Histochemical and surface ultrastructural characteristics of the nasal cavity of laughing dove

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Summary

Ten apparently healthy, adult laughing doves were used to document detailed histological, histochemical and surface ultrastructural features of the nasal cavity and to investigate the structure-function relationship of the nasal cavity in this species. We observed that the nasal cavity of the laughing dove was composed of three main regions: nasal vestibule, respiratory and olfactory. Each region presented a characteristic epithelial lining. The epithelium varied along the nasal vestibule from keratinized stratified squamous rostrally to non-keratinized stratified squamous in the middle and stratified cuboidal in the caudal region of the nasal vestibule. The respiratory region was lined with pseudostratified columnar epithelium and was initially devoid of both goblet cells and cilia, but cilia then appeared and increased gradually in number close to the olfactory region. The olfactory region was lined with a pseudostratified epithelium that consisted of three different cell types: olfactory, support cells and basal cells. In conclusion, the current investigation presents new information concerning the histological, histochemical and ultrastructural features of the laughing dove’s nasal cavity. Furthermore, the findings of this study may prove to be a valuable contribution to the avian histology and pathology literature.

1 | INTRODUCTION

The laughing dove is commonly found in Africa and the Middle East, including Egypt (Ali & Ripley, 1981). It is a small type of pigeon so in the past, it was included in the genus \textit{Columba}. However, currently, it is placed in the genus \textit{Spilopelia} (Johnson et al., 2001). Recently, various avian species in addition to the domestic chicken are being used as models for biological research, which has increased interest in the histomorphology of these alternative species (Baumel, King, Breazile, Evans, & Vanden Berge, 1993; Dyce, Sack, & Wensing, 1996; McLelland, 1990).

The respiratory organs of birds differ from those of mammals with respect to many specific features (Dar, Krishnan, Chungath, & Pillai, 2014). The anatomical and histological architecture of the nasal cavity of several avian species have been well documented in the literature, including domestic fowl (McLelland, 1990), Japanese quail (Çevik-Demirkan, Kür tôl, & Haziroğlu, 2007), the hooded crow (Hassan, 2012) and domestic ducks (Dar et al., 2014). However, there are few published investigations of the histochemical features and ultrastructure of the nasal cavity of the laughing dove.

It is known that some avian species do not have a well-developed sense of olfaction. However, other birds have highly developed olfactory senses and even use olfaction in flight navigation (homing pigeons), searching for food (kiwis, turkey vultures and petrels) and assisting with inter-individual communication (domestic chickens) (Balthazart & Taziaux, 2009; Bonadonna, Miguel, Grosbois, Jouventin, & Bessiere, 2007; Hagelin, 2006; Nevitt, Losekoot, & Weimerskirch, 2008; Wallraff, 2004).

Air enters the nasal cavity via the nares or external nostrils of the bird, which are positioned at the dorsal aspect of the base of the beak.
TABLE 1 Nasal conchae varies in several avian species

<table>
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<th>Structure</th>
<th>Birds</th>
<th>Authors</th>
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<tr>
<td>The avian nasal cavity is usually separated by three conchae, namely the anterior concha, maxillary (or middle) concha and posterior concha or olfactory concha.</td>
<td>Domestic chicken (red jungle fowl, Gallus gallus), Japanese quail and Ostrich</td>
<td>Bang, (1971); Bang &amp; Wenzel, (1985); Çevik-Demirkan et al., (2007); Ali, (2015).</td>
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Some species of birds, such as diving birds and parrots, may have a keratinized projection called an operculum to protect the nares. In other birds, such as woodpeckers, the nares are protected by bristle feathers. Air passes through the nasal cavity to the oropharynx via a slit in the hard palate called the choana (McLelland, 1990). Birds do not have a soft palate. Three conchae are typically observed in the nasal cavity in most avian species, but wide variation exists with respect to the number and shape of the nasal conchae (see Table 1 for a summary). The three conchae are described as rostral, middle and caudal, implying homology to conchae observed in mammals and other vertebrates (Baumel et al., 1993; Nickel, Schummer, & Seiferle, 1977). The rostral concha is located in the vestibular region and the middle concha, which is typically the largest of the three conchae, is located in the respiratory region (Yokosuka et al., 2009). The caudal concha is typically located in the olfactory region and is sometimes called the olfactory concha (Yokosuka et al., 2009). An accessory concha, also called the septal nasal concha, appears to be unique to Petrels (Bang, 1971). Functions of the conchae vary depending on their location in the nasal cavity and the epithelial layers they support (Bourke et al., 2014).

