

**Gross and histological study of the larynx in red sokoto goat (*Capra hircus*) from Sokoto, Nigeria****^a Danmaigoro Abubakar*, ^b Abdullahi Usman Yusuf, ^c Mahmud Abdullahi Muhammad, ^d Kabeer Abubakar,****^a Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto-Nigeria,****^b Bauchi State Ministry of Agriculture and Natural Resources, Bauchi – Nigeria****^c Department of Animal Health and Production Technology, Niger State College of Agriculture, Mokwa, Niger State, Nigeria****^d Department of Human Anatomy, Faculty of Medicine, Federal University of Lafia, Maraba Akunza, Lafia, Nasarawa-Nigeria****Authors' Contribution**

Abubakar, D & A.U Yusuf designed the experiment, A. U. Yusuf & K. Abubakar analyzed the gross and histology of the larynx in red sokoto goat, D. Abubakar & M A. Muhammad evaluated the photomicrographs, A. U. Yusuf & K. Abubakar analysed the data and conducted statistical analysis

Corresponding Author's Email Address: abubakar.danmaigoro@fnu.ac.fj*ABSTRACT****Review Process: Peer review**

Small ruminants are very important in securing food security and are economically significant in the rural community especially in the west African country. The larynx serves as both airway passage and organ of vocalization, which are associated with their own pathology and diseases. In the current study, 9 specimens of larynxes from apparently healthy red sokoto goats were obtained from Sokoto Modern abattoir, dissected, and morphologically and histologically evaluated. The results showed the larynx is a firm irregular tubular structure situated in between the ramii of the mandible with the laryngeal muscles are intertwined in between the cartilages. The rostral aspect of the larynx forms the caudal border of the pharynx while the caudal end of the larynx continues as the trachea. The thyroid cartilage is the largest while arytenoids are the smallest. Histologically, the thyroid, arytenoids and cricoid are of the hyaline cartilage while the epiglottis is made up of the elastic cartilage. This research work focus on evaluating the gross and microscopic anatomy of the larynx, thus, providing information for future use in both anatomical studies and clinical applications.

Keywords: Larynx, cartilage, hyaline, elastic, epiglottis, cricoid, arytenoid.

INTRODUCTION: In Nigeria, goat is kept by many rural dwellers in small herds to serve as sources of financial stability and supply of meat and milk products (Byanet *et al.*, 2014), thus Red Sokoto Goat (RSG) *Capra hircus L.* are the most useful goat for these purposes. The RSG is the predominant and most important breed of goat found mainly in the Sudan and Sahel savanna zone in the Northwestern zone of Nigeria. This breed is well adapted to the arid zones and its population has been estimated to be 17.3 million, which accounts for about 70% of the 34.50 million goats in Nigeria (Byanet *et al.*, 2014). The larynx is cartilaginous structure that forms the link between the pharynx and the trachea-bronchial tree (Dyce *et al.*, 2010). It lies caudal the pharynx and behind the mouth, suspended from the cranial base by the hyoid apparatus; in most species it is partly contained between the rami of the mandible and partly extended into the neck. Since its connect the tongue and hyoid apparatus, the larynx shifts its position during animal swallowing (Dyce *et al.*, 2010). The larynx develops from the endodermal lining and the adjacent mesenchyme of the foregut between the fourth and sixth branchial arches. It is a complex valvular apparatus which regulates the volume of air respiration, prevents aspiration of foreign bodies and is the chief organ of voice (Sisson, 1910). The architecture of the larynx comprises several individual cartilages and muscles (Auer and Stick, 2018). The five laryngeal cartilages are the epiglottis, thyroid cartilage, cricoid cartilage, and paired arytenoid cartilages (Langley-Hobbs *et al.*, 2013). The external laryngeal muscles elevate or depress the larynx during swallowing while the internal laryngeal muscles move the individual components of the larynx, playing an important role in breathing and phonation (MacPhail, 2014). The larynx is innervated by branches of the vagus nerve which gives rise to the cranial laryngeal nerve that bifurcates into an internal and external branch (Langley-Hobbs *et al.*, 2013). Arterial blood supplied to the larynx is through the caudal laryngeal artery and branches of the ascending pharyngeal arteries (Auer and Stick, 2018). Venous drainage is provided by the caudal laryngeal and ascending pharyngeal veins, which emptied into the external jugular vein through the thyroid vein. The lymphatic chains that serve the laryngeal area include retropharyngeal, cranial, and deep cervical lymph centers (Auer and Stick, 2018). The lumen being lined by a mucous membrane composed of stratified squamous and pseudo – stratified columnar ciliated epithelium. The larynx is lined by respiratory epithelium with the lamina propria as a continuation of the perichondrium of the laryngeal cartilages. The vocal cords are covered by stratified squamous epithelium (Aughey and Frye, 2001). Supra-basally in this epithelium, dendritic antigen-presenting Langerhans cells (LCs) can be found (Sataloff *et al.*, 2017). The epiglottis is the elastic type while the thyroid, cricoids and arytenoids are the hyaline type (Weidner *et al.*, 2009).

