were examined using competitive ELISA and 150 commercial chicken flocks were examined by reverse-transcription PCR. Antibodies against aMPV were detected in 5 out of 23 broiler flocks (21.7%), 6 out of 8 layer flocks (75%), and all 7 broiler breeder flocks (100%). These findings revealed the varying prevalence of aMPV antibodies among different types of chicken flocks, with broiler breeder flocks showing the highest seropositivity.

Rousan *et al.* (2008) conducted a study in which tracheal swabs were collected from 115 commercial broiler chicken flocks in Jordan to detect selected respiratory pathogens using RT-PCR and PCR. The results revealed that 6.0% of the flocks were co-infected with Mycoplasma gallisepticum (MG) and avian metapneumovirus (aMPV), while 2.6% of the flocks were co-infected with infectious bronchitis virus (IBV), Newcastle disease virus (NDV), and aMPV. Notably, no flock was found to be solely infected with aMPV.

Chacon *et al.* (2011) investigated 228 farms experiencing respiratory and reproductive disturbances. Among these, 15 farms tested positive for aMPV subtype A, 23 farms tested positive for aMPV subtype B, and 1 farm was found to be positive for both subtypes using RT-PCR.

Park *et al*. (2011) conducted a serological survey to detect antibodies against avian metapneumovirus (aMPV) in Korean chicken flocks. Serum samples were collected from 20 flocks, comprising 197 birds, and tested using ELISA. Overall, 73.1% (144/197) of the samples tested positive for aMPV antibodies. The

seroprevalence was notably higher in broilers (97.3%; 36/37) compared to layers (67.5%; 108/160). They concluded that the widespread presence of aMPV antibodies, particularly in older broilers and layers, in Korean chicken flocks.

Rahimi (2011) investigated the seroprevalence of avian metapneumovirus (aMPV) infection in broiler and broiler breeder chickens in Iran. A total of 435 blood samples were collected from 30 commercial flocks (24 broiler flocks aged 6–8 weeks and 6 broiler breeder flocks aged 56–72 weeks) and tested for antibodies against aMPV subtypes A, B, and C using a commercial ELISA kit. Among broiler chickens, 48.1% (167/347) of samples were seropositive, representing 83.3% (20/24) of flocks. In broiler breeder chickens, 93.2% (82/88) of samples were positive, covering all breeder flocks (100%). The seropositivity was notably higher in broiler breeder flocks (100%) compared to broiler flocks (83.3%). A higher rate of seropositivity was observed in the northwest region.

Homayonfar *et al.* (2013) reported Avian Metapneumovirus infection in chicken flocks from Azerbaijan province. They collected swabs from the choanal cleft, trachea, and turbinates from 50 commercial chicken flocks and diagnosed aMPV in 8 (16%) flocks by performing RT-PCR. On partial sequence analysis of the G gene, it confirmed that the RT-PCR positive samples belong to aMPV subtype B.

Eswaran *et al*. (2014) studied prevalence of aMPV antibodies in broiler breeder flocks in Tamil Nadu, India. Blood samples were randomly collected from 20 broiler breeder farms in the Tirupur district, totaling 485 samples from flocks aged 4 to 72 weeks. The serum samples were tested for aMPV antibodies using a commercial ELISA kit (IDEXX APV Ab Test, Liebefeld-Bern, Switzerland) capable of detecting antibodies against subtypes A, B, and C of aMPV. The results revealed that 34.02% (165/485) of the serum samples were positive, representing 70% (14/20) of the broiler breeder flocks tested.

Al-Shekaili *et al.* (2015) conducted Molecular detection of infectious bronchitis and avian metapneumovirus in Oman backyard poultry. They collected

Orophrayngeal swab from 2317 birds within 243 different backyard flocks in Oman. Thirty-nine chicken flocks were positive for IBV. Thirty two of these were genotyped and they were closely related to 793/B, M41, D274, IS/1494/06 and IS/885/00. 793/B-like IBV was also found in one turkey and one duck flock. Five flocks were positive for aMPV subtype B.

Seifi and Boroomand (2015) conducted a study to screen 35 commercial broiler flocks from northern Iran over a six-month period, from October 2012 to March 2013, focusing on flocks exhibiting respiratory signs. tracheal or nasal turbinate swabs were collected from the 35 flocks. Result showed that 8 flocks (23%) were positive by RT PCR amplifying 115 bp product.

Zahirabadi *et al*. (2017) conducted a study to investigate the prevalence of Avian Metapneumovirus (aMPV) in commercial broiler chicken farms in Qazvin province. They collected tracheal swabs from 20 farms and employed RT-PCR to test for the virus. The results revealed that 13 out of the 20 flocks were infected with aMPV, indicating an infection rate of 65%.

Mayahi *et al.* (2017) detected avian metapneumovirus using reverse transcriptase polymerase chain reaction (RT-PCR). They collected choanal cleft swabs (n=630) from 63 flocks from iran for RNA isolation out of 26 samples from three flocks (4.10%) were positive for viral RNA and all of the viruses were found to be subtype B of aMPV.

Chaboki *et al.* (2018) investigated prevalence of avian metapneumovirus subtype B in live bird market in Gilan province, Iran by RT-PCR. According to the data they analysed aMPV was detected in 30.60% of the examined birds including chickens (37.00%), turkey (33.00%), Eurasian teal (25.00%), common blackbird (33.00%), and Eurasian woodcock (25.00%).

Tucciarone *et al.* (2018) investigated the presence of avian metapneumovirus (aMPV) in Italian broiler farms using subtype-specific multiplex real-time RT-PCR. Their study analyzed 313 oropharyngeal and tracheal swabs collected from 46 commercial broiler farms located in the high poultry-density

region of Northern Italy. The results showed that 27 out of 46 farms (58.7%) tested positive for aMPV. A notably high prevalence (95%) was observed in broilers older than 26 days.

Nagy *et al*. (2018) studied the seroprevalence of avian metapneumovirus (aMPV) in non-vaccinated chicken and duck flocks across six provinces in Egypt. Serum samples were collected from 40 non-vaccinated, apparently healthy chicken flocks (23 broilers and 17 layers) and 8 duck flocks (6 Pekin and 2 Muscovy) and tested using a commercial indirect ELISA. Positive results were detected in 5 out of 23 broiler flocks, 6 out of 17 layer flocks, 1 out of 6 Pekin duck flocks, and 1 out of 2 Muscovy duck flocks.

Ali *et al*. (2019) conducted a serological survey to investigate the prevalence of avian metapneumovirus (aMPV) infection in chickens in Bangladesh between 2014 and 2016. A total of 1,929 chicken serum samples were analyzed using an indirect enzyme-linked immunosorbent assay (ELISA), of which 1,028 samples (53.29%) tested positive for aMPV antibodies. The prevalence was notably higher in broiler breeder flocks aged over 41 weeks with seasonal peaks occurring during the rainy and winter months. This study concluded that the widespread distribution of aMPV infection and its association with specific geographic and seasonal factors.

Darebaghi *et al.* (2021) conducted molecular detection of avian Metapneumovirus in semnan broiler farms. They collected upper part of the trachea, choana, and sinuses of broiler chickens having upper respiratory distress and SHS signs from 85 broiler flocks. Out of 85 tested broiler flocks, 30 (35.3%) were positive for aMPV.

Nguyen *et al.* (2021) conducted a serological and molecular characterization of avian metapneumovirus (aMPV) in chickens from Vietnam. Samples were collected from 138 farms, including 256 tissue/swab samples for aMPV nucleic acid detection and 327 serum samples for antibody analysis. RT-Nested PCR and ELISA were used to identify aMPV subtypes A and B. The results showed that 44

out of 256 samples (17.2%) tested positive for aMPV subtype B by RT-PCR, while no samples were positive for subtype A. The virus was detected in 26.8% (33/123) of farms. Serological analysis revealed that 123 out of 327 serum samples (37.6%) contained anti-aMPV antibodies.

Kumar *et al.* (2022) conducted a survey to assess the prevalence of avian metapneumovirus (aMPV) infection in layer farms across selected regions of Madhya Pradesh, India. The study focused on laying birds aged 20–72 weeks and included both serological and molecular investigations. A total of 263 serum samples were collected and analyzed using ELISA, revealing that 23 samples (9.88%) tested positive for aMPV antibodies, indicating exposure to the virus. Additionally, a molecular investigation was conducted on 169 choanal cleft swabs using RT-PCR to detect the aMPV genome. However, all samples tested negative. These findings highlight the presence of aMPV antibodies in layer flocks despite the absence of detectable viral RNA, emphasizing the need for continued surveillance and monitoring in the region.

Conan *et al*. (2023) conducted a serological survey to assess avian metapneumovirus (aMPV) in vaccinated and unvaccinated broiler chickens in Hong Kong. Blood samples were collected from chickens aged 53–93 days across 24 chicken farms, and serum samples were analyzed for aMPV antibodies using ELISA. Among unvaccinated chickens, the seroprevalence was 80.6% with significant variation observed between different batches. The high seroprevalence and elevated antibody titers suggested frequent and repeated exposure of broiler chickens to aMPV in the region.

Salles *et al.* (2023) conducted surveillance of Avian Metapneumovirus in 100 non-vaccinated chickens and co-infection with avian pathogenic Escherichia coli Brazilian poultry farms. They performed Enzyme-Linked Immunosorbent Assay (ELISA) kit to detect antibodies to aMPV subtypes in the collected sera and RT-PCR. The serological analysis revealed that 20% of the broiler chicken batches tested positive for aMPV. Results showed two farms positive for aMPV-B, none for aMPV-A, and one farm co-infected with APEC.

Escobar-Alfonso *et al.* (2024) detected subtypes of aMPV in samples from breeders, broilers, laying hens, and wild birds in Colombia. A total of 273 samples, including swabs from the upper respiratory and reproductive tracts, were collected from commercial poultry and wild birds. They performed nested RT PCR. aMPV was detected in 23 samples (8.42%).

2.7 Gross and histopathological observations

Jones *et al*. (1988) conducted an experimental study investigating the effects of rhinotracheitis virus infection in laying turkeys. In this study, twenty-four turkey hens, confirmed to be free of antibodies against turkey rhinotracheitis virus, were inoculated intranasally with a viral isolate. The infected turkeys exhibited characteristic respiratory symptoms, including nasal discharge, conjunctivitis, and swelling of the infraorbital sinuses, accompanied by a noticeable reduction in egg production. On gross examination, clear to greyish exudate—initially watery and later transitioning to a mucoid consistency—was observed in the turbinates. Excess mucus accumulation was also evident in the trachea. In the oviduct, whitish masses of inspissated albumen were scattered across various regions of the organ. Histopathological findings in the respiratory tissues revealed epithelial degeneration, loss of cilia, and necrosis of the epithelial lining. Additionally, the submucosal layers showed infiltration by lymphoid and inflammatory cells, predominantly heterophils.

Cook *et al*. (1991) inoculated 8-day-old turkey poults with turkey rhinotracheitis virus (TRTV) at the AFRC Institute for Animal Health, Houghton Laboratory, in Houghton, Huntingdon, England. Five days post-inoculation, they observed mild respiratory infection symptoms, but no post-mortem lesions were detected in poults inoculated with either virus strain alone. However, when poults were co-inoculated with bacteria and the virulent TRTV strain, most exhibited thickened air sacs, with severe thickening observed between 7 and 14 days post-inoculation. In poults inoculated with the attenuated TRTV strain and *Bordetella avium*, differential counting of *B. avium* in nasal tissue was impossible due to the replication of commensal organisms in large numbers in the presence of TRTV.

Majo *et al.* (1995) investigated the histopathological changes and distribution of turkey rhinotracheitis virus (TRTV) antigen in the respiratory and reproductive tracts of experimentally infected chickens, turkey poults, and broiler breeders in Spain. Mild respiratory signs, such as nasal discharge, gasping, and ocular discharge, were observed in all TRTV-inoculated birds. Gross examination revealed excessive mucoid exudates in the upper respiratory tract and conjunctivitis. Histopathologically, changes included catarrhal purulent rhinitis with mucopurulent exudate composed of heterophils, mucus, erythrocytes, and epithelial cells, along with focal epithelial discontinuity, hyperemia, and a dense mononuclear inflammatory infiltrate in the submucosa of the nasal turbinates. Severe tracheitis was marked by an inflammatory exudate in the tracheal lumen, epithelial exfoliation, edema, hyperemia, and mononuclear inflammatory infiltration in the submucosa. In the bronchial lumen, inflammatory exudate was present, with hyperplasia of the bronchial epithelium and an abundant mononuclear infiltrate in the submucosa of the bronchi.

Majo *et al*. (1997) conducted an experimental study to investigate the effects of co-inoculation of turkey rhinotracheitis virus (TRTV) and Escherichia coli (E. coli) in pathogen-free chickens. They employed eyedrop and intranasal routes to administer the virus and bacteria. The study aimed to evaluate the presence of E. coli, histopathological changes, and tissue distribution of viral antigen in the respiratory tract. The results showed that all 36 chickens inoculated with TRTV and E. coli developed severe rhinitis. Additionally, 3 out of the 36 chickens exhibited periorbital edema, fibrinous airsacculitis, and pericarditis. These findings revealed that TRTV may act as a primary agent that enhances E. coli multiplication, leading to increased severity of respiratory disease.

Nakamura *et al*. (1997) reported lesions in broiler chickens affected with Avian Metapneumovirus, IB virus, ND virus, and *E. coli* .The aMPV infected birds showed gross facial subcutaneous tissue swelling (especially in the periocular area) as well as submandibular subcutaneous tissue swelling. Microscopic findings included purulent exudation in the nasal cavity, cystic dilation of crypts containing

purulent exudates in the mucosal membrane, cellular infiltration in the lamina propria of mucosa, hyperplasia of epithelial cells and cellular infiltration in the lamina propria of the trachea, fibrinopurulent exudates in the air spaces of the cranial bone, purulent exudate in the middle ear and panophthalmitis.

Catelli *et al*. (1998) performed the dissemination of a chicken isolate of Avian Pneumovirus in the UK. They observed nasal exudate which was sometimes copious and/or turbid. Microscopically, it revealed hyperemia, edema, and mild mononuclear cellular infiltration in the lamina propria of the nasal turbinate, thickening of the tracheal mucosa, due to congestion, edema and mononuclear cell infiltration in the lamina propria.

Cook (2000) studied Avian Pneumovirus Infections in Turkeys and Chickens. Clinical signs included apathy, swelling of the face and infraorbital sinuses, cerebral disorientation, torticollis and opisthotonus. Grossly revealed the presence of excessive exudates in the respiratory tract, swollen infra orbital and periorbital sinuses, rhinitis. Histopathological examination showed destruction of the epithelium and loss of cilia, hyperaemia and mild mononuclear infiltration in the submucosa of nasal turbinate, transient lesions were noticed in the trachea, but other tissues, including the conjunctiva and lungs, are rarely affected.

Panigrahy *et al*. (2000) conducted an investigation into the pathogenesis and serological response of turkeys experimentally infected with the avian pneumovirus (APV/turkey/Colorado/97) isolate. They observed clinical signs in the infected birds, including nasal discharge, swollen infraorbital sinuses, conjunctivitis, and mild depression. Despite these clinical manifestations, no gross lesions were observed. Histopathological examination revealed acute, multifocal, mild to moderate rhinitis in the nasal turbinates. This inflammation was characterized by congestion, edema, infiltration of lymphocytes and heterophils, as well as loss of cilia and degeneration and sloughing of epithelial cells. Similar inflammatory changes were detected in the mucosa of the infraorbital sinuses.In the tracheal mucosa, inflammation was accompanied by a multifocal loss of the ciliated

epithelium, with the proximal trachea exhibiting more pronounced epithelial damage than the distal trachea.

Georgiades *et al*. (2001) conducted a study to screen 50 commercial broiler flocks in Greece for respiratory diseases, with particular attention to signs of swollen head syndrome (SHS). Clinical signs consistent with SHS were observed in eight of the flocks. Affected birds exhibited symptoms such as sneezing, nasal discharge, swollen eyelids, and pronounced swelling of the head due to subcutaneous edema. Post-mortem examinations of eight affected birds revealed serous rhinitis, characterized by clear nasal inflammation. Additionally, subcutaneous tissue in the head displayed inflammation accompanied by gelatinous material. Serous exudate was also observed within the infraorbital sinuses.

Alkhalaf *et al.* (2002) investigated the effects of inoculating avian metapneumovirus (aMPV) and *Bordetella avium* in 2-week-old commercial turkey poults sourced from Wooster. Microscopically, the respiratory epithelium of the infraorbital sinuses in the aMPV-inoculated group displayed intermittent and mild hyperplasia, accompanied by scattered submucosal lymphoid infiltrates that formed aggregates within the surrounding connective tissues. The ciliated epithelium remained intact. In the dually infected group, the infraorbital sinus epithelium exhibited intermittent hyperplasia with variable disruption of the cilia and hyperplastic mucous glands. The submucosa and connective tissues showed varying degrees of lymphoid infiltrates. Additionally, sinus lumens contained exudates characterized by mucus, cellular debris, and scattered bacteria.The respiratory epithelium of the infraorbital sinuses in the *B. avium*-inoculated group also showed intermittent hyperplasia with variable disruption of the overlying cilia and different levels of lymphoid infiltration. Similar to the dually infected group, exudates composed of mucus, cellular debris, and scattered bacteria were observed overlying the epithelial lining. No significant microscopic changes were identified in the trachea or lung sections from any of the inoculated groups.

Jirjis *et al*. (2002) studied the pathogenesis of avian metapneumovirus (aMPV) in turkeys. They inoculated cell culture–propagated aMPV into the

conjunctival spaces and nostrils of 4-week-old poults. Clinical signs observed were open mouth breathing, snicking, foamy or frothy discharge in the medial canthi of the eyes, nasal discharge, swelling of the infraorbital sinuses, and frothy ocular discharge. Grossly, there was swelling of the infraorbital sinuses, slight reddening of the nasal turbinates, and excessive frothy mucoid fluid in the nasal cavity. Microscopically, there was moderate infiltration of lymphocytes, macrophages, and plasma cells, with a few heterophils infiltrating the mucosa and submucosa of the nasal turbinates and infraorbital sinuses. Loss of cilia, subepithelial hyperemia, and infiltration of intraepithelial heterophils and lymphocytes were also observed in the trachea.

Cha *et al.* (2007) inoculated aMPV subtype C in turkeys from Minnesota. Gross examination revealed excessive exudates in the respiratory tract, indicating conditions such as rhinitis, conjunctivitis, sinusitis, laryngitis, and tracheitis. Microscopically, there was evidence of necrosis and damage to the upper respiratory mucosa, loss of cilia, severe infiltration of inflammatory or lymphoid cells in the submucosa, and significant tracheitis.

Aung *et al*. (2008) compared the pathogenesis of subtype A and B of avian metapneumovirus (aMPV) in commercial broilers from Germany. They observed clinical signs such as depression, coughing, nasal exudates, and frothy eyes at 4 days post-inoculation. Gross examination revealed swelling of the periorbital sinuses and clear nasal exudates. Microscopic examination showed infiltration of lymphoid cells in the epithelium, hypertrophy of the mucous glands of the nasal turbinates, thickening of the trachea, infiltration of inflammatory cells in the epithelium, focal exfoliation, and destruction of the epithelium in the infraorbital sinus. Additionally, there was infiltration of lymphocytes and formation of lymphoid follicles around the secondary collecting duct of the Harderian gland, as well as infiltration of lymphoid cells in the interstitial and interlobular tissues of the lachrymal gland.

Roussan *et al.* (2008) diagnosed aMPV in broiler chicken flocks in Jordan. They observed Clinical sign such as severe gasping, coughing, conjunctivitis, nasal

and ocular discharge, depression, and weakness, with the chickens being reluctant to move. Gross examination revealed moderate to severe congestion of the trachea with or without mucopurulent exudates, airsacculitis, and pericarditis or perihepatitis.

Hosseini and Ghalyanchi-Langeroudi (2012) reported Avian Metapneumovirus in broiler flocks from Iran and characterized the aMPV genome based on G glycoprotein. Gross findings in affected birds were swollen head syndrome and hyperemia of the trachea. Microscopically, revealed loss of cilia, increased glandular activity, hyperaemia and mild mononuclear infiltration in the submucosa of nasal turbinate.

Wei *et al*. (2013) investigated the effects of avian metapneumovirus subgroup C (aMPV-C) in yellow-feathered chickens from China after experimental inoculation. Among the inoculated chickens, some displayed clinical symptoms, including nasal discharge, sinus swelling, and watery eyes, primarily between 3 to 7 days post-inoculation (dpi). Histopathological analysis revealed a range of inflammatory changes in the trachea and lungs. In the trachea, these included mild to severe inflammation characterized by disruption of epithelial architecture, sloughing of epithelial cells, loss of ciliation, and infiltration of inflammatory cells. Additional findings included hemorrhage, lymphoid cell infiltration, and epithelial hyperplasia in both the tracheal epithelium and lamina propria. In the lung peribronchial lymphoplasmacytic infiltrates, edematous thickening of the bronchial submucosa, and hyperplasia of lymphoid cells. There was also a diffuse, mild expansion of the alveolar interstitium caused by mononuclear cell infiltration and edema. The bronchial lumens contained sloughed epithelial cells, heterophils, macrophages, and amorphous debris.

Hartmann *et al*. (2015) investigated the interaction between avian metapneumovirus (aMPV) and host tissues using tracheal organ cultures from chickens and turkeys. Their findings revealed notable structural alterations in tracheal tissues following aMPV infection. Grossly, the pathological changes were more pronounced in turkey tracheal organ cultures compared to those of chickens,

indicating greater susceptibility in turkeys. Histopathological analysis demonstrated significant damage to the tracheal epithelium in both species. Key observations included epithelial desquamation, ciliary loss, and epithelial cell degeneration, which are characteristic features of aMPV-induced tissue damage. The severity of epithelial destruction was markedly higher in turkeys than in chickens.

Hristova and Petrova (2017) found coinfection of chicken anaemia virus, mycoplasma gallisepticum, avian metapneumovirus and avian reovirus in fancy chicken breeds of age 2 weeks with clinical signs included difficult breathing, whistling sounds and conjunctivitis. Grossly, revealed fibrinous airsacculitis with gathering caseous exudate and serous tracheitis, thymus atrophy. Microscopically showed hypoplasia of the bone marrow, depletion of lymphocytes from subcapsular thymic cortex.

Kaboudi and Lachheb (2021) conducted a study on avian Metapneumovirus (aMPV) infection in turkeys, documenting a range of clinical, gross, and microscopic pathological findings. Clinically, affected birds exhibited respiratory symptoms, including dyspnea, nasal discharge, rales, sneezing, and coughing. Additional signs included periorbital edema, conjunctivitis, and infraorbital sinusitis.Gross pathological examination revealed catarrhal inflammation of the upper respiratory tract, manifesting as rhinitis, laryngitis, and tracheitis. Secondary bacterial infections exacerbated the lesions, leading to more severe conditions such as airsacculitis and pneumonia. Subcutaneous exudate and osteomyelitis in the cranial bones were also noted in turkeys infected with aMPV.Histopathologically, severe infiltration of lymphoid cells into the submucosa and damage to the epithelial lining of the turbinates were observed, accompanied by multifocal loss of the ciliated epithelium. Intracytoplasmic inclusion bodies were identified in the epithelial cells of the conjunctiva, nasal turbinates, and the upper portion of the trachea.

Abd El-Ghany (2023) provided a comprehensive review of avian metapneumovirus (aMPV) infection in poultry. Infected birds exhibit a variety of clinical signs, including nasal discharge, conjunctivitis, coughing, sneezing, and

swelling of the infraorbital sinuses. In broiler breeders and laying hens, the disease is also characterized by a significant reduction in egg production. The gross pathological lesions commonly observed in aMPV-infected birds include inflammation of the upper respiratory tract, mucosal exudates within the nasal cavities and trachea, and swollen infraorbital sinuses. In severe cases, particularly when secondary bacterial infections are present, excessive mucus and fibrinous exudates may be observed in the air sacs. Histopathological examination of tissues affected by aMPV reveals extensive necrosis and damage to the upper respiratory mucosa. This includes the destruction of cilia, significant infiltration of inflammatory or lymphoid cells within the submucosal layers, and marked tracheitis.

