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Histomorphometry and histochemical studies on the trachea of local poultry of Poonch region of UT of J & K

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ABSTRACT

The present study was carried on trachea of 06 healthy poultry of Poonch region. Trachea was composed of tunica mucosa, submucosa, cartilage rings and adventitia. Mucosa comprised of pseudo-stratified ciliated columnar epithelium with two cell types. Columnar cells had oval to elongated nuclei while the basal cells had smaller, rounder nuclei. Intra-epithelial alveolar mucous glands were observed. Apical region of glands had foamy cytoplasm. Lamina propria merged with submucosa and consisted of collagen, elastic and reticular fibers with abundant lymphocytes. Tracheal rings made up of hyaline cartilage consisted of two parts, a much thicker inner cartilage ring and outer thin ring. The glassy ground substance had lacunae containing single chondrocyte and few isogenous groups. Loose connective tissue, blood vessels, and nerves were reported in tunica adventitia. Striated muscles were connected to the trachea containing blood vessels and nerve plexus. Histochemically, mucous glands exhibited a strong reaction for PAS-AB. Perichondrium covering the cartilage ring was moderately to strongly positive for basic proteins whereas the striated muscle fibers associated with trachea displayed moderate reaction. A strong reaction for glycogen was seen in the extra-cellular matrix surrounding the lacunae containing chondrocytes. Epithelium and mucous glands were moderately positive for lipids, lamina propria was weak and tunica adventitia was strongly reactive for lipids.

Keywords: *Cartilage, Histology, Mucopolysaccharides, Poonch, Trachea*

Introduction

The Union Territory of J&K belongs to the greater Himalayan Mountain range which has considerable impact on its agro-climatic conditions. Poonch's somewhat high elevation and northerly location result in a humid subtropical climate that is significantly cooler than the rest of India. Winters are cool with short daytime and characterized by rainfall. During the months of January and February, snowfall is fairly frequent. Temperature falls below freezing at night and the average temperature in January is 2.5 °C (36.5 °F). Summers are short and usually pleasant. Poonch region's native fowl can endure temperatures as low as minus 40 degrees, and their rearing ensures the survival of the local inhabitants. Indigenous bird of Poonch region weighs about 2.1-2.5 kg (cock) and 1.6-1.8 kg (hen). Females are combless whereas males present red coloured comb.

Throughout their life, poultry are exposed to a variety of environmental stressors including unfavorable weather circumstances (Torki *et al* 2015). Low ambient temperature has been shown to have a significant negative impact on an animal's health and welfare (Dhanalakshmi *et al* 2007). According to Durmus and Kamanli (2015), the temperature-neutral zone for metabolic and productive activity in adult laying hens ranges between 18 to 23.9 °C. Similar to this, Pawar *et al* (2016) reported that the ideal temperature for the thermos-neutral zone was between 19 and 22 °C for laying hen.

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However, throughout the winter, the ambient temperature varies from -5 to +5 °C in various parts of the world (Sahin *et al* 2002). Animal productivity is significantly affected adversely by cold temperatures below 16°C. One such system is a respiratory system which is crucial for the exchange of gases, thermoregulation of body temperature, and voice production (Al-Mahmodi 2012). The upper respiratory tract, which includes the nose, nasal cavity, and throat, and the lower respiratory tract, which includes the larynx, trachea, lungs, and syrinx, are both essential for gas exchange (AL-Mussawy 2010). Mammal and fowl respiratory systems are structurally very different from one another. Birds have complete cartilaginous tracheal rings and the presence of air sacs.

Literature is available on the histomorphology of trachea in Japanese quail (Pourlis *et al* 2018), turkey (Al-Mussawy *et al* 2012), coot bird and guinea fowl (Waad 2015), duck (Al-Ahmed and Sadoon 2020), pigeon (Al-Taai 2021), partridge (Rajathi *et al* 2021) and Uttara fowl (Yadav *et al* 2022). Micrometrical studies had been done on the trachea of Turkey (Al-Mussawy *et al* 2012) and Uttara fowl (Yadav *et al* 2022). Data is also available on the histochemistry of trachea of Uttara fowl (Yadav *et al* 2022). Regarding indigenous poultry of Poonch region, anatomical data is available on the proventriculus (Sasan *et al* 2022) and gizzard (Suri *et al* 2024), but no data is available on the histomorphometry and histochemistry of trachea. Therefore, the present study was undertaken to generate information on histomorphometry and histochemistry on the trachea as this bird can survive in cold weather by utilizing locally available feed. The study will further help to unveil the distribution and localization of various histochemical components. Further, the anatomical data generated would be useful to the veterinarians and poultry breeders.

