RESEARCH
O&G Forum 2024; 34 – 3s: 2010-2021

COMPARATIVE HISTOLOGICAL, HISTOCHEMICAL AND SCANNING MICROSCOPY STUDY OF THE LUNG IN MALE MYNAH BIRDS (ACRIDOTHERES TRISTIS) AND STARLING BIRDS (*STURNS VULGARIS*)

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Abstract
This study was conducted in a postgraduate labs in college of veterinary Medicine in Al-Muthanna University to investigated the comparative histological , histochemical and scanning electron structure of the lung in male mynah and starling birds . Twelve individuals' birds (6 mynah and 6 starling birds) were subdivided into groups (8 birds for histological and histochemical study) and (4 birds for scanning electron examination). The histological and histochemical study were carried out by Routine Histological Technique and Histochemistry analysis. Histological Results showed that the lungs of both mynah and starling had distinct characteristics. The histological sections of the lungs of both birds reveals the absence of a pleura. The lung tissue is composed of numerous lobules that are separated by trabeculae. The lungs are composed of secondary bronchi, para bronchi, atria, infundibula and air capillaries, all lined with simple squamous epithelium. The histochemical results of lungs in both birds revealed positive response to Masson's trichrome stain, indicating the existence of connective tissue. Conversely, a positive response to van Gieson's stain suggests the presence of collagen fibers that are stained red. The elastic inter-parabronchial septa of mynah birds and the outer wall of the lung of the starling bird are observed using scanning electron microscopy.
Key Words : mynah , starling birds , lung , scanning electron .

Introduction:
The term migration originates from the Latin word migrate which refers to the periodical movement of birds from one location to another , the migratory lifestyle is a longstanding behavioral characteristic observed in numerous avian species , facilitating their ability to monitor and adapt to variations in climate and habitat conditions on a global scale (1) . The family Sturnidae comprises two groups of birds, namely mynah and starling birds which are indigenous to Asia (3) . Individuals relocate to various areas based on seasonal factors , food accessibility, and climatic circumstances (5) (6) . The avian respiratory system consists of two primary elements: the inflexible bronchial lungs, which facilitate gas exchange between air and blood, and the neovascularized ventilatory air sacs. The respiratory system is crucial for thermoregulation, as it is responsible for the sensation of smell and voice. The respiratory system of birds comprises several anatomical structures, including the nasal cavity, larynx, trachea, primary bronchi, syrinx, and lungs (7) .The avian lungs have a higher degree of specialization. This specialization encompasses three significant characteristics:
the expansion of the respiratory epithelium, namely the Pseudostratified ciliated columnar epithelium, and adequate ventilation mechanisms and circulation (2). Furthermore, it is very diminutive compared to the whole body size and inflexible when fully inflated(3) (4) . From a histological perspective, the bird's lungs comprise a central mass of pulmonary lobules occupied by parabronchi and connected via inter-parabronchi septa and anastomosing (8). The interparabronchial barrier causes ostriches to lose their lungs. The parabronchial wall is composed of three layers: cuboidal or squamous epithelia, which do not contain secretory units; lamina proper, which means that there is no cartilage and no loss of connective

tissue; and muscular, meaning that there are smooth muscle fibres in the third layer (9). The secondary bronchi are named by the areas of the lung they supply and branch out from primary bronchi in different locations and numbers. (10) Parabronchi, which anatomize with each other and branch from secondary bronchi, make up most of an avian lung (11,12). They divide the centres of the pulmonary lobules. In birds such as chickens, turkeys, ducks, and quails (Coturnix coturnix), there are multiple openings in the inner wall of each parabronchus. These openings lead into chambers called atriums, which are air vesicles. The atriums are lined with a squamous to cuboidal epithelium that is continuous with the air capillary lining and contains two types of epithelial cells: granular and squamous. Interatrial septa pieces of loose connective tissue separate the atriums. On the arterial openings, there is a spiral of smooth muscle bundles (13,14). There has been a dearth of research comparing the histological and histochemical characteristics of the lung in mynah and starling. Hence, the current study sets out to fill that gap.

Material and Methods:

Light microscopic studies:

This study utilized a sample of fourteen male mynah and starling birds in a healthy and mature state. seven mynahs and seven starlings were collected during winter, specifically from October to December. The birds were sourced from Al-Basrah city. The weight of all birds were equal (in mynah about 82-140 gr , in starling ranged 75-100 gr) . Inhalation anaesthesia was administered using chloroform (19) . Subsequently , longitudinal dissection was performed within the thoracic cavity and extraction of the lung samples . The specimens were preserved in a 10% formalin solution . The dehydration method involved the utilization of various concentrations of alcohol (ethanol) (70%, 80%, 90%, and 100%) , followed by the subsequent cleaning procedure employing xylene. The samples were immersed in paraffin wax, and slices with a thickness of 5-6 were prepared using a microtome. These sections were stained using the standard Hematoxylin and eosin stains and other specific stains, such as Masson's trichrome and Van Gieson stains (15).

Scanning electron microscope:

This study used four birds: two mynah birds and two starling birds. The lung fixed in a solution of 3% glutaraldehyde and 0.2mol/l cacodylate. The solutions were at a temperature of 4ºC and a pH of 7.4 for 4 hours. The specimens underwent processing according to the Jones method, followed by dehydration and critical point drying. In a vacuum, they were then coated with gold. Then, using image analysis, they were examined in a scanning electron microscope (TSEM) by Tesla (BS340). (16).