Al-Hially *et al*. (2023) provided an extensive review of avian Metapneumovirus (aMPV) infections in broiler chickens. Clinically, infected birds exhibit symptoms such as foamy eyes, nasal discharge, and swollen sinuses. Gross pathological findings included gelatinous to caseous exudates beneath the skin of the enlarged skull, as well as infectious peritonitis in laying hens. Histopathological examination revealed significant changes. The nasal turbinates showed multifocal deciliation of the epithelium, hyperemia, and mononuclear cell infiltration in the submucosa. In the tracheal epithelium, focal exfoliation of epithelial cells was observed, accompanied by thickening due to edema and infiltration of inflammatory cells. Additionally, the presence of intracytoplasmic inclusion bodies was noted in the tracheal epithelium.

Mernizi *et al*. (2023) reported various clinical manifestations in chickens infected with avian metapneumovirus (aMPV), including depression, coughing, nasal exudate, frothy eyes, and swelling of the periorbital and infraorbital sinuses, submandibular region, and neck. Additionally, affected birds exhibited prolapse of the nictitating membrane, torticollis, disorientation, and opisthotonos. Macroscopically, lesions such as periocular subcutaneous edema, blepharitis, conjunctivitis, caseous otitis, maxillary arthritis, and periostitis were observed. Microscopically, revealed the presence of intracytoplasmic eosinophilic inclusion

bodies within the nasal turbinate and trachea of infected birds, Progressive ciliary malformations and deciliation were noted, with replacement of epithelial cells by non-ciliated cells in aMPV-positive broiler flocks, Experimental studies on aMPV mono-infection demonstrated that nasal turbinate damage predominantly occurs along the inner surface of the turbinate spiral, by two days post-infection (PI), changes include mild edema in the lamina propria accompanied by mononuclear cell infiltration, however, these lesions do not extend uniformly across the entire mucosa, by four days PI, tracheal mucosal thickening and vascular congestion become evident, along with an increase in mononuclear cell infiltration. by seven days PI, deciliation is observed in multiple tissues due to persistent inflammatory infiltration, recovery of the nasal tissue is generally complete by 18 days PI, suggesting a self-limiting inflammatory process, in cases where bacterial co-infection is involved, such as in swollen head syndrome, changes are more severe, this condition is characterized by granulomatous inflammation of the subcutaneous cranial tissue, necrosis, and the presence of bacterial colonies. These findings suggest a more complex disease progression when bacterial co-infections accompany aMPV, leading to exacerbated inflammatory responses and tissue damage.

Salles *et al.* (2023) investigated the surveillance of avian metapneumovirus (aMPV) in non-vaccinated chickens and the impact of co-infection with avian pathogenic *Escherichia coli* (APEC). Chickens infected with aMPV exhibited typical respiratory symptoms such as nasal discharge, conjunctivitis, and swelling of the infraorbital sinuses. These signs were significantly exacerbated in birds co-infected with APEC. Co-infected chickens demonstrated more severe respiratory distress, lethargy, and higher morbidity compared to those infected with aMPV alone. In chickens infected with aMPV alone, gross lesions were predominantly observed in the upper respiratory tract, including excessive mucus in the trachea and mild congestion in the nasal passages. In cases of dual infection with APEC, the gross lesions were more pronounced, with fibrinous airsacculitis, purulent exudates in the trachea, and consolidation in the lungs. The combination of these pathogens intensified the severity of the respiratory lesions. Microscopic

examination revealed that aMPV-infected chickens exhibited epithelial desquamation, loss of cilia, and necrosis of the tracheal epithelium. There was also submucosal infiltration of lymphoid and inflammatory cells, primarily heterophils. Co-infection with APEC resulted in more severe histological damage, including thickening of the air sac membranes, hemorrhages, and extensive infiltration of heterophils and macrophages in the respiratory tissues.

2.8 PCR detection of avian metapneumovirus

Nakamura *et al*. (1997) reported lesions in broiler chickens affected with Avian Metapneumovirus, IB virus, ND virus, and *E. coli* from Japan, confirmed by nested PCR. aMPV was confirmed by employing nested RT-PCR using two sets of primers targeting the fusion (F) protein gene of the TRT virus 3B strain. The primer set of Fl (GATCAAGTGGCTAAGGAAAG) and F3 (CCATCATAAACACCAATCAA) was used for the first PCR, and F2 (ATTAGAGGGAGAGGTGAAGG) and F5 (ACATCAAACTAATCTGTCCT) for the second PCR (nested PCR).

Cavanagh *et al*. (1999) monitored 13 commercial broiler flocks and 30 additional flocks in eastern and northern England, as well as Scotland, for aMPV and IBV using molecular methods. RT-PCR targeting the *G* gene was performed for aMPV subtypes A and B, while multiplex RT-PCR was used for IBV detection. IBV was identified in 11 of 13 flocks (85%). For aMPV, field infection with subtype B was detected only in the final week in flocks that had not received an aMPV-A vaccine or had only received aMPV-A. In contrast, no field aMPV-B was found in flocks vaccinated with aMPV-B. Among 30 additional flocks, mostly unvaccinated against aMPV, the incidence of aMPV-B and aMPV-A was 50% and 3%, respectively.

Alkhalaf *et al*. (2002) inoculated avian Metapneumovirus (aMPV) and *Bordetella avium* alone or in combination in 2 week old commercial turkey poults from Wooster. They extracted RNA with Trizol LS reagent (GibcoBRL, Grand Island, NY) and performed the RT-PCR with the primers were designed from the

M protein sequence. The samples from sinuses, tracheas, and lungs were positive for APV by both reverse transcriptase–polymerase chain reaction and virus isolation. They concluded that the combination of both aMPV and *Bordetella avium* infected flocks showed severe clinical sign and virus persisted longer, also bacteria invaded more respiratory tissues.

Banet-Noach *et al*. (2005) screened five broiler flocks, two breeder flocks, and three layer flocks with respiratory symptoms for avian metapneumovirus strains. Samples (trachea, lungs, turbinates, and dry tracheal swabs) were collected from December 2002 to August 2004. Total RNA was extracted using the QIAamp viral RNA extraction kit (Qiagen, Germany) and subjected to RT-PCR with Nd/Nx primers targeting the N gene amplifying 115 bp product.

Gharaibeh and Algharaibeh (2007) investigated 150 commercial chicken flocks in Jordan with respiratory disease, identifying aMPV in 20 flocks through RT-PCR using the Nd/Nx primer pair. The affected flocks displayed mild respiratory symptoms, with some birds showing head swelling, reduced feed consumption, and decreased egg production.

Roussan *et al.* (2008) diagnosed aMPV in broiler chicken flocks in Jordan through RT-PCR using a pair of specific primers, Nd (5′-AGC AGG ATG GAG AGC CTC TTT G-3′) and Nx (5′-CAT GGC CCA ACA TTA TGT T-3′), which amplified a PCR product of 115 bp. They performed RT-PCR for AIV subtype H9N2, IBV, NDV, and APV, and PCR for MG. From these tests, they revealed that 13% and 14.8% of these flocks were infected with NDV and IBV, respectively.

Ongor *et al*. (2010) identified avian metapneumovirus (aMPV) subtypes in turkeys using reverse transcription polymerase chain reaction (RT-PCR). They collected 624 tracheal tissues and 20 tracheal swabs from 23 flocks experiencing respiratory issues in California. RNA was extracted from homogenized tracheal tissue and swab samples using guanidinium isothiocyanate with the EZ-RNA kit.

The flocks were screened with RT-PCR utilizing the primer pair Nd/Nx, specific to the nucleocapsid of aMPV, producing a fragment of approximately 115

bp in positive samples. The assessment revealed that out of 624 tracheal tissue samples, 36 (5.6%) and out of 20 tracheal swabs, 18 (90%) tested positive for aMPV.

Chacon *et al* (2011) investigated poultry farms with respiratory and reproductive disturbances. Samples of nasal turbinate and trachea were taken from birds showing respiratory disease, while reproductive organs (oviduct or testis) were obtained from cases of decreased egg production and fertility disturbances. They performed RT PCR with the use of primer pair Nd2 (59-AGC ARG RYR GAR AGC CTS TTT GT-39) and Nx2 (59-A CAT GGC CCA ACA TKA TRT T-39) respectively amplified a 116 bp product.

Hosseini and Ghalyanchi-Langeroudi (2012) reported Avian Metapneumovirus in broiler flocks from Iran and characterized the aMPV genome based on G glycoprotein. They collected oropharyngeal swabs from affected flocks and total RNA was extracted from sterile swabs with the use of RNAeasy Mini Kit (QIAGEN, USA). They performed RT-PCR and screened the flocks with the primer pair Nd (5'AGC AGG ATG GAG AGC CTC TTT G3') Nx (5'CAT GGC CCA ACA TTA TGT T3').

Wei *et al*. (2013) inoculated avian metapneumovirus subtype C (aMPV/C) into BALB/c mice. Total RNA was isolated from lung tissue using the RNeasy Mini kit (Qiagen) for RT-PCR. They performed RT-PCR using specific primers: sense primer (5'-GTCAATTCAGCCAAGGCAGT-3') and antisense primer (5'-GGGGCAATCCTAGCTTGAGT-3'), targeting the M gene to amplify a 200 bp product.

Abdel Azeem *et al*. (2014) studied avian Metapneumovirus (aMPV) subtype A in turkeys in Egypt. RNA was extracted from pooled swabs using the High Pure RNA Isolation Kit (Roche Diagnostics, Italy). RRT-PCR was performed to differentiate aMPV subtypes A and B, partially amplifying the G and F genes. Additional tests using RRT-PCR and RT-PCR for avian influenza virus (AIV) and

Newcastle disease virus (NDV), respectively, showed no detection of these viruses. However, aMPV subtype A was identified in the pooled swabs through RRT-PCR.

Rivera-Benitez *et al.* (2014) detcted aMPV in pullets and breeder hens in two zones of high poultry production in Mexico. A total of 105 tracheal tissue samples and 968 serum samples were collected from pullets and hens 2 to 73 weeks of age. They performed nRT-PCR amplified a fragment from the G gene of aMPV subtype A (268 base pairs [bp]) and subtype B (361 bp). Results showed that 23.8% were nRT-PCR-positive.

Sid *et al*. (2015) detected infection of MG, MS, IBV, AIV, and aMPV B by molecular analysis. They collected tracheal and cloacal swabs from six broiler flocks, two turkey flocks, and one layer flock in Algeria. They performed qRT-PCR to detect AIV targetting matrix gene, PCR to detect MG targetting mgc2 gene and RT PCR to detect aMPV subtype B and IBV. They performed RT PCR for aMPV by using oligonucleotide primers (SH1 5’ GCTTTGATCTTCCTTGTTGC SH 3’ and 5’G6− CTGACAAATTGGTCCTGATT 3’) which were designed based on a 953 bp segment of the aMPV Glycoprotein (G) gene.

Seifi and Boroomand (2015) conducted a study to screen commercial broiler flocks from northern Iran over a six-month period, from October 2012 to March 2013, focusing on flocks exhibiting respiratory signs. Tracheal or nasal turbinate swabs were collected from the 35 flocks and extraction of RNA was performed on the pooled material for swabs from each flock with RNX TM -Plus reagent (CinnaGen, Iran). They performed RT-PCR and screened the flocks with the primer pair Nd (5’AGC AGG ATG GAG AGC CTC TTT G3’) Nx (5’CAT GGC CCA ACA TTA TGT T3’).

Mayahi *et al.* (2017) detected avian metapneumovirus using reverse transcriptase polymerase chain reaction (RT-PCR) with the use of primer pair Nd (5’-AGCAGGATGGAGAGCCTCTTTG -3’) and Nx (5’-CATGGCCCAACATTATGTT -3’) respectively. They collected choanal cleft swabs (n=630) from 63 flocks from iran for RNA isolation.

Zahirabadi *et al.* (2017) investigated the infection of aMPV in commercial broiler chicken farms from Qazvin province. They collected tracheal swabs from 20 broiler chicken farms and tested the samples by RT-PCR with the use of specific primer pair Nx (5'CAT GGC CCA ACA TTA TGT T3') and Nd (5'AGC AGG ATG GAG AGC CTC TTT G3') for aMPV. 13 flocks out of 20 flocks were infected with aMPV, which accounted for 65% infection rate of the flocks.

Tucciarone *et al.* (2018) reported aMPV in 27 italian broiler farms ((58.7%), Forty-six commercial broiler farms, distributed over the high poultry density region of Northern Italy. The samples were collected by oropharyngeal or tracheal swabbing. The RNA was extracted using a High Pure RNA Isolation Kit and aMPV confirmed by A and B subtype-specific multiplex real-time RT-PCR. The high prevalence of aMPV was confirmed in broilers older than 26 days (95%) and phylogenetic analysis revealed all aMPV belong to subtype B.

Darebaghi *et al.* (2021) conducted molecular detection of avian Metapneumovirus in semnan broiler farms with the use of primer pair Nd(5'-AGCAGGATGGAGAGCCTCTTTG -3') and Nx (5'-CATGGCCCAACATTATGTT -3') respectively. They collected upper part of the trachea, choana, and sinuses of broiler chickens having upper respiratory distress and SHS signs from 85 broiler flocks.

Al-Hasan *et al*. (2022) detected aMPV in 16 (23.8%) poultry frams out of the 67 broiler farms that reported typical SHS from September 2018 to August 2019 by using RT PCR. Molecular typing using primers specific to the attachment glycoprotein (G) gene showed that all positive samples belonged to subtype B.

Kumar *et al*. (2022) conducted molecular investigation of avian Metapneumovirus infection in layer chicken farms of age group 20-72 weeks from Madhya Pradesh, India. A total of 169 choanal cleft swabs were collected for RT-PCR. All the samples were found negative for aMPV genome by RT-PCR assay. They performed RT-PCR with the use of specific primer pair ND 5'

AGCAGGATGGAGA GCCTCTTTG 3’ and NX 5’ CATGGCCCAACATTATGTT 3’ targetting the N gene.

Salles *et al*. (2023) conducted molecular surveillance on 100 unvaccinated broiler chicken farms in Brazil with respiratory tract infections to investigate aMPV and its co-infection with APEC. RNA was extracted using the RNeasy® Mini kit (QIAGEN, Hilden, Germany) and subjected to RT-PCR with primers targeting the G gene for subtypes A and B. Subtype A primers (F: 5′-GGACATCGGGAGGAGGTACA-3′, R: 5′-CACTCCTCTAACACTGACTGTTCAACT-3′) produced a 116 bp amplicon, while subtype B primers (F: 5′-TCATCCCGGAAGCCTCCCTCACTAT-3′, R: 5′-TAGCGTTTGCTGCACTGGCTTCTGATAC-3′) generated a 135 bp product. Results showed two farms positive for aMPV-B, none for aMPV-A, and one farm co-infected with APEC.

Escobar-Alfonso *et al.* (2024) detected subtypes of aMPV in samples from breeders, broilers, laying hens, and wild birds in Colombia. A total of 273 samples, including swabs from the upper respiratory and reproductive tracts, were collected from commercial poultry and wild birds. They performed nested RT PCR with the use of two sets of primers taegetting G gene that allowed the simultaneous identification of subtypes A and B. In the first amplification, the primers G6-(5′ -CTGACAAATTGGTCCTGATT-3′) and G1+(5′ -GGGACAAGTATCYMKAT-3′) were used. For the nested PCR, forward primers G8+A (5′ -CACTCACTGTTAGCGTCATA-3′) and G9+B (5′ -TAGTCCTCAAGCAAGTCCTC-3′) were used in combination with the G5-(5′ -CAAAGARCCAATAAGCCCA-3′) reverse primer, which anneals with a conserved region of both subtypes. RT PCR was performed with the use primers Nd2 (59-AGC ARG RYR GAR AGC CTS TTT GT-39) and Nx2 (59-A CAT GGC CCA ACA TKA TRT T-39) respectively targetting N gene which allowed detection of all subtypes. aMPV was detected in 23 samples (8.42%). Each of these positive samples displayed an amplification product of 116 bp on agarose gel electrophoresis for the N gene, as well as a 360 bp product for the G gene.

2.9 aMPV with associated pathogen

Droual and woolcock (1994) observed swollen head syndrome associated with E. coli and Infectious Bronchitis Virus in thirteen 27 day old chickens from central valley of California with clinical signs included a snick, swollen heads and severe depression. Grossly, revealed swollen heads due to facial cellulitis, swollen eyelids, airsacculitis, mucosa of the nasal cavity was wet, yellow caseous exudates present within air spaces of the cranial bones, middle ear and on the sclera of the eyes. Microscopically displayed mild to moderate inflammation with lymphoplasmacytic infiltration of the lamina propria of tracheal epithelium and heterophilic infiltration, in the nasal cavity, the epithelium had hyperplastic mucous glands and inflammatory cell infiltration and the lumen contained mucoid exudate and heterophils, The epithelium of the infraorbital sinus showed mild multifocal deciliation and mild heterophilic infiltration which appeared to be an extension of inflammation from the underlying connective tissue, The air spaces of skull bones and middle ear contained inflammatory exudates consisted of necrotic heterophils often associated with rod shaped bacteria and bordered by multinucleate giant cells, Subcutis and eyelid showed severe cellulitis with stromal oedema, heterophilic infiltration, mononuclear cell aggregations, and occasional necrotic and granulomatous foci.

Majo *et al.* (1997) examined the experimental co-infection of Turkey Rhinotracheitis Virus (TRTV) and *Escherichia coli* in chickens in Spain. Following TRTV inoculation, the chickens displayed clinical signs including lethargy, ruffled feathers, mild mucous nasal discharge, slight palpebral and periorbital edema on the right side, and ocular discharge. Gross examination revealed nasal congestion, abundant fibrinous airsacculitis, and thick mucus in the nasal cavity and infraorbital sinuses. Microscopically, severe purulent rhinitis and sinusitis were observed, with purulent exudate in the nasal cavity and infraorbital sinuses, epithelial desquamation, extensive cilia loss, and an intense mixed inflammatory infiltrate of lymphocytes and heterophils in the submucosa of the middle turbinate and infraorbital sinuses. Additionally, purulent material was noted in the air spaces of

the cranial bones at the skull base. The tracheal epithelium showed cilia loss, and heterophilic infiltration was present in the interlobular septa of the lungs, within the lumen of some parabronchi, and in the submucosa of the main bronchi.

Al-Ankari *et al* (2001) inoculated APV and E. coli in day old chicks 10 birds were inoculated with E. coli, 40 with APV and 10 with both agents. Respiratory signs were observed at 3 days of post inoculation. They observed macroscopic lesions such as congestion with serous or mucoid exudate in nasal turbinates, rhinitis, oedema at back of the neck and congested brain. Microscopically birds infected with pneumovirus showed predominant mononuclear infiltration with focal epithelial extension, mucous gland hyperplasia.

Van de Zande *et al*. (2001) studied the clinical, pathological and microbiological outcome of an *Escherichia coli* O2:K1 infection in avian pneumovirus infected turkeys from Germany. Specific pathogen-free (SPF) and conventional (CON) turkeys were inoculated oculonasally with virulent APV subtype A, *E. coli* O2:K1, or both at different intervals. In dually infected turkeys clinical signs were more severe like nasal exudate with mild swollen infra-orbital sinuses and frothy eyes, Gross pathology revealed fibrinous airsacculitis, pneumonia, and congestion in the lungs. Histopathological analysis showed extensive epithelial damage, hyperaemia, inflammatory cell infiltration, and bacterial colonization in the respiratory tract.

Jirjis *et al*. (2004) investigated the effects of bacterial co-infections on the pathogenesis of avian pneumovirus (aMPV) in turkeys. Four- and nine-week-old poults were inoculated with cell culture-propagated aMPV via the conjunctival space and nostrils, followed three days later by inoculation with *Escherichia coli*, *Bordetella avium* (BA), *Ornithobacterium rhinotracheale* (ORT), or a combination of all three (EBO).Turkeys infected with aMPV alone exhibited mild clinical signs, such as nasal discharge and swollen sinuses. Microscopically, mild lymphocyte and macrophage infiltration was observed in the nasal turbinates, infraorbital sinuses, and tracheal mucosa, along with excessive mucus in the nasal cavities.In turkeys co-infected with aMPV and EBO, clinical signs and lesions were more severe.

Grossly, the lungs were reddened and wet, and the air sacs contained foamy fluid and tan-white deposits (airsacculitis). Microscopically, extensive cilia loss, epithelial flattening, and marked inflammatory cell infiltration were observed in the nasal turbinates, trachea, and air sacs. The lungs exhibited fibrinous inflammation and necrotic foci surrounded by multinucleated giant cells and macrophages.Turkeys co-infected with aMPV and BA or ORT showed variable inflammatory changes, with BA causing more severe cilia loss and fibrin deposition compared to ORT. Co infection with *E. coli* caused mild to moderate inflammatory changes in the nasal cavities and air sacs. Overall, these findings underscore the significant role of bacterial co-infections in exacerbating respiratory disease in turkeys infected with aMPV, resulting in more severe pathological changes and clinical outcomes compared to infection with aMPV alone.

Rubbenstroth *et al*. (2009) investigated the pathogenesis of *Riemerella anatipestifer* (RA) in turkeys following experimental mono-infection via respiratory routes or dual infection with avian metapneumovirus (aMPV) in Germany. Clinical signs in all aMPV-infected groups were primarily characterized by swollen infraorbital sinuses, nasal discharge, and foamy ocular exudates, which are typical of turkey rhinotracheitis (TRT).Gross pathological examination of the dually infected group revealed mild fibrinous airsacculitis, adhesive pericarditis, and epicarditis. No gross lesions were observed in uninfected control groups or turkeys with single aMPV infections. Microscopically, the dually infected group exhibited mucosal damage in the nasal turbinates, including lymphoid and heterophilic cell infiltration, ciliary loss, and epithelial desquamation.

Hristova and Petrova (2017) investigated the co-infection of chicken anemia virus (CAV), *Mycoplasma gallisepticum*, aMPV, and avian reovirus in fancy chicken breeds aged 2 weeks. The clinical signs observed included laboured breathing, whistling sounds, and conjunctivitis. The presence of CAV was confirmed by PCR, and antibodies against avian reovirus and avian metapneumovirus were detected using an ELISA test. Gross examination revealed fibrinous airsacculitis with caseous exudate accumulation and serous tracheitis,

along with thymus atrophy. Microscopic examination showed hypoplasia of the bone marrow and depletion of lymphocytes from the subcapsular thymic cortex.

Abdelmoez *et al*. (2019) carried out isolation and identification of aMPV, *E. Coli, P. mirabilis, P. aeruginosa* in broiler chickens from Egypt. Four samples were confirmed aMPV positive by one-step RT-PCR using both subtype A and B (G gene) primers Ga (gf)-forward CCGGGACAAGTATCTCTATGG for all subtypes, G2 (gaf)-reverse CCACACTTG AAAGATCTACCC for A subtype and G12 (gb)-reverse CAGTCGCCTGTAATC TTCTAGGG for B subtype. While bacterial isolation revealed chickens had co infection of *E. coli, P. mirabilis* and *P. aeruginosa*.

Ruger *et al*. (2021) studied host pathogen interaction of *Mycoplasma gallisepticum* and Avian Metapneumovirus in Tracheal Organ Cultures of Chicken from Germany. TOCs were inoculated with either *M. gallisepticum*, aMPV, or both in different sequences with a 24-hour interval. Histopathological analysis was conducted to examine ciliary loss and epithelial damage over time. Single infections caused minimal ciliary loss, but dual infections led to significantly more severe epithelial damage. This suggests that aMPV weakens the epithelial barrier, allowing for more aggressive bacterial colonization.

Sharifi *et al*. (2022) observed concurrent infections of Infectious Bursal Disease (IBD), Newcastle Disease (ND), and Avian Metapneumovirus (aMPV) in broilers. They selected 20 broiler farms from various regions in West Azerbaijan Province, Iran. A total of 800 samples were collected from the trachea, spleen, cecal tonsils, and bursa of Fabricius from each flock. RNA was extracted from pooled tissue samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany).They performed RT-PCR using specific primers: for IBDV, the primer pair B3F (CCCAGAGTCTACACCATA) and B4R (TCCTGTTGCCACTCTTTC) targeting the VP2 gene to amplify a 474 bp product; for aMPV, the primer pair Nd (AGCAGGATGGAGAGCCTCTTG) and Nx (CATGGCCCAACATTATGTT) targeting the N gene to amplify a 115 bp product; and for NDV, the primer pair F (GGTGAGTCTATCCGGARGATACAAG) and R

(TCATTGGTTGCRGCAATGCTCT) targeting the F gene to amplify a 202 bp product. Molecular results showed that 45% (9/20), 30% (6/20), and 15% (3/20) of the tissue samples were positive for IBDV, NDV, and aMPV, respectively.