OBJECTIVES: This study is aimed at evaluating the gross anatomy and histology of the larynx with emphasis on the laryngeal cartilages in Red Sokoto Goat. Since it serves as both airway

passage and organ of vocalization which has its own pathology and other associated conditions. Hence there is a need for extensive study of this important organ to understand its anatomy to provide information for the diagnosis of its diseases, which is scanty in scientific literature.

MATERIALS AND METHODS: Nine specimens of larynx of apparently healthy RSG were obtained from Sokoto Modern abattoir. The skin and superficial fascia on the ventral midline of the neck were excised to expose the laryngeal muscles as earlier described by Igbokwe and Ezeasor (2015). The color, shape and positioning of the larynx were studied. The cranial aspect of the larynx was severed just around the pharynx and the joint with hyoid bone. The caudal aspect was also severed from the trachea at the first tracheal ring. The individual cartilage was separated with the aid of Anatomical atlas for guidances. However, the relationships with other were noted and images were captured using PAXcam™ HD (China). Digital weighing scale was used measure the weights of the cartilages. The entire landmarks recorded on each larynx were measured using twine and meter rule, measurements were recorded in centimeter (cm). The thyroid length (TL) was considered from the tip of the cranial cornua to the end of the caudal cornua. The thyroid width (TW) was recorded from the two laterals of the cartilage. The length from thyroid notch to posterior margin (TN) was measured. The lengths of the cranial cornua (CC) and caudal cornua (CD) were as well recorded. The epiglottis length (EL) was measured from the caudal aspect to the tip of the upper lips of the epiglottis cartilage. The epiglottis width (EW) was taken from the lateral commissures of the lips. The length of the arytenoid cartilage (AL) was measured from its apex. The arytenoid width (AW) was taken as the base width. The width of the cricoid cartilage (CW) was measured across the two laterals while the transverse diameter (TD) is measured across the ring transversely. The dorsal lamina (DL) is the length across the proximal lamina of the cartilage. The standard method of tissue paraffin embedding tissue processing method was adopted as earlier described by Igbokwe and Ezeasor (2015). The cartilages were fixed in 10% buffered neutral formalin for 48 h, and then dehydrated in a series of graded alcohol (70% for 8 h., 95% for 4 h., 95% for 4 h., 100% for 4 h. and 100% for 4 h.). They were then cleared using xylene and alcohol (1:1) for 3 h. and using xylene only for 4 h. The tissues were further embedded in wax and kept in an oven with temperature of 63°C for 8 h. Blocking was done using paraffin wax and the block were sectioned at 6 m with a rotary microtome HistoCore AUTOCUT (Lieca™, China). The transverse section of the tissues was floated onto a water bath heated to 45 °C. Following positioning on glass slides they were then placed on a hotplate at 60 °C to melt the wax and allow sections to adhere. Series of graded alcohol was used to clear the wax. Staining with haematoxylin and eosin was used in all specimens. All sections were examined and recorded. Every section that was observed under microscope was photographed using digital Amscope light

microscope to evaluate the histology of the samples.

RESULTS AND DISCUSSION: The larynx is a firm irregular tubular structure situated in between the ramii of the mandible. It is suspended by the hyoid bone, ligaments, and muscles. Laryngeal muscles are intertwined in between the cartilages. The rostral aspect of the larynx forms the caudal border of the pharynx while the caudal end of the larynx continues as the trachea. The larynx was found to be made up of a paired arytenoid cartilage and three unpaired cartilages: thyroid, epiglottis and cricoid. Morphologically, the arytenoid is paired cartilages that articulate cranially with the epiglottis, proximally with the thyroid cartilage and caudally with the cricoids cartilage. They have an irregular shape which resembles a pyramid with apex and a base. They are the smallest of the four cartilages (figure 1).