Result and discussion:

The light microscope of the lung:

The histological examination of the lungs of both mynah birds and starlings revealed the absence of a pleura. The lung tissue consisted of numerous lobules, which were separated by trabeculae. Within these lobules, the bronchial tree and pulmonary blood vessels branched out. The lobules themselves consisted of secondary bronchi, para bronchi, atria, infundibula, and air capillaries, along with connective tissue and lymph nodes.Fig (1,2).Similar findings were reported by(37,36 ,and19 inwoodpigeon,andinpoultry,respectively).

The secondary bronchi in both birds are located within the lung tissue and are covered by highly folded mucous membranes composed of pseudostratified columnar ciliated epithelium. The epithelium exhibited significant folding in comparison to the principal bronchi. These findings were corroborated by (22and23)in studies conducted on guinea fowl and chicken. The lamina propria consisted of loose connective tissue containing diffuse lymphatic tissue, delicate collagen fibers, and cartilage plates. Fig (3,4). These findings align with the findings reported by (21and20).

The parabronchi, which are air passages in the lungs, connect to atria, which are dilated chambers. From there, the air passes through ducts called infundibula, which lead to a small network of air capillaries. This network includes veins, arteries, and the epithelium of the air capillaries. The epithelium of the air capillaries and the endothelium of the blood capillaries are separated by a basement membrane. Fig (5,6). The lining of the structure consists of a simple squamous epithelium supported by loose connective tissue and thick, smooth muscle bundles. The atria, infundibula, and air capillaries are all coated by a similar kind of epithelium and connective tissue. Fig (3,4). This assertion aligns with the findings of (37,36,19,24 and 41 in the context of wood pigeons, ,poultry, swan geese, respectively).

The inter-parabronchial connective tissue separates each lobule, with interparabronchial septa leading to atria, dilated chambers. Arteries, veins, and lymph nodes pass through these Sept. Fig (5,6). The outcome was consistent with the findings reported by(36,10 and39 about Wood pigeons, regarding the local Indian pigeon, and Aseel and Vanarajia)as well as several studies conducted on different species of birds (28,33,31and30). The inter-parabronchial connective tissue partitions each lobule, resulting in a highly delicate structure. This finding is consistent with the research conducted by(25,26 in Bulbul, and ducks,respectvily) as well as the study conducted by (29 on osticha). The study revealed the existence of smooth muscle fibers in the parabronchi openings of the atria, potentially enabling the contraction and relaxation of the atrial opening during respiration.

Furthermore, the outcome corresponded with the findings found in domestic chicken (34).
The study discovered that the blood capillaries and air capillaries are intricately connected and create a network of tissue in the parabronchi, with many chambers separating them. Additionally, it enables the continuous and one-way ventilation of the exchange tissue in the invaginated avian respiratory system, resulting in remarkably high respiratory efficiency. (32and35).
The histochemical results of the lung in both birds revealed a favorable response to connective tissue when stained with Masson's trichrome stain. Additionally, blood vessels and muscle fibers exhibited a pink color, whereas collagen fibers appeared blue. Fig (7,8). Conversely, a favorable response to van Gieson's stain indicates the existence of collagen fibers that are dyed red in color.Fig (9,10).

Electron microscope:
Scanning electron microscope:

The result of mynah showed elastic inter para bronchial septa (septa inter para bronchial) these septa contain arterioles, venules and nerve fiber. Fig (11) polygonal out pouches (atria) bulge into the parabronchial wall and branch through funnel-shaped ducts (infundibula) into the air capillaries and spirally arranged bundles of smooth muscle fibers found under the connective tissue. Fig (12,13). The parabronchi and surrounding tissue are the lung unit (22), and the result of starling birds. Fig(15) the outer wall of the lung is a thin interior lung containing numerous thin groups and the longest septa.Fig(14) that are attached to the lung wall and their free ends serve to define the margins of the axial air channel that divides the internal surface of the lung into a polygonal structure which subdivided by the shortest septa into shallow slip downward to form air sacs. The cores of septa consist of collagenous elastic fiber together with lesser amounts of smooth muscle fibers. (17, 18).