Salles *et al.* (2023) conducted a comprehensive evaluation of avian metapneumovirus (aMPV) infections in non-vaccinated broiler chickens, focusing on the pathology and the impact of co-infection with avian pathogenic *Escherichia coli* (APEC). The study examined 100 batches of broiler chickens distributed across Brazil. Birds infected with aMPV exhibited respiratory symptoms such as nasal discharge, sneezing, conjunctivitis, and infraorbital sinus swelling. In cases of co-infection with APEC, clinical signs became more severe, including lethargy, open-mouth breathing, cyanosis, and reduced feed intake, indicating systemic involvement. aMPV-infected birds displayed swollen infraorbital sinuses filled with mucopurulent exudates, nasal congestion, and mild to moderate tracheal inflammation. In co-infected birds, lesions were significantly exacerbated, including fibrinous airsacculitis with thick fibrinous exudates covering the air sacs, hepatomegaly, splenomegaly, and thickened vascularized air sacs, reflecting chronic inflammation.

Secato *et al*. (2024) conducted a study on the occurrence of *Mycoplasma gallisepticum* (MG) and avian metapneumovirus (aMPV) in commercial broiler flocks from the Southeast and Midwest regions of Brazil. The research involved collecting nasal and tracheal swabs between 2017 and 2018 from 87 batches of broiler chickens originating from 15 commercial production farms that exhibited respiratory clinical signs. For sample processing, RNA was extracted from nasal and tracheal swabs using the Trizol RNA extraction protocol (Invitrogen, United States of America). DNA extraction from the swab samples was carried out using the QIAamp DNA Mini Kit (QIAGEN). They employed the RT-Nested-PCR technique with specific primer oligonucleotides to detect and differentiate subtypes A and B of aMPV. Additionally, conventional PCR targeting the mgc2 gene was utilized to identify MG. The results revealed that two out of the 87 batches of broiler chickens analyzed (2/87; 2.3%) tested positive for MG, whereas none of the

batches were found to be positive for aMPV. These findings underscore the low prevalence of MG and the absence of aMPV in the studied broiler flocks, despite the presence of respiratory clinical signs.

Mo and Mo (2025) reviewed the pathogenesis, clinical manifestations, and pathological findings of infectious laryngotracheitis virus (ILTV) and avian metapneumovirus (aMPV). ILTV causes severe respiratory distress, gasping, coughing, nasal discharge, and, in severe cases, the expulsion of blood-tinged mucus. In contrast, aMPV infections present milder symptoms, including nasal discharge, conjunctivitis, infraorbital sinus swelling, and foamy ocular secretions. Gross lesions of ILTV are primarily found in the trachea, characterized by hemorrhagic and necrotic debris, inflamed mucosa, and caseous plugs obstructing the airways. aMPV lesions are milder, including mucoid to mucopurulent exudates, sinus swelling, and tracheal congestion.

Chapter - III

Materials and Methods

CHAPTER – III
MATERIALS AND METHODS

The present study was conducted in the Department of Veterinary Pathology at PGIVAS, Akola. The samples for this study were obtained from the necropsy facility of the Department of Veterinary Pathology at PGIVAS, Akola, the Veterinary Polyclinic, Akola, and various poultry farms in and around Akola. A detailed account of the entire study, including sample collection and the various methods and protocols employed to achieve the study's objectives, is provided in the subsequent section of this chapter.

3.1 Materials

3.1.1 Glassware and plasticware

The glassware used in the present study was procured from Borosil (India) and underwent a standardized cleaning protocol. Following an overnight treatment with 0.01% diethyl pyrocarbonate (DEPC)-treated water to inactivate DNase and RNase, the glassware was subsequently sterilized by autoclaving to ensure aseptic conditions.Consumables, including plasticware, microcentrifuge tubes, PCR tubes, filter tips, pipettes, and tissue collection vials, were sourced from Tarson (India), Genaxy (India), and Eppendorf (India). All plasticware was either pre-certified as DNase- and RNase-free or subjected to treatment to eliminate DNase and RNase activity, as described earlier.

3.1.2 Equipment and instruments

Standard equipment and instruments available in the departmental laboratories of Veterinary Pathology, Veterinary Microbiology, Pharmacology and Toxicology, and Animal Genetics and Breeding of the institute were utilized for essential experimental procedures. The details of the equipment and instruments are provided in Table 3.1.

Table 3.1 Equipment and instruments used in experiments

Equipment and Instruments	**Make**
Weighing balance	Sartorious, Germany
Micropipette (2.5ul,10ul,50ul,100ul,1000ul)	Eppendorf research plus (Germany) and Accupipet (India)
Vortex	Remi (India)
Spinner	Tarson (India)
Centrifuge	Remi (India)
Cooling centrifuge	Remi (India)
Water bath	Remi (India)
Double distillation unit	Millipore (U.S.)
Thermal Cycler	TaKaRa (India)
Agarose gel Electrophoresis unit	Scie-Plas (England)
UV transilluminator	BioEra (India)
Rotary shaker	Remi (India)
Rotary microtome	Tarson (India)
Autoclave	Equitron (India)
Gel Documentation System	Vilber

3.1.3 Buffers and reagents

The compositions of the buffers and reagents used in the present study are detailed below. All buffers and reagents were prepared using Nucleases free water to ensure molecular integrity and prevent nucleic acid degradation.

1. 10% Neutral Buffered Formalin (NBF)

Monobasic Sodium dihydrogen Phosphate (NaH_2PO_4)	4.0 gm
Dibasic Disodium hydrogen Phosphate (Na_2HPO_4)	6.5 gm
37% Formaldehyde	100 ml
Distilled water	900 ml

2. 10% Nitric Acid (HNO3)

Nitric acid (HNO_3)	15.4 gm
Distilled water	100ml

3. Agarose (1.5%)

Agarose	1.5gm
TAE (1X)	up to 100ml

4. Ethidium bromide stock solution (10mg/ml)

Ethidium bromide	0.1gm
NFW	10 ml

The solution was mixed and stored at 4°C and 0.3 µg/ml was used in preparing agarose gel.

5. 50x TAE buffer

Tris base	242 gm
Acetic acid	57.1 ml
0.5 M EDTA (pH 8.0)	100 ml
Add NFW to make a volume of up to	1000 ml

6. 75% ethanol

Absolute ethanol	75 parts
NFW	30 parts

7. 80% ethanol

Absolute ethanol	80 parts
NFW	20 parts

8. 95% ethanol

Absolute ethanol	95 parts
NFW	5 part

9. RNAiso plus, Takara (Japan) Ready to use

10. Isopropanol (Sisco Research Laboratory) Ready to use

11. Chloroform (Sisco Research Laboratory) Ready to use

3.1.4 Chemicals, molecular biology reagents, enzymes and kits

All the chemicals, molecular biology reagents, enzymes, and kits used in the study were procured from TaKaRa Bio, Sisco Research Laboratory, Thermo Scientific, and Promega.

3.1.5 Oligonucleotide primers

Oligonucleotide primers employed within the current investigation were procured through commercial synthesis services offered by Eurofins. The nucleotide sequence as well as other particulars are provided in Table 3.2 and table 3.3.

Table 3.2 avian metapneumovirus oligonucleotide primers used in this study

Name of virus	Target gene	Primer Sequence (5`-3`)		Size (bp)	Source / Reference
avian Metapneumovirus (aMPV)	Nucleoprotein *(N)* gene	Nd-F	5'AGC AGG ATG GAG AGC CTC TTG 3'	115	Bayon Auboyer *et al*., 1999
		Nx-R	5'CAT GGC CCA ACA TTA TGT T 3'		

Table 3.3 List of primers used for amplification of different genes of associated viral and bacterial pathogens.

Pathogen	Target Gene	Primer Sequence(5'..... 3')	Product Size(bp)	Reference
Viral pathogens				
NDV	*F*	F- GCAGCTGCAGGGATTGTGGT R-TCTTTGAGCAGGAGGATGTTG	356	Ottiger (2010)
IBV	*N*	F-AATTTTGGTGATGACAAGATGA R-CATTGTTCCTCTCCT CATCTG	402	Farsang *et al.* (2002)
ILTV	*p32*	F- CTACGTGCTGGGCTCTAATCC R- AAACTCTCGGGTGGCTACTGC	588	Ottiger (2010)
Bacterial pathogens				
M. gallisepticum	*Mgc2*	F- CGC-AAT-TTG-GTC-CTA-ATC-CCC-AAC-A R- TAA-ACC-CAC-CTC-CAG-CTT-TAT-TTC-C	300	Rasoulinezhad *et al.*(2017)
APEC	*ecp*	F:TGAAAAAAAAAGGTTTTCTGGCAATAGC R:CGCTGATGATGGAGAAAGTGAA	500	Avelino *et al.* (2010)
ORT	*16S rRNA*	F-GAGAATTAATTTACGGATTAAG R-TTCGCTTGGTCTCCGAGAT	784	Amonsin *et al.* (1997)
Infectious Coryza	*HPG-2*	F- TGAGGGTAGTCTTGCACCCGAAT R- CAAGGTATCGATCGTCTCTCTACT	500	Chen *et al.* (1998)

3.2 Methods

3.2.1 Sampling and Specimen Collection:

The visit was arranged twice in a week to the different poultry farm (including broiler, layer and backyard birds) for collection of dead birds in and around Akola. The three recently died birds from each flock were collected and necropsied and were thoroughly screened for avian Metapneumovirus infection during the period of 9 months i.e., from April 2024 to December 2024. Also, chickens received for the necropsy to the Department of Veterinary Pathology,

PGIVAS, Akola were included in the study. During said period, a portion of nasal turbinate, trachea, lung and choanal slit swab or tracheal swab from dead and ailing birds showing gross lesions or signs of respiratory tract involvementwere collected in aseptic and sterile conditionsand stored in -20 ^{0}C until use.

A detailed necropsy was carried out on dead birds at Veterinary Pathology Department, PGIVAS, Akola. The examination focused on the respiratory system, thoroughly assessing both the upper and lower respiratory tracts. A total of 42 flocks having 134 chickens were in and around akola were screened. The birds showing gross lesions like swollen head, swollened peri orbital and infra orbital sinuses, having mild to moderate nasal discharge from this birds sample like nasal turbinate, trachea, lung and choanal slit swab or tracheal swabs were collected and were carefully documented and photographed. All the samples for RNA extraction were stored in -20^{0}C until use. Likewise, portion of nasal turbinate, trachea, lung, heart, spleen, and liver from the suspected birds preserved in 10% Neutral Buffered Formalin (NBF) for subsequent histopathological analysis.From each flock, one or more samples were collected based on the flock size and severity of symptoms. If one or more of the collected samples tested positive for avian Metapneumovirus (aMPV), the entire flock was considered positive for aMPV. The samples that tested positive for aMPV were further screened for concurrent or associated pathogens using Polymerase Chain Reaction (PCR) techniques and the same samples were also processed for histopathological examination.Details of samples collected during current study is given in the table no. 3.4.

Table 3.4: Details of the samples collected from different poultry farms in Akola district.

Sr. no.	Name of Poultry farms	Type of Chickens	No. of dead Chickens collected
1	Department of Poultry science, PGIVAS, Akola.	Broilers	6
		Layers	3
		Backyard birds (Vanashri)	2
2	Akram Sheikh, Anand Nagar,Akola	Backyard birds	3
3	Usaman Khan, Barshi Takli, Akola	Broilers	3
4	Vitesh Rokade, Murtizapur,Akola	DP cross	3
5	Dhananjay Zumbale, Lakhanda Budruk, Akola	Backyard Birds	3
6	Sunil Kamble Poultry Farm, Akola.	Backyard Birds	3
7	Baba Poultry Farm, Yevda Road, Akola.	Layers	3
8	Anil Wankhede, Sonara, Akola	Backyard birds	3
9	Amit Kamdi Poultry Farm, Girjapur, Akola.	Broiler	3
10	Raj Poultry Experiment, PKV, Akola.	Backyard birds (Giriraj)	3
11	Udayrao Deshmukh, malakapur area, Akola	Backyard birds	3
12	Jilha Kukkut Vikas Sahkari Sanstha Maryadit, Akola.	Broilers	3
13	Swapnil Kankal Poultry Farm, Shivani Road, Akola.	Broilers	3
14	Obaroy Poultry Farm, Chikli, Akola.	Broilers	3
15	Pramod Patel Poultry Farm, Shivani, Akola.	Backyard birds	3
16	Dipak Mali Poultry Farm, Akola.	Backyard birds	3
17	Girish Ingle Poultry Farm, Shivani, Akola.	Backyard birds	3
18	Aaradhya poultry farm, mase sisa, Akola	Broilers	3
19	Gajabe Poultry Farm, Paranda, Akola.	Broilers	3
20	Pankaj Takvale Poultry Farm, Yelvan, Akola.	Broilers	3
21	Gaurav Vargat, Pimplekhuta, Akola	Broilers	3
22	Vijay Dhotre Poultry Farm, PKV, Akola.	Broilers	3
23	Nitesh Bhojane, Shivar, Akola	Backyard birds	3

Sr. no.	Name of Poultry farms	Type of Chickens	No. of dead Chickens collected
24	Rameshwar Daberao Poultry Farm, Nimgaon, Akola.	Backyard birds	3
25	Pavan Dahibhare Poultry Farm, Babhulgaon, Akola.	Backyard birds	3
26	Ajay Zombade, Visora, Akola	Broilers	3
27	Sandip Patil Poultry Farm, Barshi takli, Akola	Broilers	3
28	Ibrahim Khan Poultry Farm, barshi takli, Akola	Layers	3
29	Salim Chaudhari, Shivapur phata,Akola	Broilers	3
30	Shubham Ingale, Manarkhed, Balapur,Akola	Backyard birds	3
31	Anant Kathole, Poultry Farm, Balapur, Akola.	Backyard birds	3
32	Manish Surjase,Lasur,Akola	Broilers	3
33	Hirulkar Poultry Farm, Balapur, Akola.	Broilers	3
34	Pramod Raut Poultry Farm, Nimakardha, Akola	Broilers	3
35	Anil Patil, Paras phata, Akola	Backyard birds	3
36	Javed Pathan.Kumbhari, Akola	Backyard birds	3
37	Shrikrushna, Alanda,Akola	Backyard birds	3
38	Amol Bhagat Poultry Farm, Dapki Road, Akola.	Backyard birds	3
39	Rajesh Gawai, Dapki road, Akola	Broilers	3
40	Kuldip Bhojne Poultry Farm, Mahan, Akola.	Backyard birds	3
41	Dilip Sardar, Akola	Backyard birds	3
42	Prakash Pahare Poultry Farm, Kanerisarap, Akola.	Backyard birds	3
Total number of flocks= 42			**Total birds=134**

3.2.3 Molecular diagnosis of avian Metapneumovirus and selected associated pathogens

The tissue samples utilized for molecular identification were stored at -20 ^{o}C for molecular diagnosis of aMPV targeting partial fragment of N gene and selected associated pathogens.

a) Total RNA Extraction

A total RNA was extracted from stored nasal turbinate/ lung/ trachea and choanal slit swab/tracheal swab. Approximately 100 mg of tissue were minced using RNAiso plus, Takara in a sterile mortar and pestle, and then transferred into 2 ml Eppendorf tubes. The mixture was vortexed and incubated at room temperature for 5 minutes. Next, 200 µl of chloroform (Sisco Research Laboratory) was added to the mixture, thoroughly mixed by shaking, and incubated for 2 to 3 minutes at room temperature. The mixture was then centrifuged at 12000 rpm for 15 minutes in a refrigerated centrifuge (Remi, India) at 4°C. Following centrifugation, the upper aqueous colorless layer was carefully transferred to another DEPC treated 2 ml Eppendorf tube by angling the tube at 45° and pipetting the solution out. To this, 500 µl of chilled isopropanol (Sisco Research Laboratory) was added and incubated at 4°C for 10 minutes for precipitation. The mixture was then centrifuged at 12000 G for 10 minutes at 4°C, and the supernatant was discarded. The RNA pellet was washed with 1000 µl of chilled 75% ethanol, briefly vortexed, and then centrifuged at 7500 G for 5 minutes. After discarding the supernatant, the pellet was air dried and finally suspended in 25 µl of nuclease-free water. The RNAs were stored at -20°C until further use or immediately utilized for the synthesis of complementary DNA (cDNA).

b) Synthesis of first strand cDNA from RNA

The cDNA was synthesized from the extracted RNA by using GoScript Reverse Transcription Kit (Promega). The protocol used for first–strand cDNA synthesis was as per the manufacturer's instructions with slight modifications. First of all, the 5µl reaction of RNA and primer mixture was prepared with following component (Table 3.5).

Table 3.5 Component of RNA and Primer mixture

Sr. No.	Component	Volume (µl)
1	Extracted RNA	3
2	Random primer	1
3	NFW	1
Final volume		**5**

This was incubated at 70^{o}C for 5 min in a Thermal Cycler (Takara) and then snap chilled on ice water for at least 5 min and microcentrifuge for 10 sec. Then stored on ice until reverse transcription mixture was added. After that 15 µl of reverse transcription reaction mixture was prepared with following component (Table 3.6)

Table 3.6 Component of Reverse transcription mixture

Sr. No.	Component	Volume (µl)
1	GoScript 5X Reaction Buffer	4
2	$MgCl_2$	3
3	PCR Nucleotide Mix	1
4	Recombinant RNasin	0.5
5	GoScript Reverse Transcriptase	1
6	Nuclease-Free Water	5.5
Final volume		**15**

A combination of 5 µl of RNA and primer was mixed with a reverse transcription reaction mixture of 15 µl to yield a final volume of 20 µl of cDNA. These were mixed well and centrifuged briefly for few second followed by annealing at 25^{o}C for 5 min and extended at 42^{o}C for 60 min. Before proceeding with PCR, the reverse transcriptase was inactivated at 70^{o}C for 15 min. Finally, the cDNA thus, synthesized was stored at -20^{o}C till future use.

c) Total DNA extraction

For the selected associated DNA pathogens, DNA extraction was conducted. Genomic DNA was extracted from tissue suspensions using the DNeasy Blood & Tissue Kit (Qiagen) in accordance with the manufacturer's protocol. Briefly, 100 mg portion of nasal turbinate, trachea, lung tissue was homogenized in 1 mL of Nuclease Free Water (NFW) using a sterile pestle and mortar. A 400 µL aliquot of the homogenized tissue suspension was transferred into a 1.5 mL microcentrifuge tube, followed by the addition of 180 µL of Buffer ATL and 20 µL of Proteinase K. The mixture was thoroughly vortexed and incubated at 56°C until complete tissue lysis was achieved, as indicated by a colorless appearance, with intermittent vortexing during the incubation process. Upon complete lysis, the sample was vortexed for 15 seconds, and 200 µL of Buffer AL was added. The mixture was homogenized and incubated at 56°C for 10 minutes. Subsequently, 200 µL of ethanol (96–100%) was added, and the solution was thoroughly vortexed. The entire mixture was then transferred into a DNeasy Mini spin column seated in a 2 mL collection tube and centrifuged at 8000 rpm for 1 minute. The flowthrough and collection tube were discarded. The spin column was placed into a fresh 2 mL collection tube, and 500 µL of Buffer AW1 was added, followed by centrifugation at 8000 rpm for 1 minute. The flowthrough and collection tube were discarded once again. The spin column was subsequently transferred to a new collection tube, and 500 µL of Buffer AW2 was applied, followed by centrifugation at 14000 rpm for 3 minutes. The resulting flowthrough and collection tube were discarded.For elution, the spin column was placed into a sterile microcentrifuge tube, and 200 µL of Buffer AE was carefully applied to the center of the column membrane. After a 1-minute incubation at room temperature, the spin column was centrifuged at 8000 rpm for 1 minute. The eluted DNA was collected into a microcentrifuge tube and stored at -20°C for subsequent analysis.

d) RT-PCR detection of aMPV

The PCR assay was conducted in standard 25µl reaction volume in 0.2 ml tubes for the detection of targeted virus i.e. aMPV using published primer sets by

using Green Taq Master mix (TaKaRa Bio, India). Thermal cycling conditions were standardized by performing PCR for target pathogen based on the Tm value of the primers using known positive controls for target pathogen. The composition of the reaction mixture for performing PCR (TaKaRa, India) in a 25 µl reaction volume is mentioned in Table 3.7. The contents were mixed thoroughly and subjected to the mentioned thermal cycling conditions in Table 3.8.

Table 3.7 Composition of the PCR reaction mixture

Sr. No	Component	Volume (µl)
1	Green Taq Master mix	12.5
2	Forward primer (Nd-F)	1
3	Reveres primer (Nx-R)	1
4	Nuclease free water	7.5
5	cDNA Template	3
	Final volume	**25**

Table 3.8 Thermal cycling conditions for aMPV

Sr. No.	Steps	Temperature/time	No. of Cycles
1	Initial Denaturation	94^{o}C / 15 min.	1
2	Denaturation	94^{o}C /20 sec.	35
3	Annealing	54^{o}C /15 sec.	
4	Extention	72^{o}C /15 sec.	
5	Final Extention	72^{o}C /10 min.	1
6	Hold	4^{o}C forever	

e) RT-PCR/ PCR detection of selected associated pathogens

Selected pathogens with concurrent infection were detected by following PCR assay in birds having avian Metapneumovirus infection.The composition of the reaction mixture for performing PCR (TaKaRa, India) for associated pathogens in a 20 µl reaction volume is mentioned in Table 3.9

Table 3.9 Composition of the PCR reaction mixture

Sr. No	Component	Volume (µl)
1	Green Taq Master mix	10
2	Forward primer (Nd-F)	1
3	Reveres primer (Nx-R)	1
4	Nuclease free water	5
5	Template DNA/cDNA	3
	Final volume	20

Following cyclic condition were used for amplification of different genes of concurrent pathogens

Table 3.10 Thermal cyclic conditions for associated viral pathogens

Steps	Newcastle disease virus, F gene (356 bp)	Infectious bronchitis virus, N gene (402 bp)	Infectious Laryngotracheitis Virus, p32 gene (588 bp)
Initial Denaturation	95°C / 4 min.	95°C / 5 min.	95°C / 5 min.
Final Denaturation	94°C /40 sec.	95°C /45sec.	94°C /45sec.
Annealing	57°C /50 sec.	56°C /45 sec.	56°C /45 sec.
Extention	72°C /50 sec.	72°C /45 sec.	72°C /45 sec.
Final Extention	72°C /5 min.	72°C /7 min.	72°C /7 min.
No. of cycles	35	30	35

Table 3.11 Thermal cyclic conditions for associated bacterial pathogens

Steps	*Mycoplasma gallisepticum* mgc2 gene (402 bp)	Avian Pathogenic *Escherichia coli* Ecp gene (500 bp)	Infectious Coryza HPG-2 gene (500 bp)	ORT 16s rRNA (784 bp)
Initial Denaturation	94ºC / 3 min.	95ºC / 5 min.	95ºC / 2.5 min.	95ºC / 5 min.
Final Denaturation	94ºC /30 sec.	95ºC /45 sec.	94ºC /1 min.	95ºC /45 sec.
Annealing	58ºC /30 sec.	60ºC /45 sec.	65ºC / 1 min.	52ºC /1 min.
Extention	72ºC /1 min.	72ºC /90 sec	72ºC / 2 min.	72ºC /1 min.
Final Extention	72ºC /7 min.	72ºC /10 min.	72ºC /10 min.	72ºC /10 min.
No. of cycles	35	35	25	35

f) Gel electrophoresis

A 1.5% agarose gel (HiMedia) was prepared by mixing 100 ml of 1X Tris-Acetate-EDTA (TAE) buffer. After heating to 60°C and subsequent cooling, 5 µl of Ethidium bromide (Sisco) was added to the molten agarose and thoroughly mixed. The agarose-Ethidium bromide mixture was then poured into a gel casting tray using an acrylic comb and left to solidify. After approximately 30-35 minutes, the acrylic comb was cautiously removed to create wells in the gel. The solidified agarose gel was placed in a horizontal gel electrophoresis system (Scie-plas, England) filled with 1X TAE buffer. The first lane was loaded with 2 µl of 100 bp ladder (Takara, Clontech), while the remaining wells were loaded with 10 µl of the PCR sample. Electrophoresis was conducted for 45 minutes at a rate of 80V. Subsequently, the gel was examined and photographed under UV light using a UV Trans-illuminator (Bio Era, India). The sizes of the bands were confirmed by comparison to a quantitative DNA ladder (Takara, Clontech). Presence of a band in an appropriate position indicated a positive outcome.

3.4 Pathological studies

3.4.1 Gross pathology

Detailed necropsy examination was carried out from birds died of different poultry farms. Systemic examination was carried out for the presence of any lesions in nasal turbinate, infra and periorbital sinuses, trachea, lungs, liver, heart and spleen and lesions of pathological significance were recorded.

