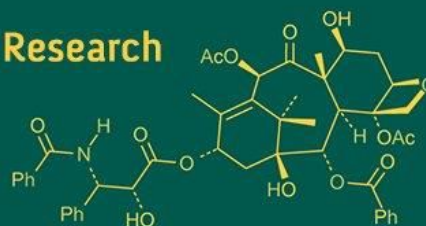
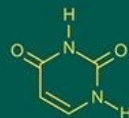


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Gross and histopathological changes in chickens infected with *Ornithobacterium rhinotracheale*

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Abstract

The present study aimed to investigate gross and histopathological changes in chickens that died with a history of respiratory distress, attributed to ornithobacteriosis. On gross examination, most frequently affected organs found were trachea, lungs and air sacs. The trachea revealed congestion, mild to moderate catarrhal exudation, caseous plugs in the lumen accompanied by haemorrhages. Lungs were most severely affected organs, revealed severe congestion, haemorrhages, edema, inflammatory exudation, areas of consolidation and unilateral and or bilateral pneumonic changes. Air sacs showed varying degrees of airsacculitis, foamy white exudate, predominantly targeting abdominal air sacs. Histopathologically, trachea revealed marked vascular congestion, epithelial necrosis, goblet cell hyperplasia, mucosal destruction, deciliation, fibrin deposition, and inflammatory infiltration. Lung sections showed fibrinopurulent pneumonia, vascular congestion, emphysema, lymphoid aggregates, interstitial fibrosis, and lymphoid hyperplasia. The lung section showed fibrinopurulent pneumonia, vascular congestion, emphysema, heavy infiltration of MNC's in and around bronchioles in the form of lymphoid aggregates, fibrin deposits in lung parenchyma, thickening of the interalveolar septa and lymphoid hyperplasia. Air sacs exhibited epithelial degeneration, hyperplasia, edema, hemorrhages, fibrinous thickening, and lymphocyte infiltration. Liver lesions included congestion, hepatocellular degeneration, necrosis, bile duct hyperplasia and lymphocytic infiltration were also noted. Spleen showed lymphocyte depletion, corpuscular atrophy, and arterial thickening. Heart exhibited myocardial edema, myofibrillar dystrophy, disorganisation and fragmentation of muscle fibers and mononuclear infiltration. Kidneys showed vascular congestion, hydropic degeneration, glomerulitis, and coagulative necrosis.

Keywords: Trachea, lungs, air sacs, congestion, hemorrhage, infiltration, *Ornithobacterium rhinotracheale*, inflammatory cells, edema

Introduction

Respiratory diseases in poultry stem from various pathogens including bacteria, viruses and fungi. Some of the most common and severe bacterial agents responsible for respiratory infections includes *Mycoplasma gallisepticum*, *Staphylococcus* spp, *Bordetella avium*, *Pasteurella multocida*, *Riemerella anatipestifer*, *Chlamydophila psittaci* and *Escherichia coli* (Yehia *et al.*, 2023) [26]. Amongst respiratory bacterial diseases, Ornithobacteriosis is an emerging respiratory bacterial disease caused by *Ornithobacterium rhinotracheale* (ORT) (Abd El-Ghany, 2021; Yusefinejad, 2024) [1, 27]. It is a gram-negative, rod-shaped, pleomorphic, microaerophilic, non-motile, non-sporulating, catalase-negative and oxidase-positive bacterium that was first reported in domesticated poultry and wild birds (Amonsin *et al.*, 1997) [2]. ORT infects numerous avian species particularly chickens and turkeys, and is recognized as a major economic concern due to its impact on growth performance, feed and water intake, egg production and mortality rate (Chansiripornchai *et al.*, 2007; Rasheed *et al.*, 2023) [6, 18]. ORT belongs to the family *Weeksellaceae* and was first detected in turkeys with respiratory disease syndrome in early 1990s in Germany (Charlton *et al.*, 1993) [7] before being named in South Africa in early 1994 (Vandamme *et al.*, 1994) [2]. It is frequently recognized as a primary or secondary infection in conjunction with other respiratory pathogens, such as bacterial and viral agents and its virulence is increased in presence of other infectious and non-infectious agents (Van Empel and Hafez, 1999; Barbosa *et al.*, 2019; Welchman *et al.*, 2013; Kursu *et al.*, 2022) [22, 4, 25, 12]. The disease has been observed in various avian species, with nearly 18 ORT serotypes identified.

Notably, chicken isolates are predominantly homogeneous and belong to serotype A (95%), while turkey isolates exhibit greater heterogeneity (Mourad *et al.*, 2023) ^[14].

Materials and Methods

The present study was conducted at the Department of Veterinary Pathology, College of Veterinary and Animal sciences, Parbhani, Maharashtra Animal and Fishery Sciences University, Nagpur. During the study period, regular post mortem examination was performed for studying the *Ornithobacterium rhinotracheale* infection in chicken. During the period of 6 months (September 2024 to February 2025), total 310 necropsies of chickens were conducted in and around Parbhani region including necropsies at various poultry farms and at the Department of Veterinary Pathology, College of Veterinary and Animal sciences, Parbhani. All the necropsied chickens were examined critically, for noting pathological lesions. The chickens that died and having history of respiratory system affections, showing varieties of clinical signs of respiratory distress were considered as suspected chicken for the research work and considered as the total number of chickens affected with respiratory diseases. These samples were considered as suspected for ORT.

Collection of samples: Systematic post mortem examination of chicken carcasses was carried out as per routine necropsy protocol. Tracheal tissues of the suspected chickens were collected and stored at -20 °C for further molecular studies.

Gross and histopathological analysis: From the affected organs, notable observations pertaining to gross pathological findings were recorded, and representative tissue samples were collected and preserved in 10 percent neutral buffer formalin for subsequent histopathological analysis using the standard procedure for routine histopathology (Luna, 1968) ^[13].

Results

Gross pathological alternations

In the present study, a total of 104 suspected chickens with clinical signs of respiratory distress were necropsied to study the pathological manifestations of *Ornithobacterium rhinotracheale* (ORT) infection. On gross examination, the most frequently affected organs found to be trachea, lungs and air sacs. The most consistent observations associated with the trachea of positive ORT cases were characterized by congestion and mild to moderate catarrhal exudation. In certain instances, tracheitis was noted, with caseous plugs in the lumen of trachea accompanied by tracheal haemorrhages (Plate No: 1, 2 & 3). Comparable findings, including manifestations of catarrhal to caseous tracheitis, were documented by Niresh, (2015) ^[15] in layer chickens located in the Namakkal district of Tamil Nadu. Also, the observations were comparable with the findings documented by Soriano *et al.* (2002) ^[21], Rahimi and Banani, (2007) ^[17] and Hauck *et al.* (2015) ^[10] who elucidated the manifestation of tracheal congestion, tracheitis and concomitant mucous exudate in avian species. Also, Kapakin *et al.* (2013) ^[11] reported the occurrence of edema, congestion and localized

hemorrhages within the mucosal layer of the trachea in quails.

