



Macro and Microanatomical Studies on the Choanal Slit of Turkey (*Meleagris gallopavo*)

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ABSTRACT

This work was carried out to describe the morphological characteristics of the choanal slit of the turkey through gross, light, and scanning electron microscopy. The choanal slit measures 27.62 mm long, and constitutes 38.30 % of the total length of the palate. The edges of the narrow part of the choanal slit is smooth rostrally but slightly thickened caudally due to the presence of 2-3 small papillae. The edge of the wide part is thickened because of presence of 5-7 conical and wedge shaped papillae. SEM indicates the presence of median fold within the choana, which represents the direct continuation of the median palatine ridge. After a short distance, this fold bifurcates into right and left folds. Several openings of the palatine salivary glands are demonstrated on the palate at the level of the choanal slit. The epithelium of the oral roof at the level of the choanal slit is stratified squamous epithelium showing intraepithelial sensory corpuscles. This epithelium transforms at the edge of the choanal slit into pseudostratified ciliated columnar epithelium that interrupted by intraepithelial mucous glands surrounded by lymphatic infiltration and nodules. Altogether, this study provides inclusive information on the macroscopic and microscopic morphological features of the choana in the turkey in comparing with those of the other birds.

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Introduction

In the birds there is no median union of the so-called orbital folds which are analogous with the palatine processes of the mammal. Similarly the paired pharyngeal folds remain separate throughout their entire length. For this reason the roof of the mouth has a cleft (choanal cleft) which is narrow at its apical end and then broadens out. It is very long in the fowl and pigeon but short in the duck and goose. In the bird this slit represents a permanent communication between the oral and the nasal cavities (Nickel *et al.*, 1977).

The choana is a median fissure in the palate connecting the oropharynx to the nasal cavity. In most species the palate is ridged both lateral and rostral to the choana. The arrangements of the ridges of the palate are especially well developed in seed-eating passerines which use them to remove the shell (King and McLelland, 1984).

In fowl, pigeon, duck, goose and partridge, the hard palate is incomplete because of the presence of a choanal slit that

extends longitudinally in the midline of the caudal half of the palate (McLelland, 1975; Nickel *et al.*, 1977; Rossi *et al.*, 2005).

Previous studies on the oropharyngeal structures of the turkey including upper beak (Sayed *et al.*, 2014), palate (Sayed *et al.*, 2016a), and pharyngeal roof (Sayed *et al.*, 2016b) revealed different morphological features in the turkey in comparing with other domestic and wild birds. Because of scanty information on the anatomy of the choanal slit of the turkey in the current literature, this study was applied to investigate the macro and microanatomical features of the choana in the turkey (Bronze black species), comprising its gross anatomical, light and scanning electron microscopic morphology, in addition to comparing obtained findings with other literatures.

Materials and methods

Birds

This study was conducted at Department of Anatomy and Histology, Faculty of Veterinary Medicine, Assiut University, Egypt, and applied on a total number of twenty healthy adult turkeys of both sexes of bronze black species aged about 12 months old and weighted 2.5-3 kg, collected from a local farm

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in Assiut Governorate. The birds were sacrificed by cervical dislocation.

Macroanatomy

For the gross anatomical examination, eight birds were used. The heads were cut off and washed under running tap water to remove traces of blood. To open the mouth cavity wider, the beak's angles were incised. The choanal slit was dissected and fixed in 10% formalin. The gross anatomical features of all the structures present in the choana were studied. The different measurements in millimeters (mean \pm S.E.) of the studied parts were taken using Percision Digital Vernier Caliper, and gross photographs were taken.

Light microscopic anatomy

For histological investigation, cross and longitudinal sections from the studied parts of the choanal slit were cut from eight birds just after sacrificing, washing, and fixation in 10% neutral buffer formalin. After proper fixation, the bony samples were kept in formic acid and 10% formol saline for the process of decalcification (Geoffrey, 1969). The specimens then were washed for 24 hours under running tap water, and dehydrated in ascending graded concentrations of ethanol. The samples were cleared in methyl benzoate and embedded in paraffin wax. Sections of 5 μ m thickness were cut, mounted on glass slides, and stained with Haematoxylin and Eosin (H&E) stain for general histological examination (Harris, 1900), Crossmon's trichrome stain for differentiation of connective tissue and muscle fibers (Crossmon, 1937) and Periodic Acid Schiff (PAS) for demonstration of neutral mucopolysaccharides (Gurr, 1962). The sections were examined with light microscope. All stain techniques were adopted after (Bancroft and Gamble, 2002).

