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Research Article

HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE LARYNGEAL CAVITY OF GOAT

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ABSTRACT

The present study was conducted on the laryngeal cavity of six goats of 8-10 months age of local mixed breed to explore its histology and histochemistry which may be of significance in disease conditions of the region. The laryngeal cavity was divided into three compartments i.e. anterior (vestibule or supraglottic), middle (rima glottidis) and caudal (post-glottic) compartments. The maximum portion of the laryngeal cavity was lined by stratified squamous non-keratinized epithelium except the post-glottic cavity which was lined by pseudostratified columnar ciliated epithelium with goblet cells. The sero-mucous type of glands with predominance of serous glands, collagen bundles, lymphoid aggregations, nerve bundles and venous caverns like structures were observed in propria-submucosa. Histochemically, the glands showed positive reaction for different types of mucopolysaccharides, mucin, and proteins.

Keywords: Goat, Histochemistry, Histology, Laryngeal cavity

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The laryngeal cavity extends from aditus laryngis to the cricoid cartilage, where it continues with the trachea. The laryngeal cavity is divided into three compartments i.e., vestibular or supraglottic which extends from aditus laryngis upto cranial portion of vocal cord. The middle compartment, rima glottidis, which is formed by vocal cords. which are folds of the mucous membrane responsible for sound production. The caudal compartment or post-glottic cavity is present just behind the glottis and is enclosed by cricoid cartilage and crico-thyroid membrane (Dellmann and Brown, 1987). The histology and histochemistry of the laryngeal cavity has been studied in pig (Manjunatha *et al.*, 2018; Parkash, 2016; Parkash and Kumar, 2019 and Kalita, 2014), buffalo, camel and donkey (Eshra *et al.*, 2016), cattle (Casteleyn *et al.*, 2008), mithun, yak and zebu (Kalita and Kalita, 2003) and adult goat (Kahwa and Purton, 1996). The perusal of literature revealed very less work done on the laryngeal cavity of goat which led to pursue the present study to record its normal structure which may be helpful to pathologists and surgeons in disease diagnosis.

MATERIALS AND METHODS

The tissues were collected from different compartments of the laryngeal cavity of six goats of either sex of 8 to 10 months of age of local mixed breed immediately after their slaughter and fixed in 10% neutral buffered formalin solution for 48 hours. The tissues were processed for routine paraffin technique for light microscopy and sections of 5-6 microns were stained by routine Harris' hematoxylin and eosin stain, Weigert's method for elastic fibres, Gomori's method for reticular fibres, Crossman's trichrome for collagen fibres, Bielschowsky's method for axis cylinder and dendrites, Holmes method for nerve cells and fibres, McManus' method for glycogen, Alcian blue method for mucosubstances (pH 2.5), PAS- Alcian blue for neutral and acidic mucopolysaccharides (pH 2.5), Mayers mucicarmine for mucin, colloidal iron for acid mucopolysaccharides (Luna, 1968), mercury bromphenol blue method for proteins, performic acid-Alcian blue method for proteins (Pearse, 1968) and Ayoub-Shklar method for keratin and prekeratin (Luna, 1968).

RESULTS AND DISCUSSION

The laryngeal cavity was comprised of anterior vestibular, middle rima glottidis and anterior and posterior parts of the post-glottic cavity.

Vestibular part: The vestibular part of the laryngeal cavity was lined by stratified squamous non-keratinized epithelium (Fig. 1) as reported in buffaloes (Attia and Moustafa, 1989), ruminants and equine (Dellmann and Brown, 1987), pig (Manjunatha *et al.*, 2018; Parkash, 2016; Parkash and Kumar, 2019 and Kalita, 2014), buffalo, camel and donkey (Eshra *et al.*, 2016), mithun, yak and zebu (Kalita and Kalita, 2003), cattle (Casteleyn *et al.*, 2008) and adult goat (Kahwa and Purton, 1996). The epithelium was comprised of varying number of rows in different strata. The stratum basale was having a single row of cuboidal to columnar nuclei with fine distribution of chromatin material. The nuclei of stratum spinosum cells were comparatively less basophilic than stratum basale cells. The superficially placed nuclei were comparatively larger and placed horizontally. A few nuclei with vacuolated appearance were also observed in between these nuclei. The cytoplasm of all these cells was finely granular and eosinophilic in appearance. The stratum superficiale was having 2-3 rows of nuclei of which superficially placed nuclei were rod shaped with pointed ends and majority of which showed pyknotic appearance