The lungs were one of the most severely affected organs in ORT affected chickens. The affected lungs exhibited dark coloration with firm consistency. The dark coloration of lungs might be due to the severe congestion and hemorrhages. The lungs showed edema, inflammatory exudation, areas of consolidation and unilateral and or bilateral pneumonic changes. In some cases, observations indicated thickening of the pleura over the lungs. The pneumonic lesions were often bilateral however; unilateral pulmonary involvement was also documented in some cases. The presence of hemorrhagic pneumonia was observed potentially which might be the result of combination of vascular damage during severe inflammation, and immune response leading to extravasation of blood into pulmonary tissues (Plate no: 4, 5 & 6). These results were in accordance with the findings documented by Van Empel *et al.* (1997) ^[23] and Banani *et al.* (2001) ^[3], who observed purulent and exudative pneumonia respectively, which corresponds with the manifestation of inflammatory exudate and consolidation noted in the pulmonary tissues as observed in the current investigation. Likewise, Siddiqui *et al.* (2008) ^[19] reported the presence of congestion and consolidation within the pulmonary structures, which were identified in both unilateral and bilateral manifestations in chickens afflicted by ORT. The present observations such as congestion, edema, and consolidation, which are indicative of pneumonia, corroborates with the results reported by Hauck *et al.* (2015) ^[10].

The manifestation of hemorrhagic pneumonia, as identified in the current investigation, aligns with the observations made by Pan *et al.* (2012) ^[16], who documented analogous lesions in affected birds. In contrast, unilateral pneumonia was reported with greater frequency than bilateral pneumonia, as noted by Sivaseelan *et al.* (2015) ^[20]. Furthermore, pneumonia in a broader context was documented by Rahimi and Banani, (2007) ^[17] and Churria *et al.* (2011) ^[8], which corroborates the alterations as observed in present study.

In the air sacs, airsacculitis was a ubiquitous finding in ORT affected chickens with opaque appearance of air sacs. The severity of airsacculitis varied, ranging from mild opaque to cloudy (Plate no: 7). In some cases, air sacs were with presence of foamy white exudate, predominantly targeting abdominal air sacs. The analogous observations of airsacculitis accompanied by a yogurt-like exudate in the abdominal air sacs were documented by Kapakin *et al.* (2013) ^[11], Niresh (2015) ^[15], Sivaseelan *et al.* (2015) ^[20], Banani *et al.* (2001) ^[3] and Hauck *et al.* (2015) ^[10].

Spleen on gross evaluation showed minimal congestion and mild to moderate splenomegaly (Plate no: 8). Liver revealed focal to multifocal congestion, haemorrhages and necrosis also there was hepatomegaly occasionally. Kidneys showed congestion and focal necrosis. Heart on gross assessment noted minimal focal congestion and occasional fibrinous pericarditis.

Histopathological Observations

Trachea

The sections of trachea revealed mild to severe congestion of mucosal as well as submucosal vasculature. Occasionally, there were focal haemorrhages in affected tracheal sections.

There was moderate to severe degeneration and disorganisation of epithelial cells of mucosa. In few sections, there was moderate to severe exfoliation of epithelial cells in tracheal lumen and the lumen appeared to be with inflammatory exudation. There was focal to diffuse deciliation and necrosis of epithelial cells. Infiltration of heterophils and mononuclear cells in mucosal and submucosal layer in trachea was most consistent finding. Also, the few sections were with goblet cell hyperplasia indicating increased secretory activity of them (Plates: 9 to 13). These findings were in agreement with the observations of Borysevych *et al.* (2021)^[5]; Kapakin *et al.* (2013)^[11] and Sivaseelan *et al.* (2015)^[20].

Lungs

Histopathological examination of lung tissues from chickens infected with *Ornithobacterium rhinotracheale* (ORT) revealed marked congestion, edema, hemorrhages and fibrinopurulent pneumonia, characterized by infiltration of heterophils and MNCs in air capillaries, interstitial septa and bronchioles. The interstitial spaces were edematous and congested, with MNCs and heterophilic infiltration, leading to thickened interalveolar septa. There were typical fibrin deposits in lung parenchyma. There was severe congestion characterised by engorged vasculature in many lung sections. Also, at places, the lung sections showed compensatory mild to moderate focal to multifocal emphysematous changes. A reduction in the lumen of parabronchioles, was noted due to edema, inflammatory exudation and infiltration mainly by lymphocytes. In few sections, there was characteristic thickening of interstitium as evidenced by moderate to severe fibrous tissue proliferation and atrophy of adjoining alveoli. There were multifocal lymphoid aggregates in lung parenchyma with its greater predominance at parabronchiolar spaces (Plates no: 14 to 20). Parabronchioles narrowing, extravasation of blood within the pulmonary parenchyma, vasodilatory responses of the vascular structures as documented by Borysevych *et al.* (2021)^[5] aligns with observations of the current investigation. Histopathologically perivascular interstitial pneumonia in the lung and fibrinopurulent pneumonia documented by Kapakin *et al.* (2013)^[11]; Niresh, (2015)^[15] and De Rosa *et al.* (1996)^[9] supports the current observations.

Air Sacs

The air sacs exhibited epithelial degeneration, with thickening of the submucosa due to fibrinous exudation and heterophilic infiltration. The presence of mononuclear and heterophilic infiltration in the air sacs, coupled with fibrous thickening was observed (Plates: 21 to 23). Similar histopathological changes have been documented in previous studies by Kapakin *et al.* (2013)^[11] and Hauck *et al.* (2015)^[10].

Heart

Histopathological sections of the heart displayed edema of the myocardial interstitium and dystrophy of muscle fibers, with evidence of fragmentation and rupture of myocardial fibers. Also, there was mild to moderate congestion, mononuclear cell infiltration in myocardial musculature (Plates no: 29 to 32).

