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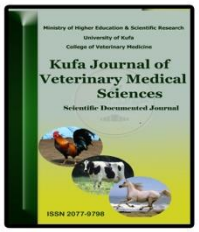

Histological Study of the Larynx in indigenous male West African guinea fowl (*Numida meleagris galeata*) In AL-Najaf province

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Summary

Microscopic investigation of the larynx in Indigenous Male West African guinea fowl *(Numida meleagris galeata*) In AL-Najaf province, the mean live weight was (1750 g ± 50 gm) our need to have a base line data on the respiratory system of this abundant species of bird in Iraq. It is expected that this work will provide a base for future research and subsequent clinical application as regards the biology of the guinea fowl. Five healthy birds utilize in this study. After bird dead the larynx dissected out and washing by normal saline solution, then were fixed immediately in 10% NBF solution, and then preparing for routine histological processing. The laryngeal mound was covered by non-keratinized stratified squamous epithelium. Toward the glottis the thickness of these epithelia decreased and some of epithelial layers were modified to epithelial glands, then epithelium became ciliated, pseudostratified columnar epithelium. Lamina propria-submucosa contained loose connective tissue connected with the perichondrium of the hyaline laryngeal cartilages (Cricoid, Arytenoid, and Procricoid).

Key words: (Male West African guinea fowl, larynx, glottis, pseudostratified columnar epithelium).

دراسة نسجية لحنجرة ذكور دجاج غينيا الغرب أفريقي البالغ المربى محليا (*Numida meleagris galeata*) في محافظة النجف

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كلية الطب البيطري / جامعة الكوفة

الخلاصة

الفحوصات المجهرية لحنجرة ذكور دجاج غينيا الجنوب افريقي البالغة المربى محليا (*Numida meleagris galeata*) في محافظة النجف الاشرف متوسط وزن (1750 ± 50 gm). لحاجتنا لامتلاك قاعدة بيانات للجهاز التنفسي لهذا النوع من الطيور الواسع الانتشار في العراق. والمتوقع من هذا العمل أن يكون محورا للأبحاث المستقبلية والتطبيقات السريرية اللاحقة فيما يتعلق بعلم أحياء دجاج غينيا. استخدم في هذه الدراسة خمسة طيور خالية من الأمراض التنفسية. بعد موت الطائر اخرجت الحنجرة وغسلت بمحلول الملح الفسلجي ثم ثبتت مباشرة في

10% من الفورمالين المتعادل (NBF) وبعد ذلك حضرت للعمليات النسجية الروتينية. المرتفع الحنجري داخل التجويف الفموي مبطن بالظهارة الحرشفية المطبقة غير المتقرنة. باتجاه مدخل الحنجرة يقل سمك هذه الظهارة تدريجيا وبعض الطبقات الظهارية تحورت الى الغدد الظهارية, والظهارة اصبحت ظهارة عمودية مطبقة كاذبة مهدبة. الطبقة تحت المخاطية من النوع النسيج الضام الرخو متصلة مع السمحاق الغضروفي لغضاريف الحنجرة الزجاجية (الغضروف الحلقي والغضروف الطرجهاري والغضروف امام الحلقي) ألمتعضمة جزئيا.

كلمات البحث: (ذكور دجاج غينيا الغرب أفريقي, فتحة المزمار, مدخل الحنجرة, الظهارة العمودية المطبقة الكاذبة المهدبة)

Introduction

The West African guinea fowl (*Numida meleagris galeata*) is sub-specie of the guinea fowl. This bird is named after "Guinea", a country on the sub-Saharan west coast of Africa. It is a medium sized bird with strong legs and a boney crest). The wild guinea fowl of West Africa is regarded as the original of the domestic stock which is consumed by the rural population in Nigeria. (1)

The purpose of the present study is to describe the histological features in details the larynx. To became ground work information utilizing in study of respiratory physiology, histopathology, also for availing in surgery and anaesthesia in turkey.