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due to degenerative changes. The nuclei were comparatively more basophilic than other cell types. The cytoplasm of the cells was finely granular and comparatively more eosinophilic in nature. The free surface of the epithelium presented papillated appearance. A few lymphoid cells were also infiltrated irregularly in the epithelium.

The propria-submucosa of vestibular part of laryngeal cavity was having loose irregular connective tissue, collagen, reticular and elastic fibres, small sized blood vessels and nerve bundles. The connective tissue became denser towards deeper part because of more distribution of collagen fibres (Fig. 2). In addition, a dense distribution of elastic fibres was also observed in the interglandular areas (Fig. 3). In the deeper portion, abundance of adipose tissue was also observed.

The sero-mucous glands with predominance of serous type were having round to oval nuclei. The cytoplasm of these cells was finely granular and eosinophilic in nature. Histochemically, the glands showed positive reaction for neutral mucopolysaccharides as demonstrated by PAS-Alcian blue method. The glandular acini showed the presence of mucin however, only a few acini showed presence of acidic mucopolysaccharides by colloidal iron method (Fig. 4). The Alcian blue method showed the presence of weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins. The performic acid-Alcian blue method showed the presence of more than 4% cysteine in the glandular acini (Fig. 5). The intra and interglandular ducts lined by simple cuboidal epithelium were mainly devoid of PAS activity.