Great diversity is found in the nasal glands of different avian species as well as reptiles. The nasal gland of birds (also called the salt gland) consists of bilaterally paired structures whose ducts open into the nasal cavity and have an osmoregulatory function (Fange, Schmidt-Nielsen, & Robinson, 1958). In marine birds, as well as in some desert birds such as the Ostrich, the salt gland plays an important role in maintaining salt and water balance by secreting highly concentrated electrolyte solutions (Schmidt-Nielsen, 1960).

As little published information exists concerning the microscopic anatomy of the nasal cavity in laughing doves, this study reported on the histological, histochemical and ultrastructural characteristics of the nasal cavity in this species and compared the features of the nasal cavity of the laughing dove with previous reports on the nasal cavity of other avian species. Findings from this investigation will be a valuable contribution to the avian histology and pathology literature.

2 | MATERIALS AND METHODS

2.1 | Specimen collection

Ten apparently healthy adult doves that included five birds of each sex (±115 g) (1–2 years old) were obtained from wild bird hunters in the Damietta governorate, Egypt. This investigation was approved by the committee of scientific research ethics, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Age determination and species identification were carried out according to Klos and Lang (1982). The adult laughing dove upper body was brownish in colour with checkered neck markings as well as a bluish-grey band along their wings. Based on plumage, all birds used in this study were adults. Birds were anesthetized using 2% xylazine HCl (3 mg/kg), given intramuscularly. The birds were then decapitated; their heads were cut sagittally into two halves using a surgical scalpel. One half head from each bird was used for light microscopy, and the remaining half head of each bird was used for the gross and scanning electron microscopic (SEM) examinations. The halves used for histological examination were cut transversally in a rostrocaudal sequence into three sections (nasal vestibule, respiratory region and olfactory region), each with a thickness of several mm.

2.2 | Gross anatomy

The nasal cavity was partitioned sagittally into two halves by the longitudinal nasal septum. On each side of the nasal septum, the nasal cavity was subdivided into two major regions or areas that were roughly oriented rostral to caudal. The most rostral region of the nasal cavity was the vestibule and the nares opened into this region. The vestibule was followed by the caudal region that contained both the respiratory region and the olfactory region, with the respiratory region located somewhat more rostral and ventral to the olfactory region.

2.3 | Histological and histochemical examination

Ten half heads were thoroughly rinsed in physiological saline and immersed in 10% neutral buffered formalin at room temperature for 1 week. The preserved specimens were dehydrated in an ascending, graded ethanol series and then cleared in three changes of xylene. Specimens were processed and decalcified using EDTA before paraffin embedding. Samples were embedded in paraffin and then the tissue blocks were sectioned at 5–7 μm thickness using a rotary microtome (YD-1508R, ZenithLab, California, USA). Deparaffinized sections were stained with haematoxylin and eosin (H&E) for morphological evaluation. Additional deparaffinized sections were stained with a combination of periodic acid Schiff (PAS) and alcian blue to detect neutral and
around 5–10 μm in length and their diameter is about 0.2 μm. It found on ciliated epithelial cells, such as those in the respiratory region of the nasal cavity and they wave rhythmically to move dirt and mucus out (sweeping).