The respiratory system of avian is more complex than the mammalian, and described as non-tidal. It is consist of the nasal cavity, larynx, trachea, syrinx, bronchi, lungs, and air sacs. Anatomy, physiology, and mechanics of the avian, and it is has principal function of exchanging oxygen and carbon dioxide between atmosphere and blood, involved in temperature regulation, and phonation these features in common with the respiratory system of mammals (2; 3; 4; and 5). But different by the larynx which does not have vocal cords, therefore is not involved in voice production and epiglottis is not present (6). At the bifurcation the trachea into the right and left primary bronchi lies the source of sound (syrinx) is partly tracheal and bronchial in origination and is highly variable in structure between species, basically it is composed of variable ossified cartilages, vibrating membranes, and muscles (7; and 8).

In the West African guinea fowl (*Numida meleagris galeata*) Laryngeal mound (mons laryngealis) appears as a conspicuous elevation, it is roughly triangular in shape (1), while it is heart-shaped in the Chicken, Turkey (*Meleagris gallopava*), and Long-legged buzzard (*Buteo rufinus*) (9; 10; 11; 12; and 13). In the Duck and Goose tends to be relatively elongated, being lozenge-shape rather than heart-shaped (14). Whereas in Ostriches the larynx protrudes from the pharynx and have not laryngeal mound (15).

The inlet (glottis) of the larynx of the Domestic fowl (*Gallus gallus var. domesticus*) such as West African guinea fowl, Chicken, Duck, and Ostriches appear as slit-like opening limited by the arytenoid cartilages, and continuous caudally by narrow groove called the laryngeal fissure (*Sulcus larynges)* (4; 10;12; and 15). The laryngeal fissure in the West African guinea fowl and Ostriches has an inverted triangle shape (1; 13; and15).

Larynx in the birds consists of (cartilages, muscles, and ligaments). Cricoid cartilage is a single and hyaline type which consists of body and the left and right wings. (16; 7; 2; and 9). The body of cricoid cartilage has median ventral crest which projects dorsally into the lumen of the larynx (17)

Procricoid cartilage is a single and hyaline type in Chicken, Turkey non-passerine species (*Spheniscids*, *Gallus*, and *Columba*), several *Corvids*, and Long-legged buzzard, it is appear as small comma-shaped, intervenes between the two arytenoid cartilages, and between the two cricoid wings, completely ossified in the adult. It consists of a dorsal body and

a ventral tail (12), Arytenoid cartilage is a paired and hyaline type in Chicken, Duck, Turkey, and Long-legged buzzard they meet together dorsocaudally. It consists of the body, rostral process, and the caudal process. (7; 12; and 17).

In birds the intrinsic skeletal laryngeal muscles are superficial muscles which run from the wing and body of cricoid cartilage to the arytenoid cartilage and the deep muscles which run from caudal midline of the larynx to the cricoid and arytenoids cartilages (14; and 12).

The skeletal extrinsic laryngeal muscles are, rostral extrinsic, The caudolateral extrinsic muscle, and the caudomedial extrinsic muscle (14).

The epithelia which lining the air ways (nasal cavity, larynx, trachea, syrinx, primary bronchi) is ciliated, pseudostratified columnar epithelium with different components, and called respiratory epithelia (17).

The larynx is lined partly by a stratified squamous epithelium and partly by a ciliated, pseudostratified columnar epithelium. Numerous elastic fibers are present in the lamina propria. Glands (serous or mucous or mixed) occur in the lamina propria and submucosa, but are lacking in the vocal and vestibular folds. Hyaline and elastic cartilages provide support of the laryngeal wall (7; and 18).