Scanning electron microscopic anatomy

For the scanning electron microscopical examination (SEM), the choanal slit of four birds was washed for several times in normal saline and acetic acid 2%, then fixed in 4% glutaraldehyde solution for 24 hours, followed by post fixation in 2% buffered osmium tetroxide. The fixed samples were washed in 0.1 M cacodylate buffer at PH 7.3, and dehydrated in ascending grades of ethanol, critical point dried in liquid carbon dioxide, and mounted on metal stubs, then coated with gold palladium in sputtering device. Specimens were examined and photographed by using JSM_4500 LV scanning electron microscope operated at 20 KV.

The terminology used is that of Nomina Anatomica Avium (Baumel *et al.*, 1979) whenever possible.

Results

The choanal slit (Choana) in the turkey extends longitudinally in the middle of the caudal half of the palate. It is represented by a median cleft through which the oral cavity communicates with the nasal cavity. Its rostral end lies opposite to the level of the lateral angle of the nostril and 15.45 mm rostral to the angle of the mouth. The slit terminates caudally 12.17 mm caudal to the level of the angle of the mouth. The choanal slit measures 27.62 mm long. It constitutes 38.30 % of the total length of the palate. The choana consists of two parts; rostral narrow (Pars rostralis) and caudal wide (Pars caudalis). The boundary between the two parts is demarcated by the last row of the palatine papillae which lie opposite to the level of the angle of the mouth. Ventrally, this boundary is also situated opposite to the rostral commissure of the laryngeal

mount (Mons laryngealis), and about 6.56 mm behind the transverse row of the caudally directed lingual papillae (Papillae linguales).

The rostral narrow part of the choanal slit corresponds to the median longitudinal groove of the tongue. Its length is 15.43 mm. It constitutes about 55.87 % of the total length of the choanal slit. The width of the narrow part is 1.55 mm. The caudal wide part of the choanal slit lies opposite to the laryngeal inlet. It measures 12.19 mm long. As a result, it constitutes about 44.13 % of the total length of the choanal slit. Concerning to its width, it measures 2.63 mm. The caudal termination of the interrupted part of the median palatine ridge in one side and the rostral end of the narrow part of the choanal slit on the other side is demarcated by the short first transverse row of palatine papillae (Papillae palatinae).

Grossly, the edges of the narrow part of the choanal slit is smooth rostrally but slightly thickened caudally due to the presence of 2-3 small sized papillae on each side. The edges of the wide part of the choanal slit is thickened due to the presence of 5-7 caudoventrally and slight medially directed conical and wedge shaped papillae. These papillae could not be demonstrated on the edge of the most caudal area of the wide part of the choanal slit (Fig. 1).



Fig. 1. Photograph of the oral roof showing the choanal slit, its narrow part (arrow) and wide part (barbed arrow). Note that the edge of the narrow part is grossly smooth, but that of the wide part is thickened due to presence of conical papillae.

The scanning electron microscopical findings show that the edge of the narrow part of the choanal slit has 2-3 wedge shaped caudomedially directed papillae. Furthermore, the papillae of the edge of the wide part are nearly situated in regular intervals. Moreover, small sized pair of papillae can be observed nearly on the edge of the wide part directly in front of the caudal end of the choanal slit. In addition, different sized cornified papillae with wide bases and pointed apices are scattered on both sides of the choanal slit (Fig. 2A).

The scanning electron microscopical results indicate the presence of median fold within the choana. It represents the direct continuation of the median palatine ridge. This fold extends caudally for a short distance within the narrow part of the choanal slit. Then after, it bifurcates into right and left folds which complete their caudal extension within the narrow then

the wide parts of the choanal slit to terminate shortly before its caudal end. Moreover, relatively large glandular openings are located within the choanal slit lateral to the right or left fold (Fig. 2B).