Spleen

The spleen exhibited depletion of lymphocytes in the lymphoid follicles, indicative of immunosuppression. There was typical lymphoid hyperplasia and thickening of splenic arteries. Some sections demonstrated fibrous proliferative changes, characterized by newly forming fibrous connective tissue, often infiltrated by inflammatory cells. The splenic corpuscles adjacent to fibrous tissue appeared atrophied. Occasional congestion of splenic blood vessels was also observed (Plates no:33 to 36). Our findings reinforce the conclusions of De Rosa *et al.* (1996)^[9] and Borysevych *et al.* (2021)^[5].

Liver

Histopathological alterations in the liver of ORT infected chickens revealed severe congestion and hepatocellular degeneration. The hepatic sinusoids appeared dilated, compressing adjacent hepatocytes. Some sections revealed mild to moderate necrotic changes, with perivascular inflammatory cell infiltration. In few sections, coagulative necrosis of hepatocytes, particularly at the periphery of hepatic lobes, was observed. Mild bile duct hyperplasia and perivascular lymphocytic infiltration were also noted (Plate no: 24 & 25). Comparable lesions have been previously reported by Borysevych *et al.* (2021)^[5] and De Rosa *et al.* (1996)^[9] in their studies.

Kidneys

Renal sections on histopathological examination revealed focal to multifocal congestion, cellular swelling, hydropic and cystic degeneration of renal tubular epithelial cells. Also, at places, there were coagulative necrotic changes and glomerulitis as evidenced by MNC infiltration. The infiltration of MNC in sections of kidneys was consistent finding. The few sections showed multifocal to diffuse congestion at corticomedullary junctions (Plate no: 26, 27 & 28).

Our observations are consistent with the findings of Borysevych *et al.* (2021)^[5]. These findings of the current study indicated that ORT infection in poultry primarily affects the respiratory system but could also cause lesions in organs such as liver, spleen, heart, and kidneys.



Plate 1: Note tracheal lumen showing pale to slightly translucent serous exudate



Plate 2: Tracheal lumen showing presence of caseous, yellow exudative plugs



Plate 3: Haemorrhagic trachea with severe congestion and mucoid exudate in the lumen



Plate 4: Note lungs with thin fibrinous coating, congestion and areas of focal consolidation



Plate 5: Lungs with congestion, petechiae, focal areas of consolidation and emphysematous changes



Plate 6: Lungs with congestion, petechial haemorrhages and consolidation



Plate 7: Airsacculitis along with presence of foamy, white to yellowish yoghurt like exudate on abdominal air sacs



Plate 8: Spleen showing marked splenomegaly and severe congestion

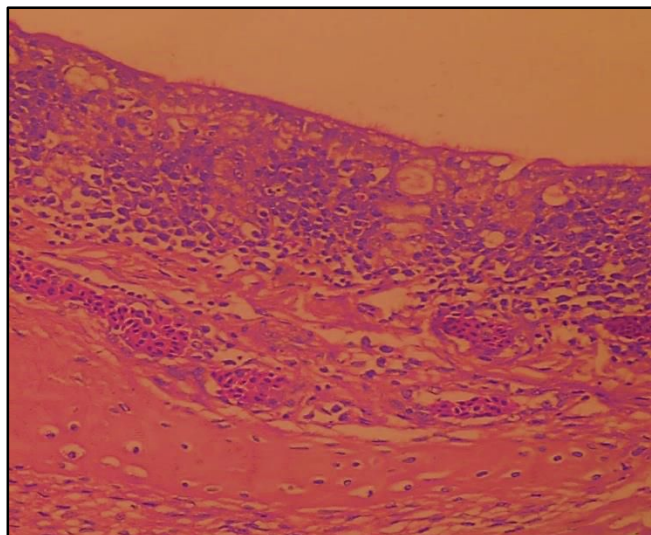


Plate 9: Section of trachea showing deciliation of epithelial cells of mucosa, congestion and inflammatory cell infiltration (H & E-400X)

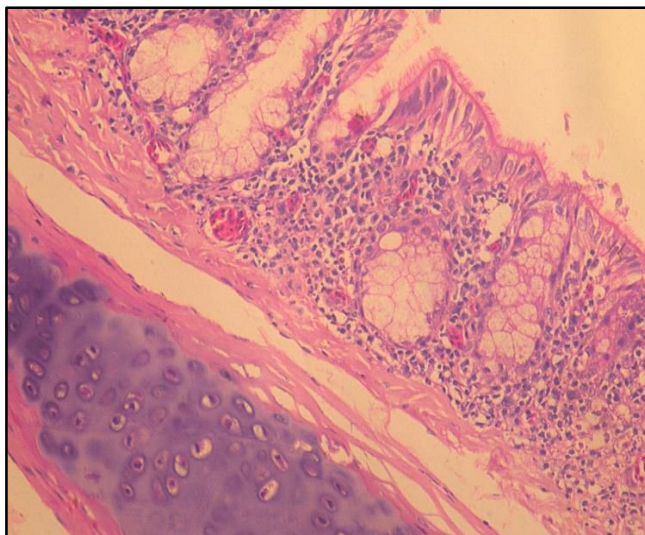


Plate 12: Section of trachea showing hyperplastic as well as hypertrophic goblet cells with inflammatory cell infiltration in mucosa (H & E-400X)

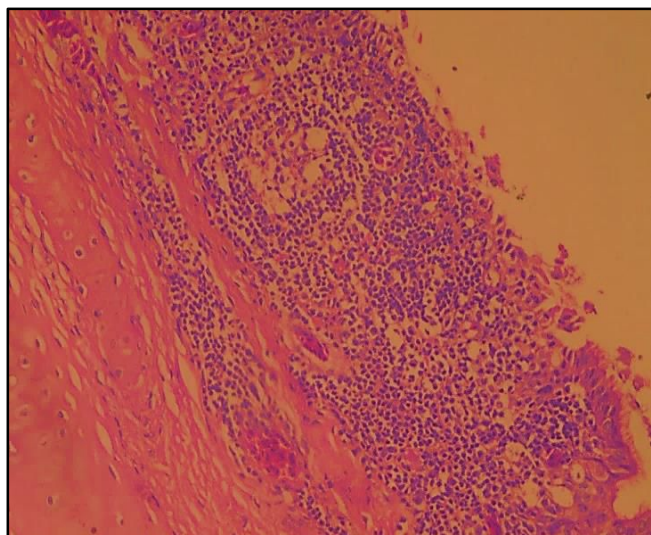


Plate 10: Section of trachea showing sloughing of epithelial layer and heavy infiltration of inflammatory cells replacing normal mucosal histoarchitecture (H & E-400X)

