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Volumetric and Volume Fractional Comparison of the Nasal Structures of the Stork (*Ciconia ciconia*) and Seagull (*Larus fuscus*) Using Computed Tomography Images

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BO and MK designed the study. BO and BS performed sterological analysis. BO and NGI collected samples. BO, MK, BS and NGI wrote the article.

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Cavalieri principle, Computed tomography, Nasal cavity, Seagull, Stork.

ABSTRACT

The rostral part of the nasal cavity is cooler to prevent the loss of water that is crucial for the fluid balance of birds. This function may be related to the structure of the nose of flying birds. For this reason, we decided to analyze the nasal structure of two different birds using volume and volume fraction approach of the stereology. In this study, 14 seagulls and 7 storks were examined and volume fractions of the nasal cavity and nasal structures were estimated. Heads of animals were scanned in computed tomography. The images were sampled randomly at a 1/5 sampling fraction. 11-16 sections for the stork and 16-20 sections for the seagull were examined. The sectional surface areas of the total nasal cavity, nasal septum, nasal space and conchae were estimated using the Cavalieri principle of the stereological methods. The volume and volume fractions of structures were assessed. Results obtained from the CT images were compared between stork and seagull. The volume fractions of the conchae, nasal cavity and nasal septum were 30.24%, 55.22%, 14.54%, and 33.03%, 53.23%, 13.73%, in stork and seagulls, respectively. The volume fractions of structures did not show statistical difference between two species. Our findings suggest that the nasal structures of the two-different species have similar architecture, which may be linked to the same function in flying birds.

INTRODUCTION

The anterior part of the nasal cavity, which is important in preserving water and energy, is colder than the posterior part in avian species. Due to this temperature difference, loss of water is prevented by regaining water from the humid air during exhalation. Saving water this way is particularly important for migratory birds (Getty, 1975; Ocal and Erden, 2002). There are large venous plexuses in the lamina propria of the conchae located in the nasal cavity. These plexuses are filled with blood in every 20-30 min and swell the nasal mucosa so that the airflow is reduced. These structures increase in surface area and warm the inhaled air by changing the direction of airflow (Ahishali *et al*., 2006).

Cavalieri principle is one of the methods used in stereology for calculation of volume and volume fraction (Diab *et al*., 1998). Reliable and objective numerical results can be obtained by using this method (Glaser and Glaser, 2000) that was developed by Italian mathematician Bonaventura Cavalieri for estimating the volume of 3-dimentional subjects (Roberts *et al*., 1997; Odaci *et al*., 2003). Effectiveness of the method has been proven in a number of studies (Calmon and Roberts, 2000; Roberts *et al*., 2000; Onuk *et al*., 2013).

Physiological and anatomical structures of the nose have been studied by Grutzenmacher *et al*. (2011). Geometrical measurements of the nasal cavity were made by using acoustic rhinometry and computed tomography in human (Gilain *et al*., 1997), in guinea pig, and rat (Straszerk and Pedersen, 2004). Despite the extensive literature survey, no data regarding this region has been found in any species except for domestic goose (Onuk *et al*., 2013). For this reason, we decided to analyze the nasal structure of two different birds (migratory and non- migratory birds) using volume fraction approach of stereology that gives quantitative data independent of the body sizes of the examined subjects.

