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Mineralization in the syrinx and caudal tracheal rings in the ostrich

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ABSTRACT

This study documented macroscopic and microscopic features and mineralization of the caudally located tracheal rings and syrinx in two ostrich (struthio camelus) having three years of age. The syrinx and trachea of the birds were stained in toto with alcian blue and alizarin red for cartilage and mineralization. Observations on the syrinx and trachea, measurements and photography were performed under stereo-microscopy. They were stained grossly using alizarin red and alcian blue to visualize mineralization and histologically by hemotoxylin and eosin (H&E) to detect ossification areas, if any. Results revealed incomplete tracheal rings located caudally in-between the intact ones. Alizarin red and alcian blue staining displayed mineralized regions grossly. Histological slides by H&E staining showed no ossification. Overall results proposed that alizarin red and alcian blue double staining is a good toll to determine mineralization which is calcium accumulation in the tissue before the formation of bone cells.

Key words: Mineralization, Ostrich, Syrinx, Trachea.

INTRODUCTION

Trachea is a tubular organ which is formed by the hyaline cartilage rings attached by short ligaments, connecting the cranial and caudal larynx (Bradley and Grahame 1950; McLeod *et al.* 1964). The rings lay in a unique pattern where the big ones overlap and alternate with small ones (Bradley and Grahame 1950; Romanoff 1960). Contrary to the case in mammals, the tracheal rings are intact in birds (McLeod *et al*. 1964; Yildiz *et al.* 2003). They are thick in the middle and thin towards edges (Bradley and Grahame 1950). Diameters of the rings are unequal allowing its adaptation while moving the neck. Trachea in birds is relatively longer due to the long neck, compared to that in mammals, with a wider diameter.

Syrinx, a voice organ located between the trachea and the primary bronchi, has mostly been organized with both tracheal and bronchial rings, hence called tracheobronchial type.

Mineralization begins earlier in birds, compared to that in mammals (Hogg 1982). Yet, cartilaginous features in the trachea and syrinx of birds tend to ossify as time passes (McLeod *et al* 1964; Salt and Zeuthen 1960). Indeed, early anatomists have used such classification in birds.

Ossification in the trachea of birds begins caudally and subsequently expands at cranial direction in general (Hogg 1982; Salt and Zeuthen 1960). Even though the mineralization occurs in the caudal component of trachea, bronchioles of the syrinx and the tympaniform membrane remain cartilaginous, thus allowing the vibration for voice production (Hogg 1982). On the other hand, ossification has also been reported in the syrinx of various birds (Salt and Zeuthen 1960; McLeod *et al.* 1964; Duncker 1971; Piperno and Peirone 1975; Hogg 1982).

Hogg (1982) has suggested that it is better to call the red spots obtained by Alizarin red staining as mineralization, instead of ossification. Since there is no study conducted on mineralization of the trachea and syrinx of the ostrich, this study aimed at revealing, if any, the ossification and mineralization of the trachea and syrinx of this species, using alizarin red and alcian blue staining and histological observations.

MATERIALS AND METHODS

The syrinx and trachea of the two ostriches (*Struthio camelus*) with three years of age and died upon natural courses, were fixed in 10% formaldehyde solution. The materials were then put in 95% ethanol and acetone to make them ready for staining procedures. They were then stained using alizarin red and alcian blue to visualize mineralization, as indicated by two references (Inouye 1967; Atalgin and Kürtül 2009).

Measurements were taken using a digital compass (Mitutoyo, Tokyo, Japan).

For histological observation, the tissue was fixed in 10% formaldehyde for at least 72 h. After routinely

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processing and embedding in paraffin, 6 ìm tissue sections were cut, stained with H&E, 10 ìm sections were cut and stained with Pincus Acid Orcein-Giemza for the elastic fibres surrounding cartilages (Luna 1968). Sections were investigated under a light microscope and were photographed.

Nomina Anatomica Avium (1993) was applied for the terminology.

RESULTS AND DISCUSSION

It does not seem bone cells in histological sections (Fig.1). The cartilage components of the tracheal rings appeared to be blue and mineralized zones red in alizarin red and alcian blue staining (Fig. 2). The mineralized areas were particularly intensified in the tracheal cartilages forming the syrinx (Fig. 3). In fact mineralization was highly prominent in the third cartilage.

The third tracheosyringeal cartilage pointed ventrally at median plane; thus, forming a "V" shape. Three tracheal rings anterior to this cartilage also had a slight resemblance and were stained red indicating mineralization. Similarly, these rings formed "V" shape dorsally at median plane, eventhough the third tracheosyringeal ring had neither dorsal fusion at median plane nor mineralization at the dorsal edges. From the lateral point of view, the mineralized components of the tracheal rings located before the primary bronchial division prolonged dorsally while getting thinner.

The cranio-caudal length of the third tracheosyringeal cartilage was shorter and measured 1.91 mm. The second cartilage had 3.20 mm length while the first one was of 4.50 mm. The length of the tracheal rings decreased cranially, extending from 3.86 mm to 2.17 mm.