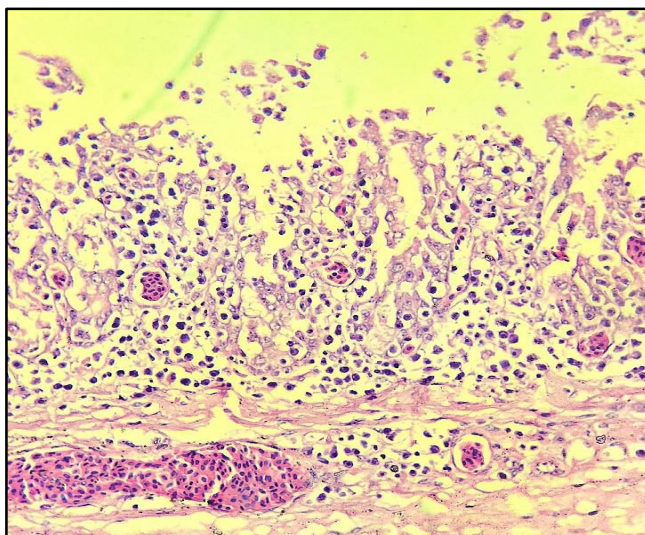


Plate 13: Section of trachea showing severe congestion, MNC infiltration and disorganization of mucosal epithelial cells (H & E-400X)

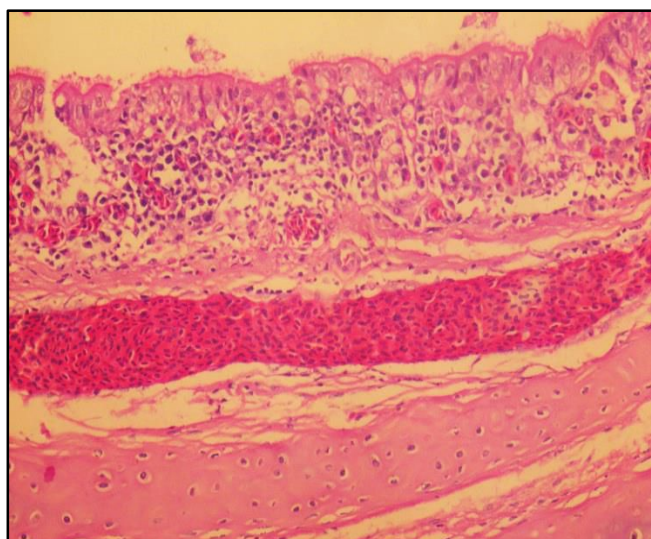


Plate 11: Section of trachea showing severe congestion, MNC infiltration and hyperplasia of epithelial cells (H & E-400X)

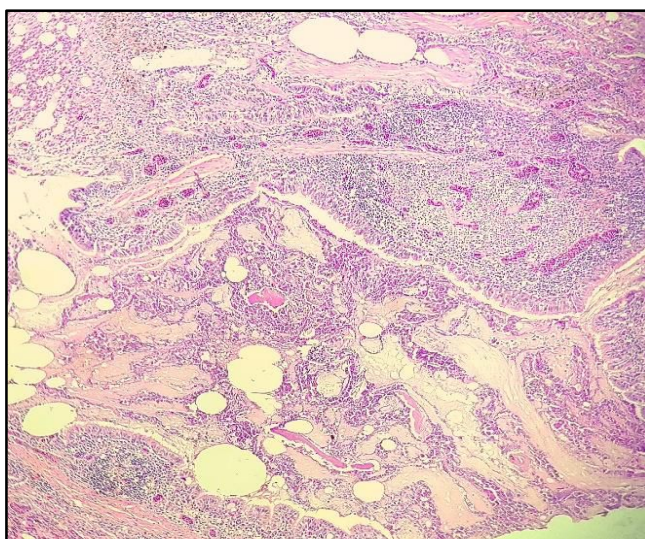


Plate 14: Section of lung showing diffuse congestion, moderate edema, mild to moderate emphysematous changes, infiltration of inflammatory cells in parenchyma (H & E-100X)

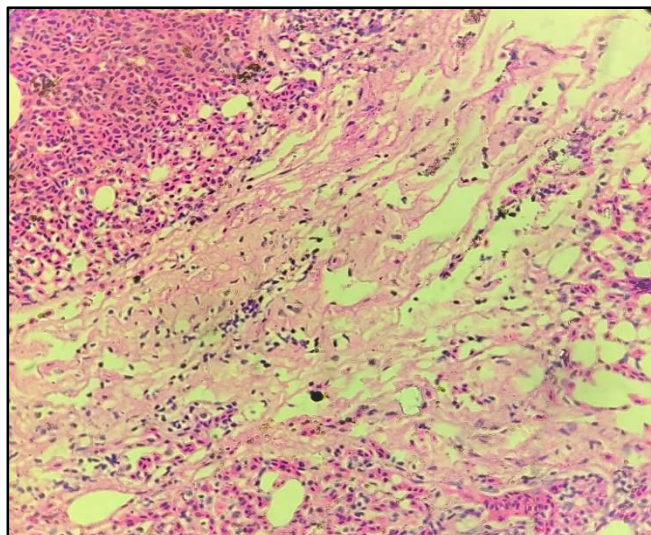


Plate 15: Section of lung showing thickening of interstitial layer with infiltration of inflammatory cells and multifocal hemorrhages (H & E-400X)

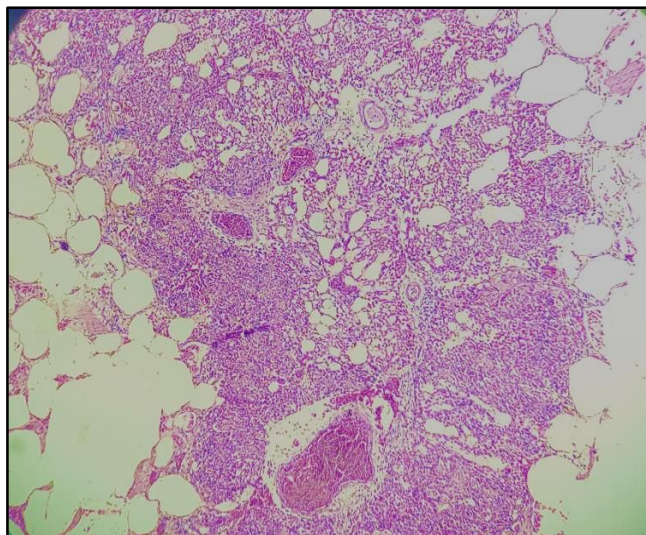


Plate 18: Section of lung showing severe congestion, edema, presence of MNC infiltration with emphysematous changes (H & E-100X)