Materials and Methods

Sample collection: Carcasses of 06 birds were obtained from the Division of Animal Genetics and Breeding, F.V.Sc & A.H., SKUAST-Jammu. Immediately following collection, respiratory tracts were thoroughly examined and tracheal tissue samples were obtained and preserved for 24 hours in 10% Neutral Buffered Formalin.

Sample processing for light microscopy

Tissue samples were processed and 5 µ thick sections were obtained. The sections were subjected to various histological and histochemical methods as detailed here under.

1. Hematoxylin and Eosin stain for routine histomorphology
2. PAS-AB (pH 2.5) for neutral and acid mucin
3. Best Carmine method for glycogen
4. Bromphenol blue for basic proteins
5. Sudan Black B for lipids

Micrometrical parameters recorded

Different micrometrical parameters were recorded:

a. Thickness of epithelium (µ)
b. Height of cilia (µ)
c. Size of nucleus of columnar cells (µ)
d. Thickness of cartilage rings (µ)
e. Longer and shorter alveolar glandular diameter (µ)
f. The eccentricity of alveolar glands was calculated as per Zedda *et al* (2016). The shorter diameter of glands was considered as minor semiaxis of ellipse (b) and longer diameter was considered as major semiaxis of ellipse (a). The degree of eccentricity was calculated according to the following mathematical formula:

$$e = \sqrt{1 - b^2/a^2}$$

The value of eccentricity lies between 0 and 1 ($0 < e < 1$). When the eccentricity is 0, the figure is round and when the value of eccentricity is towards 1, the figure becomes more elongated.
g. Size of mucous cells (µ)

Statistical analysis: The micrometrical results were presented as Mean ± Standard Error. The data was subjected to standard statistical analysis (Snedecor and Cochran 1994).

Results

Histo-morphometry

Trachea was a long tube started at the end of the cricoid cartilage and was accompanied by oesophagus. Histologically, trachea was composed of tunica mucosa, submucosa, cartilage rings, and adventitia (Fig. 1a). Mucosa was composed of pseudo-stratified ciliated columnar epithelium with oval nuclei (Fig. 1b). The mean height of cilia in trachea ranged from 3.36 to 4.04 µ with an average of 3.68 ± 0.11 µ. The epithelial layer contained two different cell types i.e. columnar cells with oval to elongated nuclei (Fig. 1b) and the basal cells with smaller, rounded nuclei. The nucleus size of columnar cells was 5.99 ± 0.31 µ. Goblet cells (mucus-producing cells) were also observed that created little depressions within the lining epithelium.

Intra-epithelial mucous glands were simple alveolar type (Fig. 1c) and lined by pyramid-shaped cells with wide basal part and narrow apical part. The lumen was not appreciable. The apical region had foamy cytoplasm and was lightly stained with H&E (Fig. 1c). The mean height of surface epithelium was 35.96 ± 0.90 µ.

The average longer and shorter alveolar glandular diameter was 75.18±5.84 µ and 43.87±3.27 µ, respectively. Eccentricity was 0.76±0.08 indicating more elongated shape of alveolar glands. The height of glandular epithelium was 16.25±1.40 µ.

Lamina propria merged with submucosa and consisted of loose connective tissue having collagen (Fig. 2a), elastic and reticular fibers (Fig. 2b) with abundant lymphocytes and few plasma cells (Fig. 1b). The thickness of lamina propria-submucosa ranged from 208.16 µ to 280.36 µ with mean of 240.82 ± 10.06 µ (Table 1).