2.4 | Morphologic criteria for cell identification

The different cell types discussed in this study were not identified using antibodies and immunohistochemistry, but they were identified using morphological, histological and histochemical criteria based on the observations published by Graziadei (1971); Yamada (1983). Cilia were identified according to Grimstone (1962); it was based on the observations published by Graziadei (1971); Yamada (1983). Cilia were identified according to Grimstone (1962); it was around 5–10 μm in length and their diameter is about 0.2 μm. It was located between the conchae and nasal cavity roof; the second

2.5 | Scanning electron microscopy examination

The remaining ten half heads were processed for scanning electron microscopy. First, each half head was cut transversally into three to five mm slices. The slices were fixed in 2.5% glutaraldehyde for 24 hr at 4°C, rinsed with phosphate buffered saline (pH: 7.4), post-fixed in 1% aqueous osmium tetroxide for 4 hr and washed in phosphate buffered saline (pH: 7.4). Subsequently, the specimens were dehydrated using ascending grades of ethanol and then underwent critical point drying. Dried samples were mounted on metal stubs using double-sided adhesive tape. Then, the specimens were coated “sputtered” with a 100 nm thick layer of gold using a BIO-RAD sputter apparatus (Cambridge, England) and scanned at various angles using a scanning electron microscope (SEM, Model-JEOL ASID-10, Cambridge Ltd., England) located at the National Researches Center, Cairo, Egypt.

3 | RESULTS

3.1 | Gross anatomy

Both male and female laughing doves exhibited similar morphological features of the nasal cavity, so male and female data were combined. Each half of the dove’s nasal cavity revealed the presence of three main regions: a rostrally positioned nasal vestibule and the proper or respiratory region and olfactory region that were enclosed in the caudal part of the nasal cavity. The nasal cavity contained three conchae: rostral, middle and caudal. The rostral concha was the largest and located opposite to the nares, between the rostral and middle third of the nasal cavity (Figure 1). The middle and caudal conchae were in close proximity to each other and located in the caudal third of the nasal cavity (Figure 1). The middle concha was positioned rostral and ventral to the caudal concha. The nasal cavity enclosed four nasal meatuses that coursed between the various conchae. The first meatus was located between the conchae and nasal cavity roof; the second meatus was situated between the rostral and middle concha. The third meatus was located between the middle and caudal conchae, and the fourth meatus was situated between the caudal concha and the caudal wall of the nasal cavity (Figure 1).

3.2 | Nasal vestibule

Microscopically, the vestibular region was further subdivided into three parts: rostral, middle and caudal. The rostral part of the nasal vestibule, which included the rostral nasal concha, was lined by highly keratinized stratified squamous epithelium resting on a prominent, wavy, basement membrane (Figure 2a,b). The degree of keratinization decreased gradually towards the caudal aspect of the nasal vestibule (Figure 2c). The most superficial epithelial layer was composed of dead, keratinized, squamous epithelial cells that presented wide intercellular spaces where the cells began to desquamate or exfoliate, causing the surface to have the appearance of isolated, leaf-like flakes (Figure 2a,d). The wide subjacent lamina propria-submucosa complex was predominantly cellular and highly vascularized and was characterized by a moderately alcianophilic intercellular matrix (Figure 2b).

The middle part of the nasal vestibule was lined with stratified squamous epithelium but with little to no keratinization (Figure 3a). The caudal part of the nasal vestibule was lined with stratified cuboidal epithelium and contained numerous, strongly alcianophilic intra-epithelial mucous glands, which opened onto the mucosal surface and released acidic mucin (Figure 3b,c). The surfaces of the most superficial epithelial cells of the middle nasal vestibule appeared as irregular, densely packed cells with polygonal outlines. The surface of the caudal nasal vestibule presented numerous orifices of the nasal mucous glands among the lining cells as well as dome-shaped apices separated by deep microgrooves (Figure 3d). The underlying subepithelial lamina propria-submucosa complex of...
both middle and caudal parts of the nasal vestibule became a narrower cellular zone than that observed in the rostral part of the nasal vestibule (Figure 3a–c).