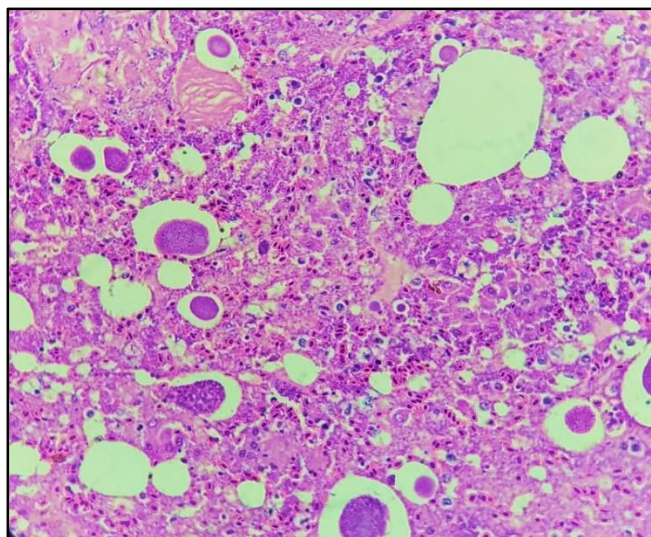


Plate 16: Section of lung showing diffuse infiltration of inflammatory cells and inflammatory exudation with necrotic tissue (H & E-400X)

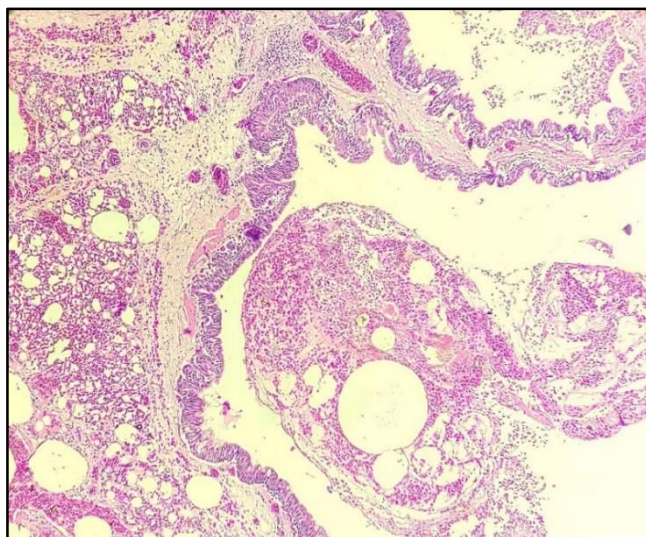


Plate 19: Section of lung showing bronchiolar epithelial cell hyperplasia, presence of inflammatory exudate in bronchiolar lumen and hemorrhage in lung parenchyma (H & E-100X)

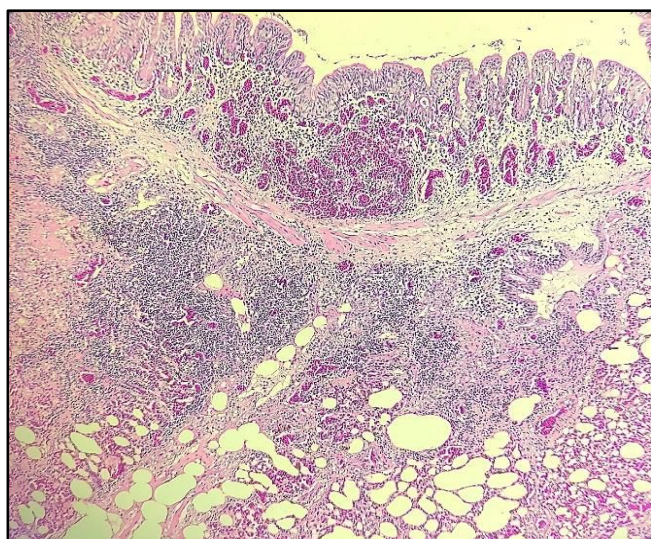


Plate 17: Section of lung showing congestion in the subepithelial mucosa of bronchioles along with infiltration of inflammatory cells around peribronchiolar space, and diffused emphysematous changes (H & E-100X)

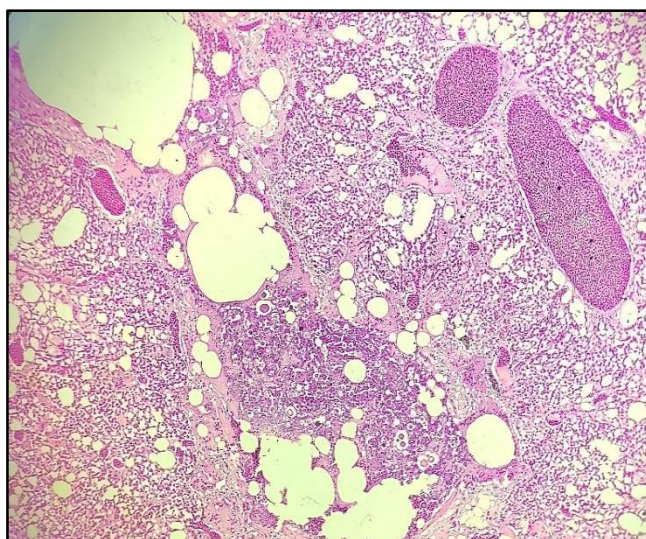


Plate 20: Section of lung showing severe congestion with engorged vasculature, edema, multifocal mild to moderate emphysematous changes and lymphoid aggregates in parenchyma (H & E-100X)

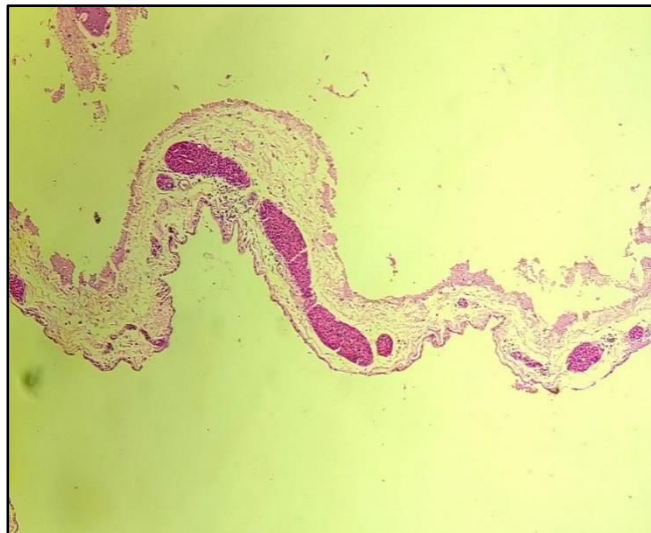


Plate 21: Section of air sac showing severe congestion, degeneration and desquamation of the epithelial cells (H & E-100X)