Tracheal rings were made of hyaline cartilage and consisted of two parts separated by loose connective tissue (Fig. 1a). Inner cartilage ring was much thicker (209.08 ± 4.50 µ) than the outer ring (88.52 ± 2.20 µ). Cartilages did not show any sign of ossification. The perichondrium of the cartilage was clearly defined and had thick connective tissue on the outside with flattened nuclei. The ground substance had chondrocyte located within lacunae and either singly or in isogenous groups, which had tiny chondrocyte clusters. The cartilage cells were elongated with eccentric nucleus. The ground substance was devoid of any type of fibers and had typical frost-glass appearance in H&E stained sections (Fig. 3a). Tunica adventitia contained loose connective tissue containing blood vessels and nerves. Striated muscles were scattered around the trachea along with blood vessels and nerve plexus (Fig. 3b).

Histochemistry

In different layers of the trachea, the histochemical distribution of neutral and acid mucopolysaccharides, basic proteins, glycogen, and lipids was recorded. The histochemical distribution of basic proteins, glycogen, neutral and acid mucopolysaccharides, and sudanophilic lipids in different components of the trachea has been summarized in Table 2. A PAS positive reaction indicated the existence of neutral mucopolysaccharides and an Alcian Blue stain at pH 2.5 revealed acid mucopolysaccharide reactivity. Bromophenol Blue technique was used to demonstrate basic proteins. Positive results for the Sudan Black B method indicated the presence of lipids, whereas positive results for the Best Carmine method suggested the presence of glycogen.

Neutral and acid mucopolysaccharide: Intra-epithelial mucous glands exhibited a strong reaction for PAS-AB (Fig. 4a). The columnar cells of lining epithelium were moderately positive for Alcian Blue (Fig. 4a). Tracheal cartilage shown strong sensitivity to PAS. Alcian Blue was seen in the perichondrium that encircled the cartilage (Fig. 4b) indicating the presence of AMPS.

Basic proteins: Tracheal epithelium showed a strong reaction for basic proteins (Fig. 4c). Perichondrium covering the cartilage ring showed a moderate to strong reaction for basic proteins whereas the striated muscle fibers associated with trachea displayed a moderate reaction (Fig. 4d). Basic proteins were absent from the ground substance of cartilage.

Glycogen: Mucous glands showed mild to moderate reaction (Fig. 5a). In the extra-cellular matrix surrounding the lacunae containing chondrocytes, glycogen showed strong reaction (Fig. 5b). All the other layers were negative for Best Carmine indicating the absence of glycogen.

Lipids: Epithelium and mucous glands showed moderate reaction to Sudan Black B (Fig. 5c). Lamina propria was weakly positive.

Discussion and Conclusion

Histo-morphometry

Histologically, trachea was composed of tunica mucosa, submucosa, cartilage rings and adventitia which was comparable to the trachea of Japanese quail (Rajathi *et al* 2009), guinea fowl and coot bird (Waad 2015), duck (Al-Ahmed and Sadoon 2020) and patridge (Rajathi *et al* 2021). Mucosa was composed of pseudo-stratified ciliated columnar epithelium with oval nuclei similar to the findings of Ghosh (2004) in poultry bird, Al-Badri and Al-Salman (2015) in hen and Waad (2015) in guinea fowl and coot bird. However, the tracheal epithelium was found to have non-ciliated cells in Japanese quail as reported by Pourlis *et al* (2018). According to Nakamura *et al* (2007), ciliary activity of mucous layer aids in cleaning the respiratory tract by expelling unwanted objects. The mean height of cilia in trachea was comparatively lower than in turkey (4.5 ± 0.9 µ) (Al-Mussawy *et al* 2012). The epithelial layer contained two different cell types. The columnar cells had oval to elongated nuclei while the basal cells had smaller, rounder nuclei as also observed by Rajathi *et al* (2021) in patridge and Yadav *et al* (2022) in Uttara fowl. Goblet cells were also observed that created little depressions resembling mucous crypts in domestic birds as recorded by Liebich (2019).