The median mucosal ridge of the cricoid cartilage contained abundant small mucous glands alveoli, and large amount of submucosal connective tissue with sporadic lymphoid tissue. The mean thickness of epithelia and cricoid cartilage at the median mucosal ridge were (420 ± 33 µm) and (1070 ± 73 µm) respectively (19)

Materials and methods

The present study used on five healthy male West African guinea fowl at the first year of their age collected from the center of AL-Najaf AL- Ashraf city, Specimens were prepared by bleeding of birds with the cutting of the major neck blood vessels after making an skin incision in the neck and separation of trachea away from the site of cutting to avoid aspiration of blood and damaged of the respiratory system (19). Then opened the mouth and the larynx identified with reported the morphological features.

Each larynx were dissected out and washed with normal saline solution (0.9% NACL), then put in the special casket were fixed immediately in neutral buffer formalin (NBF10%) at room temperature for 12-24-24 hrs seriatim. The routine histological processing was:

1- Tissue processer Histoline, Italy : Its contain the serial steps
   a- Dehydration: seven serial steps of deferent concentrations of ethanol, two hours for each step.
   b- Clearing: two steps of xylene, one hour for each step.
   c- Impregnation: two steps of melted paraffin wax (58-60 ^{o}C) two hours for each step.
2- Embedding Center TEC – 2800 Histon, Australia: contain special molds for formed wax blocks.
3- Semi-digital rotary Microtome Histoline, Italy: Sectioning measures 5 micrometer thickness.
4- Staining by:
   a- Harris Hematoxylin and Eosin Stains, routine stains used to demonstrate the general histological structure.
   b- Periodic acid-shiff (PAS) Stain used this stain to demonstrate the type of glands secretions. (20).

Morphometric Measurements:

Five sections of each larynx were taken for studied by use of ocular micrometer and the following data recorded:-

-Thickness of the
   a- Epithelium of the laryngeal mound at the oral cavity.
   b- Epithelium of the laryngeal cavity.

c- Bodies of cricoid and arytenoid cartilages (21)

And for purposes of photography used Digital camera TB – 2w Optica, Italy with Microscope B-6TI System Optica, Italy.

Results

The investigations of the larynx of West African guinea fowl revealed that the larynx protruding toward the oropharyngeal cavity as elongated triangular mound, apex toward the tongue, and base toward interance of the esophagus, several caudal pointed papillae at the caudal part of the mound, the glottis elongated triangle rostrocaudally direction. The building unites of the larynx were hyaline cartilages (single cricoid and Procricoid and paired arytenoid cartilages they connected with each other by the ligaments, and articulations) skeletal muscles (intrinsic and extrinsic laryngeal muscles). The laryngeal mound covered by non-keratinized stratified squamous epithelium (Fig. 1&2), the mean thickness of this epithelium at the thicker part was (165 ± 1.4µm), thickness of this epithelium decreased gradually toward the glottis. The submucosa composed of loose connective tissue rich by collagen bundles (Fig. 1&2). Epithelial glands initial formation at the beginning of the laryngeal cavity as small irregular alveoli, then typical epithelial gland was complete formation toward the laryngeal cavity became the simple branched tubular mucous glands opened via epithelium toward laryngeal cavity which lined by pyramid cells contain mucous droplets and basal circular nuclei and gave the positive reaction with PAS stain (Fig. 1&3). And in the same steps the squamous cells layers decreased gradually and replaced by the ciliated, pseudostratified columnar epithelium, toward laryngeal cavity (Fig. 1&3), the mean thickness of epithelia at the laryngeal cavity were (105 ± 5 µm).

Hyaline arytenoid cartilages were observed under the submucosa near the laryngeal inlet on the left and right side (Fig. 1), the mean thickness of the body was (600 ± 20 µm), and at the lateral and basal side of the laryngeal cavity there were body and wings of cricoid cartilage was hyaline type (Fig. 3), the mean thickness of body was (495 ± 6 µm). Procricoid cartilage caudal part of the larynx rostral to the cricoid wings was hyaline type.

