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RESEARCH ARTICLE

Nasal Conchae in Nandanam Chicken- Gross, Histological and Immunohistochemical Study

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ABSTRACT

Unlike mammals, birds have a unique respiratory tract that conditioning of inspired air takes place only within the nasal cavity alone. Nasal conchae are scroll-like structures situated on either side of the nasal cavity and lined by mucous membrane. Nasal conchae perform this function by counter-current heat exchange mechanism. The study was performed to document the gross and histoarchitectural details of rostral, middle and caudal conchae in Nandanam Chicken. The framework of the conchae is made of hyaline cartilage. The mucosa is lined by stratified squamous keratinized epithelium, pseudostratified ciliated columnar and olfactory epithelium in rostral, middle and caudal conchae respectively. Intraepithelial glands are made of mucus type in rostral and middle conchae serous variety located in propria-submucosa in caudal concha. Nasal cavity associated lymphoid tissue is made of CD3+ T lymphocytes.

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INTRODUCTION

Birds are endothermic vertebrates that possess various adaptations at the structural, behavioural or physiological level for their survival in the environment. The avian respiratory system is the one that shows uniqueness in both structural and physiological aspects when compared to mammals (Geist, 2000). Unlike mammals, the direction of inspired air within the respiratory tract follows a unidirectional flow. As the nasal cavity is the gateway to the respiratory passage, it possesses certain anatomical structures such as nasal conchae (turbinates) which subdivides the cavity into nasal meatuses (Cevik-Demirkan *et al.,* 2007). In mammals, the conchae are functionally important structures as they increase the available surface area for the respired air to pass over, makes them to perform counter-current heat exchange mechanisms as well as odorant analysis (Mason *et al.,* 2020). A wide variation exists between the number, location and epithelial lining among the avian species (Bourke *et al.,* 2014).

In the majority of the avian species, the nasal conchae are three in number *viz.,* rostral, middle and caudal nasal conchae homologues to that of mammals and vertebrates (Baumel *et al.,* 1993). There is wide variation exist with regard to the number of conchae among the birds. In addition, the nasal mucosa serves as the first line of defense against the entry of micro-organisms (Nochi *et al.,* 2018). A majority of research work has been carried out in the nasal cavity of various avian species such as Japanese quail (Cevik- Demirkan *et al.,* 2007); Japanese Jungle Crow (Kondoh *et al.,* 2011); Hooded crow (Hassan, 2012); Ostrich (Ali, 2015); Turkeys (Bourke and Witmer, 2016); Laughing dove (Farouk *et al.,* 2017); Geese (Harem *et al.,* 2018). The nasal mucosa consists of lymphocyte aggregations named as nasal cavity associated lymphoid tissue (NALT) which is similar to gut-associated lymphoid tissue (GALT) in the gastro-intestinal tract (Kang *et al.,* 2014).

Nandanam chicken is a dual purpose, coloured variety with strong disease resistance. This popular strain is very well adopted for backyard farming among the poultry farmers in the state of Tamil Nadu. This strain was developed in Institute of Poultry Production and Management, Tamil Nadu Veterinary and Animal Sciences University, Chennai (Kannan *et al.,* 2015*).* Due to paucity of literature available in Nandanam chicken, the present basic research on the gross, histological, histochemical and immunohistochemical studies of nasal conchae was carried out.

MATERIALS AND METHODS

The present study was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai. Twenty-four adult healthy Nandanam chicken heads were procured from the local slaughterhouse in Chennai. The lower beak was removed. A midsagittal section was made through the cartilaginous nasal septum in the upper beak. The rostral, middle and caudal nasal conchae were identified in the nasal cavity. Gross morphological study was carried out. The nasal conchae were collected and fixed with Carnoy's fixative for 6 hours and then transferred to 70 per cent alcohol for preservation. The tissue pieces were processed for routine paraffin embedding as per Kannan *et al.* (2015). The sections were cut at 3-5 µm thickness. For histological observation routine Haematoxylin and Eosin, Masson's trichrome for collagen fibers (Luna, 1968) and for histochemical studies, McManus's method for neutral mucins (PAS), Alcian blue (pH 2.5) for demonstration of acidic mucins and combined PAS-Alcian Blue were done (Singh and Sulochana, 1996). Immunohistochemical localization of T lymphocytes (CD3+) was done using anti-CD3+ monoclonal primary antibody (Pathn Situ Co.) and counterstaining was done using Gill's Haematoxylin. The terminologies used in the present study were as per *Nomina Anatomica Avium* (Baumel *et al.*, 1993). Histomorphologic observation of the stained sections were done using a Leica microscope (CH 9435 Heer brugg) under different magnifications.