### 3.3 | Respiratory region

The caudal part of the respiratory region included the middle nasal concha. The respiratory region, including the middle nasal concha, was lined by pseudostratified non-ciliated columnar epithelium, which became densely ciliated towards the olfactory region (Figure 4a,b). The respiratory epithelium extended caudally from the caudal part of the nasal vestibule and contained numerous intra-epithelial mucous glands. These glands were identified as accumulations of mucous-secreting goblet cells. SEM examination revealed few to no cilia and numerous surface openings for the intra-epithelial mucous glands. The surface extending towards the olfactory region contained fewer mucous glands, but higher numbers of cilia began to appear (Figure 4c) until the entire surface was covered with densely packed cilia (Figure 4d). The alcian blue positive intra-epithelial mucous glands were located among the respiratory epithelial cells. Additional PAS-positive glands
were observed in the lamina propria-submucosa complex that surrounded the respiratory region (Figure 4e,f).

3.4 | Olfactory region

The olfactory region, which included the caudal nasal concha, was lined by pseudostratified columnar epithelium that was composed of three different cell types: olfactory, supporting and basal cells (Figure 5a). The olfactory cells rested on the basement membrane and included closely packed cell bodies that formed a layer that included a majority of the olfactory epithelium. The cell bodies of the olfactory cells contained large, ovoid, pale euchromatic nuclei that were located mainly in the central portion of the epithelial lining. These cells extended processes of varying lengths up to the free epithelial surface (Figure 5b). With SEM, the surface of the olfactory epithelium showed numerous spherical olfactory vesicles that bulged over the epithelial surface. Each vesicle was formed by the enlarged apical end of an olfactory cell and gave rise to numerous olfactory cilia of different lengths that coursed in all directions to cover the entire surface of the olfactory epithelium (Figure 5d,e). The support cells were tall columnar cells located among the olfactory cells, with large spherical, dense, heterochromatic nuclei located above the level of the olfactory cell nuclei. The basal cells rested on the basement membrane and had large spherical nuclei. The basal cells did not reach the luminal epithelial surface (Figure 5b). Additional glands were observed in the lamina propria-submucosal complex and exhibited moderate-to-strong PAS and alcian blue positive reactivity, which was indicative of the presence of a mixture of acidic and neutral mucins (Figure 5c).

4 | DISCUSSION

Functions carried out by the nasal cavity include olfaction, air filtration, water economy and thermoregulation. The different regions of the nasal cavity are adapted to accomplish these various functions. The vestibule and respiratory regions primarily function in air filtration and water and thermoregulation, while the most caudal and dorsal region of the nasal cavity is primarily devoted to olfaction.

The gross anatomy of the nasal cavity in several avian species is well documented, including the chicken (Meng-fei, Hai-hong, & Qian, 2014), Japanese quail (Çevik-Demirkan et al., 2007), hooded crow (Hassan, 2012) and domestic ducks (Dar et al., 2014). The nasal cavity contains several nasal conchae that vary in number and size in avian species, and a summary of some of the published accounts is included in Table 1.
Identifying the nasal meatuses is not the same in birds compared to mammals as in birds the three main conchae lie in a more or less rostrocaudal series, as do the meatuses (Baumel et al., 1993). Therefore, the mammalian terms, "dorsal, middle and ventral meatuses," are not suitable to use with avian species. In this study, we documented the presence of four nasal meatuses, which were located between the respective conchae.

The avian nasal cavity is described as generally consisting of three main regions: the rostral vestibular region, which is covered with stratified squamous epithelium; a middle respiratory region, which is covered with respiratory epithelium; and a caudal olfactory region that is covered with olfactory epithelium (Bang & Wenzel, 1985). Similar findings were observed in this study. The lining epithelium of the nasal cavity in the laughing dove also varied according to the different regions: nasal vestibule, respiratory and olfactory. Samuelson (2007) mentioned that the epithelium of the nasal cavity in birds was similar to mammals. Cormak (1978) stated that the human nasal cavity was divided into a vestibule portion and a respiratory portion with the olfactory area as part of the respiratory portion.