On the lateral aspect of each side of the laryngeal mound at the submucosa, there were simple tubular branched mucous salivary glands which open toward the oral cavity. It consisted of mucous cells which were pyramidal in shape, and these glands gave the positive reaction with PAS stain (Fig. 2)

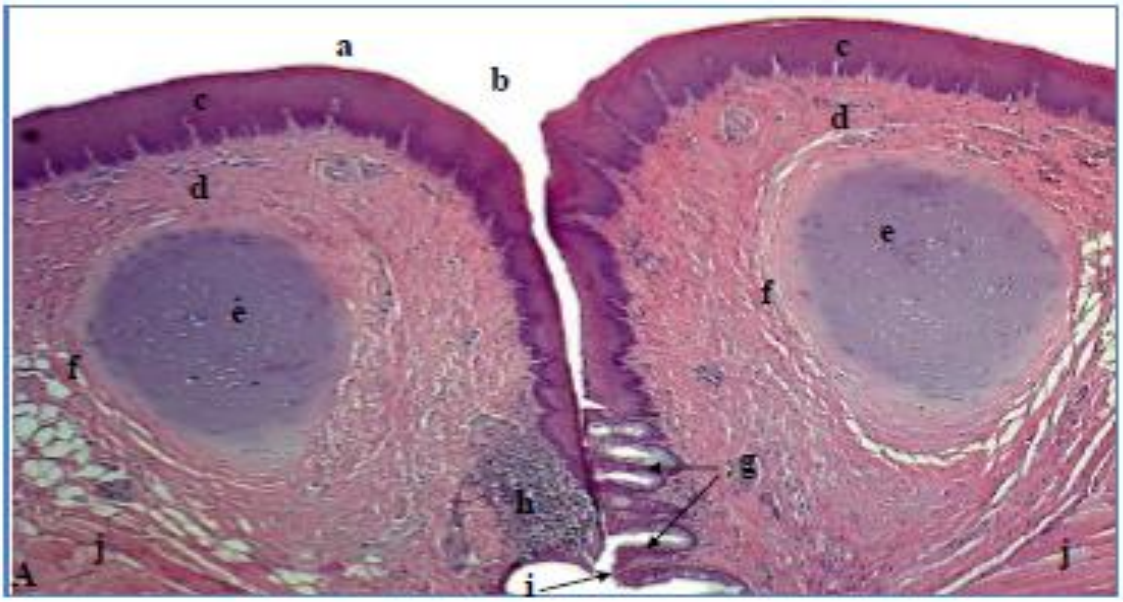

Fig. (1): Cross section in the laryngeal glottis in the West African guinea fowl showing: Oral cavity (a) laryngeal glottis (b), stratified squamous epithelium (oral mucosa) (c), lamina propria-submucosa rich by large bundles of collagen fibers (d), body of arytenoid hyaline cartilage (e), perichondrium (f), primary tubular mucus laryngeal glands (g), subemucosal lymphoid tissue (h) ciliated, pseudostratified columnar epithelium at the initial part of laryngeal cavity (i), bundles of skeletal muscle (deep intrinsic laryngeal muscle) (j).
H & E stain X 100 A