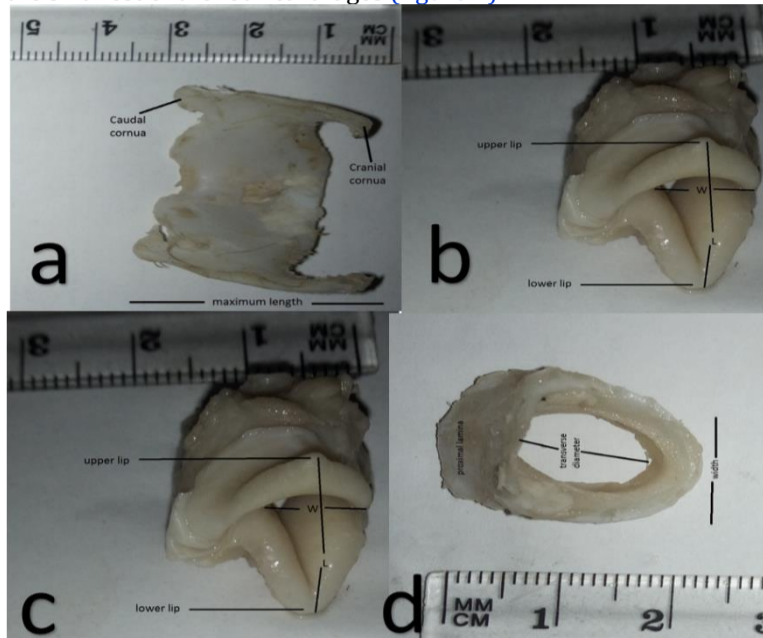


Figure 1: Cranial view of arytenoid cartilages (a), dorsal view of cricoid cartilage (b), cranial view of the Epiglottis cartilage (c) and caudal view of cricoid cartilage of red sokoto goat.

Thyroid cartilage is a semi-circular structure covering the ventral aspect of the laryngeal boundary. It's the largest among the laryngeal cartilages. Rostrally, it attaches with the proximal part of the epiglottis. It has cranial cornua which attaches with the hyoid bone and caudal cornua attaches with the prominence of the cricoids cartilage (figure 1). However, Epiglottis forms the most cranial aspect of the larynx and extends into the pharynx. Its cranial border has a proximal and distal two-pointed leaf - like lips with the proximal lip being the largest. The caudal end of the epiglottis joins with the arytenoids distally and proximally with the thyroid cartilage. The epiglottis is flexible and relatively softer than the other laryngeal cartilages (figure 1). Cricoids cartilage appears ring shaped cartilage that forms the caudal extremity of the larynx. Caudal to it, continues the first tracheal ring. It has a broad proximal lamina. The arytenoids attach to its cranial lower part. The transverse diameter of the ring is longer than the width across the ring in all the samples dissected (figure 1). The average weight of thyroid cartilage in Red Sokoto goat was found to be 1.31 ± 0.49 grams. The length was 2.97 ± 0.36 cm, the maximum thyroid width was found to be 2.44 ± 0.57 cm, the length from thyroid notch to posterior margin was 1.87 ± 0.19 cm, the lengths of cranial and caudal cornua were found to be 0.62 ± 0.31 cm and 1.14 ± 0.25 cm as shown in table 1. The epiglottis weighed 2.06 ± 0.85 grams; however, the average length and width were 2.40 ± 0.58 cm and 1.61 ± 0.60 cm respectively (table 1). The arytenoids weighed 0.39 ± 0.20 grams, average length of 1.48 ± 0.31 cm with a base width of 0.99 ± 0.09 cm (table 1). The cricoid cartilage weighed 1.53 ± 0.64 grams and transverse diameter measures 1.94 ± 0.43 cm, the maximum width has a measurement of 1.22 ± 0.36 cm while the width of dorsal lamina measures 1.94 ± 0.11 cm (table 1). The thyroid, arytenoid and cricoid cartilages all possess hyaline cartilage characteristics. The perichondrium envelops the cartilage and within the matrix are sparsely lies multiple lacunae that houses chondrocytes. The matrix in thyroid cartilage is densely populated with lacunae while that of cricoid and arytenoids are sparsely populated (figure 2-5). Epiglottis is made up of elastic cartilage with a matrix surrounded by perichondrium. The matrix is heavily infiltrated with elastic fiber and the lacunae are surrounded by dense elastic fibers. The chondrocytes are each placed within a lacuna (figure 1).