It is noteworthy that the epithelium of the nasal vestibule in the laughing dove varied from stratified squamous epithelium that was keratinized to non-keratinized stratified squamous to stratified cuboidal in the rostral, middle and caudal regions of the nasal vestibule, respectively. The epithelium lining of the respiratory and olfactory regions was pseudostratified columnar, which is similar to the observations of Meng-fei et al. (2014) for the chicken. The most superficial keratinized cells in the rostral vestibule showed desquamation or exfoliation that appeared as isolated leaf-like flakes of keratinized material. This observation suggests the presence of continuous wear and tear of that surface area. Banks (1993) reported that the epithelium of the nasal cavity in birds is similar to that of mammals. However, instead of the individual goblet cells that are present in the nasal cavity of mammals, numerous intra-epithelial mucous glands, which are composed of collections of goblet cells, were observed in the nasal cavity of the laughing dove. These glands first appeared in the caudal part of the nasal vestibule and extended into the respiratory region. Bacha and Bacha (1990) also reported the presence of intra-epithelial mucous glands in the avian nasal cavity. The intra-epithelial mucous glands showed a strong alcianophilic reaction and opened directly onto the luminal surface. The potential significance of secretions by intra-epithelial glands may be to moisten the nasal cavity and trap dust and other foreign bodies, especially during flying. Also, mucins secreted by such glands may play an important role in protection against antigens (Derrien et al., 2010).

In several avian species, the olfactory system appears to play a major role in their daily life. Olfaction is related to many physiological processes including homing, reproduction, distinguishing their food and carrying out foraging or predatory behaviours (Balthazart & Taziaux, 2009; Hagelin & Jones, 2007; Harriman & Berger, 1986; Nevitt et al., 2008; Wenzel, 1968). Similar to reports by Banks (1993) and Randall and Reace (1996) for birds other than laughing doves, the olfactory region of the laughing dove was lined with olfactory epithelium similar to that of mammals except for the shape of Bowman's (or olfactory) glands, which were nearly ovoid in shape in the laughing dove and not flask-shaped. Also, Naessen (1971); Moran, Rowley, and Jafek (1982a); Pyatrina (1982) and Nakashima, Kimmelman, and Snow (1984) have reported that the olfactory epithelium of most vertebrates is pseudostratified and consists of olfactory (or neurons), supporting and basal cells. Recently, a fourth cell type, the microvillar cell, has been identified in the human olfactory epithelium (Moran, Rowley, & Jafek, 1982b; Moran et al., 1982a), but these cells were not observed in the olfactory epithelium of the laughing doves included in this study. Ultrastructurally, Graziadei (1975); Mygind (1975); Lenz (1977);...
Menco (1977); Wang and Halpern (1980); Ohno, Ohyma, Hanamure, and Ogawa (1981); Larsen and Tos (1982) and Yamada (1983) mentioned that olfactory cells are bipolar neurons with apical dendrites that terminate in ciliated knobs at or above the epithelial surface. The projections or knobs observed above the surface of the olfactory epithelium in this study presented many cilia.

Support cells have been observed to partially or completely surround the olfactory cells and their processes (Costanzo & Morrison, 1989; Graziadei, 1971; Graziadei & Monti-Graziadei, 1976). The relationship between support cells and olfactory cells resembles that of glial cells and neurons in the central nervous system (Connors, Bernardo, & Price, 1984). Support cells are thought to provide physical and metabolic support for the olfactory cells. The upper part of the support cells is reported to have a broad, columnar shape with microvilli located on the apical surface. However, we did not observe microvilli because the entire surface epithelium was covered completely by the olfactory cilia. The function of the microvillar cells is unknown. We observed in this study that the lamina propria/submucosa of the three nasal cavity regions of the laughing dove attached the respective lining epithelium to underlying bone and cartilage, similar to descriptions by Randall and Reace (1996) for other birds.

5 | CONCLUSION

Based on the histological, histochemical and ultrastructural features obtained in the current study, we concluded that the gross anatomy and histological features of the nasal cavity of laughing dove are similar to other birds. The nasal cavity was differentiated into three main regions that were lined with different types of epithelia that matched the function of each region. A protective keratinized stratified squamous epithelium was located in the vestibular region, a respiratory epithelium in this study presented many cilia.

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CONFLICT OF INTEREST

None of the authors has any financial interest or any possible conflict of interest related to this manuscript.

REFERENCES


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