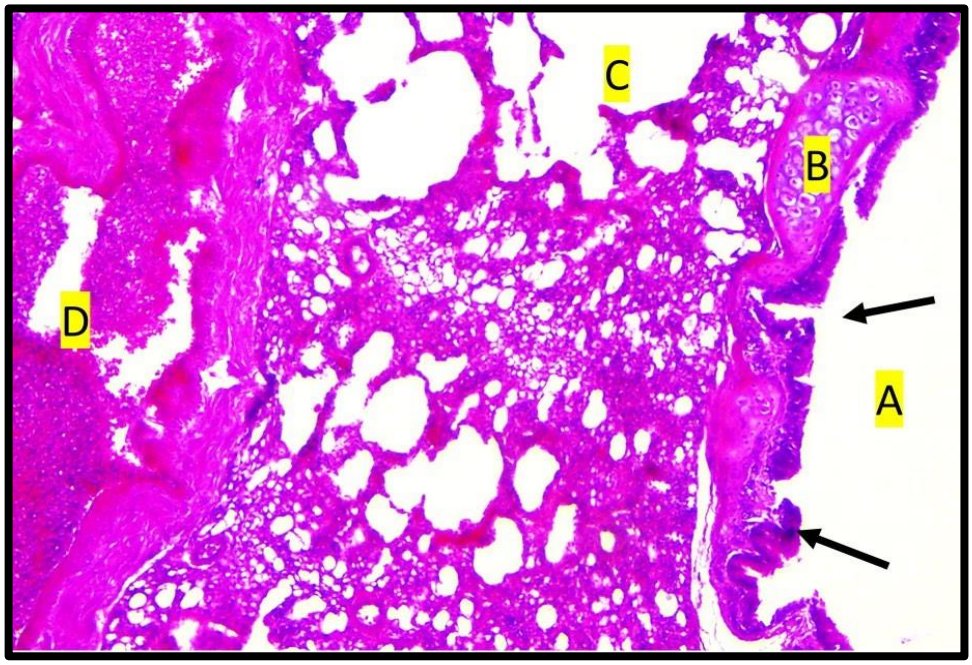

Figure (1): cross section of lung mynah birds show the: A. lumen secondary bronchi, folded epithelium (black arrow), B. hyaline cartilage, C. Parabronchi lumen, D. blood vessel, E. air capillary. (H&E 10X)

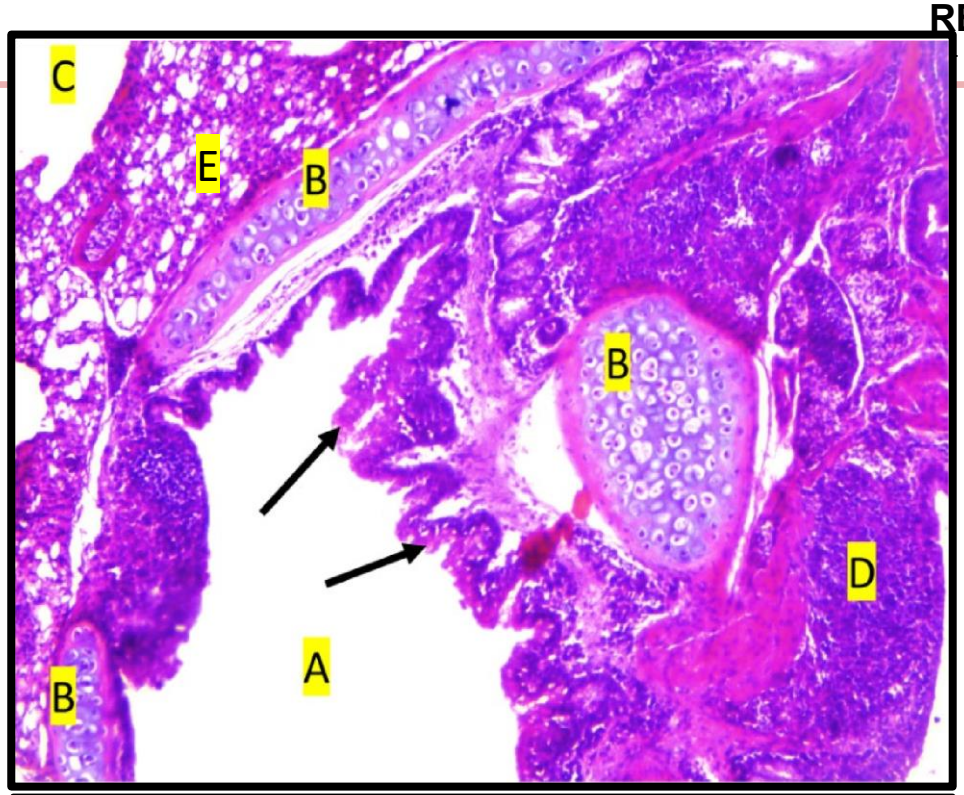

Figure (2): cross section of lung starling birds show the: **A**. lumen secondary bronchi, folded epithelium (black arrow) **B**. hyaline cartilage, **C**. Parabronchi lumen, **D**. lymph node, **E**. air capillary.(H&E 10X)

