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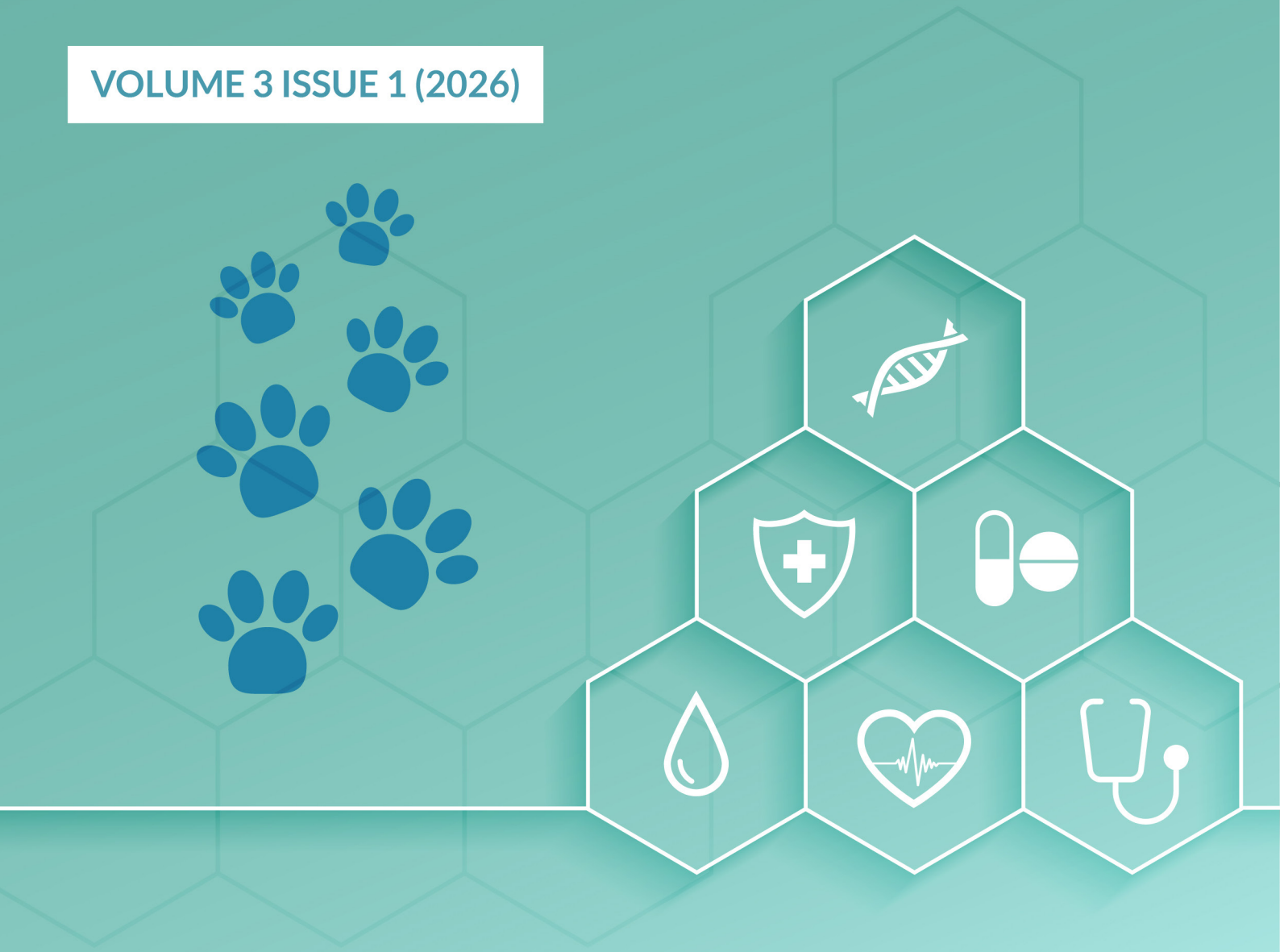

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Morphological and Histological Characteristics of Trachea in Iraqi Pin-tailed Sandgrouse