The mucous glands were simple alveolar type and located intra-epithelial as also reported in domestic fowl (Bello *et al* 2018) and Uttara fowl (Yadav *et al* 2022). The apical region of glands had foamy cytoplasm and was lightly stained with H&E as earlier reported in partridge (Rajathi *et al* 2021). The mean epithelium height was comparable to the average height of epithelium in Uttara fowl of 28 days of age (39.90 ± 1.5 µ) (Yadav et al., 2022). Average epithelium height ranged between 9.18 to 12.20 µ in pigeon (Rajathi *et al* 2018), 225.06 ± 1.50 in geese, 185.20 ± 110 in cattle egret and 216.60 ± 1.30 µ in sparrows (Sakr *et al* 2022).

Lamina propria merged with submucosa and consisted of loose connective tissue as reported earlier in turkey (Khaksar *et al* 2012), coot bird (Waad 2015) and European starlings (Gazi 2017). Cevik-Demirkan *et al* (2007) in Japanese quail and Yadav *et al* (2022) in Uttara fowl observed a very thin layer of lymphocyte infiltration in lamina propria. Similar to the Uttara chicken (Yadav *et al* 2022), the lamina propria submucosa was non-glandular. However, the lamina propria submucosa of adult Iraqi pigeons included many seromucous glands (Al-Taai 2021).

In the present study, the thickness of lamina propria-submucosa was higher than in Uttara fowl of 112 days old where the width of lamina propria submucosa was 125.74 ± 9.4µ (Yadav *et al* 2022). However, in pigeon thickness varied between 220 to 316 µm (Rajathi *et al* 2018) while its was 323 to 405 µm in partridge (Rajathi *et al* 2021).

Tracheal rings were made of hyaline cartilage as reported by Dellmann and Eurell (1998) in chicken and consisted of two parts. The inner cartilage ring was much thicker than the outer ring. Similar findings were made by Al-Ahmed and Sadoon (2020) in duck. Cartilages did not show any sign of ossification similar to the trachea of guinea fowl (Waad 2015). Tracheal cartilage of turkey was partially ossified (Al-Mussawy *et al* 2012). However, trachea of coot bird showed ossification (Waad 2015). In present study, the cartilage cells were elongated with eccentric nucleus similar to the observation made by Ghosh (2004) in poultry birds. The ground substance was devoid of any type of fibers and had typical frost-glass appearance in H&E stained sections as also observed by Ghosh (2004) in poultry bird.

Tunica adventitia contained loose connective tissue containing blood vessels and nerves similar to the findings of Waad (2015) in guinea fowl and coot bird and Rajathi *et al* (2021) in patridge. Striated muscles were scattered around the trachea along with blood vessels and nerve plexus. Banks (1993) reported that in chicken longitudinally oriented striated muscles were located at the periphery of the trachea in a lateral position. Rajathi *et al* (2009) in Japanese quail reported the presence of striated muscle fibers associated with trachea as sterno-trachealis muscle. Cevik-Demirkan *et al* (2007) in Japanese quail reported that tunica muscularis presented transverse muscle layers containing blood vessels and myentric plexus.

Histochemistry

In different layers of the trachea, the histochemical distribution of neutral and acid mucopolysaccharides, basic proteins, glycogen, and lipids was recorded. Intra-epithelial mucous glands exhibited a strong reaction for PAS-AB similar to the findings of Al-Mussawy *et al* (2012) in turkey and Yadav *et al* (2022) in Uttara fowl suggesting secretion of muco-polysaccharide substances which may act as a protective barrier for the epithelium. According to Dar *et al* (2018), the kuttanad duck's mucous gland and goblet cells across its respiratory system had positive reactions to PAS and Alcian Blue. The columnar cells of the lining epithelium were moderately positive for Alcian Blue. Tracheal cartilage showed strong sensitivity to PAS which was similar to the results of Yadav *et al* (2022) in Uttara chicken. Alcian Blue was seen in the perichondrium that encircled the cartilage indicating presence of AMPS.

Tracheal epithelium showed strong reaction for basic proteins. Perichondrium covering the cartilage ring showed moderate to strong reaction for basic proteins whereas the striated muscle fibers associated with trachea displayed moderate reaction.