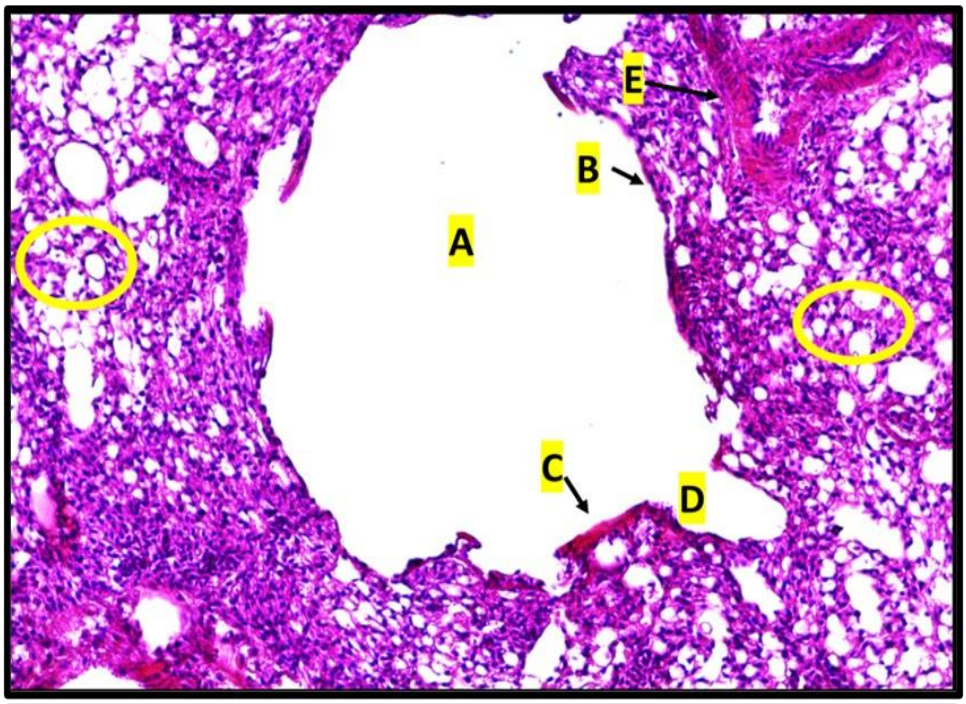

Figure (3): cross section of parabronchi in mynah birds show the: **A**. parabronchi lumen, **B**. simple squamous epithelium, **C**. smooth muscle, **D**. atria, **E**. blood vessels, air capillary (yellow circle). (H&E stain 40X)

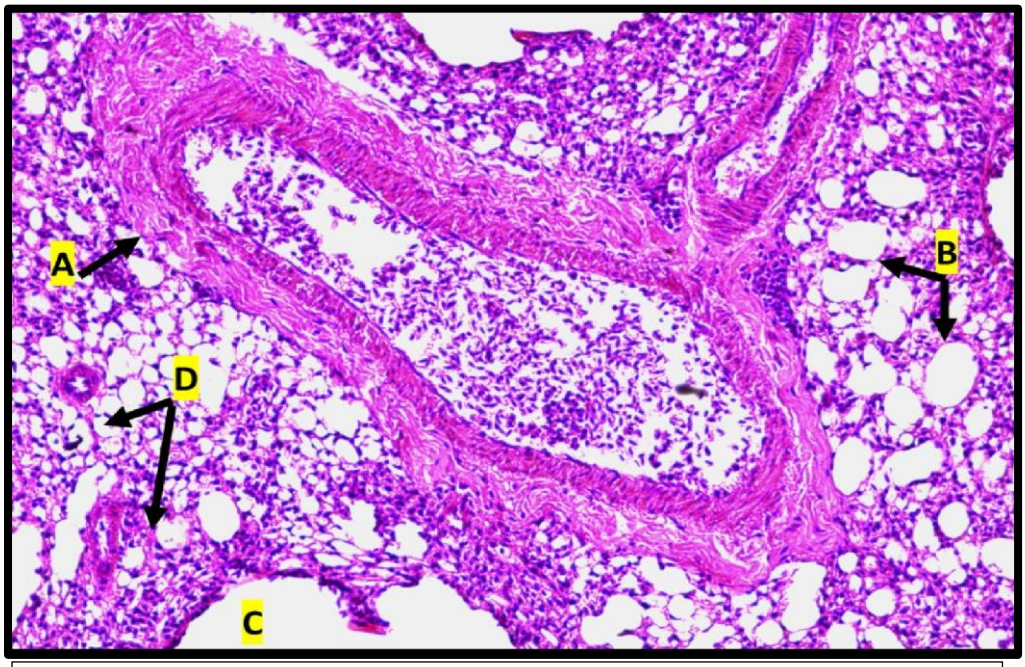

Figure (4):cross section of lung mynah birds show the: A. blood vessel, B. air capillaries, C. parabronchi, D. interparabronchial blood vessels (H&E 20X)

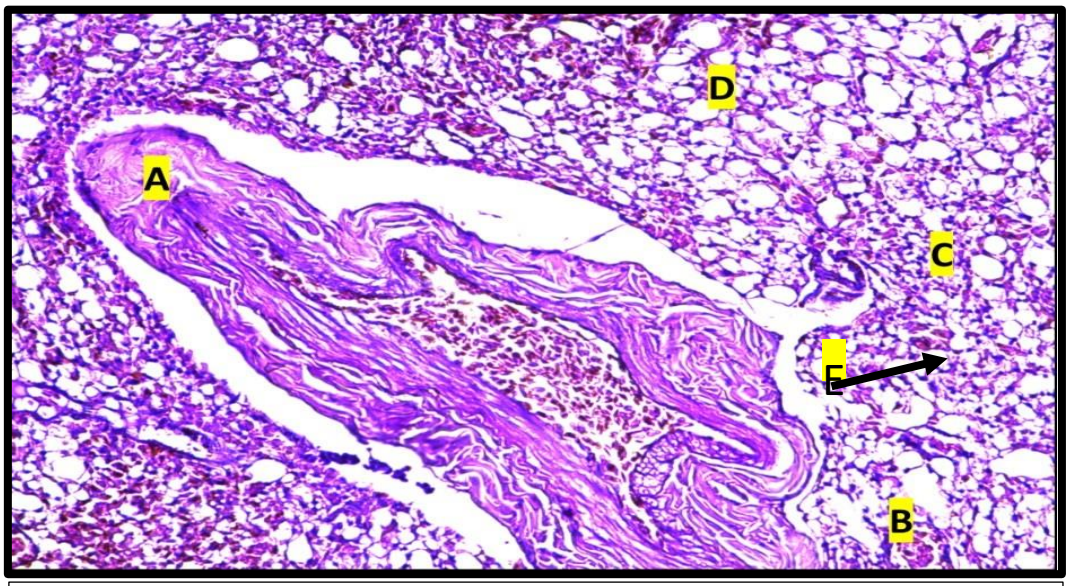

Figure (5):cross section of lung starling birds show the: A. blood vessel, B. lymph nodes, C.parenchyma , D. air capillary, E. interparabronchial blood vessels (H&E 20X)