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ABSTRACT

This research explored the physical form of the Iraqi sandgrouse, as well as the internal structure of its trachea histologically. To achieve this, five (5) male adult sandgrouse from Al-Ghazel market, Baghdad, were used. Appropriate approval was obtained to perform this research on animals. The birds were anesthetized with chloroform and euthanized before being dissected. The trachea was taken from each bird, and cleaned with 0.9% NaCl, for use in the morphology of the cartilaginous rings and in histology studies. The present findings indicate that the trachea is a flexible tube made of cartilage and measures approximately 4.5± 0.16 cm in length extending from the end of the cricoid cartilage to the thoracic cavity, where it separates into two primary bronchi behind the base of the heart. The trachea has two sections: one proximal section attaching to the larynx with a diameter of approximately 0.57± 0.06 cm and a distal section connecting to the vocal organ (syrinx) with a diameter of approximately 0.45± 0.08 cm. Histologically, the trachea is composed of three layers of tissue: mucosa, submucosa, and adventitia. Longitudinal striated muscle fibers that are multinucleated were visible supporting the adventitia. The mucosa is comprised of pseudostratified epithelium, while the submucosal layer is composed of loose connective tissue, and hyaline cartilage. The adventitia contains loose connective tissue and blood vessels. On histometric analysis, layer measurements revealed the mean thickness of the mucosa to be approximately 30.2 ± 1.6 µm, that of the submucosa to be approximately 205.8 ± 7.8 µm, while that of the adventitia was approximately 65.5 ± 6.4 µm.

INTRODUCTION

Pterocles alchata is a bird that belongs to the Pteroclidiformes and Pteroclididae families, and the common name of this bird is sandgrouse. This type of sandgrouse is found throughout the Middle East, Africa, India, and south through Iran and Pakistan. The Iraqi pin-tailed sandgrouse is the most abundant type of sandgrouse located in Iraq, and is found in vast numbers throughout the desert areas and dry grasslands (Alshammary *et al.*, 2018 ; Fazaa *et al.*, 2017).

Birds have a circular, flexible tube made of cartilage, also known as the trachea. This tube runs along the midline of the bird's neck and has a slant toward the right side of the neck (i.e. at an angle from the ventrolateral axis of the cervical vertebrae). It also runs parallel to, and follows the course of, the bird's esophagus, all the way to the end of the trachea (Getty, 1975; Khaksar *et al.*, 2012). The trachea is a tubular structure that has hyaline cartilage along its entire length, from the larynx to the point at which it divides at the syrinx, and is formed into complete rings with the appearance of an O (having a complete circle) - therefore forming complete circles rather than C or U shapes as are commonly found in mammals (Khayoon,2022; King & Mclelland,1984). The trachea bifurcates into two primary bronchi at their respective proximal ends, and appear to consist of multiple rings of hyaline cartilage arranged in a tube-like manner. Each primary bronchus extends from the point where the trachea bifurcates to the hilum within the lung (Alumeri *et al.*, 2013). The trachea of histology is organized into three different layers: one is the mucosa, which consists of pseudostratified columnar epithelial tissue with goblet cells and mucous glands; the second is the lamina propria (or submucosa), which has dense connective tissue and rings of hyaline cartilage; and the last is the adventitia, which consists of loose connective tissue and contains blood vessels (Mokhtar & Hussien, 2020; Samuelson, 2007). Despite the ecological significance and wide geographic distribution of the Iraqi sandgrouse (*Pterocles alchata*), there are no studies investigating the morphology and histology of its trachea. Building on our previous work on the lungs of this species, the present study aims to investigate the morphological and histological characteristics of the trachea and compare them with those of other avian species. This study will contribute valuable information to our understanding of the avian respiratory system.

MATERIALS AND METHODS

The study involved five sexually mature male sandgrouse (*Pterocles alchata*) from the Al-Ghazel bird market in Baghdad. Their health was verified to ensure that they were not suffering from any injury or disease before being humanely euthanized via Anesthesia (via chloroform). After feathers were plucked from each bird, their tracheas were removed by making a long incision