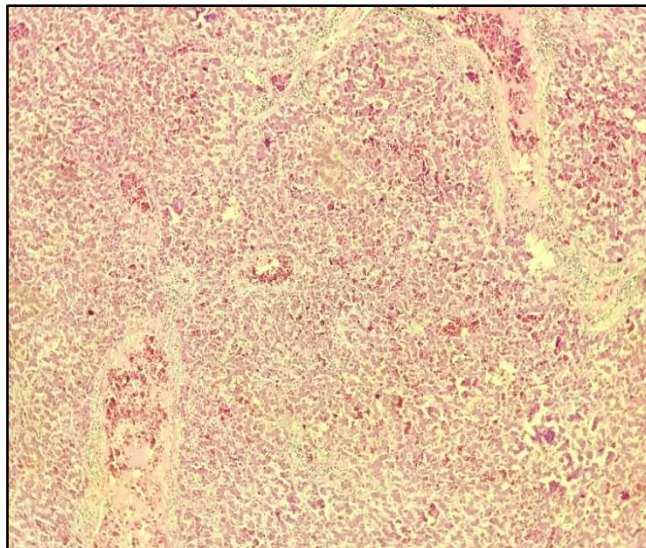


Plate 24: Section of liver showing diffuse congestion with infiltration of MNC (H & E-100X)

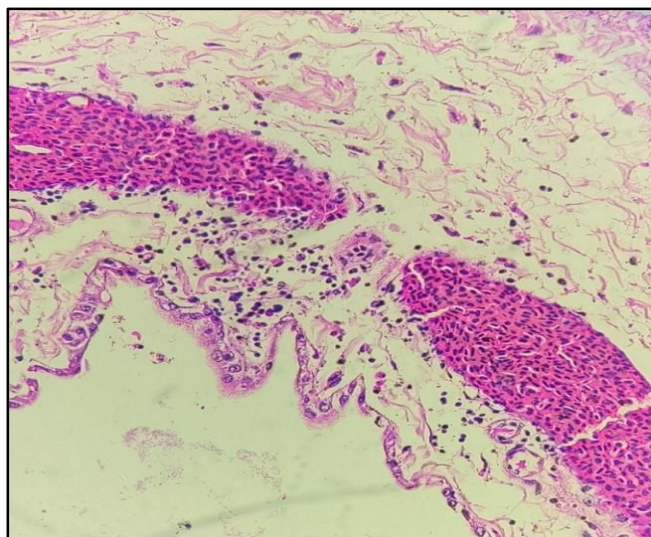


Plate 22: Section of air sac showing severe congestion, degeneration and desquamation of the epithelial cells along with infiltration of inflammatory cells (H & E-400X)

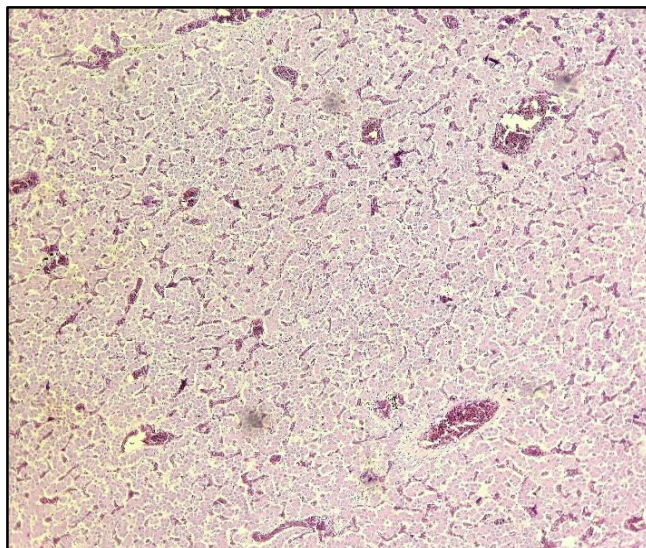


Plate 25: Section of liver showing diffuse congestion (H & E-100X)

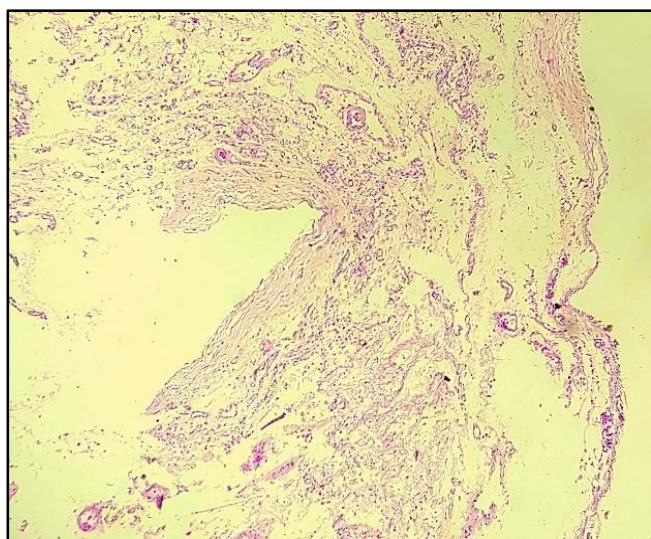


Plate 23: Section of air sac showing fibrinous airsacculitis (H & E-100X)

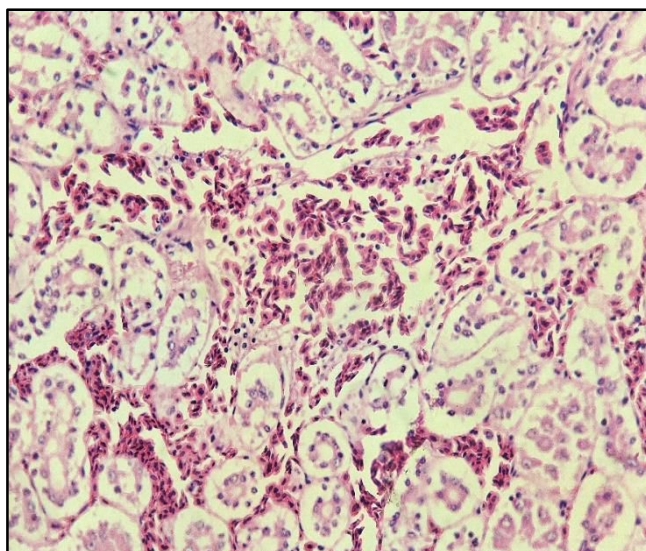


Plate 26: Section of kidney showing severe diffused congestion (H & E-400X)