At higher magnification, numerous glandular openings arranged in longitudinal rows are demonstrated on the ventral aspects of the right and left folds. These openings are concentrated on the cranial part of the folds than on their caudal parts. Furthermore, few small openings are freely distributed

in the wide part of the choanal slit between the right and left folds (Fig. 3A).

The higher magnification of the fold within the choanal slit indicates the presence of two types of epithelial cells on its lateral side. The first type is polyhedral microvillus cells provided with microvilli, while the other type is ciliated cells covered with long cilia. On the other hand, the medial sides of these folds contain polyhedral cells with microvilli (Figs. 3B & 3C).

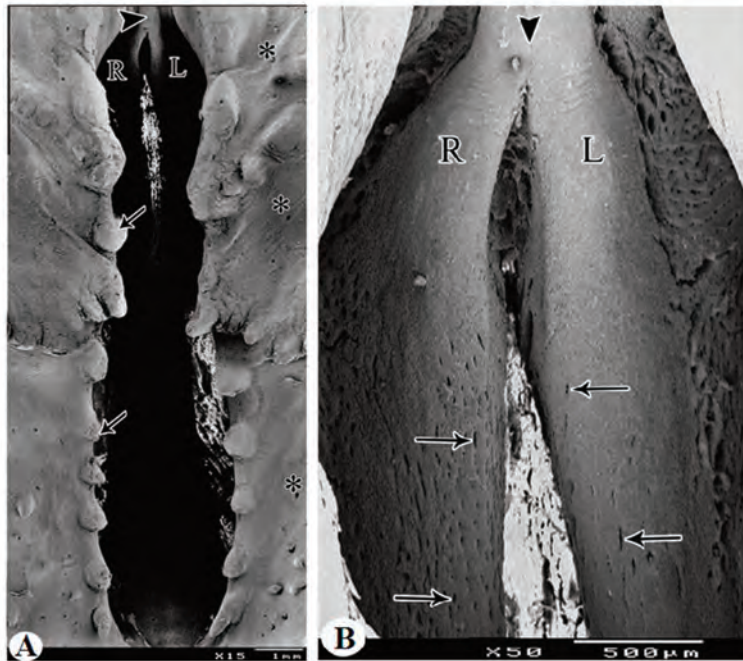


Fig. 2. Scanning electron micrograph of the choanal slit (A) showing the median fold (arrow head) and its bifurcation into right (R) and left (L) folds. Note the presence of papillae on the edge of the caudal portion of the narrow part and that of the wide part (arrows) in addition to openings of the medial palatine salivary glands (stars), bar=1 μ m. (B) Scanning electron micrograph at the beginning of the narrow part of the choanal slit showing the median fold (arrow head) and its bifurcation into right (R) and left (L) folds. Note the presence of numerous glandular openings (arrows) on the ventral aspect of the right and left folds, bar=500 μ m.