3.4.2 Processing of tissues for histopathology

Sections of nasal turbinate, trachea, lung, liver, heart and spleen tissues collected from poultry carcasses suspected for avian Metapneumovirus based on gross lesions were preserved in 10% neutral buffered formalin. The Tissues of nasal turbinate, trachea, lung, heart, liver, and spleen from PCR positive cases of avian Metapneumovirus were processed for paraffin embedding technique to observe histopathological alteration. Tissues after fixation in 10% NBF (at least 72 h), The fixed nasal turbinate tissue was then placed in a 10% Nitric acid (decalcifying solution) for 1 hr then all tissues were cut in pieces of 4-5 mm thickness and placed in tissue capsules for overnight washing, followed by dehydration in ascending grade of alcohol (Half hour each in 75%, 80%, 85%, 90%, 95% and one hour in absolute alcohol I, II, III) followed by clearing through three changes of xylene for 40 min each. The xylene was replaced completely by at least three changes of molten paraffin wax (melting point 58-60°C) for two hour. Then tissues were embedded in paraffin blocks. Tissue section of 4-5µ thickness were cut using microtome (Leica) and collected in tissue floatation water bath. The sections were stained with Hematoxylin and Eosin (Luna, 1968) for general histopathology.

Haematoxylin and Eosin (H & E) staining was done by routine method. Briefly, the tissue sections were deparaffinized using xylene I, xylene II, and xylene III for approximately 3-5 minutes each, then transferred to a mixture of xylene and alcohol (1:1) for 5 minutes. Subsequently, they were treated with absolute alcohol I, absolute alcohol II, 90% alcohol, 80% alcohol, and 70%

alcohol for 5 minutes each to remove residual xylene, followed by a thorough wash in tap water. After washing, the sections were stained with haematoxylin solution for 10-12 minutes, then washed again in tap water. Further steps included differentiation in acid alcohol, treatment with ammonia water until the sections turned blue, another round of washing, blotting with filter paper, and counterstaining with 1% Eosin for 30 seconds until they appeared light pink. Following another wash, the sections were dehydrated using graded alcohols (70%, 80%, 90%, absolute alcohol) for 5 minutes each, followed by placement in a mixture of alcohol and xylene (1:1). After washing, blotting, and air drying, clearing was performed in xylene-I and xylene-II for 15-30 minutes. The sections were then mounted in DPX with a cover glass, and the slides were dried in a dust-free environment (Luna, 1968). Finally, the stained tissue sections were examined under a microscope to record any histopathological changes.

Chapter - IV

Results and Discussion

CHAPTER – IV
RESULTS AND DISCUSSION

Avian Metapneumovirus (aMPV) primarily affects turkeys and chickens, leading to substantial economic losses due to reduced productivity, increased mortality, and concurrent bacterial and viral infections. Clinically, aMPV infection manifests as respiratory distress, serous to mucopurulent nasal discharge, swelling of the infraorbital sinuses, and a significant decline in egg production in laying hens. The virus is of particular concern, as it predisposes birds to opportunistic infections, thereby exacerbating the severity of clinical disease (Abd El-Ghany, 2023). Considering the economic and health implications of aMPV and sparse reports on its occurrence, a comprehensive study on the *"*Pathology of avian Metapneumovirus and associated pathogens in chickens*"* in the Akola district was conducted to know the incidence, pathology and concurrent infection during spontaneous outbreaks. To address the objectives the chickens showing swollen heads, swollen sinuses or having mild to moderate nasal discharge, ocular discharge, and depression were considered for the present investigation.

4.1 Molecular diagnosis and incidence of aMPV

In the present study, a total of 42 chicken flocks with suspected clinical signs/respiratory lesions were screened for aMPV and associated pathogens. Three recently died birds from each flock were collected and necropsied. Nasal turbinate and trachea from recently died birds with prominent respiratory lesions were used to extract genomic RNA and DNA for screening for aMPV and associated pathogens by PCR assay. RT-PCR assay with WOAH recommended specific primers targeting partial fragment of N gene was used to confirm the aMPV infection which showed 115 bp (Plate 4.1) specific amplicons on agarose gel electrophoresis as described earlier (Bayon Auboyer *et al*., 1999). It revealed aMPV infection in birds from 8 flocks demonstrating 19.04% (08/42) flock level incidence.

A comparative literature search revealed similar incidence rates of aMPV infection in poultry across different geographical regions. Studies by Nguyen *et al*. (2021), Salles *et al*. (2023), and Seifi and Boroomand (2015) reported prevalence rates of 17.2% in chickens from Vietnam (using RT-Nested PCR and ELISA), 20% in non-vaccinated chickens co-infected with avian pathogenic *Escherichia coli* (APEC) from Brazilian poultry farms (using RT-PCR and ELISA), and 23% in commercial broiler flocks from northern Iran (using RT-PCR), respectively.

Conversely, higher incidence rates of 35.3% and 80.6% were documented by Darebaghi *et al*. (2021) and Conan *et al*. (2023) in broiler farms in Semnan (using PCR) and unvaccinated broiler chickens in Hong Kong (using ELISA), respectively. In contrast, lower incidence rates of 8.42%, 9.88%, and 16% were reported by Escobar-Alfonso *et al*. (2024), Kumar *et al*. (2022), and Homayonfar *et al*. (2013) in breeders, broilers, laying hens, and wild birds in Colombia (using nested RT-PCR); layer farms across selected regions of Madhya Pradesh, India (using ELISA and RT-PCR); and commercial chicken flocks from Azerbaijan province (using RT-PCR), respectively. The observed variation in aMPV incidence may be attributed to factors such as the ability of the virus to replicate suboptimally in the initial days of infection (Roussan *et al*., 2008; Cook, 2000), as well as differences in breed susceptibility, timing of sample collection, seasonal variations, and geographical distribution (Aung *et al*., 2008; Gharaibeh and Algharaibeh, 2007; Park *et al*., 2011; Rahimi, 2011).

Since aMPV predisposes birds to secondary respiratory infections, the present study investigated the presence of co-infections with selected bacterial and viral pathogens. PCR assays employing pathogen-specific primers, as described by Farsang *et al*. (2002), Ottiger, (2010), Ottiger, (2010), Chen *et al*. (1998), Amonsin *et al*. (1997), Rasoulinezhad *et al*. (2017), and Avelino *et al*. (2010), were used to detect Infectious Bronchitis Virus (IBV), Newcastle Disease Virus (NDV), Infectious Laryngotracheitis Virus (ILTV), Infectious Coryza (IC), Ornithobacterium rhinotracheale (ORT), Mycoplasma gallisepticum (MG), and APEC respectively. The presence of co-infections was confirmed through agarose

gel electrophoresis of PCR amplicons, yielding specific bands of 402 bp (IBV) (Plate 4.5), 356 bp (NDV), 588 bp (ILTV) (Plate 4.7), 500 bp (IC) (Plate 4.4), 784 bp (ORT) (Plate 4.3), 300 bp (MG) (Plate 4.2), and 500 bp (APEC) (Plate 4.6), respectively.

In the current study birds from 6 aMPV positive flocks, were also concurrently infected with MG. This finding aligns with Ruger *et al*. (2021), who reported aMPV and MG co-infections and explored their pathogen–host interactions. Additionally, two aMPV-infected flocks were co-infected with ORT, corroborating previous experimental findings by Jirjis *et al*. (2004) and Marien *et al*. (2005), who demonstrated that dual infection with aMPV and ORT led to more severe respiratory lesions than single infections. Moreover, two flocks (25%) exhibited concurrent APEC infection, consistent with findings by Salles *et al.* (2023), who demonstrated the association between aMPV and APEC. Furthermore, one flock (12.5%) showed co-infection with IC, as previously reported by Murthy *et al.* (2008).

Co-infections with other respiratory viruses, including IBV, ILTV, and NDV, were also investigated. IBV was detected to be co-infected in birds of 01 aMPV (12.5%, 01/08) infected cases which is in agreement with studies by Andreopoulou *et al*. (2019) and Ardicli *et al*. (2022), who described concurrent aMPV and IBV infections. Additionally, ILTV co-infection was identified in one flock (12.5%), consistent with findings by Ardicli *et al*. (2022) and Mo and Mo (2025). Notably, no aMPV-positive flock exhibited co-infection with NDV.

Among these birds, concurrent infection of selected multiple pathogens was also noticed including aMPV + MG + IBV infection in 01 flock, aMPV+ MG + APEC in 1 flock (12.5%), aMPV + MG + ILTV in 01 flock (12.5%), aMPV + IC + ORT in 1 flock (12.5%), aMPV + ORT + APEC in 1 flock (12.5%) and aMPV + MG in three flocks.

These findings are in accordance with a previous experimental study in turkeys by Marien *et al*. (2005) who demonstrated that co-infections involving

aMPV and multiple pathogens (e.g., aMPV + MG; aMPV + ORT + APEC + MG; aMPV + ORT; and aMPV + APEC) led to exacerbated respiratory lesions in turkeys. Similarly, Majo *et al*. (1997) reported concurrent infection of turkey rhinotracheitis virus (TRTV/aMPV) and *E. coli* in chickens, while Al-Ankari *et al*. (2001) described co-infection of aMPV and *E. coli* in day-old chicks. Jirjis *et al*. (2004) documented aMPV infections in turkeys in combination with *E. coli*, *Bordetella avium* (BA), and ORT. Additionally, Hristova and Petrova (2017) reported co-infections of chicken anemia virus (CAV), MG, aMPV, and avian reovirus in ornamental chicken breeds. A molecular survey by Roussan *et al*. (2008) revealed the presence of multiple respiratory pathogens, including avian influenza virus (AIV, H9N2), NDV, IBV, aMPV, and MG, in various combinations in commercial broiler chickens. Furthermore, Sid *et al*. (2015) demonstrated that all poultry flocks tested were positive for at least one respiratory pathogen, with MG detected in every case. Co-infections involving multiple pathogens (e.g. MG + IBV, MG + aMPV, MG + AIV + IBV, and MG + Mycoplasma synoviae + AIV + IBV) increased mortality in Algerian poultry farms.

It has been well established that aMPV weakens the respiratory epithelial barrier, allowing for more aggressive bacterial colonization (Ruger *et al.*, 2021). This includes the destruction of cilia, significant infiltration of inflammatory or lymphoid cells within the submucosal layers, and marked tracheitis (Abd El-Ghany, 2023). In the present study all these findings suggest a more complex disease progression when bacterial and viral co-infections accompany aMPV, leading to exacerbated inflammatory responses and tissue damage (Mernizi *et al*., 2023). So field infections involving secondary bacterial or viral pathogens which intensify these changes and contribute to more severe morbidity and mortality rates (Suarez *et al.*, 2020).

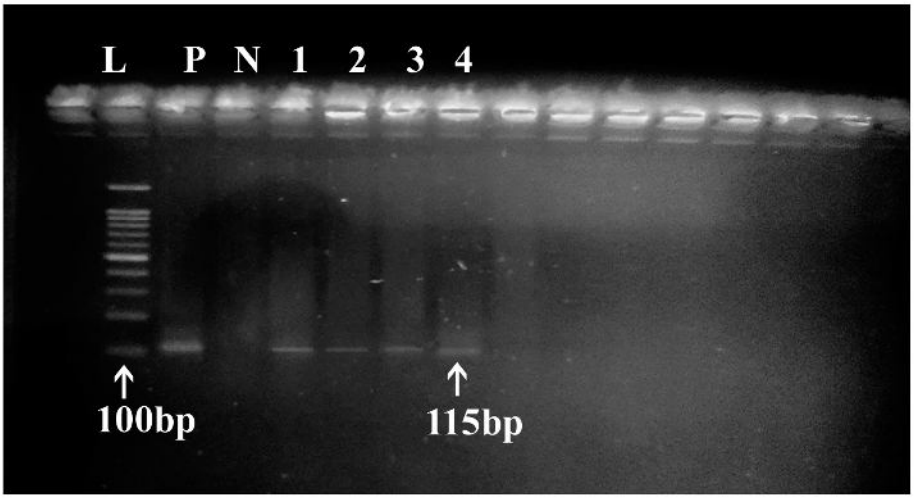

Plate 4.1 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1, 2, 3 and 4 positive sample for aMPV (115 bp, N gene) along with lane P- positive control, N-negative control and L-ladder 100bp

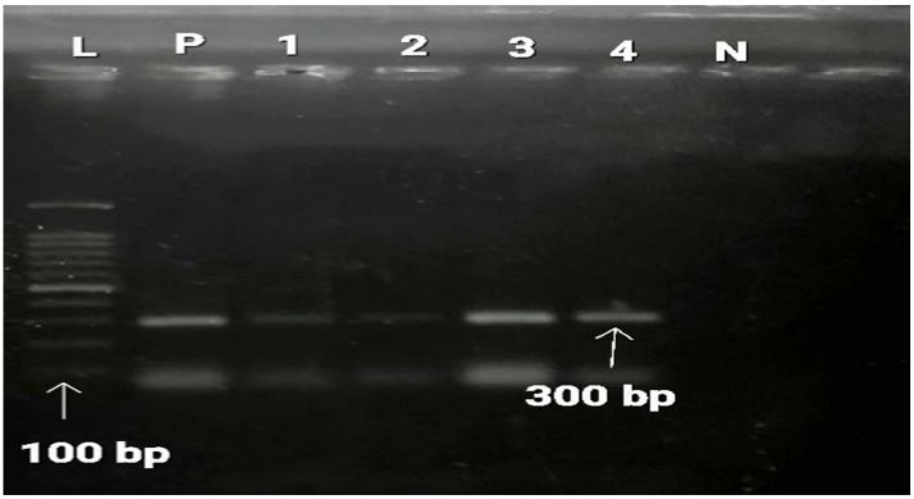

Plate 4.2 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1, 2, 3, and 4 positive sample for MG (300bp, Mgc2 gene) along with lane P- positive control, N-negative control and L-ladder 100bp

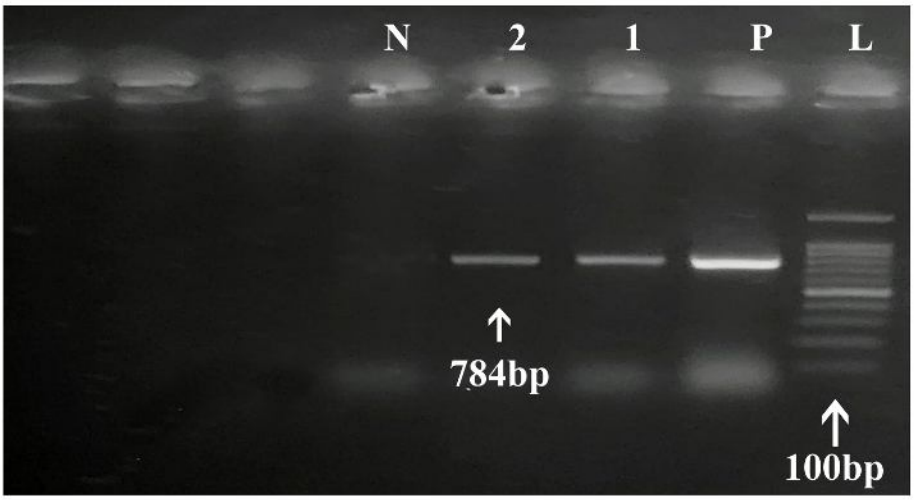

Plate 4.3 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1 and 2 positive sample for ORT(784bp, 16S rRNA) along with lane P- positive control, N-negative control and L-ladder 100bp

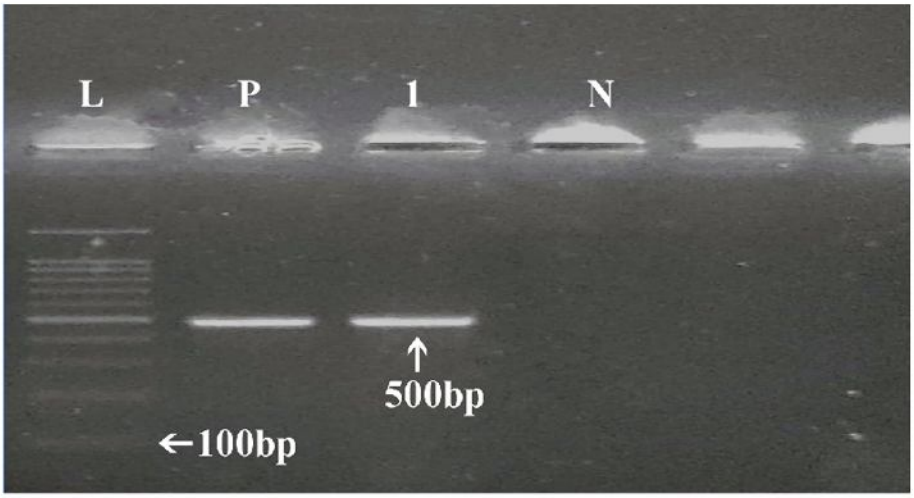

Plate 4.4 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for IC(500bp, HPG-2 gene) along with lane P- positive control, N-negative control and L-ladder 100bp

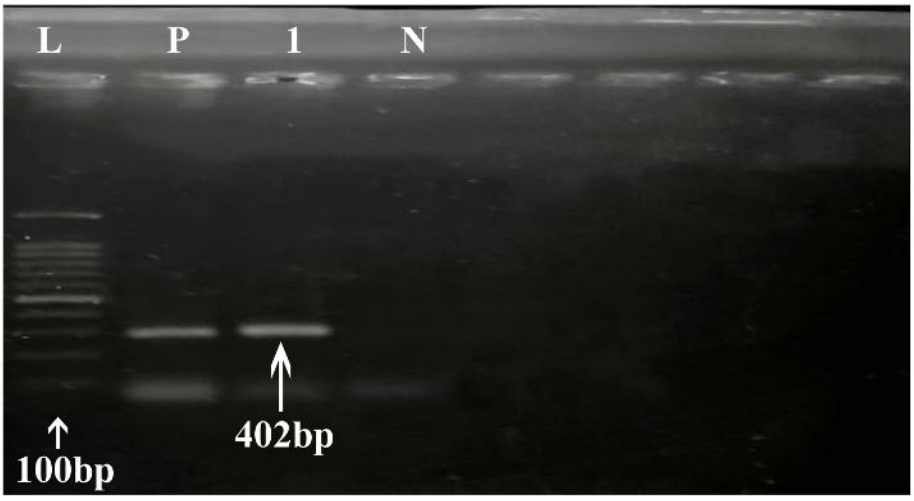

Plate 4.5 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for IBV(402bp, N gene) along with lane P- positive control, N-negative control and L-ladder 100bp

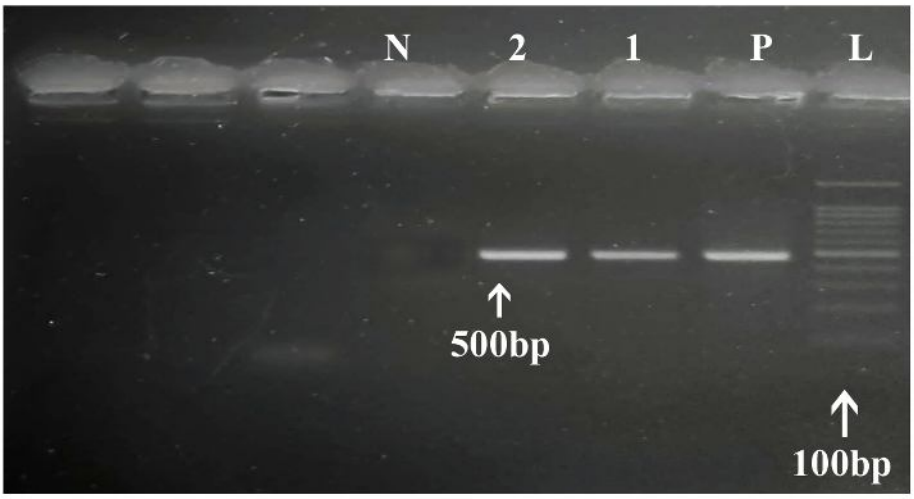

Plate 4.6 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1 and 2 positive sample for APEC(500bp, ecp gene) along with lane P- positive control, N-negative control and L-ladder 100bp

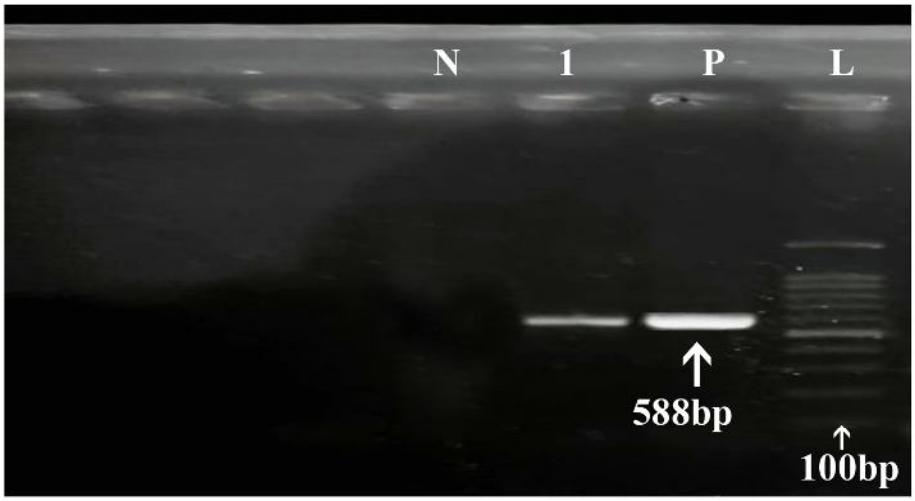

Plate 4.7 : Agarose gel electrophoresis stained with ethidium bromide showing lane 1 positive sample for ILTV (588bp, p32 gene) along with lane P- positive control, N-negative control and L-ladder 100bp

Table 4.1 Incidence of aMPV and associated pathogens with concurrent infection.

aMPV and associated pathogens with concurrent infection	Infected flock (%)
aMPV + MG	3 (37.5)
aMPV + MG + IBV	1 (12.5)
aMPV+ MG + APEC	1 (12.5)
aMPV + MG + ILTV	1 (12.5)
aMPV + IC + ORT	1 (12.5)
aMPV + ORT + APEC	1 (12.5)

4.2 Clinical signs

In the present study, all flocks infected with aMPV and co-infected with MG exhibited variable clinical signs, including mild to moderate nasal discharge, ocular discharge, dyspnea, and anorexia. In some cases, affected birds presented with severe nasal discharge, while others showed mild to moderate conjunctivitis and sneezing. These findings align with previous reports by Majo *et al*. (1995) and Panigrahy *et al*. (2000) in turkeys infected with aMPV, as well as studies in chickens infected with MG (Prajapati *et al*., 2018; Karthik *et al*., 2018; Islam *et al*., 2011). However, the severity of clinical signs observed in the present study was generally milder as compared to these reports. Flocks concurrently infected with aMPV, ORT, and IC exhibited more severe clinical signs, including swollen sinuses, severe dyspnea, gasping, profuse nasal discharge, depression, weight loss, and reduced feed intake. These observations are consistent with the findings by Jirjis *et al*. (2002) in turkeys and Georgiades *et al*. (2001) in broilers infected with aMPV. Similar clinical manifestations have been reported in cases of IC (Akter *et al*., 2013; Dwivedi *et al*., 2018) and ORT infections (Rahimi and Banani, 2007; Murthy *et al*., 2008).

Additionally, a flock co-infected with aMPV, MG, and IBV exhibited tracheal rales, gasping, sneezing, coughing, anorexia, and swollen periorbital and

infraorbital sinuses. These findings are in agreement with previous reports by Droual and Woolcock (1994) and Salles *et al*. (2023) in aMPV-infected chickens, Kleven (2008) and Bagal *et al*. (2019) in MG-infected chickens, and Singh and Dash (2008) and Dolz *et al*. (2012) in IBV-infected flocks. Another aMPV-affected flock, co-infection with ORT and APEC resulted in clinical signs such as swollen heads, severe dyspnea, swollen periorbital and infraorbital sinuses filled with caseous exudate, gasping, respiratory rales, and mucinous nasal exudates. These clinical manifestations correspond with findings from experimental aMPV infections in turkeys (Jirjis *et al*., 2004; Rubbenstroth *et al*., 2009) and reports of ORT and APEC infections in poultry (Rahimi and Banani, 2007; Murthy *et al*., 2008; Sojka and Carnavan, 1961; Bajwa *et al*., 1992). Notably, the severity of clinical signs in the present study was greater than those described in previous reports.