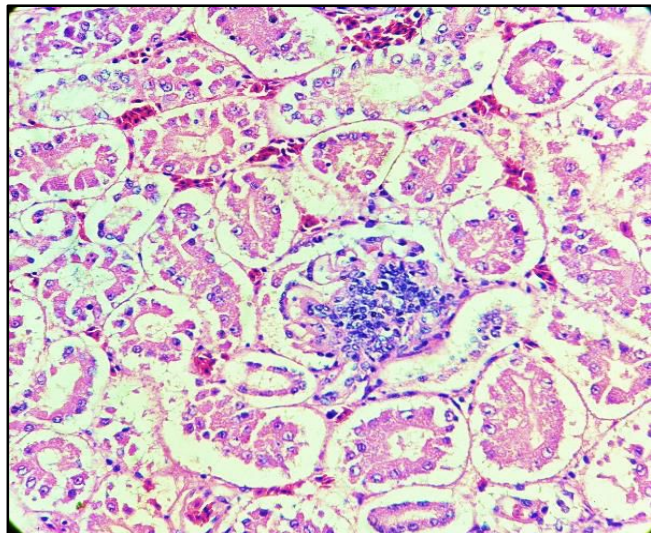


Plate 27: Section of kidney showing multifocal cellular swelling, coagulative necrosis of tubular epithelial cells and glomerulitis (H & E-400X)

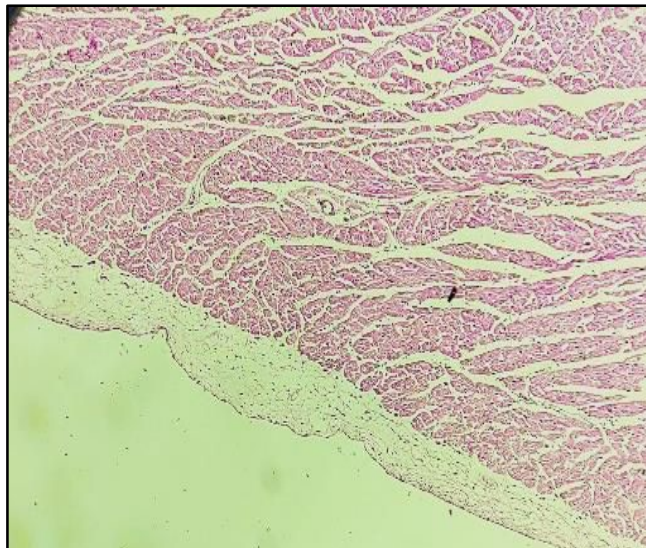


Plate 30: Note thin fibrinous pericarditis with MNC infiltration in heart (H & E-100X)

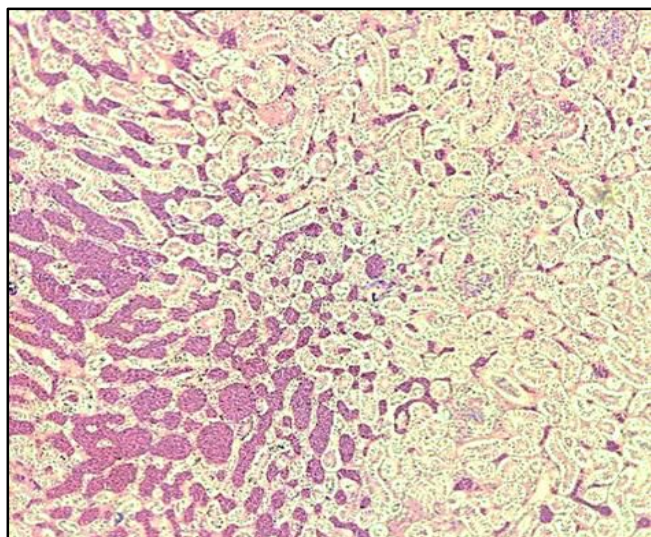


Plate 28: Section of kidney showing diffuse congestion in cortex with multifocal degenerative and necrotic changes in tubular epithelium (H & E-100X)

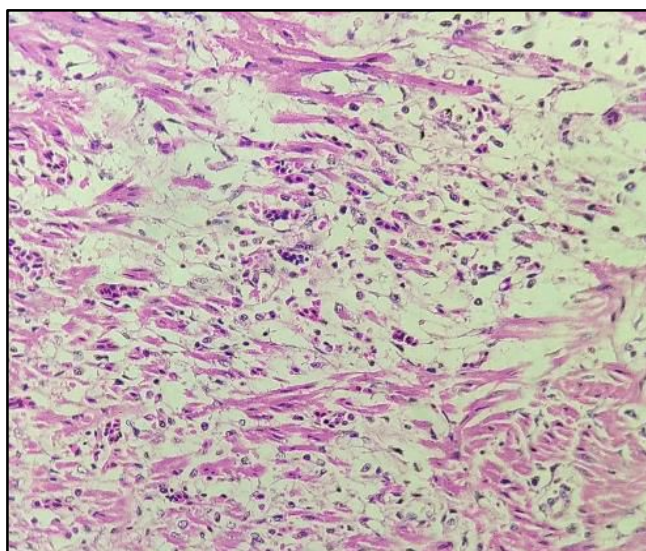


Plate 31: Section of heart showing disorganization, fragmentation and necrosis of the myocardial fibers (H & E-400X)

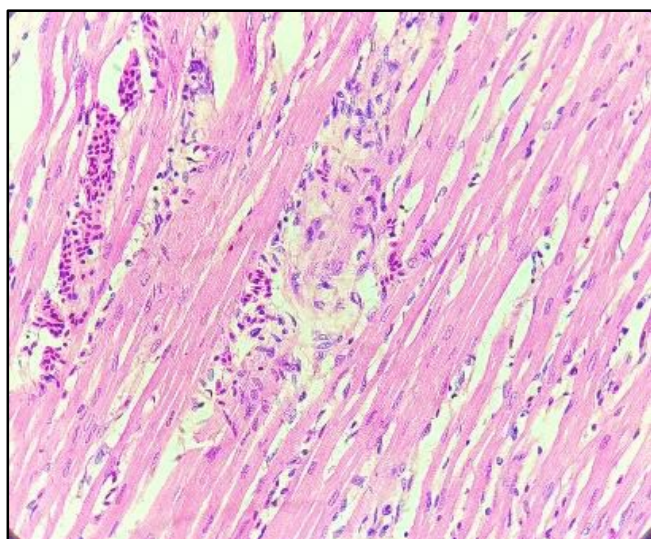


Plate 29: Section of heart showing multifocal infiltration of inflammatory cells along with areas of congestion (H & E-400X)