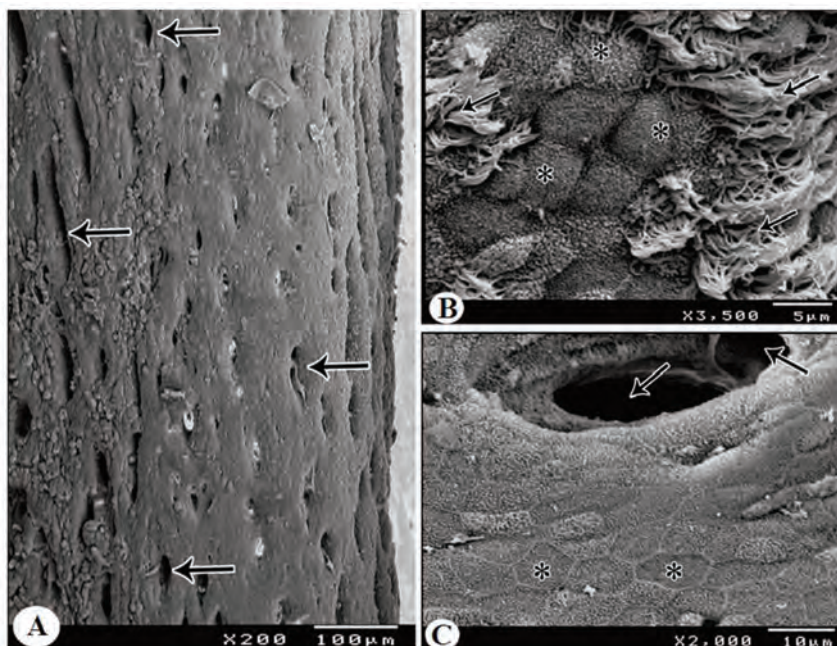


Fig. 3. Scanning electron micrograph of higher magnification of the fold within the choanal slit (A) showing longitudinal rows of the slit like excretory glandular openings (arrows), bar=100 μ m. (B) Scanning electron micrograph of higher magnification of the fold within the narrow part of the choanal slit showing presence of two types of cells; polyhedral microvillus cells provided with microvilli (stars) and ciliated cells covered with long cilia (arrows), bar=5 μ m. (C) Scanning electron micrograph of higher magnification of the fold showing polyhedral microvillus cells provided with microvilli (stars) and the excretory glandular openings (arrows) on the medial side of the fold, bar=10 μ m.

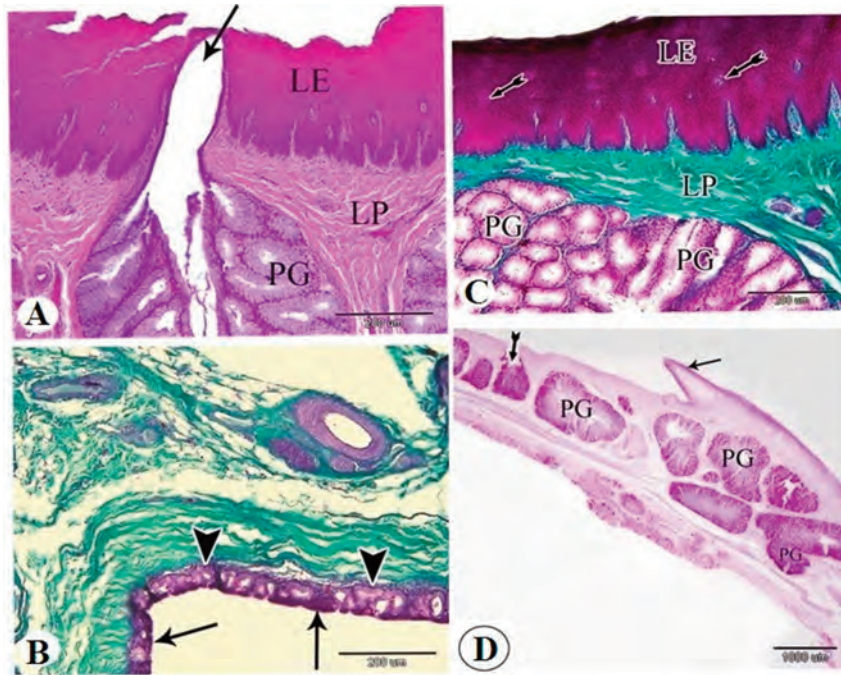


Fig. 4. Photomicrograph of cross section of the palate at the level of the choanal slit (A) showing lamina epithelialis (LE), lamina propria (LP), palatine salivary glands (PG) and their ducts (arrow) open on the epithelial surface, H&E stain, bar=200 µm. (B) Photomicrograph of lining epithelium of the choanal slit showing respiratory epithelium (arrows) containing intraepithelial glands (arrow heads), Crossmon's trichrome stain, bar=200 µm. (C) Photomicrograph of cross section of the palate at the level of the choanal slit showing lamina epithelialis (LE) contains intraepithelial sensory corpuscles (barbed arrows), dense connective tissue lamina propria (LP) and palatine salivary glands (PG), Crossmon's trichrome stain, bar=200 µm. (D) Photomicrograph of longitudinal section of the palate at the level of the choanal slit showing palatine papillae (arrow), PAS-positive palatine salivary glands (PG) and their ducts (barbed arrow), PAS stain, bar=100 µm.