Mucous glands showed mild to moderate reaction. In the extra-cellular matrix surrounding the lacunae containing chondrocytes, glycogen showed a strong reaction. All the other layers were negative for Best Carmine indicating the absence of glycogen. However, Yadav *et al* (2022) observed moderate Best Carmine reaction in the tracheal cartilage of Uttara fowl.

Epithelium and mucous glands showed moderate reaction to Sudan Black B. Lamina propria was weakly positive. These findings corroborate the findings of Yadav *et al* (2022) in Uttara fowl.

Conclusion

The present study extended the knowledge regarding the histomorphometry and histochemistry of trachea of this indigenous bird of Poonch region which further adds to the literature. This study also provided photographic evidence and can act as a useful guide for those who are wishing to understand the anatomy of trachea of poultry. It can also be helpful in better understanding of the physiology of respiration in this bird.

Conflicts of interest: The authors declare that there is no conflict of interest.

Table 1: Micrometrical parameters of trachea of indigenous poultry of Poonch region

Parameter	Range (in µ)	Mean value (in µ)
Height of epithelium	33.46 – 39.66	35.96 ± 0.90
Nuclear size of columnar cells	5.06 – 6.89	5.99 ± 0.31
Height of cilia	3.36 – 4.04	3.68 ± 0.11
Thickness of Lamina propria submucosa	208.16 – 280.36	240.82 ± 10.06
Thickness of inner cartilage ring	190.77 – 220.81	209.08 ± 4.50
Thickness of outer cartilage ring	83.16 – 97.70	88.52 ± 2.20
Length of mucous glands	62.64 – 96.23	75.18 ± 5.84
Width of mucous glands	37.88 – 57.42	43.87 ± 3.27
Eccentricity	0.395 – 0.919	0.76 ± 0.08
Size of mucous cell	11.48 – 19.90	16.25 ± 1.40

Table 2: Histochemical observations of trachea of indigenous poultry of Poonch region

Parameters	Stain	Epithelium			LP-S	Perichondrium	Cartilage
		Cilia	Columnar cells	Goblet cells			
Neutral mucopolysaccharides	PAS	-	-	+++	-	-	+++
Acid mucopolysaccharides	Alcian Blue (pH 2.5)	++	++	-	++	++	-
Glycogen	Best carmine	-	-	-	-	-	++/+++
Basic proteins	Bromophenol Blue	-	-	-	-	+++	-
Lipids	Sudan Black B	++	++	++	+	-	-