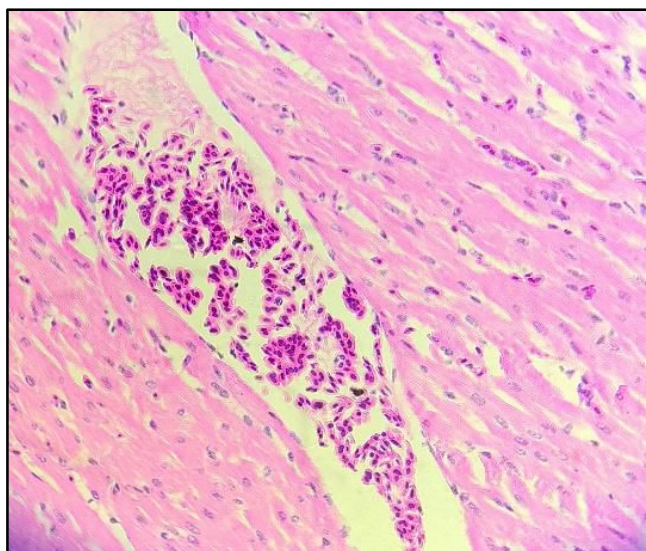


Plate 32: Section of heart showing severe congestion (H & E-400X)



Plate 33: Section of spleen showing lymphoid depletion, severe congestion and fibrous tissue proliferation (H & E-100X)

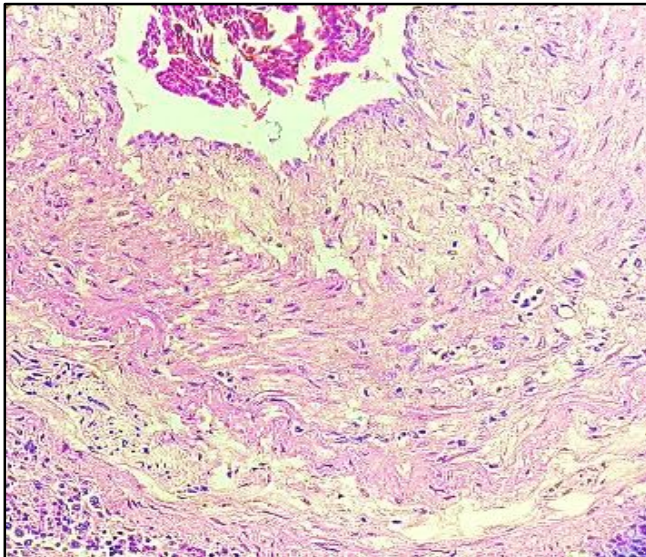


Plate 34: Section of spleen showing marked thickening of splenic artery with MNC infiltration in periarterial areas (H & E-400X)

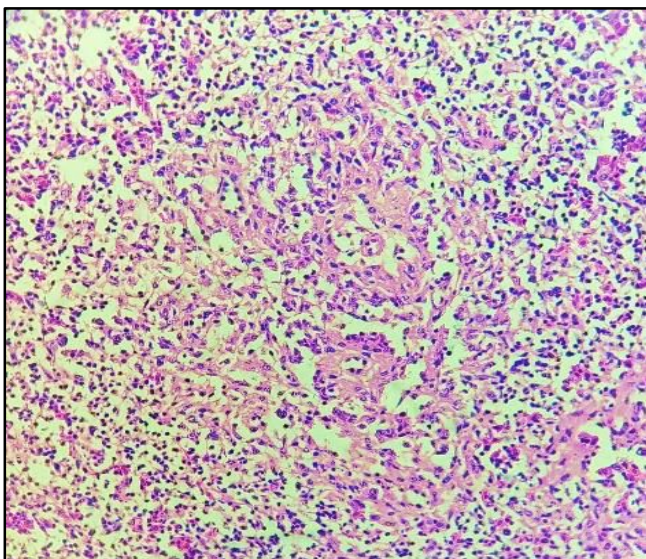


Plate 35: Section of spleen showing marked lymphoid depletion with necrotic foci scattered throughout the splenic parenchyma (H & E-400X)

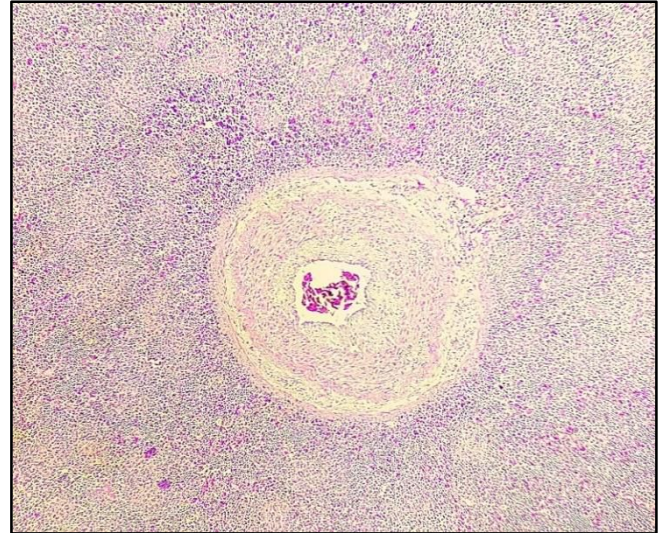


Plate 36: Section of spleen showing congestion and thickening of splenic artery (H & E-100X)

Conclusion

The study highlights the significant pathomorphological alterations caused by ornithobacteriosis in chickens particularly in the trachea, lungs, and air sacs. On gross and histopathological examination, trachea showed remarkable congestion, inflammatory changes characterised by epithelial disorganisation, exfoliation of epithelial cells with goblet cell hyperplasia and infiltration of inflammatory cells. Lungs as an important target organ showed congestion, pneumonic changes as evidenced by severe inflammatory cell infiltration, bronchiolar exudation, bronchiolar epithelial cell hyperplasia and compensatory emphysematous changes with thickening of interstitium. The study contributes to a deeper understanding of the disease pathology, which is crucial for improving diagnostic and management strategies in veterinary practice.

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