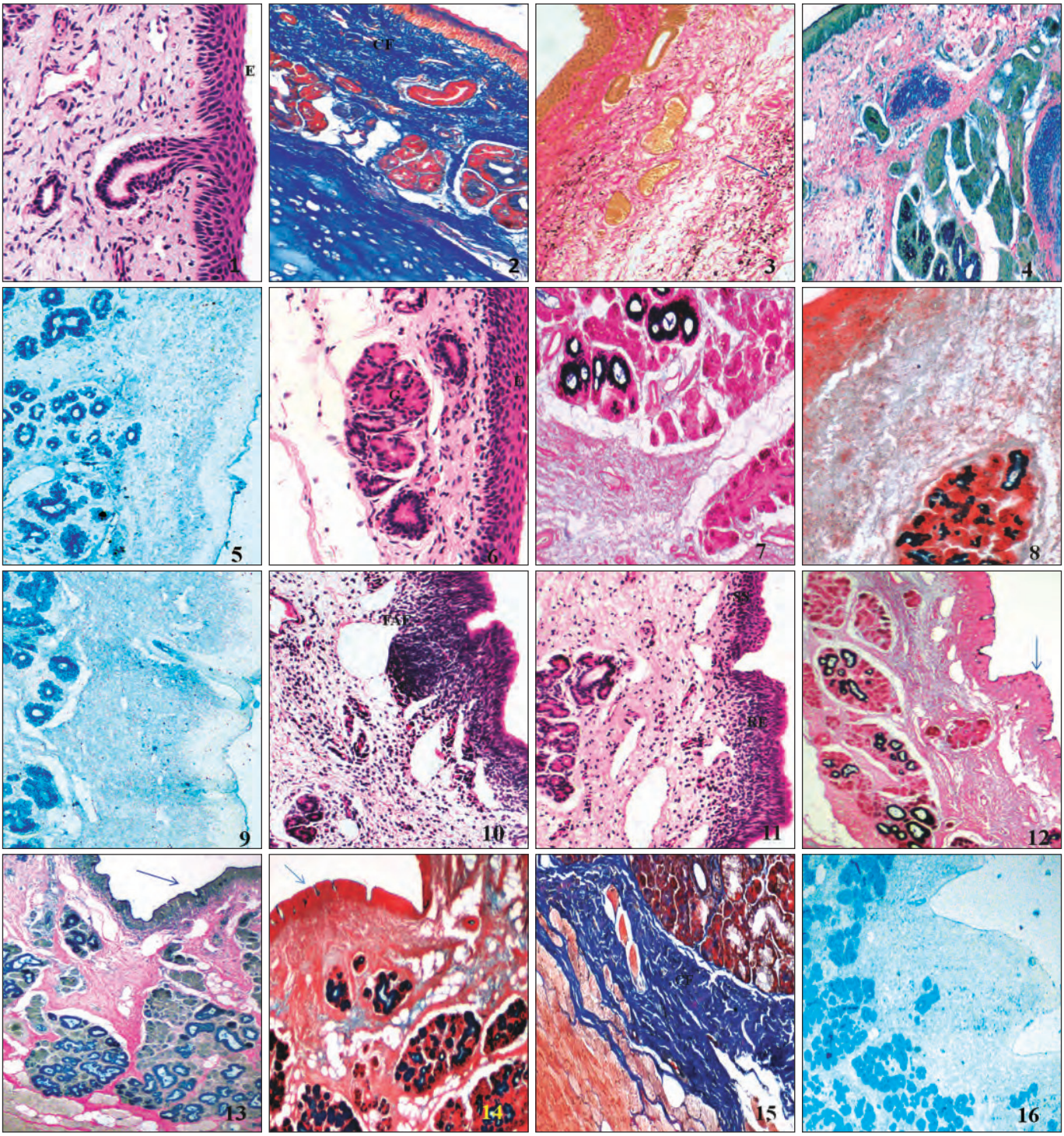
Rima glottidis: The rima glottidis of the laryngeal cavity was lined by stratified squamous non-keratinized epithelium (Fig. 6) as reported in adult goat (Kahwa and Purton, 1996), pig (Manjunatha *et al.*, 2018; Parkash and Kumar, 2019 and Kalita, 2014), buffalo, camel and donkey (Eshra *et al.*, 2016), cattle (Casteleyn *et al.*, 2008), mithun, yak and zebu (Kalita and Kalita, 2003). The histological features of different strata were similar to those of the vestibular part except variation in number of rows.

The propria-submucosa having loose irregular connective tissue was constituted by different types of fibres, nerve bundles and fine blood capillaries. In the subepithelial portion, a dense arrangement of collagen fibres was observed. The connective tissue became denser towards the deeper part because of dense distribution of elastic fibres in the interglandular areas and around the tunica intima of the blood vessels. The sero-mucous glands with predominance of serous were observed as reported in pig (Parkash, 2016; Parkash and Kumar, 2019), buffalo, camel and donkey (Eshra *et al.*, 2016). The interglandular and intra-glandular ducts were lined by simple cuboidal epithelium. Deeper to the glands, the connective tissue became again loose and irregular with abundance of adipose tissue and thick arrangement of striated muscles. The fatty tissue was present in between hyaline cartilage and striated muscles. The glands were very much reduced in the caudal half of the rima glottidis part. The sero-mucous glands showed positive activity for neutral mucopolysaccharides as compared to acidic mucopolysaccharides (Fig. 7). The mucous acini showed PAS positive reaction for the presence of glycogen, mucin, acidic mucopolysaccharides, weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins in the mucous glandular acini (Fig. 8). The glandular acini showed qualitatively more than 4% of cysteine as demonstrated by performic acid Alcian blue method (Fig. 9). The serous glands showed mild positive reaction in the caudal half of the rima glottidis.