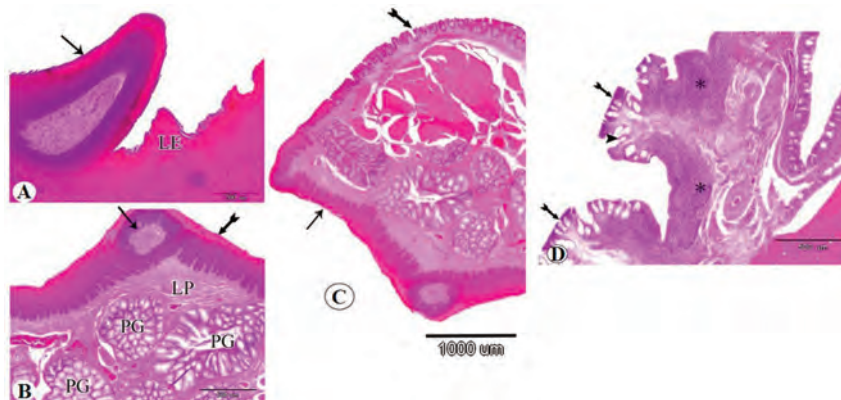


Fig. 5. Photomicrograph of longitudinal section at the edge of the choanal slit (A) showing lamina epithelialis (LE) and conical shaped palatine papillae (arrow) contain core of connective tissue, H&E stain, bar= 200 µm. (B) Photomicrograph of cross section of the palate at the level of the choanal slit showing keratinized epithelium (barbed arrow), palatine papillae (arrow) and lamina propria (LP) containing palatine salivary glands (PG), H&E stain, bar=500 µm. (C) Photomicrograph of cross section of the palate at the level of the choanal slit showing transition of the stratified squamous epithelium (arrow) into pseudostratified ciliated columnar epithelium (barbed arrow), H&E stain, bar=1000 µm. (D) Photomicrograph of cross section of the palate at the level of the choanal slit showing the pseudostratified ciliated columnar epithelium (barbed arrows) containing intraepithelial mucous glands (head arrows), lymphatic infiltration and nodules (star) in the lamina propria, H&E stain, bar=500 µm.

The covering epithelium of the oral roof of the turkey at the level of the choanal slit is stratified squamous epithelium showing intraepithelial sensory corpuscles. This epithelium at the edge of the slit transforms into pseudostratified ciliated columnar epithelium (respiratory epithelium) which interrupted by intraepithelial mucous glands. Lymphatic tissues were demonstrated in between the intraepithelial mucous glands just close to the margin of the choanal slit. These lymphatic tissues either diffuse infiltration or in follicles.

The lamina propria consists of dense connective tissue layer containing collagenous fibers, blood vessels, nerve bundles and Periodic Acid-Schiff (PAS)-positive compound tubular mucous palatine salivary glands. These glands are well developed and surrounded by a connective tissue capsule from

which connective tissue septa arise dividing these glands into lobules. Each lobule consists of secretory units lined by columnar epithelium with flat basally located nuclei and lightly stained basophilic foamy vacuolated cytoplasm. These glands are surrounded by numerous solitary lymphatic nodules. On both sides of the choanal slit at the level of its narrow and wide parts, different sized cornified palatine papillae with wide bases and pointed apices are demonstrated (Figs. 4-5).

Discussion

The present findings show that the choanal slit in the examined turkeys lies in the caudal part of the palate, correspon-

ding to that described in the chicken (McLelland, 1975), in fowl, pigeon, goose, duck, ostrich, herons, ratites and partridge (Nickel *et al.*, 1977; McLelland, 1979; Mohamed and Zayed, 2003; Ali, 2004, Rossi *et al.*, 2005; Tivane *et al.*, 2011). However, the slit was informed to extend to the rostral part of the palate in the chicken and pigeon (Mohamed and Zayed, 2003). In this respect, the choanal slit in the duck lies completely within the pharyngeal cavity (Hassouna, 2002).