RESULTS

Gross morphology: In the present study on Nandanam chicken, the nasal cavity was a cone shaped passage extended from the external nares to choanae with its wider part facing caudally and the narrow part rostrally. A cartilaginous nasal septum divided the nasal cavity completely into equal right and left halves. Each half comprised of three nasal conchae (turbinates) *viz.*, rostral, middle and caudal conchae (Fig. 1) which partitioned the nasal cavity into three regions *viz.*, vestibular, respiratory and olfactory regions from before backwards respectively. These conchae were protruded from the lateral wall of the nasal cavity.

The cranial concha was the second largest concha and located anteriorly near the external nares. It was conical in shape with slightly involuted appearance. Its broad end was attached dorsoventrally with in the nasal cavity and the long narrow end extended to the external nares. The middle concha was the largest, spirally curved in appearance. The caudal concha was the smallest one and roughly triangular in outline.

Histology and hisochemistry: Light microscopic observation revealed that the wall of the nasal conchae was made up of epithelium, propria-submucosa, and hyaline cartilage. The rostral concha was divided into cranial and caudal parts which were lined by a stratified squamous keratinized epithelium (Fig. 2). The degree of keratinization was found to be decreased and almost absent totally at the caudal part. A leaf-like flakes appearance was also observed (Fig. 3a). In addition, numerous crypt-like intra-epithelial mucous glands which

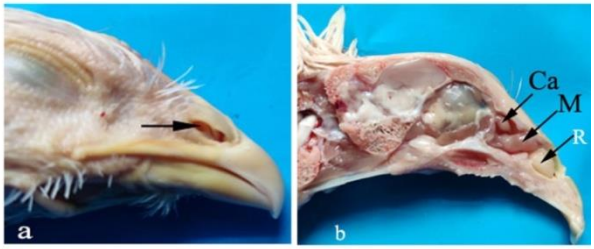

Fig. 1: Photograph showing a) Head of Nandanam Chicken. External nare (arrow) b) Gross anatomical location of Rostral (R), Middle (M) and Caudal (Ca) Nasal conchae.

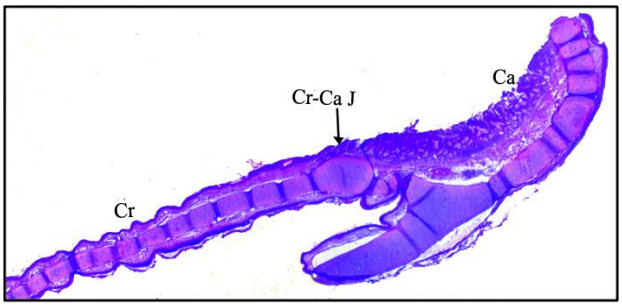

Fig. 2: Photomicrograph of the Rostral concha Cr- Cranial, Ca-caudal parts, Cr-Ca J – Cranio-Caudal Junction. H&E stain, x 12.5.

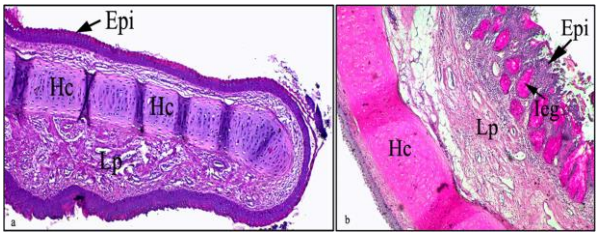

Fig. 3: Photomicrograph of a) Cranial part of the Rostral concha. Stratified squamous keratinized epithelium (Epi), Propria-submucosa (Lp), Hyaline cartilage (Hc). Haematoxylin and Eosin stain, x 100; b) PAS positive reaction in the Intra-epithelial mucus glands of caudal part of the rostral concha. Stratified squamous epithelium (Epi), Intra-epithelial mucus gland (leg), Propria-submucosa (Lp), Hyaline cartilage (Hc), PAS x 100.