Similarly, flocks co-infected with aMPV, APEC, and MG exhibited lethargy, moderate nasal discharge, anorexia, and respiratory distress. These findings are in accordance with earlier studies on experimental co-infections of aMPV and APEC in turkeys (Majo *et al*., 1997) and chickens (Salles *et al*., 2023), as well as MG infections in poultry (Bagal *et al*., 2019; Islam *et al*., 2011). Flocks co-infected with aMPV, MG, and ILTV demonstrated severe respiratory distress, coughing, nasal discharge, conjunctivitis, swollen infraorbital and periorbital sinuses filled with mucopurulent exudates, and foamy eyes. These clinical signs are consistent with findings reported by Jirjis *et al*. (2002) and Salles *et al*. (2023) in aMPV-infected poultry, as well as previous studies on MG (Ley and Yoder, 2008; Islam *et al*., 2011; Karthik *et al*., 2018; Prajapati *et al*., 2018) and ILTV infections (Couto *et al*., 2016). Compared to prior studies, the severity of clinical signs in the present study was moderate.

Flocks infected with aMPV are highly susceptible to secondary bacterial infections, which contribute to the emergence of severe clinical signs, particularly swollen periorbital and infraorbital sinuses. Majo *et al*. (1997) attributed this to the direct impact of aMPV infection, in combination with factors such as individual

bird susceptibility, high population density, elevated ammonia levels, and inadequate ventilation. Diagnosing respiratory diseases in poultry based solely on clinical signs remains a challenge, as multiple respiratory pathogens can produce overlapping symptoms. Kaore *et al*. (2018) emphasized that respiratory diseases in poultry exhibit nearly identical clinical signs, necessitating pathological, molecular and serological diagnostic methods for accurate differentiation.

4.3 Pathology of aMPV infection and associated pathogens

Although aMPV infection causes milder upper respiratory tract infection, severe lesions were recorded in the current study which could be attributed to concurrent infections of multiple respiratory pathogens (Marien *et al*., 2005). This indicates that multiple pathogens interact synergistically, amplifying the inflammatory response and causing greater tissue damage. The presence of multiple pathogens may also weaken the immune system, accelerating disease progression and contributing to higher mortality rates (Umar, 2017). The pathological alterations observed in the current study from aMPV affected birds in association with other pathogens are as follows:

4.3.1 aMPV and MG

In the present study, three flocks were identified with concurrent infections of aMPV and MG. Among the affected birds, some exhibited purulent nasal discharge (Plate 4.8). Gross examination revealed congestion and mild edema of the nasal turbinates in several birds (Plate 4.9), while a few displayed only congestion. The birds from the one flock presented with severe conjunctivitis and erythema of the head (Plate 4.10). In birds from two flocks, caseous plugs were observed in the tracheal lumen, accompanied by mild tracheal congestion (Plate 4.11). Additional lesions included airsacculitis and pulmonary congestion with edema and focal hemorrhages (Plate 4.12). In severely affected birds from one flock, fibrinous perihepatitis and pericarditis were noted, characterized by a pale yellow fibrinous exudate covering the heart and liver, along with concurrent airsacculitis (Plate 4.13). These findings align with previous studies by Hristova

and Petrova (2007), who reported co-infections involving chicken anemia virus (CAV), MG, aMPV, and avian reovirus in fancy chicken breeds. Also, Jirjis *et al*. (2002) studied the pathogenesis of aMPV infection in turkeys and reported comparable lesions. In a similar line milder lesions were described by Catelli *et al*. (1998) in aMPV affected chickens and Cook *et al*. (1991) in turkey rhinotracheitis virus (TRTV) affected turkeys. Similarly, Bagal *et al*. (2019) documented pathological changes associated with MG infections in broiler chickens from western Maharashtra. Roussan *et al*. (2008) conducted a molecular survey on avian respiratory pathogens in Jordanian broiler chickens, describing severe lesions linked to multiple respiratory pathogens, including H9N2, NDV, IBV, aMPV, and MG.

Microscopically, aMPV and MG co-infected birds revealed variable pathological lesions across multiple organs. Nasal turbinates were markedly congested in all the chickens with mild to moderate disruption of the respiratory epithelium and mononuclear cell infiltration within the mucosa and lamina propria, while two birds revealed a fibrinonecrotic exudate in the middle nasal chamber (Plate 4.14). The tracheal sections exhibited epithelial sloughing, and loss of mucous glands along with submucosal edema (Plate 4.15). Lung sections showed marked capillary congestion, perivascular edema, and thrombus formation and infiltration of inflammatory cells (Plate 4.16). Section of the lung from one bird showed fibrinocellular exudate filling the parabronchial lumen, along with marked capillary congestion (Plate 4.17). Heart sections exhibited mild to moderate congestions accompanied by focal necrosis with leukocytic infiltration between muscle fibers in two sections (Plate 4.18). In the spleen sections, severe congestion, lymphocyte depletion, and necrosis were observed (Plate 4.19). The lesions observed in nasal turbinate and trachea are in agreement with previous reports of Panigrahy *et al.* (2000) in experimentally aMPV inoculated turkeys and Hristova and Petrova (2017) in aMPV, CAV and MG concurrently infected fancy breed chickens. Furthermore Bagal *et al.* (2019) and Couto *et al.* (2016) observed similar lesions in the lung, heart and spleen from MG infected chickens. The severity of lesions in the present study was more pronounced compared to those of earlier

Plate 4.8 : Chicken showing whitish purulent nasal discharge upon pressing the nostrils

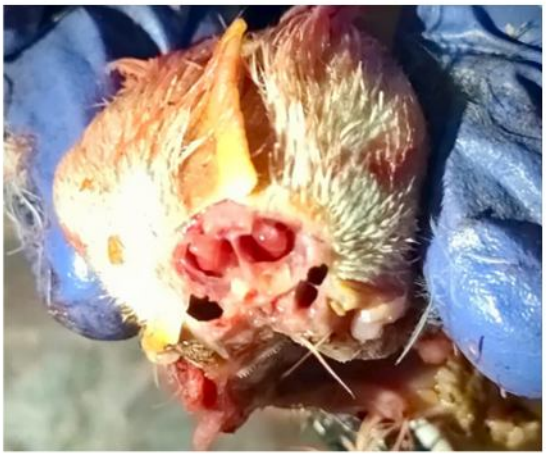

Plate 4.9 : Cross section of nasal cavity showing marked congestion and edema

Plate 4.10 : Bird showing severe conjunctivitis along with erythema

Plate 4.11: Bird showing presence of yellowish cheesy caseous exudate in the tracheal lumen

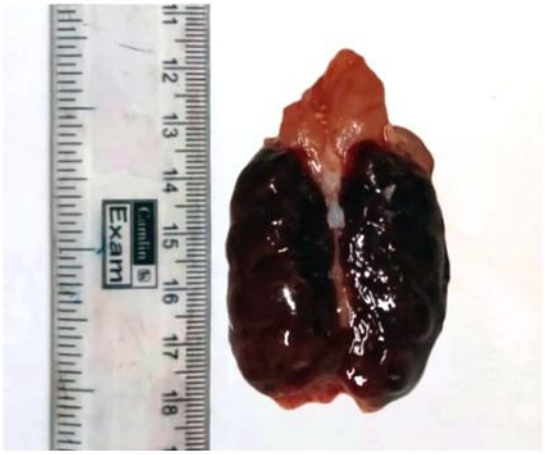

Plate 4.12 : Lungs showing dark reddish consolidation, congestion and edema with focal areas of hemorrhages

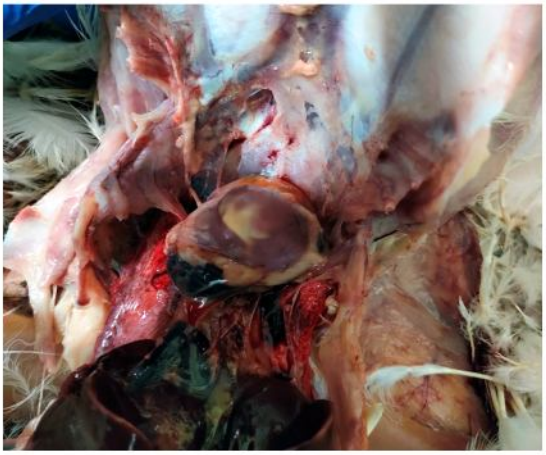

Plate 4.13 : Presence of pale yellowish fibrinous covering over heart and liver indicating fibrinous perihepatitis and pericarditis along with airsacculitis

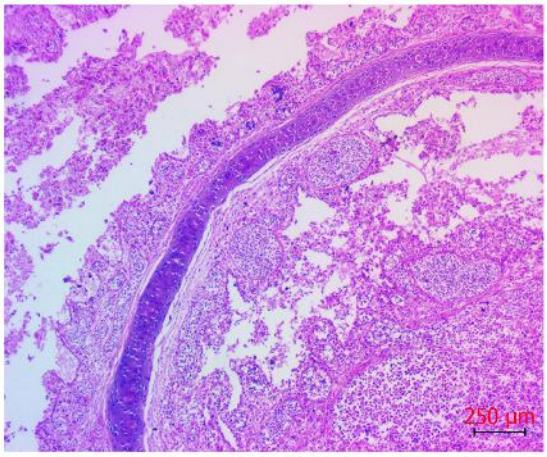

Plate 4.14 : Microphotograph of the middle nasal chamber showing fibrinonecrotic exudate filling the lumen, along with disruption of the respiratory epithelium and infiltration of mononuclear cells in the lamina propria and submucosa (H&E, 100x)

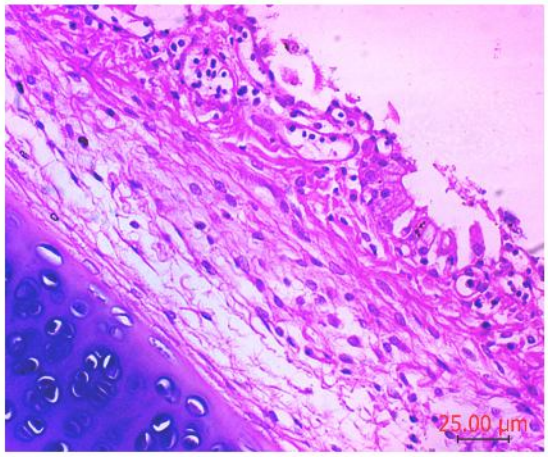

Plate 4.15 : Microphotograph of the trachea showing mucosal epithelial necrosis and sloughing, loss of mucous glands, and prominent submucosal edema (H&E, 400x)

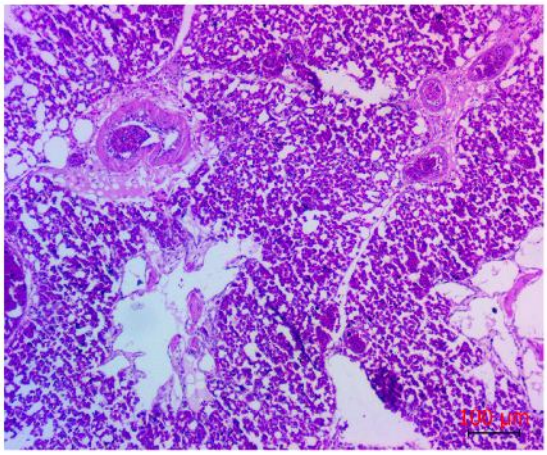

Plate 4.16 : Microphotograph of the lung showing marked infiltration of inflammatory cells, perivascular edema, and vascular thrombi (H&E, 100x)

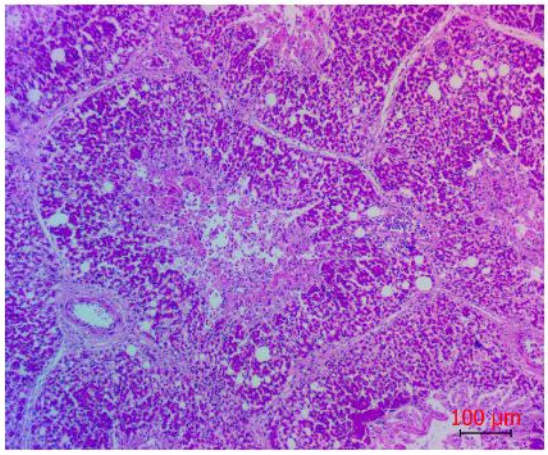

Plate 4.17 : Microphotograph of the lung showing fibrinocellular exudate filling the parabronchial lumen, along with marked capillary congestion (H&E, 100x)

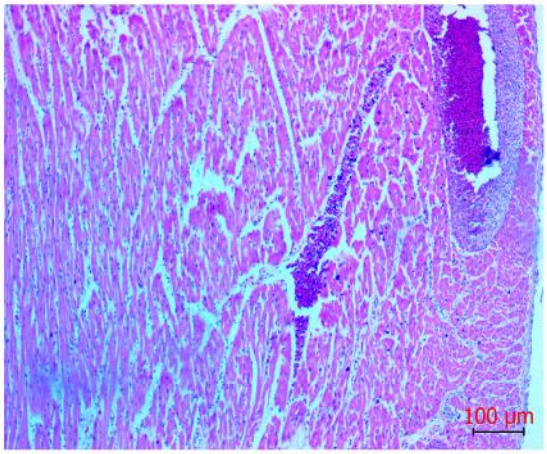

Plate 4.18 : Microphotograph of heart showing focal necrosis and leukocytic infiltration in between the muscle fibers (H&E,100x)

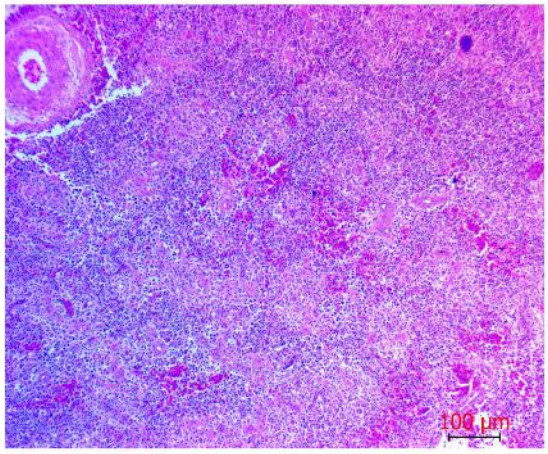

Plate 4.19 : Microphotograph of spleen showing congestion, depletion of lymphocytes and multifocal necrosis (H&E, 100x)

findings. Such severe lesions are likely due to the synergistic effect of aMPV + MG dual infection, which alters pathogen replication dynamics, accelerates lesion development, and intensifies host immune responses (Ruger *et al.,* 2021).

4.3.2 aMPV, ORT and IC

Birds from one flock infected with aMPV and co-infected with ORT and IC exhibited gross lesions with more severity. Notable findings included pronounced periorbital swelling with caseous exudates (Plate 4.20). A cross-section of the nasal cavity in those birds revealed marked congestion and the presence of cheesy flakes qq2within the lumen (Plate 4.21). The trachea contained mucoid exudates, accompanied by mucosal congestion (Plate 4.22). Additionally, fibrinous airsacculitis was observed, along with multiple areas of pulmonary consolidation and severe congestion (Plate 4.23). These findings are consistent with those reported by Murthy *et al*. (2008) in commercial layers infected with ORT and IC in Tamil Nadu, India., and Marien *et al*. (2005) who investigated the combined effects of aMPV and ORT in turkeys through experimental inoculation, highlighting the exacerbation of respiratory lesions in co-infected birds. The severity of lesions in the present study likely reflects the cumulative effects of these pathogens, which intensify tissue damage and accelerate disease progression.

Histopathological evaluation of birds infected with aMPV and co-infected with ORT and IC revealed a pronounced lesion in the nasal turbinate. Microscopic analysis of this region showed extensive epithelial damage, congestion of blood vessels, and a dense accumulation of mononuclear cells within the lamina propria. These findings indicate chronic inflammation consistent with lymphoplasmacytic rhinitis (Plate 4.24). The trachea exhibited mild to moderate multifocal epithelial degeneration, submucosal edema and infiltration of inflammatory cells (Plate 4.25). Lung sections showed lymphoid hyperplasia in peribronchiolar regions (Plate 4.26), while some lung sections displayed fibrin-rich exudates within the parabronchial lumen, accompanied by infiltration of mononuclear cells and increased fibrous tissue proliferation (Plate 4.27). Certain lung areas displayed parabronchiolar necrosis, along with inflammatory cell infiltration in both the

lamina propria and surrounding bronchial structures (Plate 4.28). The liver presented significant periportal mononuclear cell infiltration along with focal necrotic areas (Plate 4.29). These histopathological findings are in line with previous research by Panigrahy *et al*. (2000) who studied the pathogenesis and serological response in turkeys experimentally infected with aMPV, and Jirjis *et al*. (2004) who examined how ORT co-infection influenced the progression of aMPV in turkeys. Similarly, Dwivedi *et al*. (2018) investigated the pathology of infectious coryza in birds in Jabalpur, while Singh *et al*. (2013) experimentally inoculated field isolate of IC, revealed similar pathological lesions. Additionally, Marien *et al*. (2005) assessed the combined effects of aMPV, MG, ORT, and APEC in turkeys through experimental inoculation, revealing moderate respiratory lesions. aMPV primarily affects ciliated epithelial and goblet cells in the upper respiratory tract, including the nasal cavity, trachea, and sinuses. This infection causes cellular damage and necrosis in key respiratory tissues, creating an environment conducive to secondary bacterial pathogens such as ORT and IC. These pathogens can invade, colonize, and exacerbate disease progression, leading to lesion development in multiple organs (Ruger *et al.,* 2021; Al-Hially *et al*., 2023). The lung lesions observed in the present study are likely associated with ORT infection, as this bacterium produces endotoxins and proteases that contribute to coagulative necrosis of respiratory tissues. ORT also forms biofilms, allowing it to adhere firmly to damaged respiratory epithelium, ultimately leading to fibrinous pneumonia by promoting persistent inflammation and tissue damage (Kaore *et al* ., 2018; Castro *et al.,* 2019).

4.3.3 aMPV, MG and IBV

aMPV, MG and IBV concurrently infected birds exhibited muco-purulent nasal discharge, periorbital swelling, and pronounced conjunctivitis (Plate 4.30). The trachea contained fibrinous exudates within the lumen, accompanied by mild mucosal congestion (Plate 4.31). The lungs displayed marked congestion, edema, and focal areas of consolidation (Plate 4.32). The spleen was enlarged, congested, with mottled appearance (Plate 4.33). These lesions align with the findings of

Plate 4.20 : Bird showing marked periorbital swelling with presence of cheesy exudates

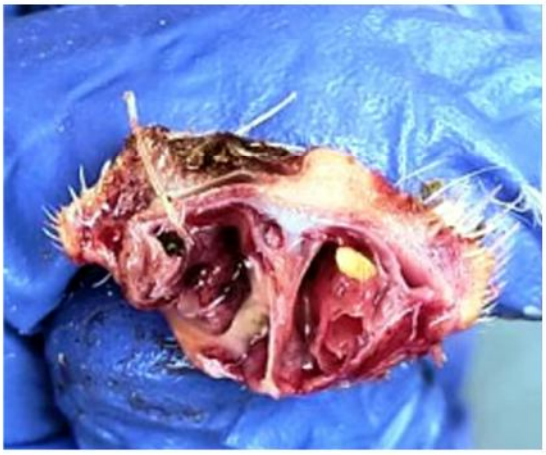

Plate 4.21 : Cross section of nasal cavity showing marked congested and cheesy flakes in the lumen

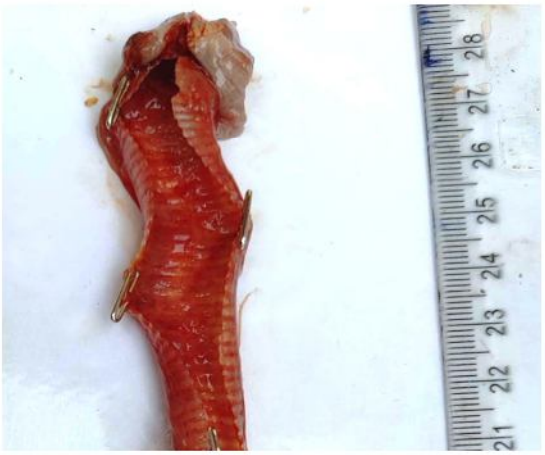

Plate 4.22 : Trachea showing mucoid exudates in the lumen and congestion of tracheal mucosa

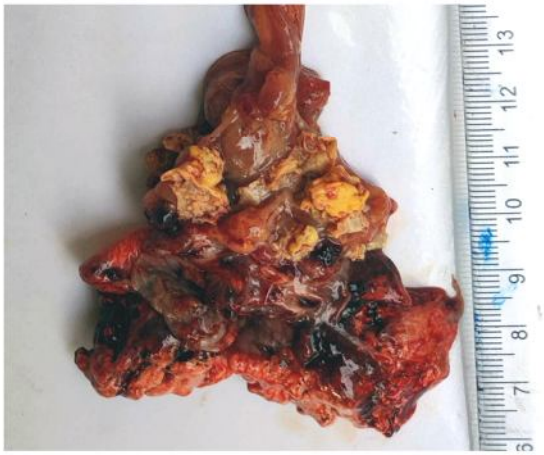

Plate 4.23 : Lung showing fibrinous airsacculitis with multiple areas of consolidation and severe congestion

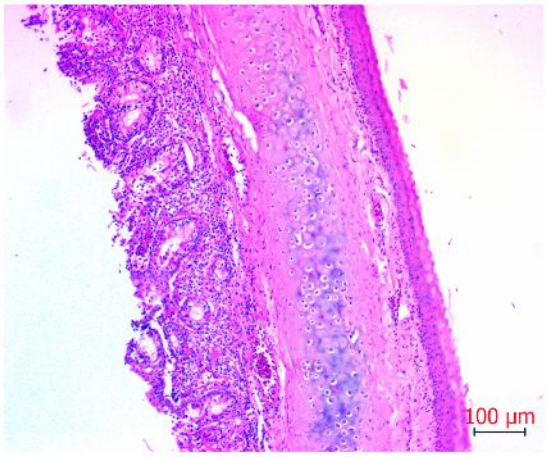

Plate 4.24 : Microphotograph of the nasal turbinate demonstrating epithelial necrosis, marked vascular congestion, and a dense mononuclear cells infiltrate in the lamina propria, consistent with chronic lymphoplasmacytic rhinitis (H&E, 100x).