	Thyroid	Epiglottis	Arytenoids	Cricoids
Weight (g)	1.31 ± 0.49	2.06 ± 0.85	0.39 ± 0.20	1.53 ± 0.64
Length (cm)	2.97 ± 0.36	2.40 ± 0.58	1.48 ± 0.31	
Breath (cm)	2.44 ± 0.57	1.61 ± 0.60	0.99 ± 0.09	1.22 ± 0.36
Length from thyroid notch to posterior margin (cm)	1.87 ± 0.19			
Cranial cornua (cm)	$0.62 \pm .31$			
Caudal cornua (cm)	$1.14 \pm .25$			
Width of dorsal lamina (cm)				1.94 ± 0.11
Transverse diameter (cm)				1.94 ± 0.43

Table 1: The morphometry of laryngeal cartilages.

The larynx is composed of mainly of four cartilages in which three of those cartilages are unpaired and they are the epiglottis, thyroid, and cricoid cartilages. The arytenoid cartilage is paired. This is similar with the findings of Manjunatha *et al.* (2018) in pigs, and the works of Erdogan and Pérez (2017) in giraffe.

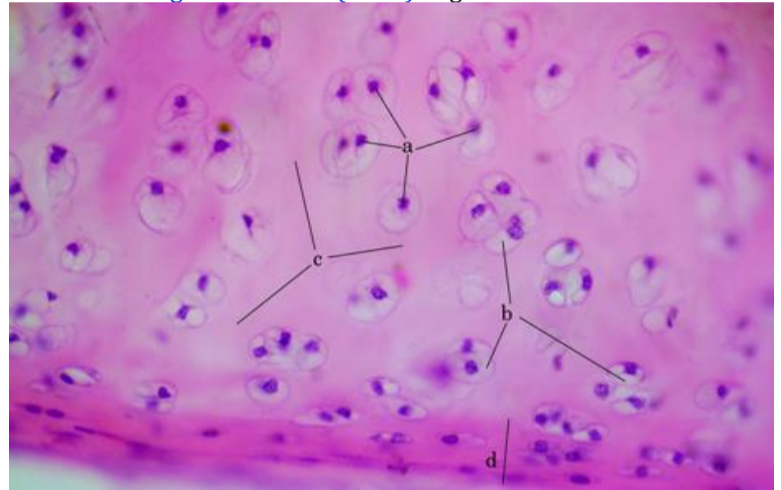


Figure 2: Photomicrograph of thyroid cartilage; a) chondrocytes sitting in lacunae b) lacunae c) Matrix d) Perichondrium. The thyroid cartilage is a hyaline cartilage (x40) in H&E.



Figure 3: Photomicrograph of arytenoid cartilage; a) Chondrocytes b) matrix c) Perichondrium d) lacunae. The arytenoids cartilages are hyaline cartilage. (x40) in H&E.