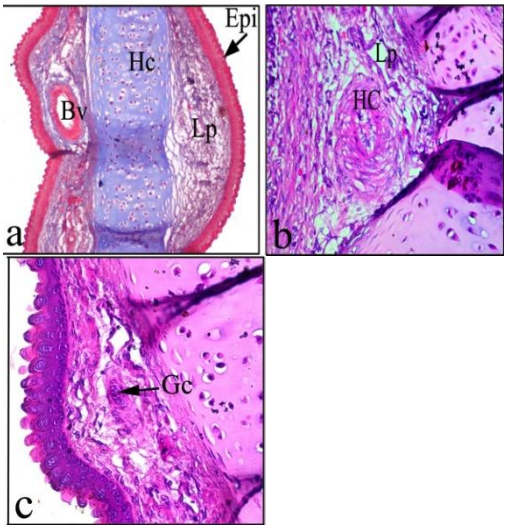

Fig. 4: Photomicrograph of a) Cranial part of the Rostral concha Stratified squamous keratinized epithelium (Epi), Propria-submucosa (Lp), Hyaline cartilage (Hc), Blood vessel (Bv). Masson's Trichrome stain, X100 b) Herbst corpuscle (HC) in the propria-submucosa (Lp), H&E stain, x 400 c) Grandry's corpuscle (Gc) in the propria-submucosa H&E stain, x 400.

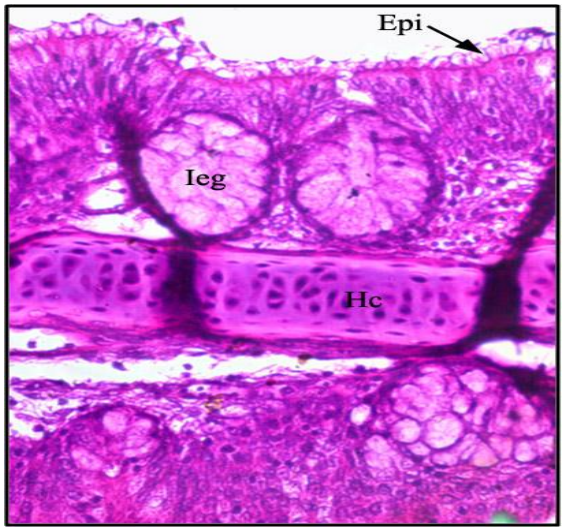

Fig. 5: Photomicrograph of the Middle nasal concha. Pseudostratified ciliated columnar epithelium (Epi), Intra-epithelial mucus gland (Ieg), Hyaline cartilage (Hc), H&E stain, x 400.

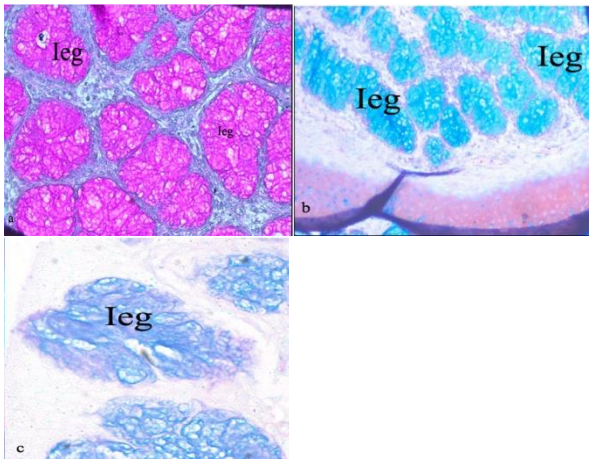

Fig. 6: Photomicrograph showing a) PAS positive reaction in the Intra-epithelial mucus glands (Ieg), PAS x 400 b) AB (pH 2.5) reactivity in the Intra-epithelial mucus glands (Ieg), Alcian Blue(pH 2.5) x 100 c) PAS- AB (pH 2.5)reactivity in the Intra-epithelial mucus glands(Ieg), Combined PAS- AB x 400.