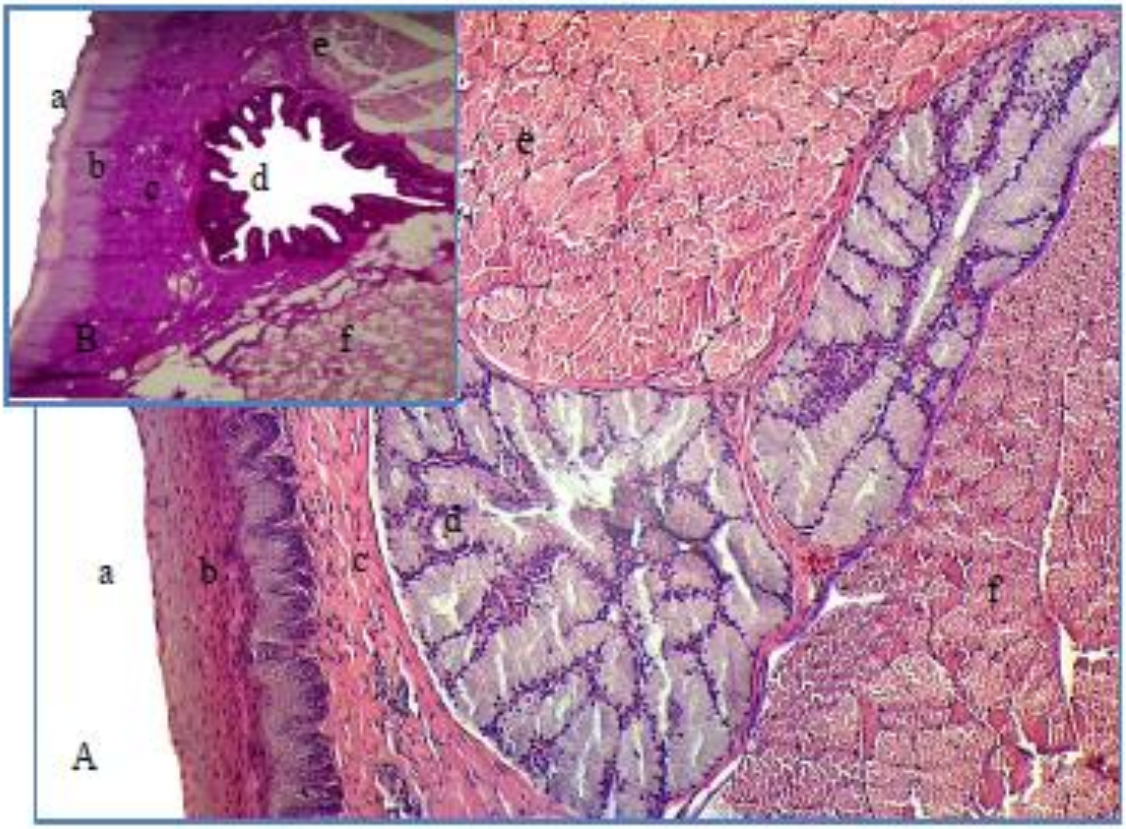

Fig. (2): Cross sections (A and B) of the laryngeal mound in the West African guinea fowl showing the laryngeal salivary glands: Oral cavity (a), non-keratinized stratified squamous epithelium (b), lamina propria-submucosa rich by large bundles of collagen fibers (c), simple