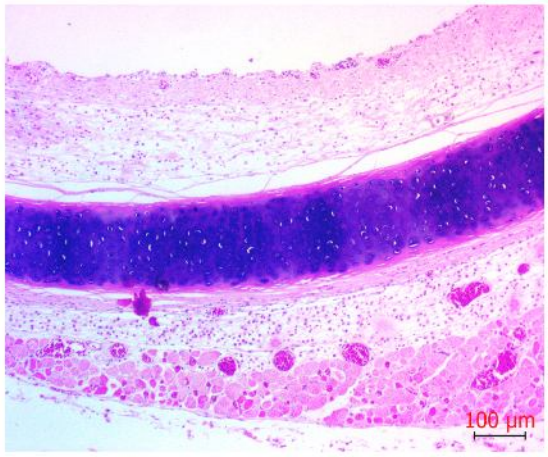

Plate 4.25 : Microphotograph of the trachea showing marked necrosis with submucosal edema and inflammatory cells infiltration (H&E, 100x)

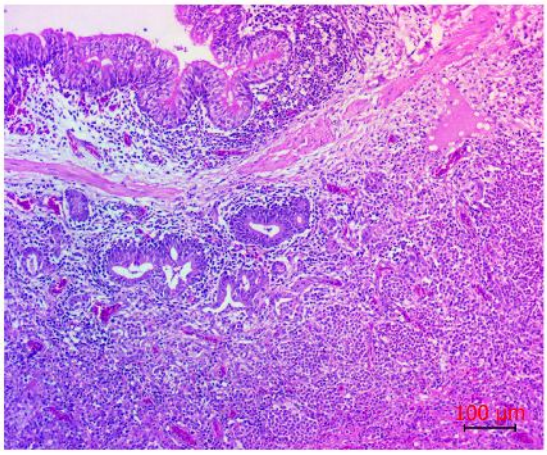

Plate 4.26 : Microphotograph of the lung showing lymphoid hyperplasia in the peribronchiolar area (H&E, 100x)

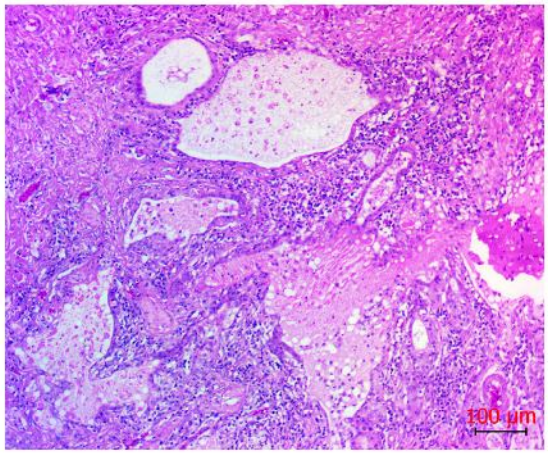

Plate 4.27 : Microphotograph of lung showing fibrinocellular exudate in para-bronchial lumen with mononuclear cells infiltration and fibrous connective tissue proliferation (H&E, 100x)

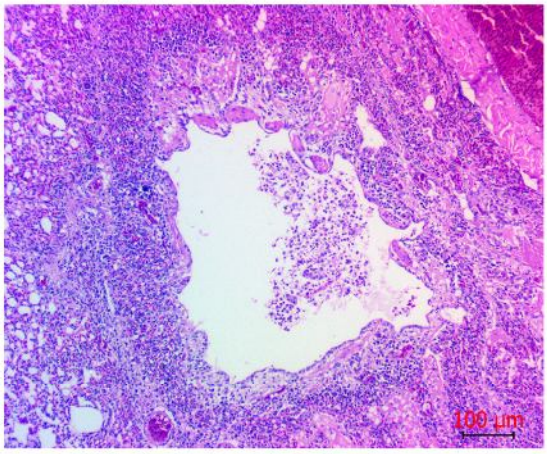

Plate 4.28 : Microphotograph of lung showing parabronchiolar necrosis and mononuclear cells infiltration in the lamina propria and peribronchiolar area (H&E, 100x)

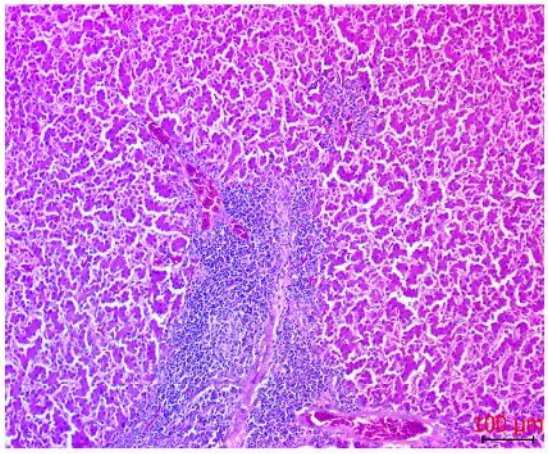

Plate 4.29 : Microphotograph of liver showing marked periportal mononuclear cell infiltration and areas of focal necro-sis (H&E, 100x)

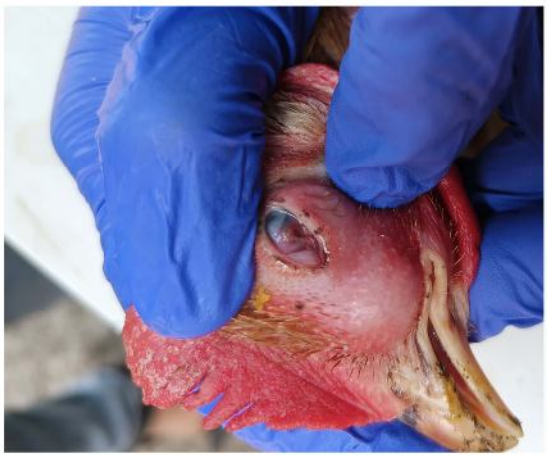

Plate 4.30 : Chicken showing severe unilateral periocular swelling with marked erythema and severe conjunctivitis

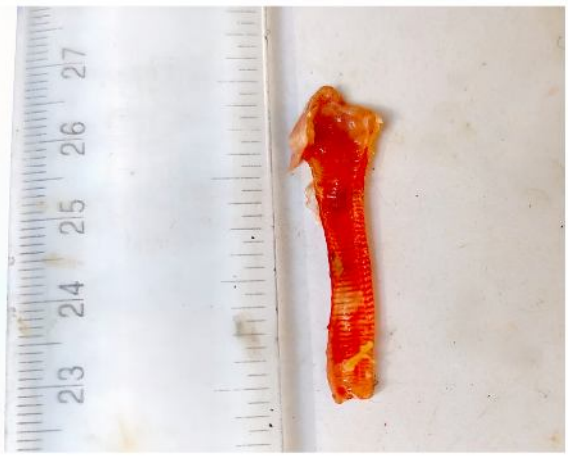

Plate 4.31 :Trachea showing fibrinous exudates in the lumen with mild congestion of mucosa

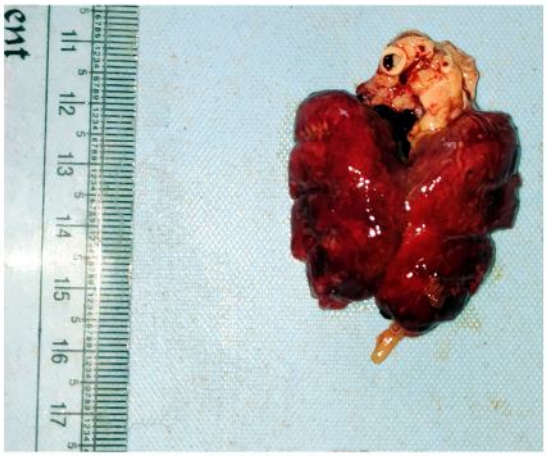

Plate 4.32 : Lung with marked congestion, edema and focal areas of consolidation

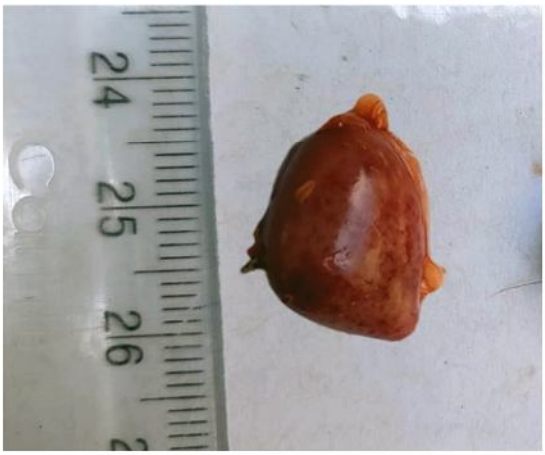

Plate 4.33 : Enlarged and congested spleen with mottled appearance

Roussan *et al*. (2008) who reported severe damage associated with multiple respiratory pathogens during a molecular survey of avian respiratory diseases (AIV, IBV, NDV, APV, and MG) in commercial broiler chickens in Jordan. Similarly, Aung *et al*. (2008) observed comparable but milder lesions in turkeys experimentally infected with aMPV. The study of Bagal *et al*. (2019), Kleven (2008), Gharaibeh and Al-Roussan (2008), and Islam *et al*. (2011) on the pathology of MG in chickens reported similar but less severe lesions. Additionally, chicken with IBV and co infected with IBV, MG exhibited comparable milder lesions in the affected respiratory organs (Dolz *et al*., 2012; Kaore *et al*., 2018) respectively. Microscopically, it revealed extensive necrosis of epithelial cells and mucous glands in the nasal turbinates accompanied by mononuclear infiltration in the lamina propria (Plate 4.34) and the nasal chamber was filled with necrotic exudates along with the destruction of the epithelium of nasal conchae (Plate 4.35). The trachea showed thickening of tracheal mucosa due to edema and mononuclear cell infiltration in lamina propria (Plate 4.36). The lung tissue revealed the presence of fibrino cellular exudate in the bronchiolar and parabronchial lumen (Plate 4.37). Spleen tissue indicated a thickened capsule with multifocal areas of necrosis and heterophilic infiltration (Plate 4.38). Liver sections had diffuse congestion, hepatocellular necrosis and heterophilic infiltration (Plate 4.39). All these histopathological changes of nasal turbinate, trachea and lung are in conformity with that described by Majo *et al.* (1995) in broiler breeder and turkey poults, experimentally infected with Turkey Rhinotracheitis Virus (TRTV/aMPV); Catelli *et al*. (1998) in experimentally inoculated aMPV chicken from UK and Hristova and Petrova (2017) in MG, aMPV coinfected fancy chicken breeds. The lesions in the lung and liver observed in the present study were also recorded in MG infected chickens by Thilagavathi *et al*. (2016), Brar *et al*. (2017) and Karthik *et al*. (2018). In comparison to the present study, previous investigations collectively reported relatively milder to moderate histopathological lesions, indicating a more severe disease manifestation in the current study. aMPV, MG and IBV show a similar tropism for ciliated cells of the respiratory tract. So that all target the ciliated respiratory epithelium, impair mucociliary clearance, and induce pro-inflammatory

responses, creating synergistic damage, while suppressing interferon-mediated defenses (Hoerr *et al*., 1994; Ruger *et al*., 2021). Also, the damage caused by IBV is significant enough for MG to infiltrate the compromised tissues, resulting in widespread infection to multiple organs (Smith *et al.*, 1985).

4.3.4 aMPV, ORT and APEC

Birds infected with aMPV and co-infected with ORT and APEC exhibited distinct gross pathological lesions. Notable findings included pronounced orbital enlargement due to the accumulation of thick, caseous exudates (Plate 4.40). The tracheal lumen contained mucinous exudates, accompanied by mild mucosal congestion around the tracheal rings (Plate 4.41). Pulmonary lesions included congestion, consolidation, and the presence of a yellowish fibrinous layer on the lung surface (Plate 4.42). Additionally, fibrinous pericarditis was observed (Plate 4.43). These findings are consistent with those reported by Marien *et al*. (2005) in turkeys experimentally inoculated with aMPV and ORT. Similarly, Salles *et al*. (2023) conducted a molecular analysis of dual infections involving aMPV and APEC, documenting comparable pathological lesions. The observed severity of lesions in the present study suggests a synergistic effect of these pathogens, leading to exacerbated respiratory and systemic disease.

Microscopic examination of birds from a single flock infected with aMPV and co-infected with ORT and APEC revealed severe pathological changes. The nasal mucosa was severely congested and the middle nasal chamber was filled with fibrinonecrotic exudate and necrosis of mucosa, accompanied by mononuclear cell infiltration in the lamina propria (Plate 4.44). One bird revealed infraorbital sinus obliterated by fibrinonecrotic debris (Plate 4.45). Similar histopathological findings in the infraorbital sinuses and nasal chamber were previously reported by Aung *et al*. (2008) in experimental aMPV infection in turkeys and by Jirjis *et al*. (2004) in turkeys co-infected with aMPV and ORT. However, the lesions observed in these studies were comparatively milder than those in the present investigation. The tracheal mucosa and submucosa exhibited marked necrosis (Plate 4.46) with heterophilic infiltration. Lung sections revealed multifocal bronchiolar necrosis and

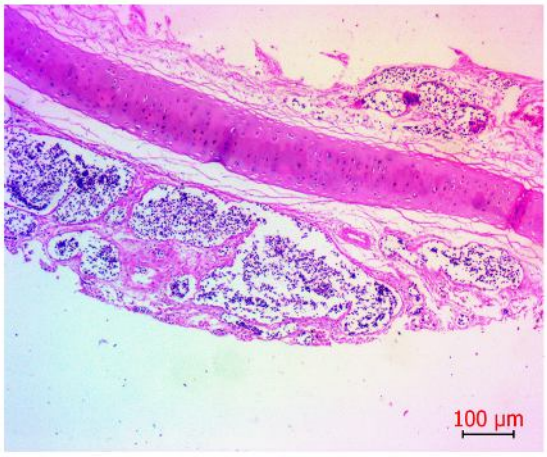

Plate 4.34 : Microphotograph of the nasal turbinate (concha) from the middle nasal chamber, demonstrating extensive necrosis of the epithelial cells and mucous glands, accompanied by infiltration of mononuclear cells (H&E, 100x)

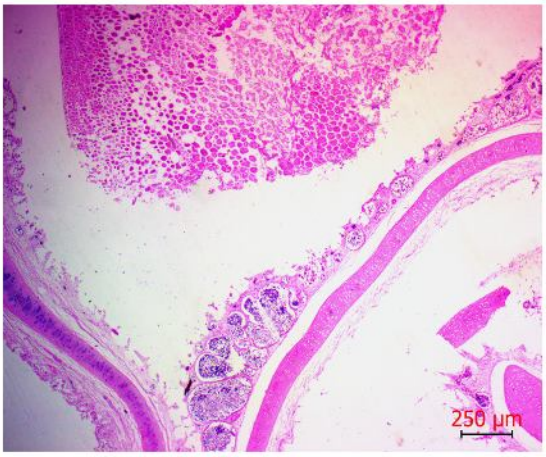

Plate 4.35 : Microphotograph of the middle nasal chamber showing necrotic exudate within the lumen, accompanied by extensive necrosis and loss of mucous glands and the nasal conchal mucosa (H&E, 40x)

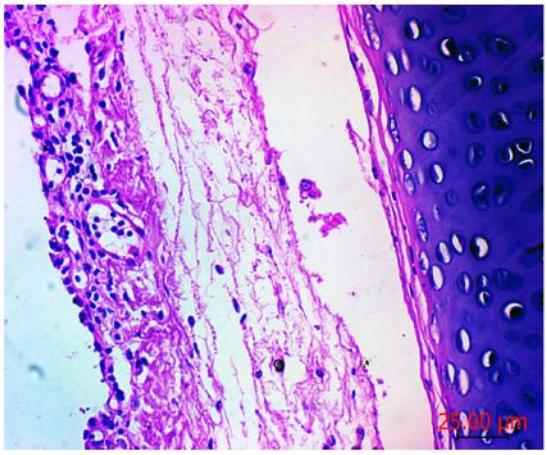

Plate 4.36 : Microphotograph of the trachea showing necrosis of the mucosal epithelium, infiltration of mononuclear cells, and edematous changes in the lamina propria (H&E, 400x)

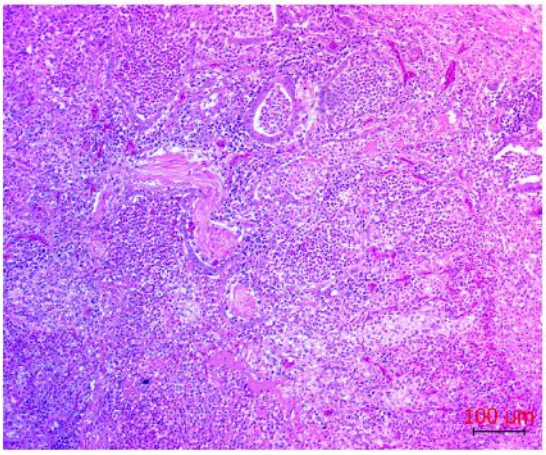

Plate 4.37 : Microphotograph of lung showing presence of fibrino cellular exudate in the bronchiolar and parabronchial lumen predominated with mononuclear cells (H&E, 100x)

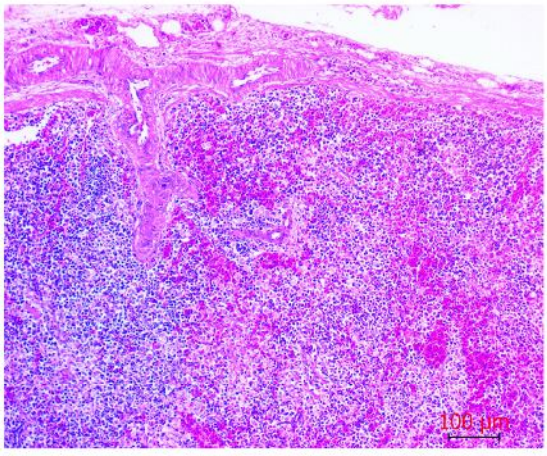

Plate 4.38 : Microphotograph of the spleen showing a thickened capsule with multifocal areas of necrosis and infiltration of heterophils (H&E, 100x).

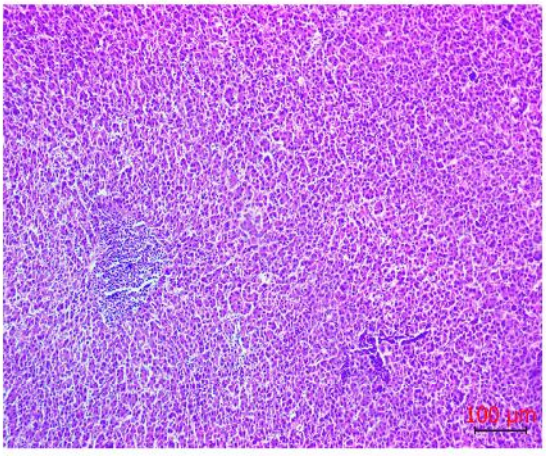

Plate 4.39 : Microphotograph of the liver showing mild vascular congestion, focal areas of hepatocellular necrosis, and infiltration of heterophils and lymphocytes (H&E, 100x)

Plate 4.40 : Bird showing severe focally extensive unilateral periorbital swelling, erythema and presence of cheesy exudate in the lumen.

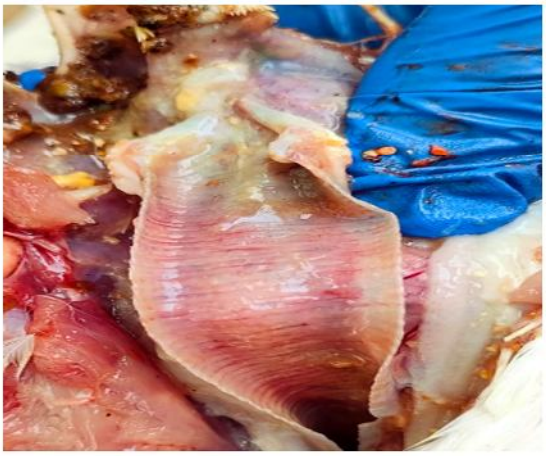

Plate 4.41 : Trachea : tracheal lumen showing mucinous exudate and mild mucosal hyperemia adjacent to the carti-laginous rings.

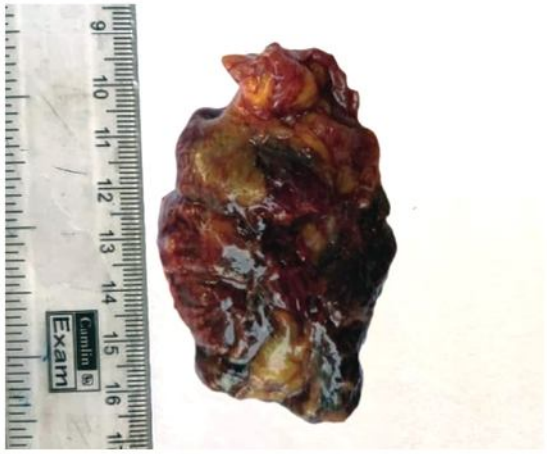

Plate 4.42 : Lung showing severe, multifocal coalescing consolidation with firm consistency, necrotizing foci, and fibrinous exudation.

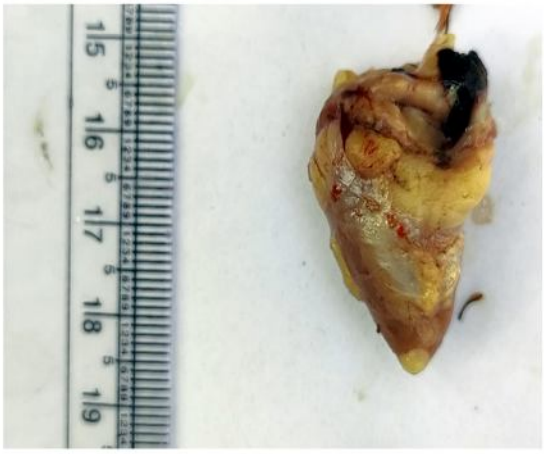

Plate 4.43 : Fibrinous pericarditis : presence of yellowish fibrinous layer over pericardium.

fibrinocellular exudate in the lumen (Plate 4.47). Further, fibrinous pericarditis (Plate 4.48) and thickening of the hepatic capsule due to the presence fibrino-cellular exudates (Plate 4.49) and multifocal areas of necrosis in the liver were consistent in the affected chicken. Comparable findings in these organs were also noted in the turkeys co-infected with aMPV, ORT and APEC (Jirjis *et al.*, 2004), the heart and liver lesions in the present study are in accordance with Riaz *et al.* (2016) documented the pathological effects of APEC in broiler birds. The aMPV invades host cells by binding to sialic acid receptors via its attachment glycoprotein (G), leading to viral replication, cellular damage, and necrosis in the upper respiratory tract. By suppressing host immunity, aMPV paves the way for secondary bacterial infections like ORT and APEC, which exacerbate disease severity. As a result of their combined viral and bacterial pathogenicity, these bacteria subsequently spread through bacteremia, resulting in serious lesions in several organs (Ruger *et al.,* 2021; Al-Hially *et al*., 2023).

4.3.5 aMPV, APEC and MG

Birds infected with aMPV and co infected with APEC, MG showed gross lesions such as purulent exudate oozing from the nostrils (Plate 4.50) and caseous cheesy exudates in the nasal turbinates (Plate 4.51). The trachea had fibrinous exudates within the lumen, along with congestion of mucosa around the tracheal rings (Plate 4.52). Additionally, fibrinous pericarditis was observed, characterized by a yellowish fibrinous layer covering the heart, along with hepatomegaly (Plate 4.53). These findings align with the observations of Shah *et al*. (2019), who investigated the pathology of APEC in broilers. Additionally, Majo *et al*. (1995) reported similar lesions following experimental inoculation of turkey rhinotracheitis virus (TRTV/aMPV) in chickens, while Riaz *et al*. (2016) documented comparable pathological changes in APEC-infected broilers. However, the severity of lesions observed in the present study was comparatively milder than those reported by Salles *et al*. (2023) and Nakamura *et al*. (1997) in aMPV and APEC concurrently affected chicken.