In one cadaver, there were four incomplete tracheal rings located caudally in-between the intact ones. The intact tracheal rings located between two incomplete rings formed a slight "S" shape to keep the integrity of the trachea. The

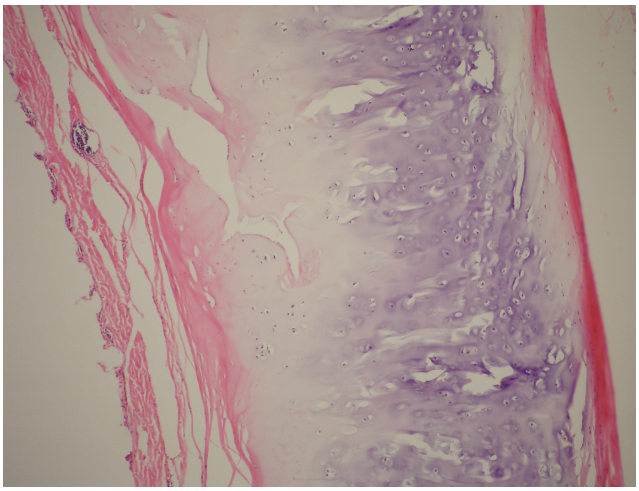

Fig 1: Histological section of the trachea.

ventral portions of the incomplete rings were mineralized (Fig. 2). There were also three incomplete tracheal rings with various mineralized portions in another cadaver.

The approximate length of the tracheal rings was 82.07 mm of which 24.55 mm was ossified. The approximate length of the rings constructing tympanum and the first 15 ones anterior to that was 89.22 mm, of which 64.57 mm mineralized. The length and mineralized portion of the rings decreased cranially.

The syrinx (Fig. 3) lay ventral to the glandular stomach, at the level of the second and third thoracic vertebra and between the terminal part of the trachea and primary bronchus. It was formed by three sets of rings; tympanum, tracheosyringeal cartilages, and bronchosyringeal cartilages. There were three tracheosyringeal cartilages with their ventral aspects mineralized mostly. Those that joined the construction of the tympanum cranially were cylindrical in

Fig 2: Ventral view of the trachea stained with alizarin red and alcian blue. (Scale bar 5 mm)

Fig 3: Ventral view of the syrinx stained with alizarin red and alcian blue.(Scale bar 5 mm)

shape. The four rings located caudal to the tympanum were composed of the syringotraheal cartilages. Eventhough, their dorsal portions were mineralized, mineralization on the ventral portions increased as descended caudally. The last tracheosyringeal cartilage was not intact dorsally. The three bronchosyringeal cartilages were crescentic, with the density of mineralization increasing as advanced cranially. The medial walls of the primary bronchi fused at the level of the tracheal bifurcation, thus forming pessulus. Neither cartilage nor mineralization was observed grossly in this structure. No syringeal bulla, which is originated in general from bronchial cartilage in syrinx, was seen.

In H&E staining, dark basophilic areas (chondrocytes in lacuna, isogenic groups) with lesser cellular content were seen in tracheal slides (Fig 1). Intense eosinophilic areas and tubular structures were observed beneath the perichondrium. In some areas and dark staining was seen around these tubular structures. Neither endrochondral ossification nor typical bony lamella, both observed with aging, was observed; however, the stained structure with alizarin red indicated mineralization in the eosinophilic regions.

Mineralization and ossification procedures in the trachea of birds have been amply studied in general (McLeod *et al*. 1964; Hogg 1982). These studies have reported that mineralization and ossification occur first at the caudal end of the trachea, near the syrinx, expanding at cranial direction. Due to the fact that no study, as to our knowledge, has been conducted on mineralization of the trachea and syrinx of the adult ostrich, this study aimed at documenting the presence of mineralization and ossification in the trachea and syrinx by applying alizarin red and alcian blue staining and histological observations. The tracheal rings near the larynx were not included in the study since it is well known in general that ossification begins at the caudal aspect, near the syrinx, of the trachea, thus advancing through the larynx.

Studies (Hogg 1982; Tasbas *et al*. 1994) have suggested that alizarin staining is not a good tool for determining ossification centers; yet, in these studies cartilage or bone tissues have been proposed to be visualized by histological procedures only. Alizarin staining applied in this study has also indicated mineralized regions in the tissues used. Since the size of the model used in this study is relatively big, no ossification centers were observed. Histological studies also confirmed the lack of ossification. The red areas seen were not because of the ossification, they are indeed mineralized regions, just as suggested by Hogg (1982). In contrary, Hogg (1982) has reported genuine bone tissue in the rings of larynx and trachea in aging, adult and old birds but histological slides showed no bone cells in the three years-old birds used in this study.

A study(Hogg 1982) has reported mineralization in the trachea of 98 days-old Golden Comet chicks. It indicated that mineralization begins at the caudal portion, expanding cranially, as also reported by Bradley and Grahame (1950); Garside (1968), and Hogg (1982). Similarly, mineralization has been densely observed caudally, indicating the fact that it began at this side and expanded cranially.

Hogg (1982) indicated in his study done on Golden Comet chicks that the caudal and cranial regions of the trachea and tympanum, respectively, were mineralized entirely. He also reported that the cranial component of the trachea was lightly stained completely. Likewise, Gross (1964) and Myers (1917) showed ossification in the tympanum and in the first ring of the bronchial cartilages of the syrinx in adult chicken, respectively. On the other hand, none of the mineralized tracheal rings in this study displayed complete staining.

Hogg (1982), depicted that he encountered rare incomplete rings in the trachea of Golden Comet chicks. Mineralization in the four incomplete rings located at the caudal edge of the trachea in the present study was similar to this earlier report.

Edges of the last tracheosyringeal cartilage were not fused dorsally at the median plane, which were not mineralized yet. This is in contrast to the recent report by Yildiz, *et al.* (2003).

Consequently, this study reported the anatomical and histological features of the trachea and syrinx of the ostrich. Alizarin red and alcian blue staining revealed mineralization but ossification, in parallel with the results of similar studies performed on smaller birds.

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