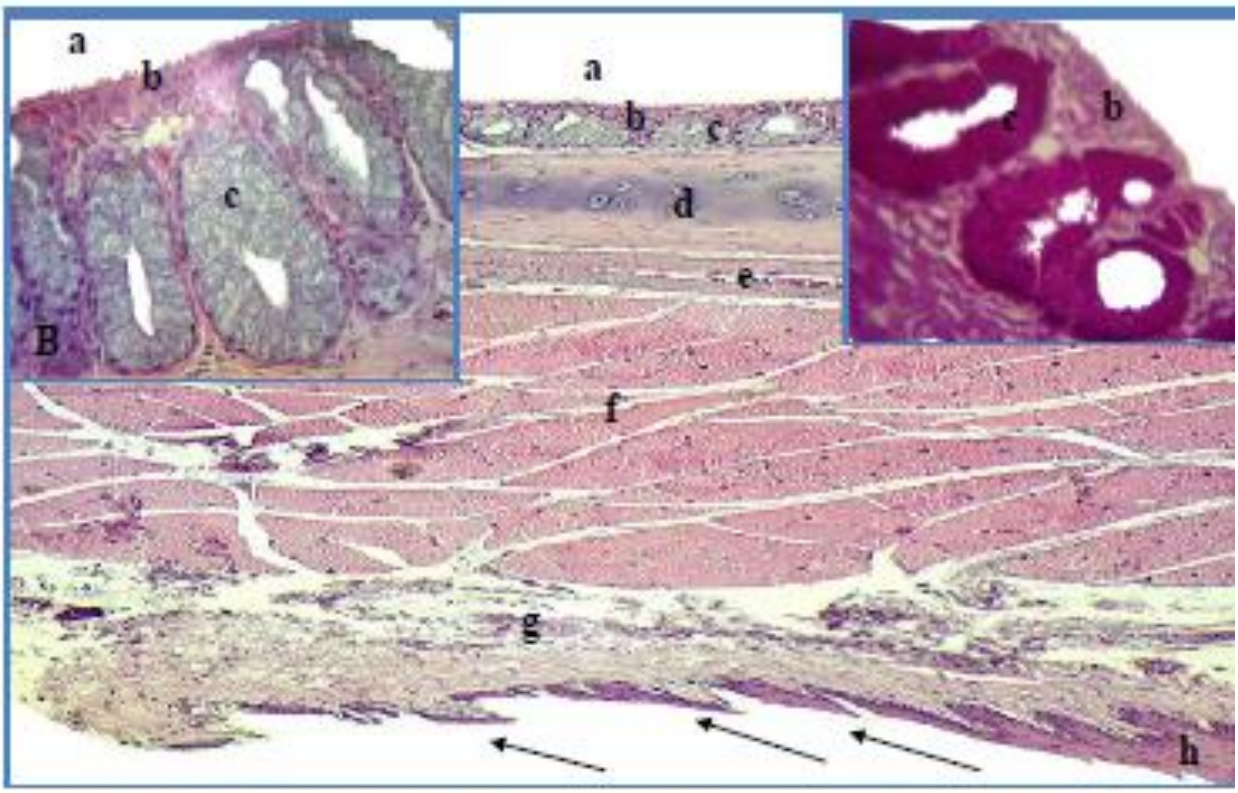

Fig. (3): Cross sections of the wall of larynx (A, B, and C) in the West African guinea fowl sho laryngeal cavity (a), ciliated, pseudostratified columnar epithelium (b), simple tubular mucous glan body of cricoid cartilage (hyaline cartilage) (d), blood vessel (e), bundles of skeletal muscle intrinsic laryngeal muscle) (f), lamina propria-submucosa of the oral mucosa (loose connective t (g), laryngeal papillae (arrows), stratified squamous epithelium (oral mucosa) (h).

H & E stain X100 A

Discussion

Histological findings of the larynx west African guinea fowl haven't focal cords like mammals, therefore it's didn't produce sound but with trachea, oral cavity and beak act as upper syringeal tract play an important role in revision of voice which produced by the syrinx. (7), (15), and (19). The laryngeal glottis different in length and width among birds species but placed at the median of the mound can be thought of as a valve atop the lungs which can prevent air flow out of the lungs or the inward flow of foreign matter like food or water into the lungs (19).

The laryngeal mound covered by non-keratinized stratified squamous epithelium this epithelium of the oral cavity which direct contact with food to avoid friction (22; and 12; 19). Close to the glottis the epithelium converted gradually to ciliated, pseudostratified columnar epithelium with abundant various sizes mucous glands opened via epithelium toward laryngeal cavity these results agree with (7), (17), and (19) in birds.

The simple tubular branched mucous salivary glands to keep the mucous membrane of the mouth moist, and provide a protective and lubricant coat of mucous, these result harmonized with (14) and (19) in turkey and not in agreement with (14) in chicken there were caudal laryngeal salivary glands (Tubuloaleveolar glands) and lateral laryngeal salivary glands (simple tubular glands). Thickness of the arytenoid

cartilage was more than the cricoid cartilage, and these cartilages not ossified at this age, in addition the laryngeal cavity discontents the median mucosal ridge (thickening of the body of cricoid cartilage and protruding toward the laryngeal cavity). These facts were incongruity with (19) in adult turkey who suggested the cricoid cartilage solider than the other laryngeal cartilages due to it was fully ossified, especially at the mucosal ridge was firstly ossified.

Numerous of the mucus laryngeal glands and lymphoid tissue at the laryngeal epithelium considered development of defense system in this species (23) and (19).

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