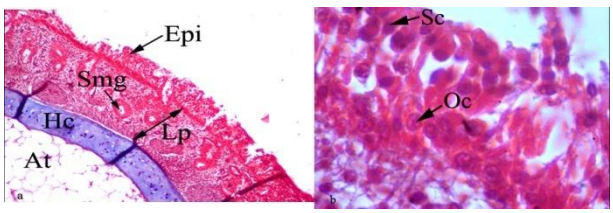

Fig. 7: Photomicrograph of the Caudal nasal concha. a) Olfactory epithelium (Epi), propria-submucosa (Lp), Submucosal gland (Smg), Hyaline cartilage (Hc), Adipose tissue (At). Masson's Trichrome stain, x 50 b) Olfactory epithelium. Supporting cell (Sc), Olfactory cell (Oc), Masson's Trichrome stain, x 1000.

which opened on the epithelial surface were noticed in the caudal part. These glands were positive for PAS (Fig. 3b) and Alcian Blue staining. The propria-submucosa consisted of numerous cells, blood vessels and collagen fibers (Fig. 4a). The presence of Grandry's corpuscle and Herbst corpuscle were observed in the propria-submucosa (Fig. 4b, c) and was more in the cranial part. The cartilage was found to be hyaline in nature.

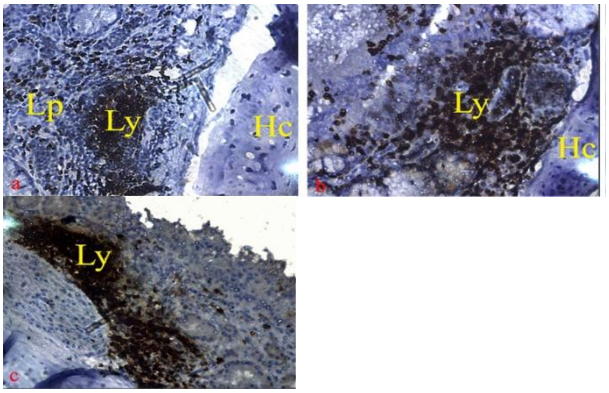

Fig. 8: Photomicrograph showing the immunohistochemical staining of rostral (a), middle (b) and caudal (c) using CD3+ MAb. Ly - T-lymphocyte aggregation x400.

The mucosa of the middle concha was lined by pseudostratified ciliated columnar epithelium. Large intra-epithelial mucous glands arranged as a single or multiple row were observed (Fig. 5). These glands opened directly on the surface epithelium and showed a positive reaction for PAS, Alcian-Blue and combined PAS-AB staining (Fig. 6a-c). The propria-submucosa was found to be minimal when compared to the rostral concha. It showed solitary lymphoid follicles and also richly vascularized. The hyaline cartilage was observed below the propria-submucosa.

The caudal concha was lined by pseudostratified columnar epithelium composed of three different distinguishable cell types *viz.*, basal cell, olfactory cell and supporting cell (Fig. 7a, b). The basal cells were arranged in a single row, consisted of the basally located spherical nucleus. The supporting cells were the most superficial ones located on the surface of the epithelium. It had a large, spherical heterochromatic nucleus. The olfactory cells were the most predominant cell type located between the basal and supporting cells. It consisted of a large, ovoid euchromatic nucleus with the cytoplasmic processes of varying length, extended in all directions and thus reached the surface of the epithelium. Unlike the other conchae, the intra-epithelial mucous glands were not observed in the caudal concha. Instead, the propria-submucosa consisted of the submucosal serous gland which opened on the surface epithelium. Distribution of adipose tissue was observed subjacent to hyaline cartilage.

Immunohistochemistry: Immunohistochemical staining of the nasal conchae using anti-CD3+ monoclonal primary antibody revealed the presence of aggregation of T-lymphocytes in the propria-submucosa of the rostral, middle and caudal conchae respectively (Fig. 8a, b, c).

DISCUSSION

The present study was carried out on the nasal conchae in Nandanam chicken with an emphasise about gross, histological, histochemical and immunohisto-chemical details. The complete division of nasal cavity into two equal halves regulates the amount of air entering into nasopharynx through the choanae (Casteleyn *et al.*, 2018).