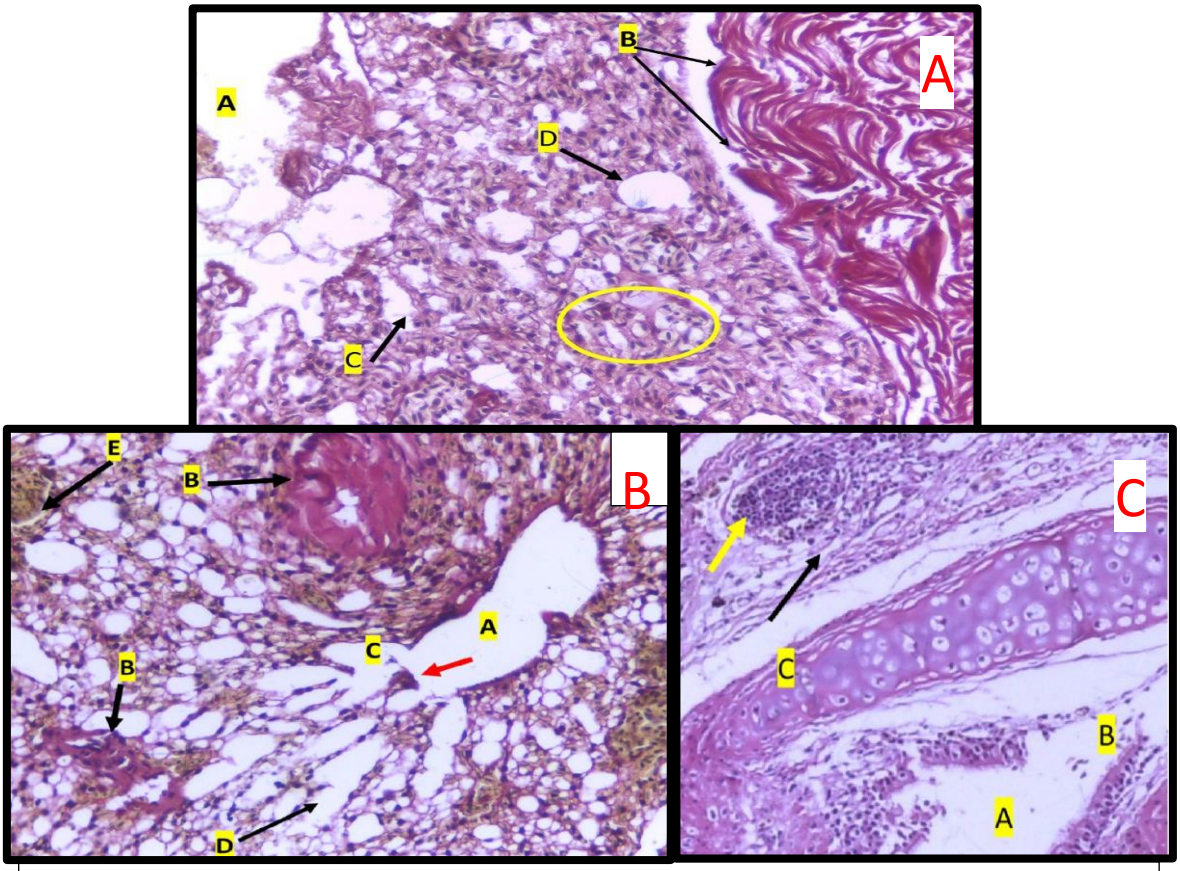

Figure (6): cross section of lung in mynah bird show the : (A), A. parabronchi lumen , B. collagen fiber , C. infundibula , D. air capillary , parenchyma (yellow circle). (B), A. parabronchi lumen, B. collagen fiber , C. atria ,D. infundibula, E. blood vessel, smooth muscle (red circle). (C), A. secondary lumen, B, , pseudostratified columnar ciliated epithelium , C.Hyaline cartilage, lamina propria (black arrow), lymphatic nodes (yellow arrow).