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along the ventral side of the trachea and cutting just above the location where the trachea branches into the bronchi. The tracheostomies collected were put into a 1% solution of normal saline to remove any debris attached to the exterior of the tracheostomies for each of the two studies. In the morphological portion of the study, the tracheas were stained with 1% methylene blue for one minute, then washed three times with 70% alcohol for viewing and counting the number of cartilaginous rings for each of the five birds. In the histological portion of the study, the trachea were fixed in 10% formalin for three days and then washed once with distilled water for 30 minutes before transferring to 70% alcohol for further histological analysis. Using a standard paraffin technique, samples were processed with fixation, clearing, infiltration and finally embedding in paraffin wax at 60°C to create histological sections for microscopy (Ahsan *et al.*, 2024). The histological sections were cut to a thickness of 6 µm using a microtome and stained for tissue detail using hematoxylin and eosin (H&E) stains. Alcian Blue stain was also used to identify mucopolysaccharide content (Luna, 1968). The prepared histological sections were examined using a microscope with a Canon camera for digital imaging. Measurements of the tracheal layer thickness were made using ImageJ software to produce histometric data, and Microsoft Excel 2016 was used for statistical analyses by measuring means ± standard deviations for the data collected (Jahan *et al.*, 2026). The experimental procedures carried out adhered to the agencies' ethical guidelines for scientific experimentation, having been granted approval from the Scientific Research Ethics Committee at the University of Baghdad, College of Education for Pure Sciences (Ibn Al-Haitham), with the approval number (EC-106) and date (25/ 3/ 2026).

RESULTS AND DISCUSSION

Study 1 is concerned with anatomy and morphology, as well as the histological make-up of the trachea.

The trachea of the sandgrouse from Iraq is shown to be a flexible, cylindrical, cartilaginous, hollow structure that is white in color and not folded. The trachea is 4.5 ± 0.16 cm long from the back of the cricoid cartilage and extends back from the back of the neck to the thorax. After entering the thoracic cavity, the tracheal tube Bifurcates posterior to the heart into the left and right primary bronchi, which enter the lungs at the hilum (a term describing the visceral surface of the lungs). (Figure 1).

The trachea is comprised of a succession of fully cartilaginous rings in an "O" shaped configuration. In total, the number of rings is around 50-51. There are two distinct segments of the trachea: the proximal part which is the superior segment and is attached to the laryngeal

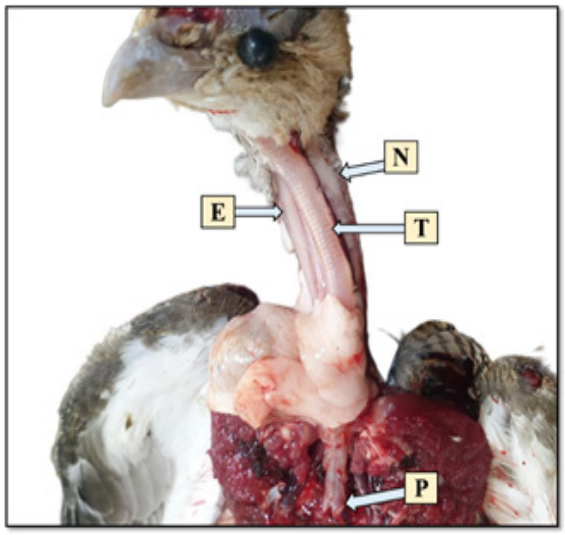

Figure 1: Ventral View In The Iraqi Pin Tailed Sandgrouse Showing: Esophagus (E), Neck (N) , Primary Bronchi (P) , Trachea (T).