Anterior part of post-glottic cavity: The anterior portion of the post-glottic cavity was lined by stratified squamous non-keratinized epithelium having varying number of rows in different strata as reported in buffalo, camel and donkey (Eshra *et al.*, 2016), mithun, yak and zebu (Kalita and Kalita, 2003), adult goat (Kahwa and Purton, 1996), pig (Manjunatha *et al.*, 2018; Parkash and Kumar, 2019 and Kalita, 2014), cattle (Casteleyn *et al.*, 2008). The epithelium was comprised of 5-7 rows of nuclei. The stratum basale cells were linearly arranged on the basement membrane. The oval to round basophilic nuclei had fine distribution of chromatin material. The cytoplasm of these cells was very less and finely granular and eosinophilic in nature. The stratum spinosum was having 3-4 layers of nuclei which were oval to elongated and directed obliquely and vertically. The typical spiny appearance of the cells was lacking because of less intercellular spaces. The stratum superficiale was having 2-3 rows of nuclei. The deeper nuclei were having features like those of stratum spinosum and the superficially placed nuclei became flat and rod shaped with tapering ends. The majority of the nuclei showed pyknotic appearance because of degenerative changes. The free surface of the epithelium was slightly undulating and the interpapillary pegs were absent towards the deeper surface. This region showed only a few lymphoid cells infiltrated in the epithelium.

The propria-submucosa was having loose irregular connective tissue along with very less distribution of lymphoid cells. Only clusters of serous acini were observed. Histochemically, the glandular acini showed presence of only neutral mucopolysaccharides, glycogen, moderate positive for mucin, negative for acidic mucopolysaccharides, positive for weakly sulfated acidic mucopolysaccharides, hyaluronic acid, sialomucins and proteins.

Posterior part of post-glottic cavity: The posterior part of the post-glottic cavity of larynx was lined by pseudostratified columnar ciliated epithelium or follicle associated



Figs. 1-16. **(1)** Photomicrograph of vestibular part of laryngeal cavity showing stratified squamous non-keratinized epithelium (E) . H. & E. × 200. **(2)** Photomicrograph showing collagen fibers (CF) in the subepithelial portion of vestibular part of the laryngeal cavity. Crossman's trichrome method x 100. **(3)** Photomicrograph showing arrangement of elastic fibers (arrow) in the vestibular part of the laryngeal cavity. Weigert's method x 100. **(4)** Photomicrograph showing moderate postitive activity of acidic mucopolysaccharides (light blue colour) in the glands of vestibular part of the laryngeal cavity. Colloidal iron method x 100. **(5)** Photomicrograph showing positive activity of more than 4% cystine (sky colour) in the glands of vestibular part of the laryngeal cavity. Performic acid AB x 100. **(6)** Photomicrograph showing stratified squamous non-keratinized epithelium (E) and glands (G) in the propria submucosa of rima glottidis part of laryngeal cavity. H&E. x 200. **(7)** Photomicrograph showing neutral mucopolysaccharides (red colour) and few acidic mucopolysaccharides (blue colour) in the glands of rima glottidis part of the laryngeal cavity. PAS AB x 200. **(8)** Photomicrograph showing positive activity of weakly sulfated acidic mucopolysachharides, hyaluronic acid and sialomucins in the glands (blue colour) of rima glottidis part of the laryngeal cavity. Alcian blue x 200. **(9)** Photomicrograph showing positive activity of more than 4% cystine (sky colour) in the glands of the rima glottidis part of laryngeal cavity. Performic acid A B x 200. **(10)** Photomicrograph showing follicle associated epithelium (FAE) like structures in the postglottic region of the laryngeal cavity. H.&E. x 200. **(11)** Photomicrograph showing transition of epithelium on anterior part of the post glottic region from respiratory epithelium (RE) pseudostratified ciliated columnar to stratified squamous (SS) epithelium. H. & E. x 200. **(12)** Photomicrograph showing neutral mucopolysaccharides in the goblet cells (arrow) and glands (red colour) and acidic mucopolysaccharides (blue colour) in propria submucosa of postglottic region of the laryngeal cavity. PAS AB x 100. **(13)** Photomicrograph showing positive activity of acidic mucopolysaccharides in the goblet cells (arrow) and the glands (light blue colour) in the postglottic region of the laryngeal cavity. Colloidal iron method x 100. **(14)** Photomicrograph showing positive activity of weakly sulfated acidic mucopolysachharides, hyaluronic acid and sialomucins in the goblet cells (arrow) and glands (blue colour) of postglottic region of the laryngeal cavity. Alcian blue x 100. **(15)** Photomicrograph showing collagen fibers (CF) in between glands and muscle fasciculi of propria submucosa of post-glottic part of the laryngeal cavity. Crossman's trichrome method x 100. **(16)** Photomicrograph showing positive activity of more than 4% cystine in the glands (sky colour) of postglottic region of the laryngeal cavity. Performic acid AB x 100.