In the middle third of the palate in chicken is located the choanal space, which serves as a communication to the nasal cavity (Koch, 1973). The short and caudally positioned choanal slit in the goose compared with the chicken and pigeon may play role to prevent the entrance of water to the slit during sieving feed particles from the water (Mohamed and Zayed, 2003). On contrast, in the ostrich the middle third of the semi-circular darker area which presents in the caudal third portion of hard palate is transformed into a triangular depression with two choanal slits obliquely in its borders. The right and left choanal slits are separated from each other by a thin median septum (Tadjalli *et al.*, 2008). In this connection, Tivane *et al.* (2011) mentioned that the choana in ostrich formed inverted V-shaped depression subdividing along the midline by a prominent mucosal ridge.

Corresponding to the present investigation, the choanal slit measures 27.62 mm long and constitutes 38.30% of the total length of the palate. In 60 days old duck, it is 26.42 mm long and constitutes 31.84% of the total length of the palate (Madkour, 2011). In the chicken, pigeon and goose, the choana measures 23, 16 and 23 mm long respectively, and constitutes 53.49, 50.00 and 27.06% of the total length of the palate respectively (Mohamed and Zayed, 2003). In the European magpie and Common raven, the length of the choanal slit reported was 17.75 and 22.29 mm respectively (Erdogan and Alan, 2011). In the ostrich, this slit measures 2.9 cm long (Tadjalli *et al.*, 2008), and 2.8-3.4 cm long (Ali, 2004). According to the before mentioned data, it is worthy to clarify that the length of the choanal slit is variable ranging from 16 to 34 mm, and constitutes about 25-50 % of the total palate length depending on the avian species.

In agreement with McLelland (1975) in fowl, Bailey *et al.* (1997) in captive bustards, Hassouna, (2002) in duck, Mohamed and Zayed (2003) in chicken, pigeon and goose and Madkour (2011) in duck, the present work shows that in the turkey the choanal slit consists of rostral narrow part and caudal wide caudal part. The narrow part may correspond to the median palatine suture of mammals (Heidrich, 1908), while the caudal part is homologous to the mammalian choana (Lucas and Stettenheim, 1972). The boundary between the narrow and wide parts of the choanal slit in the examined turkeys is demarcated by the last row of the palatine papillae which lies opposite to the level of the angle of the mouth. In duck, a transverse row of thin, pointed, caudally directed papillae were observed at the junction between the two parts of the slit (McLelland, 1975; Hassouna, 2002). This row of papillae lies shortly behind the level of the angle of the mouth in the duck (Madkour, 2011). In the goose, this transverse row of papillae is absent (McLelland, 1975).

According to the obtained literature and results of this work, the relation between the position of both parts of the choanal slit and the laryngeal inlet (glottis) differs depending on the species of the bird. The narrow part of the slit in the examined turkey corresponds to the median longitudinal groove. This part lies completely rostral to the laryngeal inlet in duck (Madkour, 2011). The wide part of the choanal slit lies opposite to the laryngeal inlet in the studied bird as that given in duck (Hassouna, 2002), in pigeon and goose (Mohamed and Zayed, 2003), in African pied crow (Igwebuike and Eze, 2010), in ostrich (Tivane *et al.*, 2011) and in house sparrow and

Columba (King and McLelland, 1975). In the contrary, the wide part of the slit is located rostral to the laryngeal cleft in chicken (Mohamed and Zayed, 2003) and in common kestrel (King and McLelland, 1975). It is suggested that during respiration the tongue is applied firmly to the roof of the mouth and the narrow part of the slit is occluded, so that only the more caudal broader part remains patent to allow the stream of air to enter the larynx (Nickel *et al.*, 1977). The anatomical position of the choanal opening at the same level of the laryngeal opening facilitates inspired air to pass directly to the respiratory passage (Hassouna, 2002).