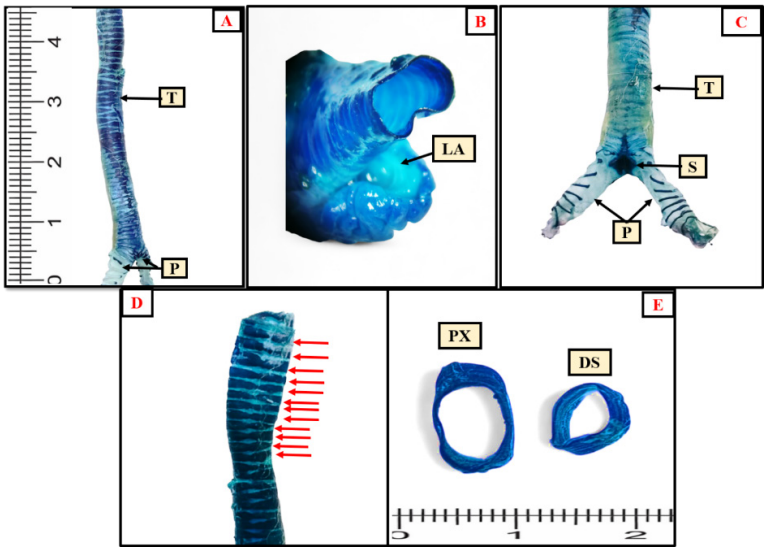

Figure 2: Morphological Image in the Iraqi pin tailed sandgrouse Trachea stained by methylene blue showing: A- The trachea ; B- The proximal part of trachea ; C- the Distal part of trachea ; D- the Cartilaginous rings; E- The Diameter of Proximal and Distal part: Distal part (DS) , Larynx (LA) , Primary bronchi (P) ,Proximal part (PX) , Syrinx (S) , Trachea (T) , Cartilaginous rings (Red Arrow).

structure, and the primary gland in the neck (thyroid) is present bilaterally; and the distal part will reside inside the thoracic cavity, with its termination being connected to the syrinx and primary bronchi.

Statistical measurements indicate the average diameters of both the proximal and distal Part to be approximately 0.57 ± 0.06 cm and 0.45 ± 0.08 cm, respectively (Figure 2).

The three layers of histology, Tunica mucosa, Tunica submucosa and Tunica adventitia are the major components of the histological structure of the Trachea (Figure 3). The three layers of histology begin with the Tunica Mucosa which is a layer of pseudostratified ciliated columnar epithelial tissue with ciliated free surfaces, with basal cells triangularly shaped located between them resting on the basement membrane. The alcian blue stain will show the presence of mucous glands that are present within this epithelial layer. These may be single in nature (goblet cells) or may exist as simple tubuloalveolar types of glands. The lamina propria supports the mucosal layer, which consists of loose connective tissue containing collagenous fibers and blood vessels (Figure 4).

The submucosa, which is the second layer of the trachea, appears thicker than the mucosa and consists of loose connective tissues, elastin, and hyaline cartilage; most of the submucosa comprises hyaline cartilage. The chondrocytes of the hyaline cartilage undergo ossification (partial bony formation), and occupy small spaces called lacunae in the cartilaginous matrix. Each lacuna may contain more than one chondrocyte, thus forming groups (isogenic groups or "cell nests"). The outer surface of cartilage is covered with perichondrium. The findings also demonstrate that there are only small ossifications present (Figures 3 , Figure 5).

The adventitia was found to contain connective tissues and blood vessels. The presence of skeletal muscle cell bundles, located longitudinally and surrounding the trachea, does not form a true layer of the tracheal wall (Figures 3 , Figure 6).

The thickness of the mucosa is approximately 30.2 ± 1.6 µm, the thickness of the tunica submucosa is

Figure 3: Cross section in the Iraqi pin tailed sandgrouse showing the tunica of Trachea: Tunica Adventitia (A) , Bone Marrow (BM), Blood vessels (BV) , Hyaline cartilage (H) , Lamina propria (L) , Mucosa with epithelia (M) , Sub mucosa (SM) , Skeletal muscles cells (SK); 10 X , by H&E stain.

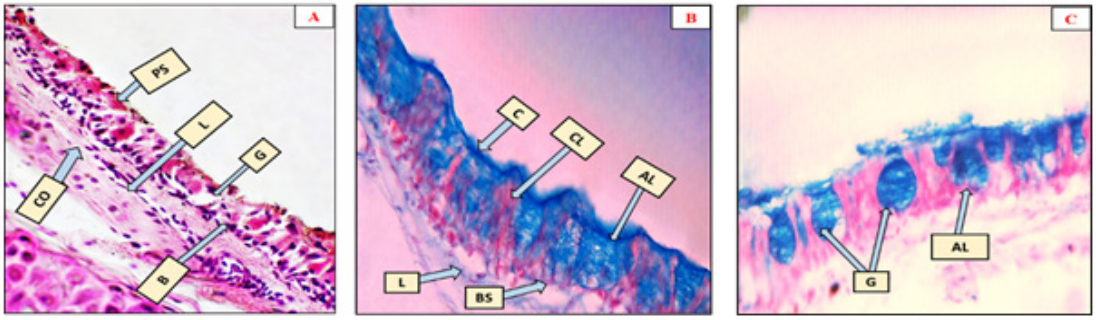

Figure 4: Cross section in the Iraqi pin tailed sandgrouse Trachea showing the tunica Mucosa : Alveolar tubular mucous gland (AL) , Basal cell (B), Basement membrane (BS) , Cilia (C) , Columnar cell (CL) , Collagen fiber (CO) , Goblet cell (G) , Lamina propria (L) Pseudostratified epithelium (PS) (40 X , A: H&E stain; B&C: Alcian Blue stain)