epithelium because of presence of lymphoid cells in the subepithelial portion (Fig. 10) as reported in adult goat (Kahwa and Purton, 1996), buffalo, camel and donkey (Eshra *et al.*, 2016), rat (Lewis and Prentice, 1980). The epithelium was having undulating appearance and was comprised of basal, supporting and goblet cells. The basal cells were having round to oval nuclei resting on the basement membrane and these nuclei were having fine distribution of chromatin material giving basophilic appearance. The cytoplasm of these cells was finely granular and eosinophilic in nature. The large number of lymphoid cells infiltrated in between the basal cells also extended into the rest portion of the epithelium. The supporting cells were having rod to elongated nuclei of varying shapes and size. The nuclei were having smaller clumps of chromatin material distributed irregularly throughout the nucleoplasm. The free surface of the cells presented cilia of uniform length. The cytoplasm of these cells was finely granular and eosinophilic in nature. The goblet cells were interspersed in between the supporting cells and showed vacuolated appearance may be because of washing of mucus during processing of the tissues. The nuclei of the cells were pushed towards the basal portion. The cytoplasm of these cells was less eosinophilic and had floccular appearance. The lymphoid cells were observed throughout the height of epithelium. The transition of epithelium from pseudostratified ciliated columnar into stratified squamous non-keratinized epithelium was observed in the anterior portion of post-glottic cavity (Fig. 11). The goblet cells showed positive reaction for neutral mucopolysaccharides (Fig. 12), acidic mucopolysaccharides as demonstrated by colloidal iron method (Fig. 13), positive for weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins as demonstrated by Alcian blue method (Fig. 14), moderately positive for mucin and proteins.

The propria-submucosa of posterior part of post-glottic cavity was having dense arrangement of lymphoid cells in the subepithelial portion, fine distribution of collagen, reticular and elastic fibres, small sized blood vessels and nerve bundles. The connective tissue became denser towards the deeper part because of dense distribution of collagen fibres (Fig. 15). The concentration of collagen fibres was drastically reduced in the deepest part where thick arrangement of muscles was present. The sero-mucous type of glands with predominance of serous were present as reported in buffaloes and donkeys (Eshra *et al.*, 2016), pig (Parkash and Kumar, 2019) and ruminants and equines (Dellmann and Brown, 1987). The lumen of these acini was generally smaller in size. These acini were surrounded by myoepithelial cells. The cytoplasm of these cells was finely granular and eosinophilic in nature. The mucous acini were having larger lumen as compared to the serous type. The serous demilunes were also observed. The laryngeal glands were more in sheep as compared to goats (Al Sadi, 2006). The interglandular and intra-glandular ducts were lined by simple to stratified cuboidal epithelium and these ducts coursed towards the surface of epithelium. Histochemically, the acini showed the positive reaction for the presence of neutral mucopolysaccharides, acidic mucopolysaccharides, mucins, glycogen, weakly sulfated acidic mucopolysaccharides, hyaluronic acid and sialomucins. The muscle fasciculi also showed mild positive reaction for glycogen. The inter and intra-glandular ducts were strongly positive for glycogen and mucin. The glandular acini showed positive reaction for proteins as demonstrated by performic acid-Alcian blue method (Fig. 16) as reported in pig (Kalita, 2014), sheep (Pathak and Rajput, 2020; Al Sadi, 2006).

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