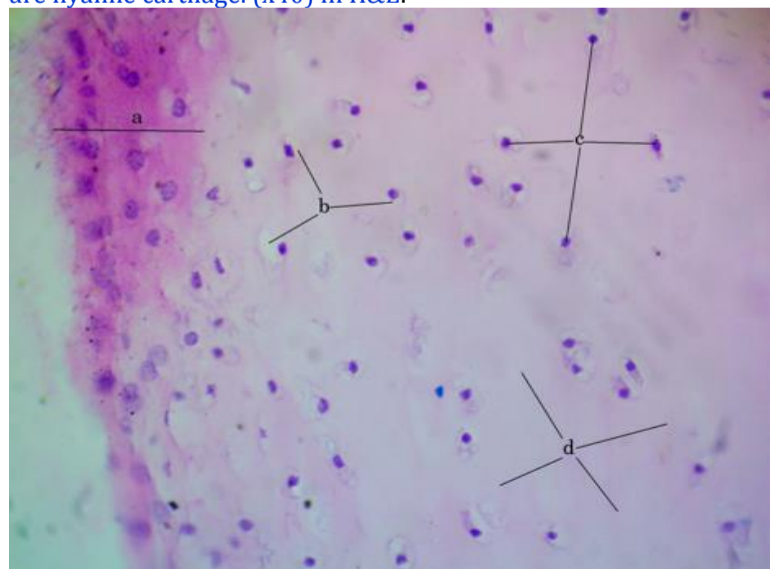


Figure 4: Photomicrograph of cricoid cartilage; a) Perichondrium b) Lacunae c) Chondrocytes d) matrix. Cricoid cartilage is hyaline cartilage. (x40) in H&E.

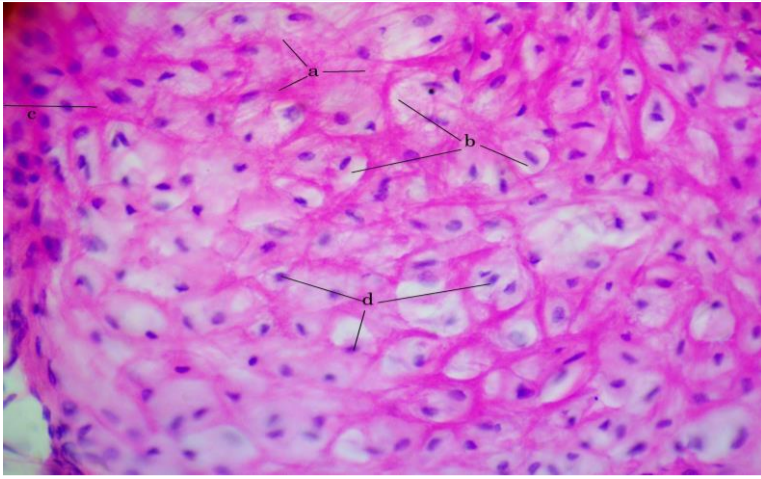


Figure 5: Photomicrograph of epiglottis; a) elastic fibres within the matrix surrounding the lacunae. b) Lacunae c) Perichondrium d) chondrocytes. Epiglottis is made up of elastic cartilage. (x40) in H&E.

The thyroid cartilage is the largest among the four cartilages and has cranial and caudal cornua which corresponds with the report of ALSadi (2006) in sheep however there is variation with that of a giraffe as there was absence of cranial cornua (Erdogan and Pérez, 2017). The arytenoids cartilages are paired having a pyramid like shape with apex and a base. They articulate cranially with the epiglottis, proximally a with the thyroid and caudally with cricoids as reported by Rajani *et al.* (2019) in elephants. Cricoid cartilage is a ringed shaped cartilage that forms the caudal extremity of the larynx. It has a broad proximal lamina. This is also reported in elephants (Rajani *et al.*, 2019) having ring-shaped and composed of a dorsal lamina and a ventro-lateral arch. Prominent median crests were observed in elephants (Rajani *et al.*, 2019) and in giraffes (Erdogan and Pérez, 2017). However median crest is absent in sheep (ALSadi, 2006). The epiglottis is a flexible cartilage that forms the most cranial aspect of the larynx and extends into the pharynx. The epiglottis has a proximal and distal two-pointed leaf-like lips with the proximal being the largest. This finding is in accordance with that of sheep and goat (ALSadi, 2006) also as that of giraffe (Erdogan and Pérez, 2017) but differs with that of Paca (Ruaro *et al.*, 2021) as it was found to be round. The histology of the thyroid, arytenoids, and cricoid shows hyaline cartilage as that of sheep (ALSadi, 2006) and *Didelphis marsupialis* (L) (Pantoja *et al.*, 2020). The photomicrograph of the epiglottis in red Sokoto goat shows an elastic cartilage with dense elastic fibers in the matrix. This is also found in sheep and goat by ALSadi (2006) to be of elastic type. This at same time goes in line with the findings in opossums (Pantoja *et al.*, 2020) that epiglottis consisted of elastic cartilage and is covered by a non-keratinized stratified squamous epithelium.

CONCLUSION: This research work focus on evaluating the gross and microscopic anatomy of the larynx, thus, providing information for future use in both anatomical studies and clinical applications.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

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