The statistical data indicate that the length of the narrow and wide parts of the choanal slit in the examined turkey is 15.43 and 12.19 mm, in 60 days old duck is 10.76 and 15.66 mm (Madkour, 2011), in chicken is 14 and 9 mm, in pigeon is 12 and 4 mm as well as in goose is 10 and 13 mm respectively (Mohamed and Zayed, 2003). In chicken, the wide part is nearly half the narrow part, but in duck the former part is longer than the latter part (McLelland, 1975). The wide part is double the narrow part in the duck (Hassouna, 2002). This clarifies that the narrow part of the choanal slit is longer than the wide part in the turkey, chicken and pigeon (walking and flying birds). On contrast, in duck and goose (swimming birds) the narrow part is slightly shorter than the wide part.

With regard to the edge of the choanal slit, it is clear grossly that the edge of the narrow part in turkey is smooth rostrally but thickened caudally due to the presence of 2-3 small sized papillae. Moreover, the edge of the wide part is thickened due to the presence of 5-7 conical and wedge-shaped papillae. Corresponding to Mohamed and Zayed (2003) the papillae are demonstrated only in the edge of the narrow part in pigeon, but in both wide and narrow parts of the slit in the chicken and goose. In the chicken, the papillae have nearly the same size at both narrow and wide parts of the slit, while in the goose the papillae are arranged into two rows, they are long, but become thinner and shorter caudally. In this respect, Hassouna (2002) described 1-2 rows of papillae on the edge of the wide part and few short papillae at the narrow part. McLelland (1975) reported that the choanal slit of the duck and goose has thin, pointed caudally directed papillae which are mostly better developed close to the edges of the wide part, while in chicken, few small papillae are irregularly distributed on the edges of the slit.

The present scanning electron microscopical findings indicate that the papillae of the edge of the narrow part are wedge-shaped and caudomedially directed. Furthermore, those of the edge of the wide part are nearly situated in regular intervals. In the goose, the papillae of the choanal slit appear conical in shape with narrow bases encircled with concentric scales. However, in chicken and pigeon they are tongue-shaped with wide bases (Mohamed and Zayed, 2003). In the duck, two rows of papillae can be identified on the edge of the wide part of the choanal slit in all studied ages from 1-60 days old. The papillae are mostly cone-shaped but some of them are dome-shaped (Madkour, 2011). The mucous membrane at the edge of the choanal slit particularly at the wide caudal part in the duck was thickened forming permanent mucosal folds. With scanning electron microscope, these mucosal folds appeared as fingers like processes with different thickness (Hassouna, 2002). In European magpie and Common raven, spinous, conical caudomedially pointed papillae were noted lined up throughout the choanal cleft at its two medial borders (Erdogan and Alan, 2011).

The present investigation as shown by electron microscopy reveals the presence of median fold within the choana which represents the direct continuation of the median palatine ridge. This fold extends within the narrow rostral part, then after it bifurcates into right and left folds. According

to the present scanning electron microscopical observation, these folds contain numerous glandular openings and two types of epithelial cells; the first type is a polyhedral microvillus cells provided with microvilli and the other is a ciliated cells covered with long cilia. Structurally, the present work shows that these glands are mucous in type and surrounded by lymphatic infiltration and nodules. In duck, Madkour (2011) supported these findings and mentioned that numerous openings of the intraepithelial glands are scattered within the epithelium of the choanal slit. At higher magnification, few fine cilia of epithelium are scattered between these openings.

In the current study, the covering epithelium at the level of the choanal slit is stratified squamous epithelium which continues with the respiratory epithelium of the nasal cavity at the edge of the choanal slit. Similar findings were reported in the fowl (King and McLelland, 1975) and in the duck (Madkour, 2011).

Conclusion

It seems apparent from the current study that the choana of the turkey shows morphological characteristic features that differ from the other domestic birds. Among these features the presence of the median fold within the choana that represents the direct continuation of the median palatine ridge. Within the choanal slit, this fold bifurcates into right and left folds, which contain numerous mucous glandular openings, polyhedral microvillus cells provided with microvilli, and ciliated cells covered with long cilia.

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