Van Gieson stain A ;B 20X ;C 40X

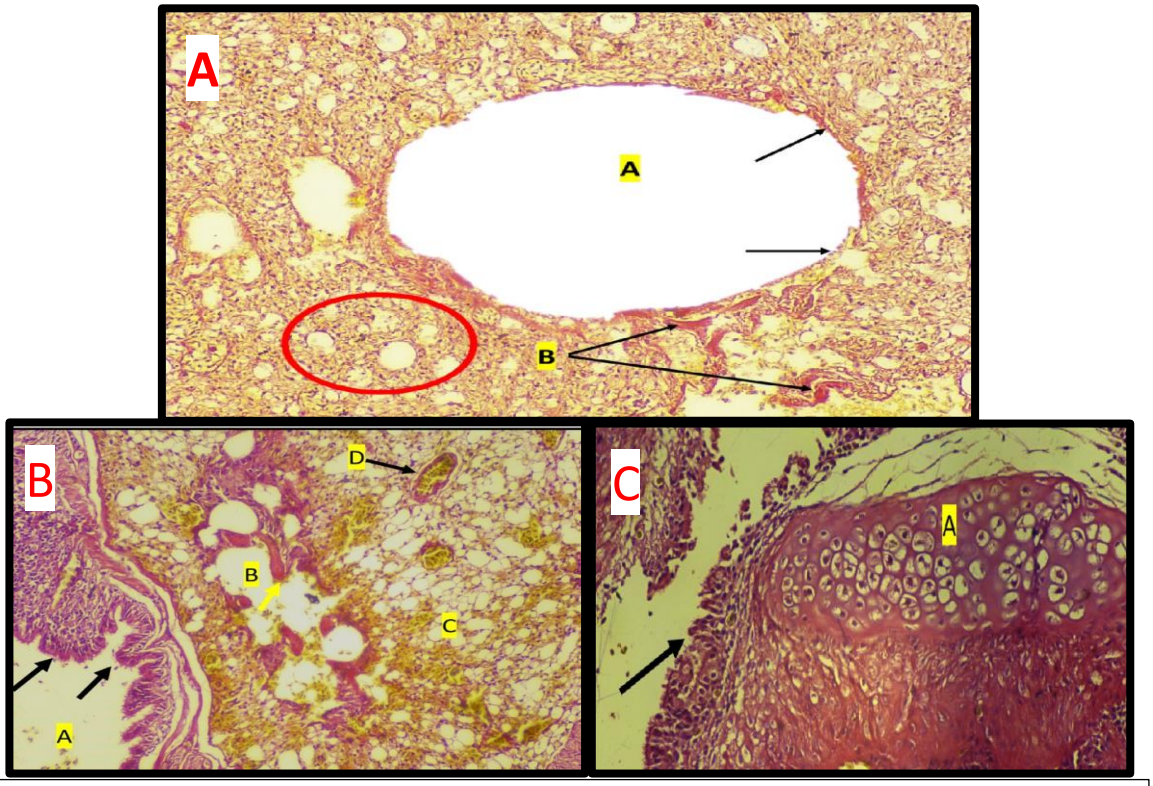

Figure (7): cross section of lung in starling show the: (A), A. para bronchi lumen , B. collagen fiber , pseudostratified columnar ciliated epithelium(black arrow) ,air capillary (yellow circle); (B), A. secondary lumen , pseudostratified columnar ciliated epithelium(black arrow), B. para bronchi, smooth muscle (yellow arrow), C. air capillary ,D. blood vessel ; (C), A. hyaline cartilage, pseudostratified columnar ciliated epithelium(black arrow).

Van Gieson stainA ;B; 20x ,C 40X

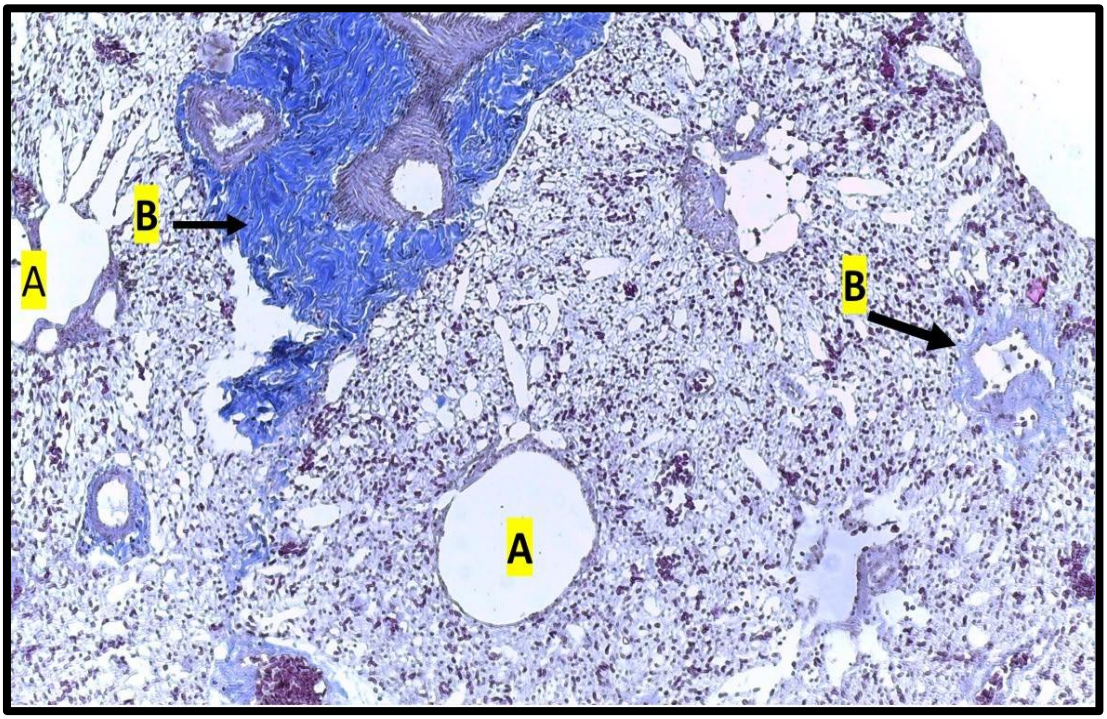

Figure (8): cross section of lung in mynah birds show the: A. parabronchial lumen, B, Collagen fiber (Masson Trichrome 20X)