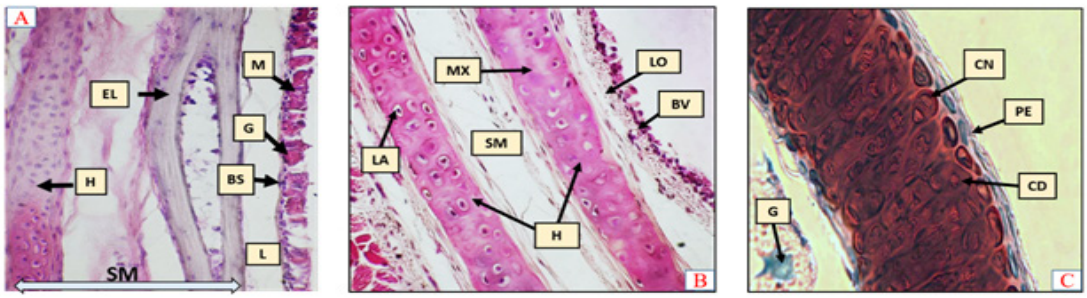

Figure 5: Cross section in the Iraqi pin tailed sandgrouse Trachea showing the tunica Submucosa : Basement membrane (BS) , Blood vessel (BV) ,Chondrocyte (CD) , Cell nest (Isogenic group cell) (CN) , Elastic fiber (EL) , Goblet cell (G) , Hyaline cartilage (H), Lamina propria (L), Lacuna (LA), Loss connective tissue (LO), Mucosa (M) , Matrix (MX), Perichondrium (PE),Sub mucosa (SM) ; (A&B: 40X , H&E ; C:40X , Alcian Blue stain.)

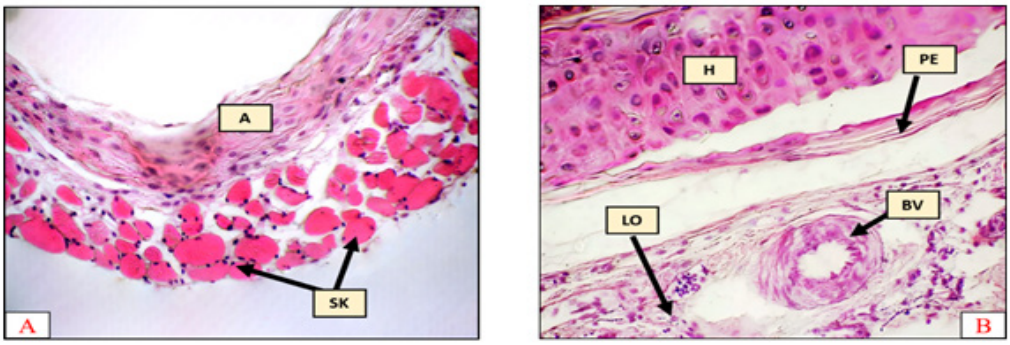

Figure 6 : Cross section in the Iraqi pin tailed sandgrouse Trachea showing the tunica Adventitia : Adventitia (A), Blood vessel (BV) , Hyaline cartilage (H), Loss connective tissue (LO), Perichondrium (PE), Skeletal Muscles Cell (SK) ; (A&B:40X , H&E stain)