Histopathological analysis of birds co-infected with aMPV, APEC and MG, revealed the presence of necrotic luminal exudates along with submucosal vascular congestion in the nasal chamber (Plate 4.54). The trachea revealed mononuclear cell infiltration within the subepithelial tissue, accompanied by epithelial hyperplasia and degeneration (Plate 4.55). Another section of the trachea exhibited destruction of ciliated epithelium, necrosis, and the presence of necrotic exudates within the tracheal lumen (Plate 4.56). In the lungs, there was marked parabronchial necrosis and a complete loss of pulmonary architecture due to infiltration of inflammatory exudates (Plate 4.57). The heart showed a distended pericardium due to fibrinocellular exudates (Plate 4.58), while the liver demonstrated infiltration of inflammatory cells, multifocal areas of necrosis, and hemorrhages (Plate 4.59). The lesions observed in the nasal turbinate, trachea, and lungs are consistent with the findings of Cha *et al*. (2007) in turkeys inoculated with aMPV subtype C from Minnesota, and Hristova and Petrova (2017) in fancy chicken breeds co-infected with MG and aMPV. Similar pathological changes were also reported by Droual and Woolcock (1994) in cases of swollen head syndrome associated with APEC and IBV in California as well as by Majo *et al*. (1997) in chickens experimentally co-infected with TRTV/aMPV and *E coli* from Spain. Additionally, Brar *et al*. (2017) and Karthik *et al.* (2018) documented comparable findings in cases of MG mono-infection. The cardiac lesions observed in the present study are in line with the findings of Bajwa *et al.* (1992), Islam *et al.* (2011), and Kaore *et al.* (2018), who investigated dual infections of MG and APEC in chickens. Liver lesions observed in this study correspond with those reported by Baliarsingh *et al*. (1993) and Bhalerao *et al*. (2013) in birds with APEC infections. The distended pericardium, cardiac edema, necrosis, and hemorrhages in the liver observed in infected birds could be attributed to the chick-lethal toxin (CLT) produced by *Escherichia coli*. This toxin is known to cause vascular damage, increased capillary permeability, and tissue necrosis, leading to fluid accumulation in the pericardium, cardiac swelling, and severe hepatic lesions (Truscott *et al.*, 1974). The aMPV, MG and APEC combination showed extensive microscopic lesion due to synergistic interaction between aMPV, MG and APEC which

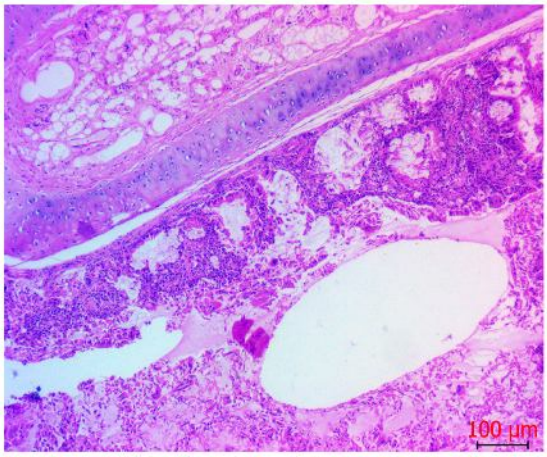

Plate 4.44 : Microphotograph of the middle nasal chamber of a chicken showing fibrinocellular exudate occluding the lumen, along with necrosis and mononuclear cell infiltration in the lamina propria (H&E, 100x)

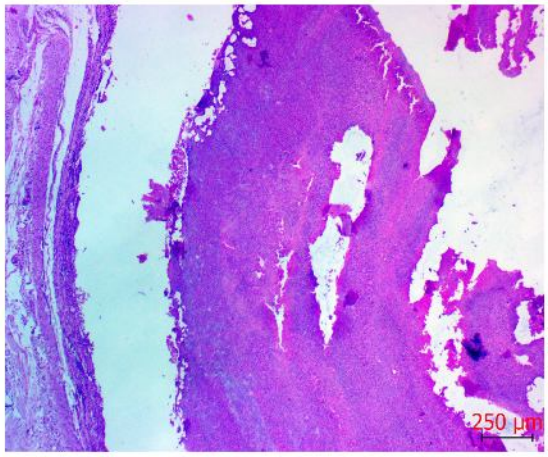

Plate 4.45 : Microphotograph of infraorbital sinus obliterated by the presence of fibrinous necrotic debris (H&E, 40x)

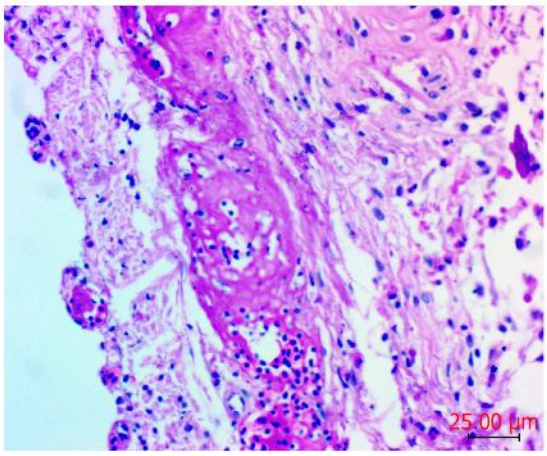

Plate 4.46 : Microphotograph of the trachea showing extensive mucosal and submucosal necrosis with inflammatory cell infiltration (H&E, 400x)

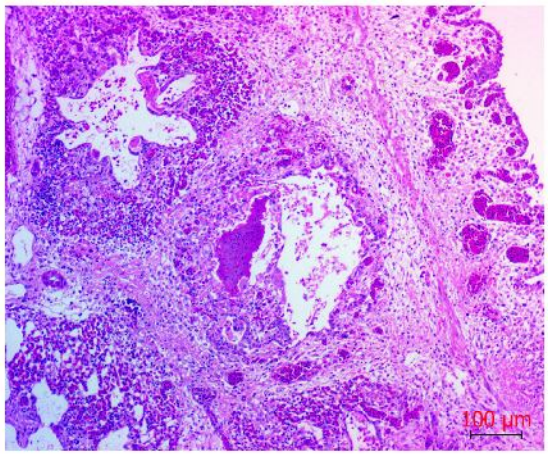

Plate 4.47 : Microphotograph of the lung showing bronchiolar necrosis, infiltration of mononuclear cells and presence of necrotic exudate in the lumen (H&E, 100x)

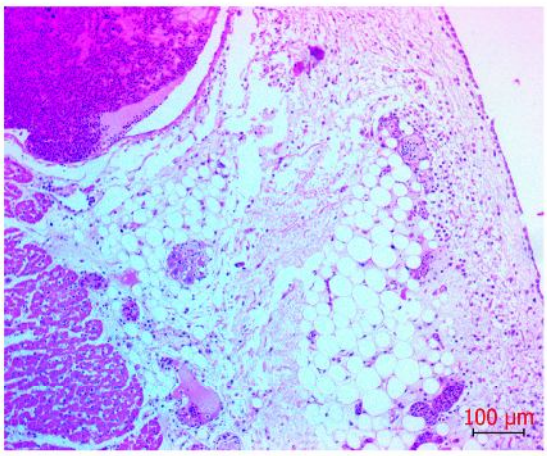

Plate 4.48 : Microphotograph of the of the heart showing fibrinous pericarditis and venous congestion (H&E,100x)

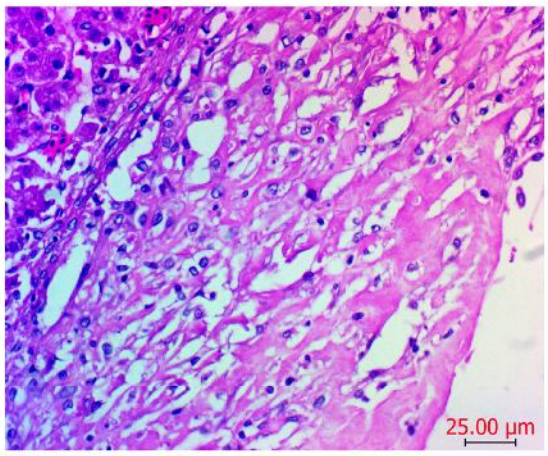

Plate 4.49 : Microphotograph of the liver showing thickened hepatic capsule due to presence fibrino-cellular exudates (H&E,400x)

Plate 4.50 : Bird showing purulent exudate oozing from nostrils

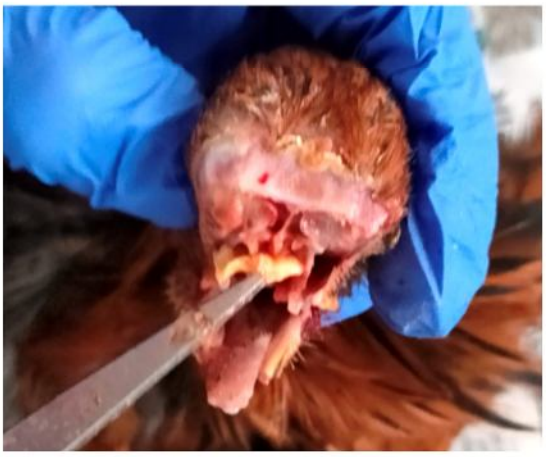

Plate 4.51 : Cross section of nasal cavity exhibiting caseous cheesy exudates

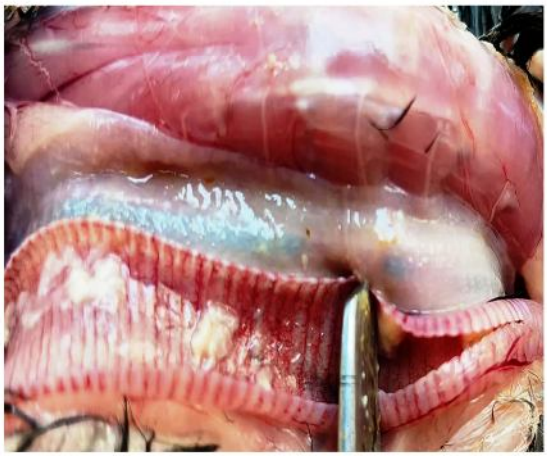

Plate 4.52 : Trachea : tracheal lumen containing fibrinous exudates and congestion of mucosa around tracheal rings

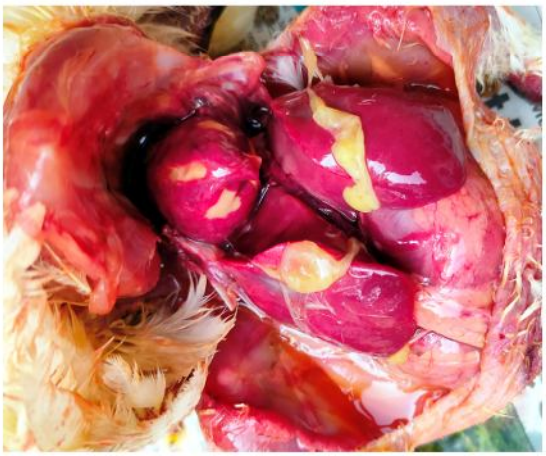

Plate 4.53 : Bird showing yellowish fibrinous layer over the surface of heart and liver and airsacculitis

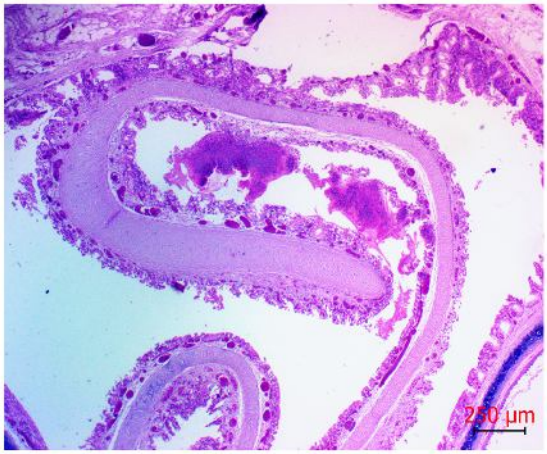

Plate 4.54 : Microphotograph of nasal concha from middle nasal chamber showing marked mucosal architecture effacement with luminal sloughing of necrotic cellular debris, submucosal vascular congestion, and inflammatory infiltrate (H&E, 40x)

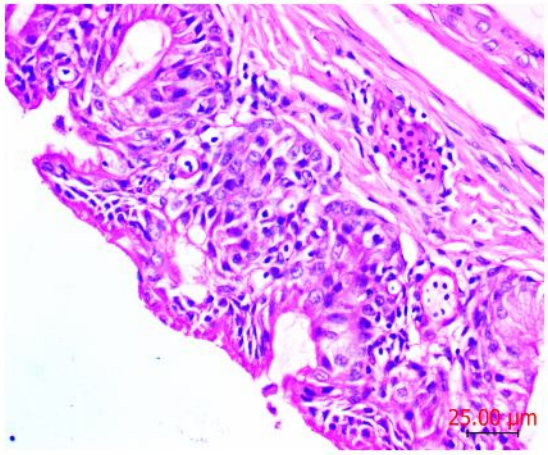

Plate 4.55 : Microphotograph of trachea showing mononuclear cell infiltration within the subepithelial tissue, accompanied by epithelial hyperplasia and degeneration, indicative of a chronic inflammatory response (H & E 400x)

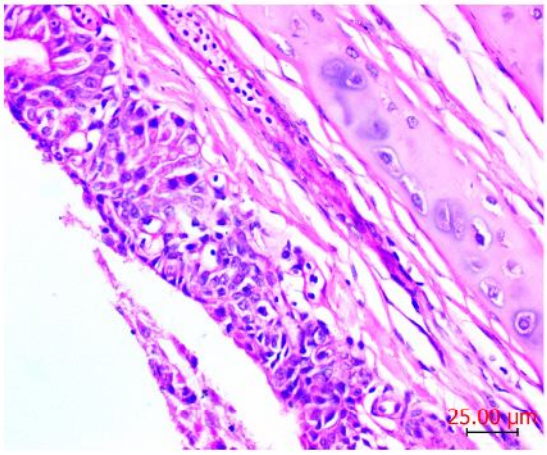

Plate 4.56 : Microphotograph of trachea showing disruption of ciliated epithelial cells with necrosis, mild hyperplasia and presence of necrotic exudate in the lumen (H&E 400x)

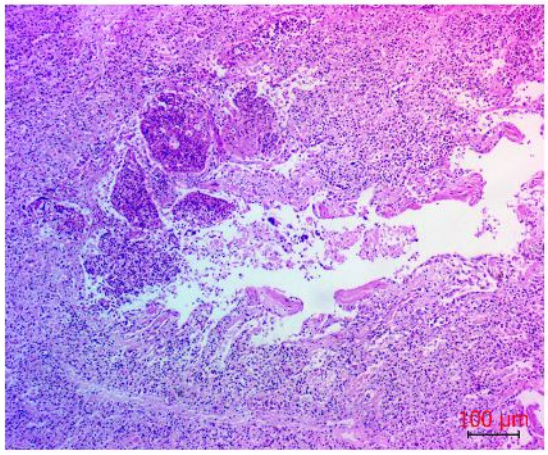

Plate 4.57 : Microphotograph of the lung showing marked parabronchial necrosis and loss of pulmonary architecture due to infiltration of inflammatory exudate admixed of mononuclear cells and heterophils (H&E, 100x)

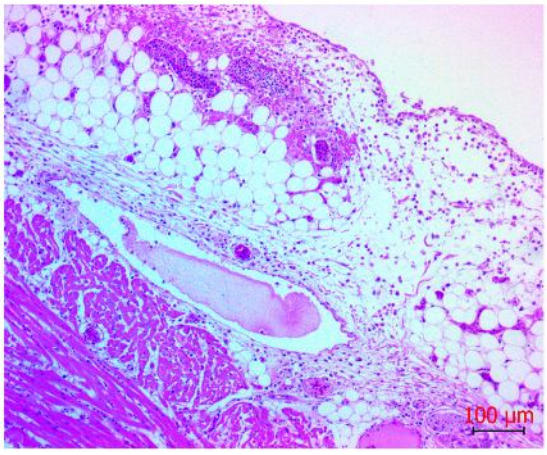

Plate 4.58 : Microphotograph of the heart showing thickened pericardium due to presence of fibrinocellular exudate (H&E, 100x)

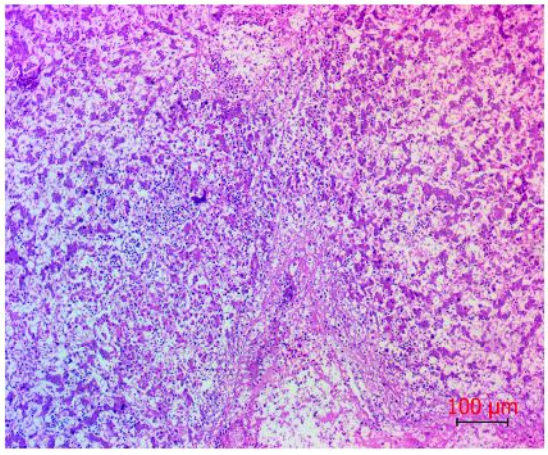

Plate 4.59 : Microphotograph of liver showing marked necrosis, inflammatory cells infiltration and haemorrhages (H&E, 100x)

exacerbated microscopic lesions. The virulence factors produced by *E. coli*, including hydrogen peroxide, nitric oxide, and various proteases induce significant necrosis of host cells, triggering an excessive release of pro-inflammatory cytokines. Moreover, *E. coli* inhibits phagocytosis and disrupts the normal functioning of B and T lymphocytes, potentially facilitating the replication of viruses (Kaore *et al*., 2018).

4.3.6 aMPV, MG and ILTV

Grossly birds infected with aMPV and co-infected with MG, ILTV, revealed swollen heads and eyes filled with caseous exudates, often leading to blindness (Plate 4.60). The tracheal mucosa displayed reddish mucoid exudates and congestion (Plate 4.61). The lungs showed consolidation and congestion (Plate 4.62), while the pericardium was covered by a thin fibrinous layer (Plate 4.63).These entire macroscopic lesion in the present study were in accordance with the findings of Couto *et al.* (2016) who reported dual infections of ILT and MG in layer chickens with respiratory lesions, Also Kaboudi and Lachheb (2021) studied aMPV in turkeys and reported comparable pathological alterations in the infected turkeys. In addition, individually in spontaneous outbreak of MG Bagal *et al.* (2019) and Kleven (2008) recorded similar gross lesions in chicken, however milder than the lesions of the present study.

Microscopic examination revealed loss of cilia, destruction and desquamation of epithelium, marked mononuclear infiltration in lamina propria and fibrino suppurative exudate (Plate 4.64) and mucous gland hyperplasia in nasal conchae of the nasal cavity (Plate 4.65). Infraorbital sinuses were infiltrated with mononuclear cells in the mucosa (Plate 4.66). The trachea revealed destruction of epithelium, sub mucosal edema, loss of cilia, submucosal vascular congestion and edema, consistent with acute necrotizing tracheitis (Plate 4.67). There was congestion, and heterophilic infiltration in the lung sections of infected birds (Plate 4.68). Heart sections from affected birds showed varying degrees of mononuclear cell infiltration along with degeneration of cardiac muscle fibers (Plate 4.69). The lesion of nasal turbinate, infraorbital sinuses and trachea are in accordance with the

findings of Catelli *et al*. (1998) who experimentally inoculated aMPV in chicken from the UK. Majo *et al*. (1995) reported similar lesions following experimental inoculation of turkey rhinotracheitis virus (TRTV/aMPV) in chickens. Couto *et al*. (2016) reported co-infection of MG + ILTV in layer chickens and reported similar lesions. The lesions in the lung and heart are in accordance with Thilagvathi *et al*. (2016); Brar *et al*. (2017) and Karthik *et al*. (2018) in MG infected birds. aMPV infection disrupts respiratory tract defenses, creating an ideal environment for secondary viral and bacterial pathogens like MG to invade which causes worsening lesions and exacerbates disease severity (Abd El-Ghany, 2023).

Plate 4.60 : Bird showing severe unilateral periocular swelling due to accumulations of fibrinous and caseous exudate within the infraorbital sinus.

Plate 4.61 : Tracheal mucosa showing reddish mucoid exudates and marked congestion

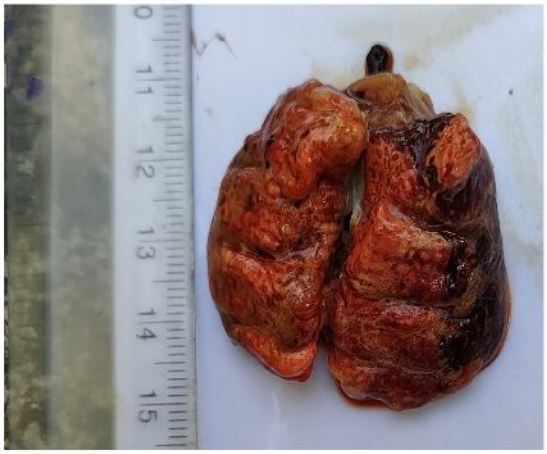

Plate 4.62 : Lung showing dark reddish consolidation at lateral portion of right lung lobe and congestion

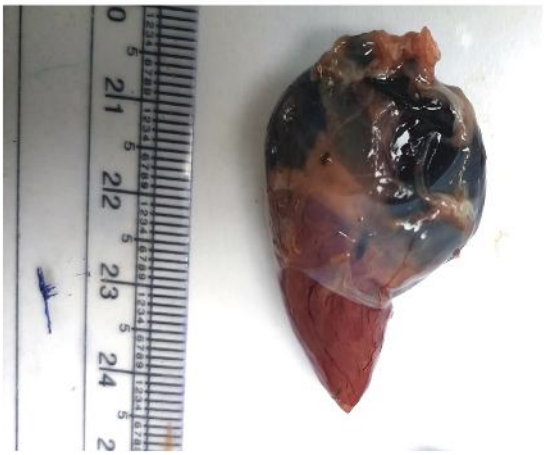

Plate 4.63 : Fibrinous pericarditis : Presence of pale whitish layer over the pericardium.

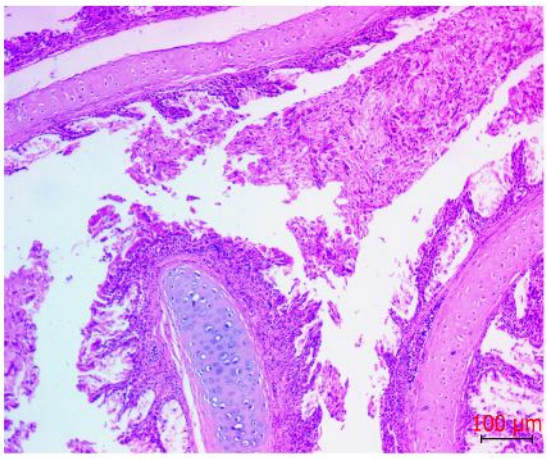

Plate 4.64 : Microphotograph of the middle nasal chamber showing extensive destruction of the respiratory epithelium with epithelial sloughing, ciliary disruption, and marked mononuclear cell infiltration in the lamina propria. Additionally, fibrinous necrotic exudate is observed within the middle nasal canal (H&E, 100x)

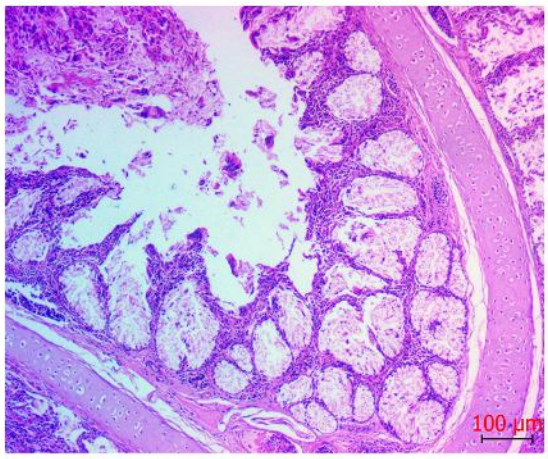

Plate 4.65 : Microphotograph of the nasal turbinate from middle nasal chamber showing mucous gland hyperplasia, epithelial desquamation with luminal sloughing, and fibrinosuppurative exudate within the airway lumen (H&E 100x)

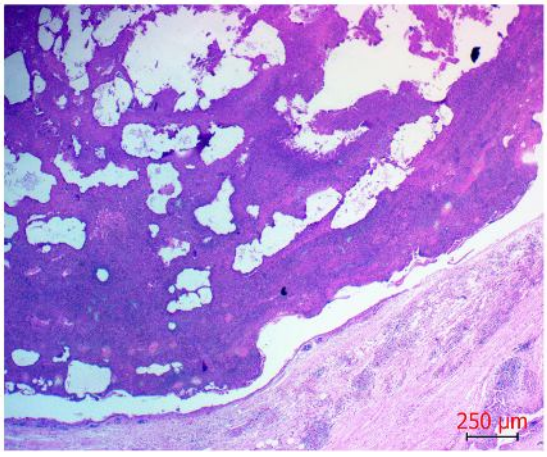

Plate 4.66 : Microphotograph of infraorbital sinus showing eosinophilic fibrino-necrotic material distending the lumen and mucosal necrosis, and dense mononuclear inflammatory infiltrates expanding the mucosa and submucosa, consistent with necrotizing sinusitis with abscess formation (H&E, 40x)

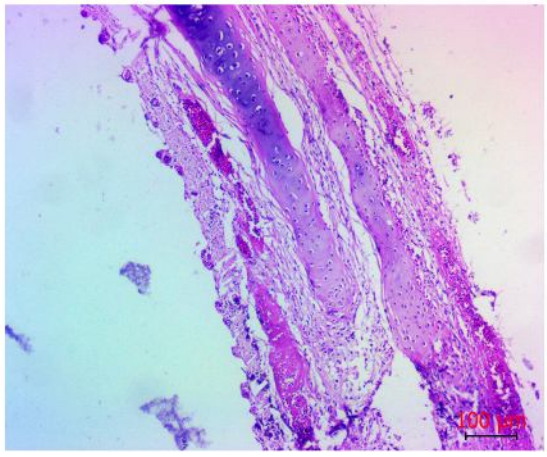

Plate 4.67 : Microphotograph of the trachea exhibiting marked necrosis of the respiratory epithelium with sloughing of cellular debris, accompanied by submucosal vascular congestion and edema, consistent with acute necrotizing tracheitis (H&E, 100x)

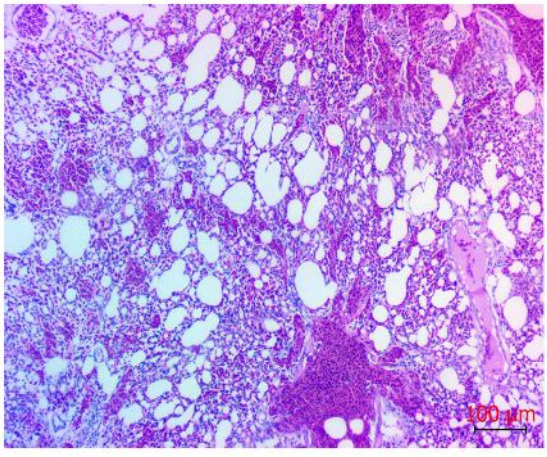

Plate 4.68 : Microphotograph of the lung showing marked pulmonary congestion, alveolar capillary engorgement, and infiltration of heterophils (H&E, 100x)

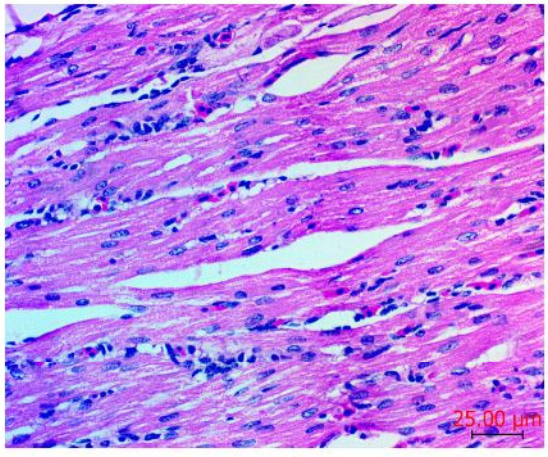

Plate 4.69 : Microphotograph of heart showing varying degrees of mononuclear cells infiltration and degeneration of cardiac muscle fibers (H&E, 400x)

Chapter - V

Summary and Conclusions

CHAPTER – V

SUMMARY AND CONCLUSION

Avian Metapneumovirus (aMPV) infection is a highly contagious respiratory disease in poultry, influenced by multiple factors, including host susceptibility, environmental stressors, and interactions with other pathogens. This disease primarily affects turkeys and chickens, leading to significant economic losses due to decreased productivity, increased mortality, and concurrent bacterial and viral infections. The present study titled "Pathology of avian Metapneumovirus and associated pathogens in chickens" was conducted in the Akola district. This research aimed to identify the pathological manifestations and selected concurrent infection along with aMPV infection.