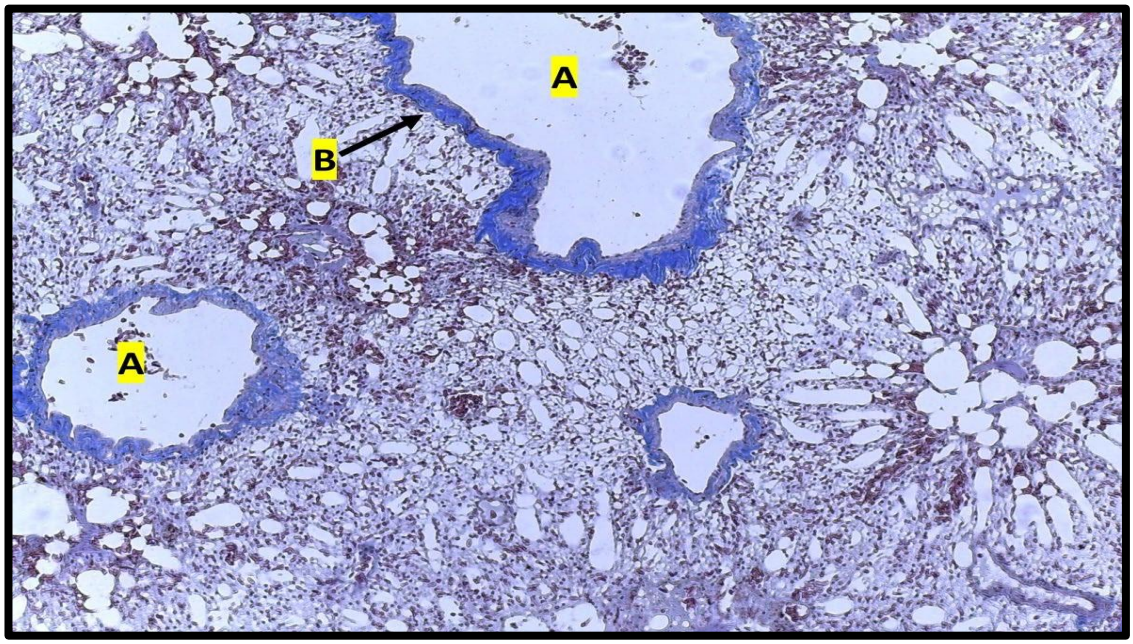

Figure (9): cross section of lung in starling birds show the: A. parabronchial lumen, B, Collagen fiber (Masson Trichrome 20X)

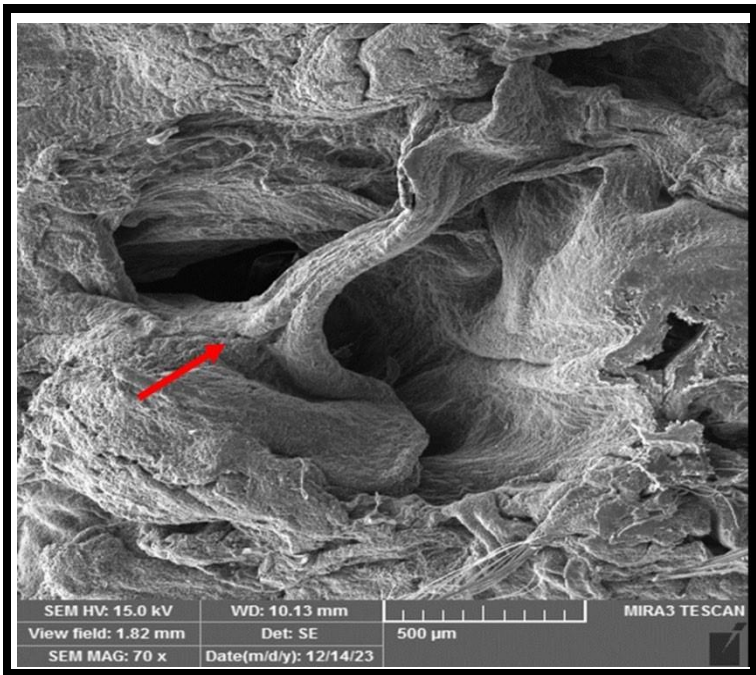

Figure (10): Scanning electron micrograph of the lung in mynah show: Elastic septa (inter parabronchial septa) (red arrow). MAG (70) X, Line

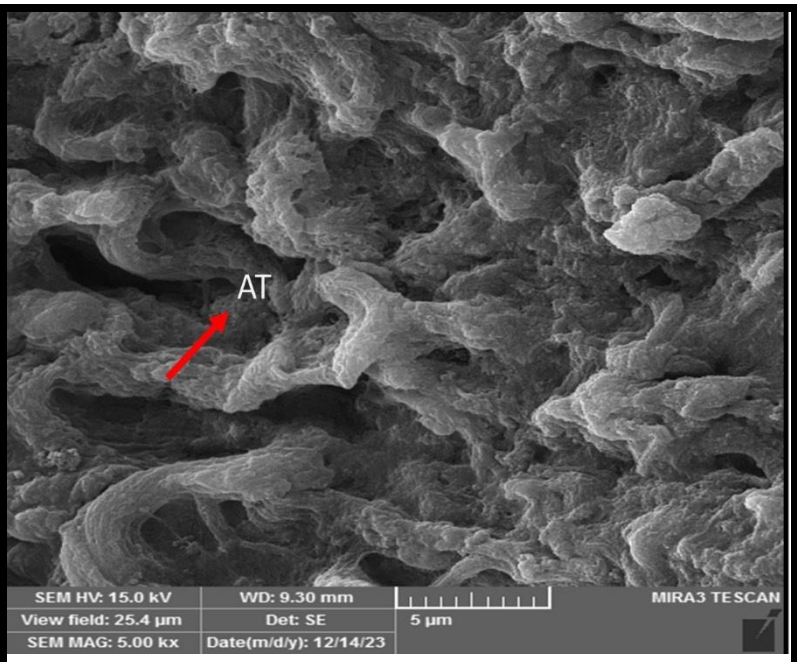

Figure (11): Scanning electron micrograph of the parabronchus in mynah show: polygonal out pouches
Atria (red arrow). MAG (5.00) X, Line scale 5um