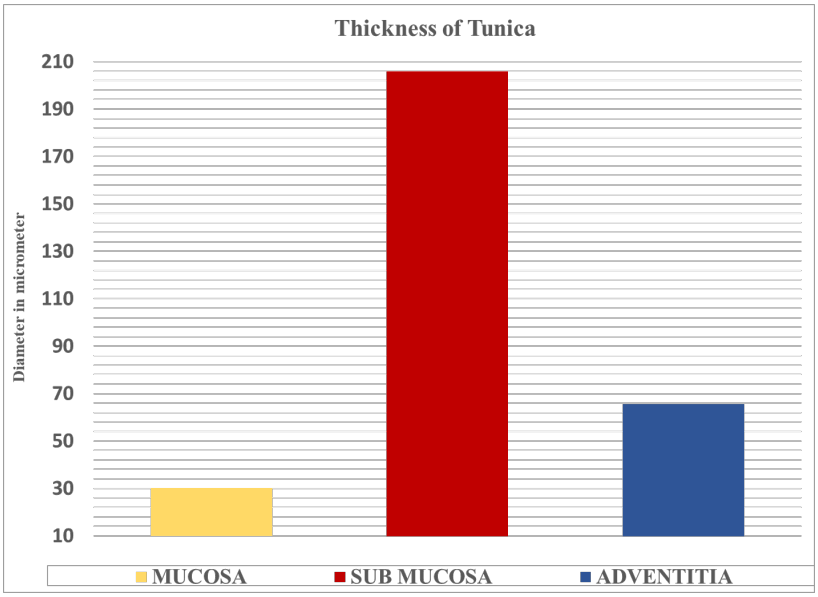

Figure 7 : Shows the mean and standard error (Mean±SE) for the thickness of three tunica of Trachea (Mucosa, sub mucosa and adventitia)

approximately 205.8 ± 7.8 µm, and the thickness of the adventitia is approximately 65.5 ± 6.4 µm (Figure 7)

Discussion

According to our research, we've now discovered that the Iraqi Sandgrouse has a trachea that appears as a rounded, colored tube created by cartilage that does not produce any visible folds and is colored white. These findings agree with what has been found by other researchers, (Al-Taai 2021; Al-Jawadi & Mutlak 2020; Ali & Mutlak, 2019 2019), but there are some differences in comparison with work done by Bello *et al.,* 2017) where they discovered a red-colored trachea in the donkey (*Equus asinus*), and Klingler (2016) where they noted that Canada geese (*Branta canadensis*) have very long tracheas which form loops prior to entering their lungs allowing for stability while they are flying to allow for easy airflow through their body.

The trachea runs from the trunk and lies to the left of the ventral aspect of the neck and is to the right of the esophagus. The reason for the tracheas being separated from the esophagus is because the crop is located between the two structures. The area between the trachea and the esophagus is significant in that it relates to how an Iraqi sandgrouse eats in their arid environment, just like the studies performed by Klingler (2016) on the two species of pigeons (*Columba livia* and *Zenaida macroura*). On the other hand, these results are in contrast with those obtained for *Sturnus vulgaris* (Al-Taai *et al.*, 2022) and *Anas platyrhynchos* (AL-Ahmed.& Sadoon, 2020) where both birds exhibited a trachea located on the dorsal area of the neck with the esophagus located to the right side of the neck.

The trachea has several complete ring rings to support itself in an O-shaped configuration. There is a variation in average number of rings per species, however in this study there are an average of approximately 50-51 rings per bird. (Sakr *et al.*, 2022) found an average of 130-140 rings for a goose, 155-160 for a cow and 45-60 for a sparrow. The average number of tracheal rings was between108-126 in the domestic fowl and between112-116 in the domestic duck (Das & Mishra 1965). The diversity in the number of tracheal rings is a result of anatomical differences that

exist such as neck length, body size, and species-specific adaptions to their environment.

This current study also supports the finding of (Sakr *et al.*, 2022) and Rajab et al (2024) who reported that the trachea is divided into two major sections when associated with the proximal laryngeal area. The proximal portion of the trachea connects via the larynx and the distal portion connects via the syrinx where it bifurcates into the main prison and bronchi of the lungs. In contrast, Yılmaz et al (2016), when studying male and female chickens, reported that the trachea is comprised of three sections (upper, medium, and lower) and Ali and Mutlak (2019) studying the rumped tomb bat observed a similar result..

The microscopic structure in the trachea is composed of three distinct tissue types: the mucosa, submucosa, and adventitia. Al-Mamoori *et al.* (2025) reported the same findings in their examination of the Iraqi quail's anatomy. According to Al-Taai *et al.* (2022), on the other hand, there are four tissue layers in the trachea (mucosa, submucosa, adventitia, and muscularis), which Bacha & Bacha (2000) also agree with. Furthermore, Al-Jawadi & Mutlak (2020) identified the fourth layer in the weasel as hyaline cartilage.