Birds showing respiratory signs from 42 flocks in and around Akola were screened for avian Metapneumovirus (aMPV) and associated pathogens. Three dead birds per flock underwent necropsy and PCR testing using WOAH-recommended primers. The findings indicated that birds from 8 flocks were positive for the presence of aMPV, resulting in a flock-level incidence of 19.04%. Further, aMPV positive birds were screened for other selected respiratory pathogens (IBV, NDV, ILTV, IC, ORT, MG, and APEC) using PCR. It revealed concurrent infection of aMPV, MG and IBV in 01 flock (12.5%), aMPV, MG and APEC in 1 flock (12.5%), aMPV + MG + ILTV in 01 flock (12.5%), aMPV + IC + ORT in 1 flock (12.5%), aMPV + ORT + APEC in 1 flock (12.5%) and aMPV + MG in 03 flocks. No co-infections with NDV were detected.

In the present study, flocks infected with aMPV, particularly those co-infected with MG, exhibited varying clinical signs, including nasal and ocular discharge, dyspnea, and anorexia. Co-infections with pathogens such as ORT, IC, IBV, APEC, and ILTV resulted in more severe manifestations, including swollen sinuses, gasping, coughing, tracheal rales, lethargy, and mucopurulent exudates. The most severe signs were observed in flocks with multiple concurrent infections, especially involving MG and ILTV. The findings highlight that aMPV-infected

birds are highly predisposed to secondary bacterial infections, which intensify clinical outcomes particularly sinus swellings due to synergistic pathogenic interactions.

Grossly, aMPV and MG co-infected birds exhibited variable gross lesions including profuse nasal discharge, congested and edematous nasal turbinates, conjunctivitis with erythema, caseous plugs within the tracheal lumen, mild tracheal congestion, air sacculitis, and congested lungs with focal hemorrhages. In severe cases, fibrinous perihepatitis and pericarditis were also noted. Histopathologically, the middle nasal chamber exhibited fibrinonecrotic exudates, epithelial disruption, and mononuclear infiltration. The trachea showed epithelial sloughing, mucous gland loss, and submucosal edema, while lung tissues revealed capillary congestion, perivascular edema, thrombi, and inflammatory infiltration. Cardiac lesions included focal necrosis and leukocytic infiltration, and the spleen showed marked congestion, lymphoid depletion, and necrosis.

Birds co-infected with aMPV, ORT, and IC exhibited gross lesions such as periorbital swelling with caseous exudate, nasal cavity congestion with cheesy flakes, mucoid tracheal exudate, and fibrinous pleuritis with lung consolidation. Histologically, nasal turbinates exhibited chronic inflammation, epithelial damage, congestion, and mononuclear accumulation. Tracheal sections revealed epithelial degeneration, submucosal swelling, and inflammatory infiltration. Lung lesions included lymphoid hyperplasia, fibrinous exudates, mononuclear infiltration, and necrosis, with periportal inflammation and focal hepatic necrosis.

In addition, aMPV, MG, and IBV co-infection showed severe nasal discharge, periorbital swelling, conjunctivitis, lung congestion, and splenomegaly. Microscopic lesions included epithelial necrosis in nasal turbinates, destruction of mucous glands, mononuclear infiltration, and necrotic exudates. Tracheal mucosa showed thickening due to edema and inflammatory infiltration, while the lungs revealed bronchiolar and parabronchial fibrinocellular exudates. The spleen had a thickened capsule with necrosis and heterophilic infiltration; the liver showed hepatocellular necrosis and hemorrhage.

Orbital swelling with cheesy exudates, tracheal mucinous discharge, pulmonary consolidation with a fibrinous layer, and fibrinous pericarditis was recorded in aMPV, ORT, and APEC positive birds. Histologically, nasal and infraorbital sinuses were filled with fibrinonecrotic debris; the trachea showed necrosis of mucosa and submucosa; the lungs and heart had severe inflammatory changes. The liver exhibited a thickened capsule and fibrinous exudate.

Similarly, birds with aMPV, MG, and APEC showed purulent nasal discharge, caseous exudates in turbinates, and tracheal fibrin. Microscopy revealed necrotic luminal debris in the nasal cavity, vascular congestion, epithelial degeneration, and loss of cilia in the trachea. Lungs showed parabronchial necrosis and disrupted architecture. The pericardium and liver were involved with inflammatory exudates and widespread necrosis. In birds co-infected with aMPV, MG, and ILTV, prominent macroscopic lesions included swollen heads and eyes filled with caseous exudates leading to blindness, tracheal congestion with reddish mucoid exudate, and consolidated lungs. Histopathologically, the nasal chamber showed cilia loss, epithelial damage, and fibrinous luminal exudate. Turbinates showed mucous gland hyperplasia and epithelial desquamation. Infraorbital sinuses and trachea had extensive mononuclear infiltration, submucosal edema, and vascular congestion. Lungs were congested with heterophilic infiltration, while the heart exhibited muscle fiber degeneration and mononuclear infiltration.

Conclusions:

1) The study confirmed a 19.04% flock-level incidence of avian Metapneumovirus (aMPV) in poultry flocks exhibiting respiratory symptoms, by PCR.
2) aMPV is not severe respiratory disease but co-infection with other bacterial and viral agents lead to severe morbidity, mortality and exacerbates clinical manifestation and lesions.
3) Bacterial co-infections with MG were most prevalent followed by ORT, APEC and IC.
4) Viral co-infections with IBV and ILTV were detected.

5) Clinical signs varied depending on co-infections, included respiratory distress, nasal and ocular discharge, conjunctivitis, and swollen infraorbital sinuses. Severe cases exhibited fibrinous pericarditis, pneumonia, and airsacculitis.
6) Due to multiple concurrent respiratory infections, the diagnosis of specific pathogens becomes challenging. Therefore, PCR-based identification of the causative agent(s), in conjunction with lesion analysis, is considered an effective approach to facilitate accurate diagnosis and guide appropriate preventive and therapeutic strategies.

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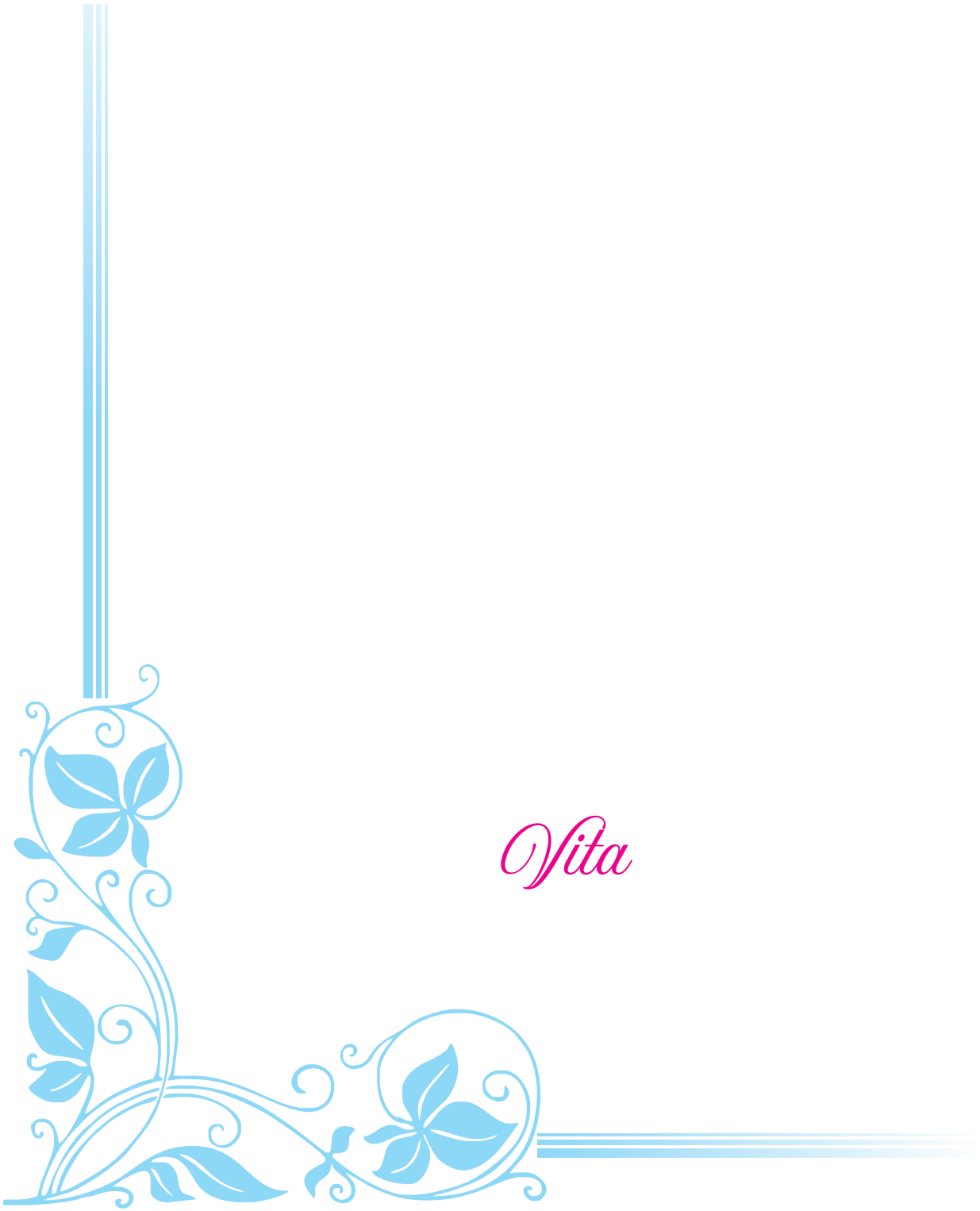


Vita

VITA

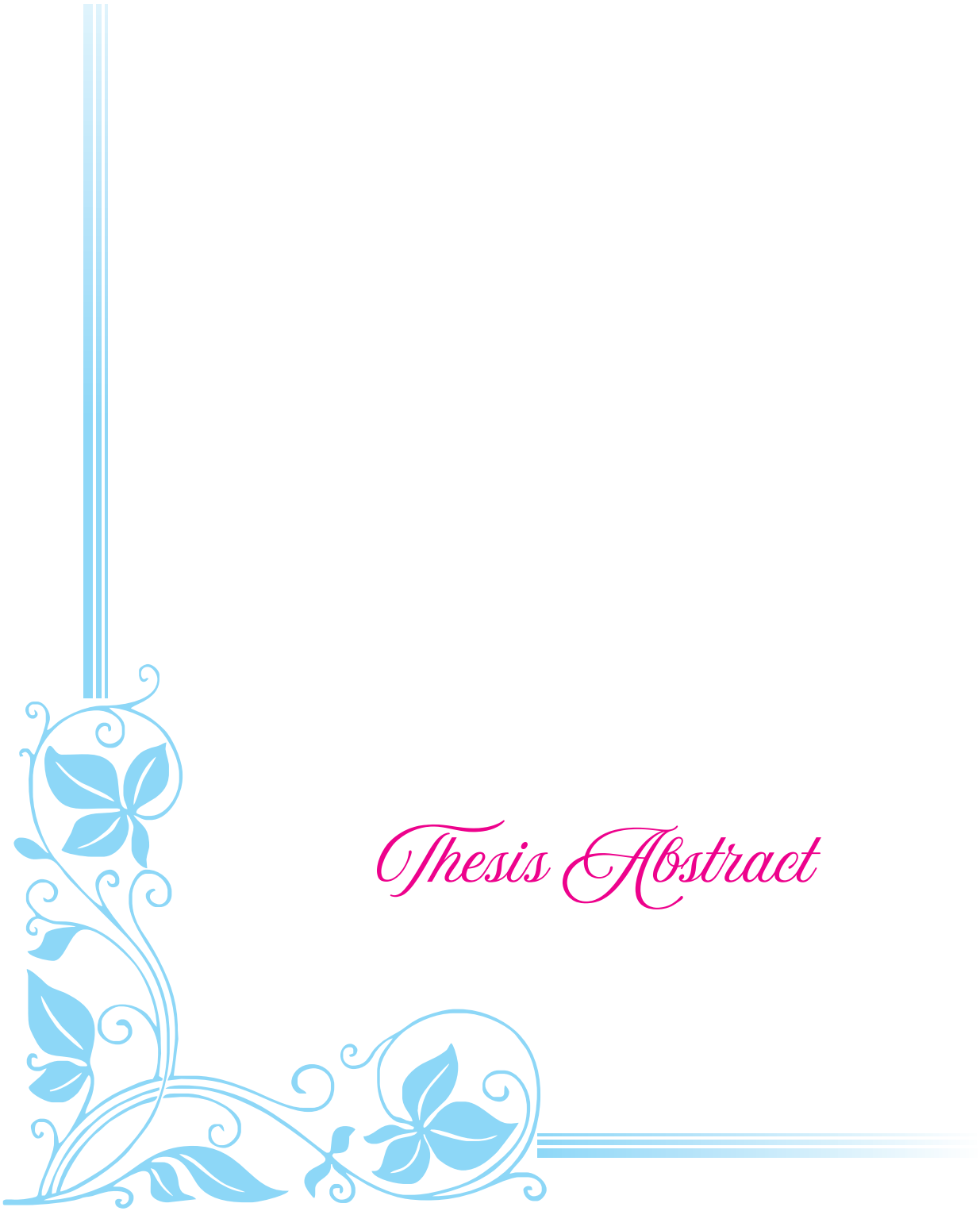
The author, **Dr. Sharda Deorao Garudi** was born on 10 August 1998 at Dist. Parbhani of Maharashtra State. She completed her early education at Bal Vidya Mandir High School, Parbhani. With a keen interest in science, she pursued her higher secondary education at Shri Shivaji College, Parbhani.

She then enrolled in the College of Veterinary and Animal Sciences, Parbhani, where she successfully obtained her Bachelor of Veterinary Science and Animal Husbandry (B.V.Sc. & A.H.) degree in 2023, graduating with first-class honors. During her undergraduate studies, she actively participated in National Service Scheme (NSS) camps and Animal Health Camps**,** contributing to community welfare and veterinary outreach programs.

To further enhance her expertise, she pursued a Master of Veterinary Science (M.V.Sc.) in Veterinary Pathology at the Post Graduate Institute of Veterinary and Animal Sciences, Akola**.** She successfully completed her coursework with first-class distinction. Throughout her postgraduate journey, she took part in various seminars, conferences, and research presentations**.** Notably, she presented both oral and poster research at the Veterinary Pathology Congress 2023**,** focusing on the pathological and molecular detection of bovine tuberculosis and caseous lymphadenitis in goats.

She is a Life Member of the Maharashtra State Veterinary Council (MSVC)**.**

Her two years of postgraduate research culminated in the successful submission of her thesis. She possesses proficiency in advanced pathological techniques, computing skills, and the effective utilization of the internet for research and data analysis.



Thesis Abstract

THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	**:**	**PATHOLOGY OF AVIAN METAPNEUMOVIRUS AND ASSOCIATED PATHOGENS IN CHICKENS**
b)	**Full name of student**	**:**	**Garudi Sharda Deorao**
c)	**Name and address of Major advisor**	**:**	**Dr. B. P. Kamdi** Assistant Professor, Department of Veterinary Pathology, PGIVAS, Akola
d)	**Degree to be awarded**	**:**	M. V. Sc.
e)	**Year of award of degree**	**:**	2025
f)	**Major subject**	**:**	Veterinary Pathology
g)	**Total number of pages In the thesis**	**:**	72
h)	**Number of words in the abstract**	**:**	281
i)	**Signature of Student**	**:**	
j)	**Signature, Name and address of forwarding authority**	**:**	

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ABSTRACT

Avian Metapneumovirus (aMPV) primarily affects turkeys and chickens, causing significant economic losses due to reduced productivity, increased mortality, and concurrent bacterial and viral infections. The present study aimed to determine the incidence of aMPV and its associated pathogens in poultry flocks within the Akola and to assess the pathological alterations in these infections. Out of 42 poultry flocks screened between

April 2024 and December 2024 in around Akola, Maharashtara, 19.04% (8/42) tested positive for aMPV using PCR with specific primers (115bp). Flocks infected with aMPV were further tested for co-infections with MG, ORT, IC, APEC, IBV, NDV, and ILTV by using PCR. The results revealed co-infections in the following proportions: 37.5% (3/8) of flocks had aMPV + MG, 12.5% (1/8) had aMPV + MG + IBV, 12.5% (1/8) had aMPV + MG + APEC, 12.5% (1/8) had aMPV + MG + ILTV, 12.5% (1/8) had aMPV + IC + ORT, and 12.5% (1/8) had aMPV + ORT + APEC.This study investigated the pathological effects of co-infections involving aMPV and other respiratory pathogens in birds. Co-infections with aMPV and other pathogens, resulted in severe and complex pathological lesions. Gross and microscopic examinations revealed lesions including periorbital swelling, airsacculitis, tracheitis, fibrinous perihepatitis, pericarditis, epithelial disruption, and mononuclear infiltration in nasal turbinate. The most severe pathological changes were observed in birds co-infected with aMPV, ORT and APEC characterized by swollen peri-orbital and infra-orbital sinuses with caseous exudate. The study highlights the high prevalence of co-infections in aMPV-positive poultry flocks and their impact on disease severity. The findings emphasize large scale molecular epidemiological studies for identification of respiratory pathogens and associated risk factors so that effective preventive and biosecurity measures can be implemented.

प्रबंध सारांश

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	: **कोंबड्यांमधील मेटान्युमो विषाणू व संबंधित रोगजनकांच्या विकृतीशास्त्राचा अभ्यास**
२.	**विद्यार्थ्याचे पूर्ण नाव**	: **गारुडी शारदा देवराव**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	: **डॉ. बी. पी. कामडी** सहाय्यकप्राध्यापक पशुविकृतीशास्त्र विभाग स्नातकोत्तर पशुवैद्यक व पशुविज्ञानसंस्था, अकोला
४.	**प्रदान केली जाणारी पदवी**	: एम.व्ही.एसस्सी.
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०२५
६.	**मुख्य विषय**	: पशुविकृतीशास्त्र
७.	**प्रबंधामधील एकूण पाने**	: ७२
८.	**प्रबंध सारांशामधील एकुण शब्द**	: ३००
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंध कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

(डॉ. र. सु. इंगोले)
विभाग प्रमुख
पशुविकृतीशास्त्र विभाग
स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था,अकोला.

प्रबंध सारांश

पक्ष्यांतील मेटाप्न्युमो विषाणू (एएमपीव्ही) हा प्रामुख्याने टर्की आणि कोंबड्यांमधील श्वसन संस्थेशी निगडीत आजारास कारणीभूत आहे. या आजारामुळे कोंबड्यांमधील उत्पादकता कमी होऊन मृत्युदर वाढतो, तसेच संसर्गित पक्षी इतर जीवाणू आणि विषाणूंच्या संसर्गामध्ये वाढ होते आणि त्यामुळे शेतकऱ्यांचे आर्थिक नुकसान होते. या अभ्यासाचा उद्देश अकोला जिल्ह्यातील कोंबड्यामध्ये एएमपीव्ही आणि त्याच्याशी संबंधित रोगजनकांच्या घटना निश्चित करणे आणि या संसर्गांच्या रोगानिदाना विषयक

प्रभावाचे मूल्यांकन करणे हा होता.महाराष्ट्रातील अकोला जिल्ह्यात एप्रिल २०२४ ते डिसेंबर २०२४ दरम्यान तपासणी केलेल्या ४२ कोंबड्याच्या गटापैकी १९.०४% (८/४२) गट पीसीआरद्वारे विशिष्ट प्रायमर (११५bp) वापरून एएमपीव्ही साठी बाधित आढळून आले. एएमपीव्हीने संक्रमित झालेल्या गटांची पीसीआर वापरून *मायकोप्लाझ्मा गॅलिसेप्टिकम* (एमजी), *ऑर्निथेबॅक्टेरियम राइनोट्रकॅले* (ओआरटी), इन्फेक्शियस कोरायझा (आयसी), एव्हियन पॅथोजेनिक *एस्चेरिचिया कोलाई* (एपीईसी), इन्फेक्शियस ब्रॉन्कायटिस विषाणू (आयबीव्ही), न्यूकॅसल डिसीज विषाणू (एनडीव्ही) आणि इन्फेक्शियस लॅरिंगोट्राकायटिस विषाणू (आयएलटीव्ही) या सर्व सह-संक्रमणांसाठी पुढील चाचणी करण्यात आली. निकालांमधून खालील प्रमाणात सह-संक्रमण आढळले: ३७.५% (३/८) गटांमध्ये एएमपीव्ही + एमजी, १२.५% (१/८) गटांमध्ये एएमपीव्ही + एमजी + IBV, १२.५% (१/८) गटांमध्ये एएमपीव्ही + एमजी + एपीईसी, १२.५% (१/८) गटांमध्ये एएमपीव्ही + एमजी + ILTV, १२.५% (१/८) गटांमध्ये एएमपीव्ही + IC + ओआरटी आणि १२.५% (१/८) गटांमध्ये एएमपीव्ही + ओआरटी + एपीईसीसह-संक्रमण आढळून आले होते. या अभ्यासात पक्ष्यांमध्ये मेटाप्न्यूमो विषाणू आणि इतर श्वसन रोगजनकांच्या सह-संक्रमणांच्या विकृतीजन्य परिणामांची तपासणी करण्यात आली. एएमपीव्ही आणि इतर रोगजनकांच्या सह-संक्रमणामुळे गंभीर आणि गुंतागुंतीच्या विकृतीजन्य जखमा निर्माण झालेल्या आढळून आल्या. स्थूल आणि सूक्ष्म तपासणीत डोळ्यांच्या भोवती सूज, वायुसांथींचादाह, श्वासनलिकेचा दाह, यकृताभोवती फायब्रिनयुक्तदाह, हृदयावरणशोथ, उपकला व्यत्यय आणि नाकाच्या आतील मुरक्यांमध्ये एकसंध अणुकेंद्रक असलेल्या पेशींची वाढलेली संख्या दिसून आली, तसेच त्याठिकाणी सूज व जखमा निर्माण झाल्या.सर्वात गंभीर विकृतीजन्य बदल एएमपीव्ही, ओआरटी आणि एपीईसीसह-संक्रमित पक्ष्यांमध्ये आढळून आला, ज्यामध्ये चिकट, पिवळसर-पांढऱ्या स्वरूपाच्या स्रावासह डोळ्याच्या भोवतालच्या व डोळ्याखालच्या हवेशीर पोकळीत तीव्र सूज दिसून आली. या अभ्यासात एएमपीव्ही कोंबड्याच्या गटांमध्ये सह-संक्रमणांचे उच्च प्रमाण आणि त्या रोगाच्या तीव्रतेवरील परिणाम अधोरेखित झाला आहे. हा शोध कोंबड्यामध्ये श्वसन रोगांचा प्रादुर्भाव नियंत्रित करण्यासाठी सुधारित जैवसुरक्षा, वेळेवर लसीकरण आणि नियमित आरोग्य देखरेखीचे महत्त्व यावर भर देत आहे.