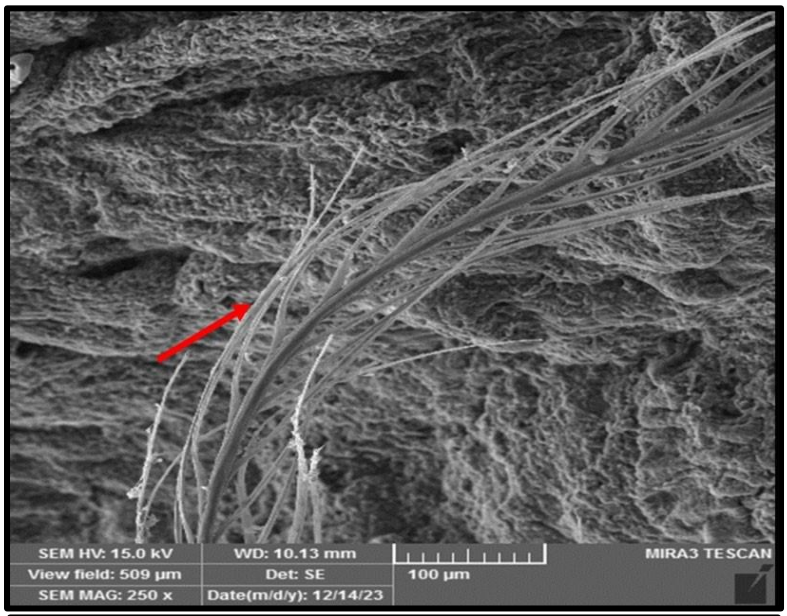

Figure (12): Scanning electron micrograph of the lung in mynah show: muscle fiber (red arrow). MAG (250) X, Line scale 100um

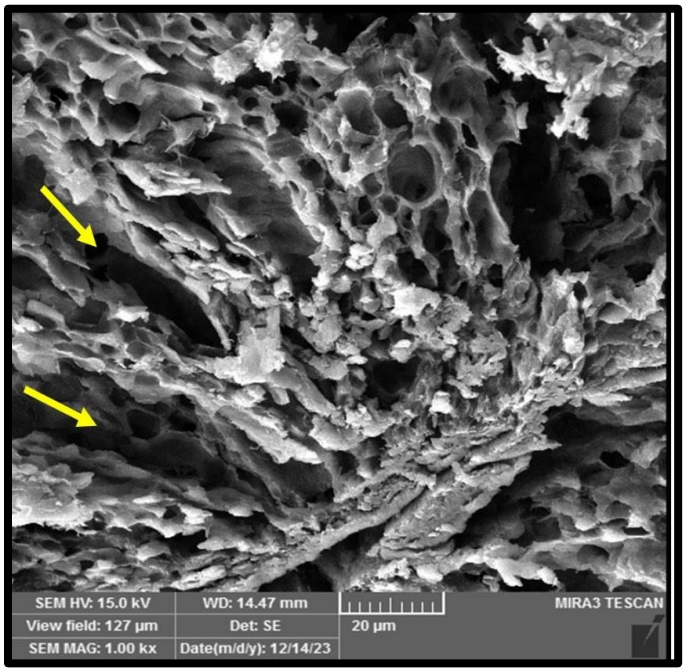

Figure (13): Scanning electron micrograph of the lung in starling show: Intrabronchial septa (yellow arrow). MAG (1.00) X, Line scale 20um

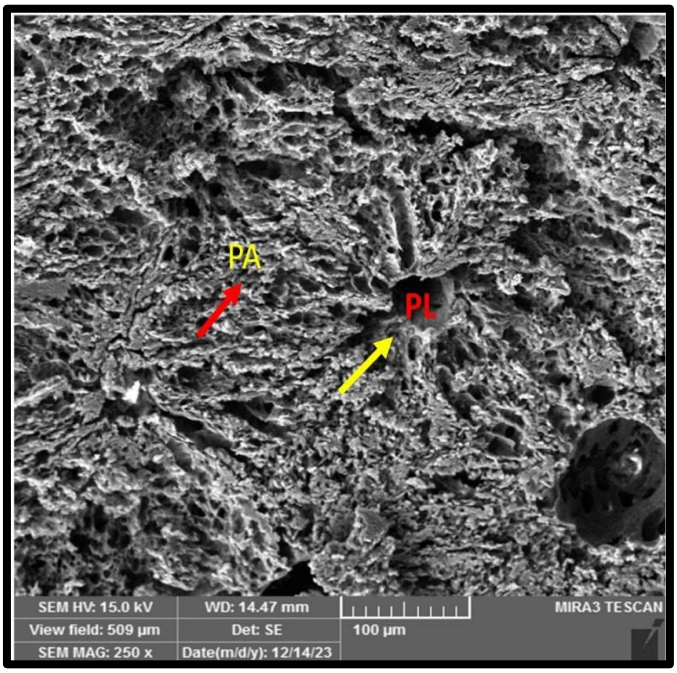

Figure (14): Scanning electron micrograph of the lung in starling show: parenchyma (red arrow) parabronchus (yellow arrow). MAG (250) X, Line

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