The mucosa of the trachea is comprised of ciliated, pseudostratified epithelium and includes columnar and basal epithelial cells, all resting on a basement membrane. These observations parallel the research findings by Rajab *et al.* (2024) and Waad (2015) performed in studies of the pigeon and guinea pig. Mucous glands are present within the mucosa; these include the two types of mucous glands (the goblet cell) and simple alveolar tubular glands as described in the studies by Frappier & Eurell (2006) and subsequently confirmed by Al-Mussawy *et al.* (2010) in turkeys. In contrast, Al-Taai *et al.* (2022) reported that only the two types of glands (mucous and serous) are found in the upper region of the trachea, which is where these glands are more prevalent than in the rest of the trachea. The mucous and serous secretions from the mucous glands contribute to the maintenance of moisture and function within the trachea.

The lamina propria sits between the mucosa and the submucosa. It is made up of loose connective tissue that provide support through collagenous fibres, as stated by (Al-Mussawy *et al.*, 2010; Ali & Mutlak, 2019). The submucosa is made up of loose connective tissue, elastic fibers, and hyaline cartilage, which exists in the shape of a cylindrical structure and is the predominant tissue type of this layer. This finding is in agreement with studies by Çevik-Demirkan *et al.* (2007) and Al-Mamoori *et al.* (2025). On the contrary, Ali & Mutlak (2019) indicated that hyaline cartilage in the rumped tomb bat forms an independent layer that will have a "C"-shaped appearance, which differs from what was observed in this study.

This article's findings align with those of Pantoja *et al.* (2020) and Al-Jawadi & Mutlak (2020), which state that hyaline cartilage comprises chondrocytes encased within lacunae in a matrix and includes a layer called perichondrium. Additionally, this study found partial ossification present in some areas of cartilage tissue. Partial ossification may be an anatomical adaptation to the desert habitat for increased rigidity in the tracheal wall to help maintain airway support during respiration in a hot and dry environment. It is expected that the results will lead to increased airflow and improved access to oxygen for the animals.

AL- Ahmed. & Sadoon (2020) in *Anas platyrhynchos*, Al-Badri & Al-Salman (2015) in *Gallus domesticus*, and Gazi (2017) in *Sturnus vulgaris* also produced similar findings regarding submucosal glands in birds. Moreover, the presence of submucosal glands, as mentioned in the present study, agrees with (Al-Taai , 2021; Rajab *et al.,* 2024) in *Columba livia*, Sakr *et al.* (2022) in domestic cattle, domestic goose, and house sparrows, and Al-Taai *et al.* (2022) in *Sturnus vulgaris*. However, Al-Jawadi & Mutlak (2020) found the submucosa to contain mucous and serous glands in the weasel's trachea, which contradict the findings of the present study.

Outer layer (adventitia) of the tracheal wall is the tunica adventitia, composed of loose connective tissue including blood vessels; the results support the observations made by Al-Mussawy *et al.* (2010) in turkeys and Al Taai (2021) in *Columba livia*. The current study also found multinucleate skeletal muscle cells oriented longitudinally encircling the adventitia; these connections support the external trachea as well as sound production, but they do not form a discrete tracheal wall layer. Al Taai *et al.* (2022) in *Sturnus vulgarus* and Al-Mussawy *et al.* (2010) in turkey each showed smooth muscle surroundng the adventitia; this is in contrast to what the current study found.

Through morphometric analysis, thicknesses of the various tracheal layers were shown to vary somewhat; the mucosa was the thinnest layer and the submucosa was the thickest laye (hylaine cartilage accounted for an appreciable increase in thickness); these observations corroborate the findings of Al-Jawadi & Mutlak (2020) on weasels and by Ali & Mutlak (2019) on Rumped tomb bats.

CONCLUSION

Through integration of data obtained from observing tracheal morphology, histology and measuring various morphometric parameters, this study provides a complete description of the trachea of the Iraqi pin-tailed sandgrouse. The study describes how the cartilaginous rings provide structural support for the trachea, while connective tissues provide flexibility. The arrangement and cellular type within each layer of the trachea help form the overall structure of the avian respiratory tract and therefore serve as an important frame reference for comparative study of anatomy, histology, and physiology of various species of birds.

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