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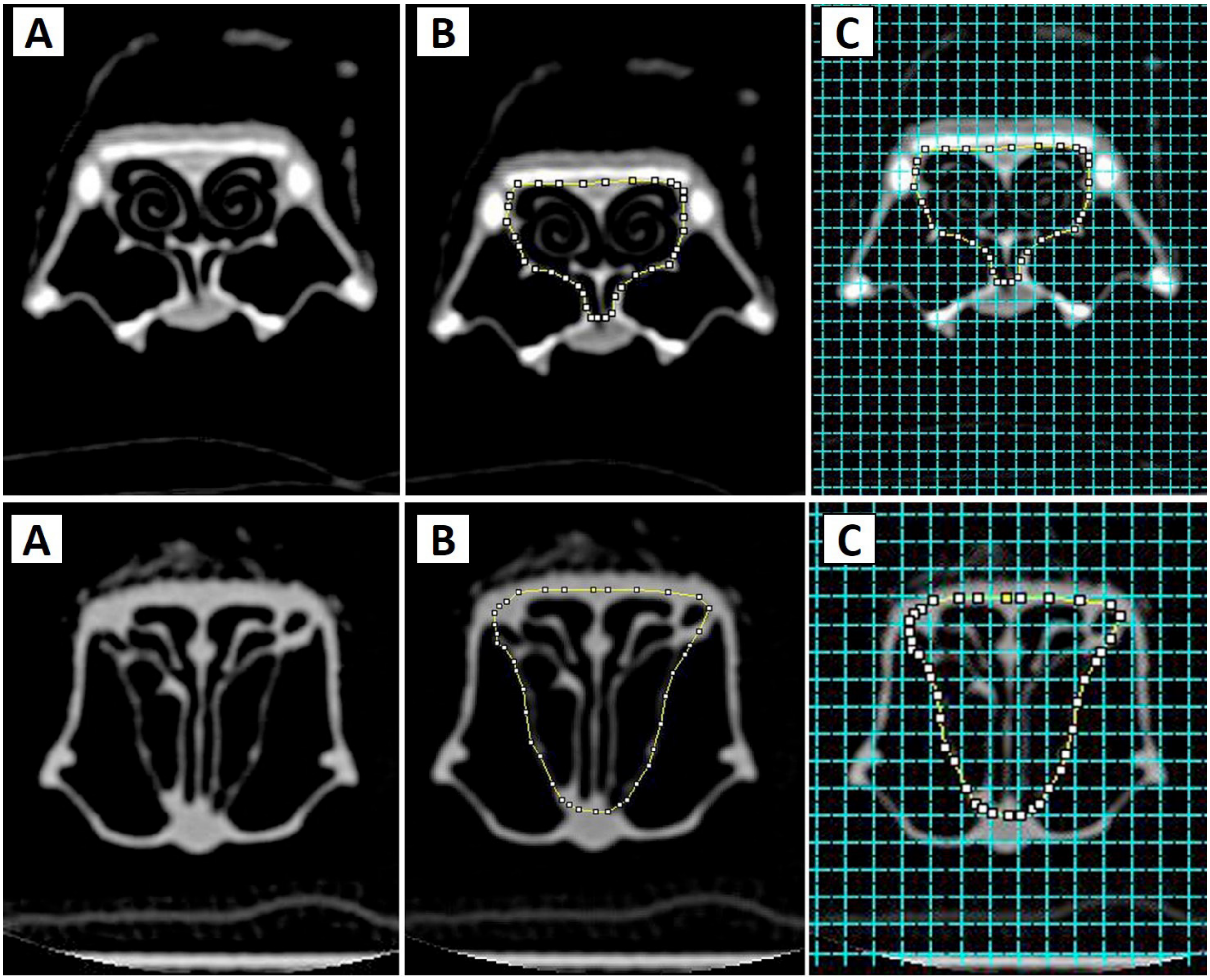

Fig. 1. Computed tomography (CT) images of nasal structures of the seagull (top panel) and stork (bottom panel). **A,** a transverse section of nasal cavity; **B,** application of manual planimetry to obtain sectional cut surface area of the region of interest; **C,** a point counting grid superimposed on a CT section.

MATERIALS AND METHODS

In the present study, 1040-1490 g live weight adult 14 seagulls (*Larus fuscus*), a non- migratory bird from Schizognath group, that were used in different studies in the Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University and 3480-3920g live weight seven adult storks (*Ciconia ciconia*), migratory birds from Desmognath group, when they were about to die and could not be saved were used for the study. In this study, materials that were free of respiratory diseases and pathological symptoms were observed. The study was carried out with the permission of the General Directorate of Nature Conservation and National Parks of the Ministry of Forestry and Water Affairs. Nasal cavities of animals were scanned using computerized tomography (CT) with transverse orientation (Fig. 1).

The 512x512 sized images with 0.5 mm thickness were obtained using Toshiba Aquilion 16 computed tomography. The images were stored as DICOM files and transferred to a CD. Using the ImageJ software, the images were sampled randomly with 1/5 fraction (a program distributed freely by the National Institute of Health of the USA). Depending on the length of nose, 11-16 sections for the stork and 16-20 sections for the seagull were examined (Fig. 1A, B in both top and bottom panels). Surface areas of the conchae, nasal septum and nasal cavity were estimated using the grid plugin of the ImageJ software. For this purpose, grids with representing areas of 0.3 and 0.4 mm^2 were randomly thrown onto sections and the number of plusses hitting the interested structure were counted (Fig. 1C in both top and bottom panels).

The volumes were estimated by multiplication of representing areas of grids with the total number of points hitting the structure as described before (Onuk *et al.*, 2013). The total volume of nasal cavity is assessed using the polygon selection toll of the ImageJ. The borders of the nasal cavity were drawn in all sections (Fig. 1B, C in both top and bottom panels) and total volume of the nasal cavity was calculated by multiplying total surface area with the section thickness. The volume fractions of the conchae and nasal cavity within the total nasal cavity were calculated using the equation:

$$\text{Vol. fraction of structure} = \frac{\text{Vol. of structure}}{\text{Vol. of total nasal cavity}} \times 100$$

To assess whether point intervals on the grid and the number of sections were adequate, the coefficient of error (CE) were calculated (Gundersen and Jensen, 1987). The volumes, volume fractions and CE values were calculated automatically by entering the number of points, representing areas per point and sectional surface areas in an excel worksheet of Microsoft.