The presence of rostral, middle and caudal conchae was similar to majority of avian species, mammals and other vertebrates. However, variation exist with regard to the number of conchae among the avian species viz., absence of rostral concha in Sulidae and quail (Aysun *et al.,* 2007); lack of middle concha in dove (Madkour, 2019) and absence of caudal concha in African grey parrots (Schmidt *et al.,* 2015). However, there are two school of thought with regard to the number of conchae in dove. Madkour (2019) stated that the middle concha was absent in dove, whereas, Farouk *et al.* (2017) mentioned in his observation that all the three conchae were present in laughing dove.

All the three conchae were covered by mucous membrane which increases the surface area of nasal mucosa which helps in conditioning the inspired air. Of the three, the middle concha was the largest one in Nandanam Chicken as in other domestic birds and Coturnix (Tasbas *et al.,* 1994). In hooded crow, the caudal concha was the largest one (Hassan, 2012).

The presence of stratified squamous keratinized epithelium in the cranial part of vestibular region of the nasal cavity might help in protecting the very first compartment of the nasal cavity. The presence of leaf-like flakes confirmed that periodic desquamation of the most superficial cornified cells as a result of continuous wear and tear. Unlike in mammals, the goblet cells were found absent in Nandanam chicken. Instead, the presence of intra-epithelial mucous glands found in huge numbers in the caudal part of the vestibular region. This is in contrast to the findings of Yan *et al.* (2014) who opined that the intraepithelial glands were composed of goblet cells in domestic chicken and Harem *et al. (*2018) in geese. These glands were positive for PAS and Alcian blue reaction confirmed the acid and neutral mucopolysaccharides nature of the secretion as reported by Dar *et al.* (2014) in domestic chicken and duck. This mucin also plays a protective role against antigens (Derrien *et al.,* 2010). These secretions kept the mucous membrane always moist and acted as a mucous blanket which helped in trapping the particulate matter and microbes in the inspired air. The richness of vascularity in the propria-submucosa might help in conditioning the inspired air.

The vestibular region consisted of two types of mechanoreceptors *viz.,* Herbst and Grandry's corpuscles which are able to receive the sense of pressure and vibration. The distribution was found to be more in the cranial part of rostral concha than caudal part in Nandanam chicken.

The lining epithelium of respiratory region was pseudo-stratified ciliated columnar variety which is in contrast to the findings of Meng-fei *et al. (*2014) in chicken and Farouk *et al. (*2017) in laughing dove. The ciliated mucosa might act as muco-ciliary blanket which assist in elimination of foreign particles as in duck (Ali and Pearson, 2007).

The pseudostratified columnar epithelium of olfactory region, composed of basal, supporting and olfactory cells and its shape, position of nuclei was similar to the observations made by Kondoh *et al.* (2011) in the Japanese jungle crow and Farouk *et al.* (2017) in laughing dove. The olfactory mucosa plays an important role in olfaction related physiological processes viz., homing, reproduction and discrimination of food (Balthazart and Taziaux, 2009). Unlike the rostral and middle conchae, the caudal concha showed the presence of serous glands in the propria-submucosa instead of intra-epithelial mucous glands as in geese (Harem *et al.,* 2018). The odour molecules may get dissolved in the serous secretion of olfactory region to facilitate transmission of olfactory nerve impulses. However, Farouk *et al. (*2017) observed mucus secreting glands in the propria-submucosa in laughing dove.

The existence of nasal mucosa associated lymphatic tissue (NALT) in the conchae serves as the main site for initiation of nasal immune response in the respiratory passage (Hiller *et al.,* 1998; Kang *et al.,* 2013). In chicken, the NALT is a well defined structure in the propria-submucosa, composed of both T and B lymphocytes (Nochi *et al.,* 2018). The presence of NALT paves way for intranasal administration of vaccines in birds (Sato and Kiyono, 2012).

Conclusions: The gross, histological, histochemical and immunohistochemical features about nasal conchae in Nandanam chicken were similar to other birds except the following unique findings. The middle concha was lined by pseudostratified ciliated columnar epithelium. Absence of goblet cells in the intra-epithelial glands of rostral and middle conchae. In addition, increased vascularity of the mucosa, large surface area and the existence of NALT makes the nasal cavity an effective route for mucosal vaccination in Nandanam chicken.

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Authors contribution: PD- Execution of the study; TAK- Designing the study and submission; RG- Photomicroscopy work; GR – Writing the manuscript; SB, NP – Manuscript editing.

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