Data obtained from CT section images were evaluated for the data structure and GENMOD (Generalized Linear Modal) procedure was used to compare the methods using SAS (2006).

RESULTS

In this study, volume (cm^3) and volume fractions (%) of structures located in the nasal cavity of seagull and of stork were calculated by applying the Cavalieri Principle to the images obtained from CT images. The mean volumes of the total nasal cavity, conchae, nasal space and nasal septum were recorded as 3.89 cm^3, 1.27 cm^3, 2.09 cm^3, 0.53 cm^3 for the seagull and 4.36 cm^3, 1.32 cm^3, 2.41 cm^3, 0.63 cm^3 for the stork, respectively (Table I). In addition, values of volume fractions for conchae, nasal space and nasal septum were determined as 33.03%, 53.23%, 13.73% for the seagull and 30.24%, 55.22%, 14.54% for the stork, respectively (Table I). Finally, all the values of volume and volume fraction were compared. The mean volumes of the total nasal cavity, conchae, nasal space and nasal septum were slightly larger in the stork than that of the seagull. However, the differences did not rise to a statistically significant level ($p \geq 0.05$).

DISCUSSION

Information about the data of the normal anatomical structures of living creatures is of importance in accurate evaluation of radiographic examinations, in understanding of the surgical procedure results (Weiglein *et al.*, 1992), and in the diagnosis of diseases widespread among avian species such as avian influenza and laryngotracheitis (Calnek *et al.*, 1991).

Anatomical structures are used in taxonomical differentiation of avian species. Based on the skull, birds are divided into four (4) groups as palaeognath, schizognath, desmognath and aegithognath (Demirsoy, 1992).

The first study on nasal cavity and size of conchae of avian species was carried out on domestic goose by Onuk *et al.* (2013). Standard values were obtained by comparing the physical sections and CT images. In addition, it has been reported that compared to the physical sections, the sections obtained from CT images better represent the real values with very minor deviation because of not being affected by the partial voluming effect depending on the section thickness (Sahin *et al.*, 2003; Onuk *et al.*, 2013). Therefore, CT was preferred to investigate the nasal cavity and conchae of the seagull and stork.

Table I.- Descriptive data for the volume (cm^3) of structures and volume fractions (%) of structures located in the nasal cavity of seagull and stork.

Measurement	Seagull		Stork	
	Volume±SD (cm^3)	Min-Max	Volume±SD (cm^3)	Min-Max
Structures in the nasal cavities				
Total volume of nasal cavity	3.89±0.13	3.12-4.53	4.36±0.31	3.56-5.46
Conchae volume	1.27 ±0.04	0.95-1.60	1.32±0.15	0.83-2.15
Space volume	2.09±0.10	1.41-2.63	2.41±0.20	1.84-3.20
Nasal septum volume	0.53±0.03	0.35-0.75	0.63±0.06	0.48-0.88
Volume fractions of structures (%)				
Conchae fraction	33.03±1.42	22.33-40.54	30.24±2.48	22.33-5.46
Space fraction	53.23± 1.28	45.03-62.20	55.22±2.44	44.53-64.83
Nasal septum fraction	13.73± 0.54	10.99-17.57	14.54±1.03	11.62-19.49

The volume and volume fraction of nasal cavity structures of the two different bird species that are taxonomically different has been determined for the first time in the current study. The results showed that these two species cannot be differentiated based on the parameters investigated in this study because of high similarity. It has been reported in literature (Getty, 1975; Ocal and Erden, 2002) that rostral part of the nasal cavity of migratory birds was colder compared to the caudal part, and the water was saved by means of this temperature difference. If the conchae volume and the nasal temperature are lower, the water loss is lesser. According to this principle, it was expected that the respective volume of the conchae in the migratory birds was lower. But, it is viewed that the conchae volumes and volume fractions were not important in preserving water, because there was no difference between the two species. Also, Ahishali *et al*. (2006) reported the presence of large plexus which increase the humidity and warmth in the expiration of air. These plexuses cause water loss by evaporating humidity in the exhaled air. If the number of plexus is less, water loss in the nasal cavity is expected to be at the minimum level. Due to lack of a significant difference between the conchae volumes and volume fractions of stork and seagull, we inferred that there will be less amount of plexus in stork's conchae than in seagull's conchae.

For better understanding of water preservation in migratory birds, more detailed studies about the conchae venous plexus density and the morphologic structure of the conchae epithelium are required. The first data on the nasal cavity and the conchae volume/volume fractions in the stork and seagull, contributes to the anatomy literature.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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