Strong: +++, Moderate:++, Weak:+, Negative: -
LP-S: *Lamina propria submucosa*

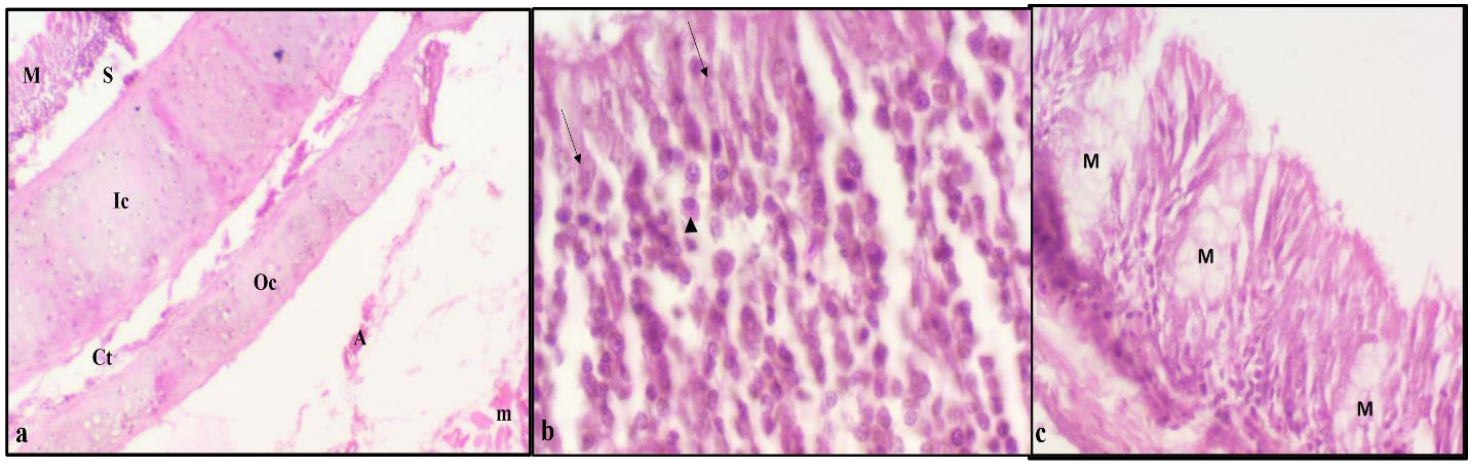

Fig. 1 (a) Photomicrograph of trachea showing tunica mucosa (M), submucosa (S), Inner cartilage ring (Ic), outer cartilage ring (Oc), adventitia (A) and muscle (m). Inner and outer cartilage rings are separated by loose connective tissue (Ct). x100 H&E stain. **(b)** Trachea showing pseudo-stratified ciliated columnar epithelium with oval nuclei (arrow). Abundant lymphocytes with few plasma cells (arrow head) were also seen. x1000 H&E stain. **(c)** Trachea showing intra-epithelial location of mucous glands (M). x400 H&E stain

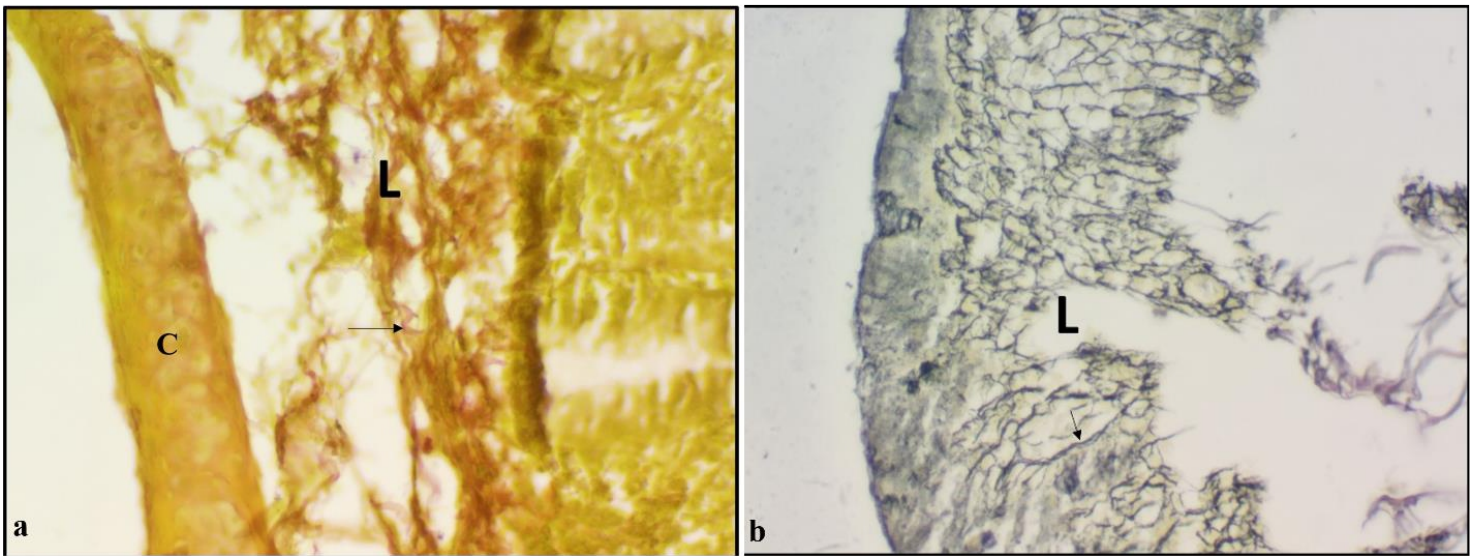

Fig. 2 (a) Photomicrograph of trachea showing collagen fibers (arrow) in lamina propria submucosa (L). Cartilage ring (C) is also visible. x400 VG & Verhoeff's stain. **(b)** Trachea showing reticular fibers (arrow) in lamina propria submucosa (L). x400 Gomori stain

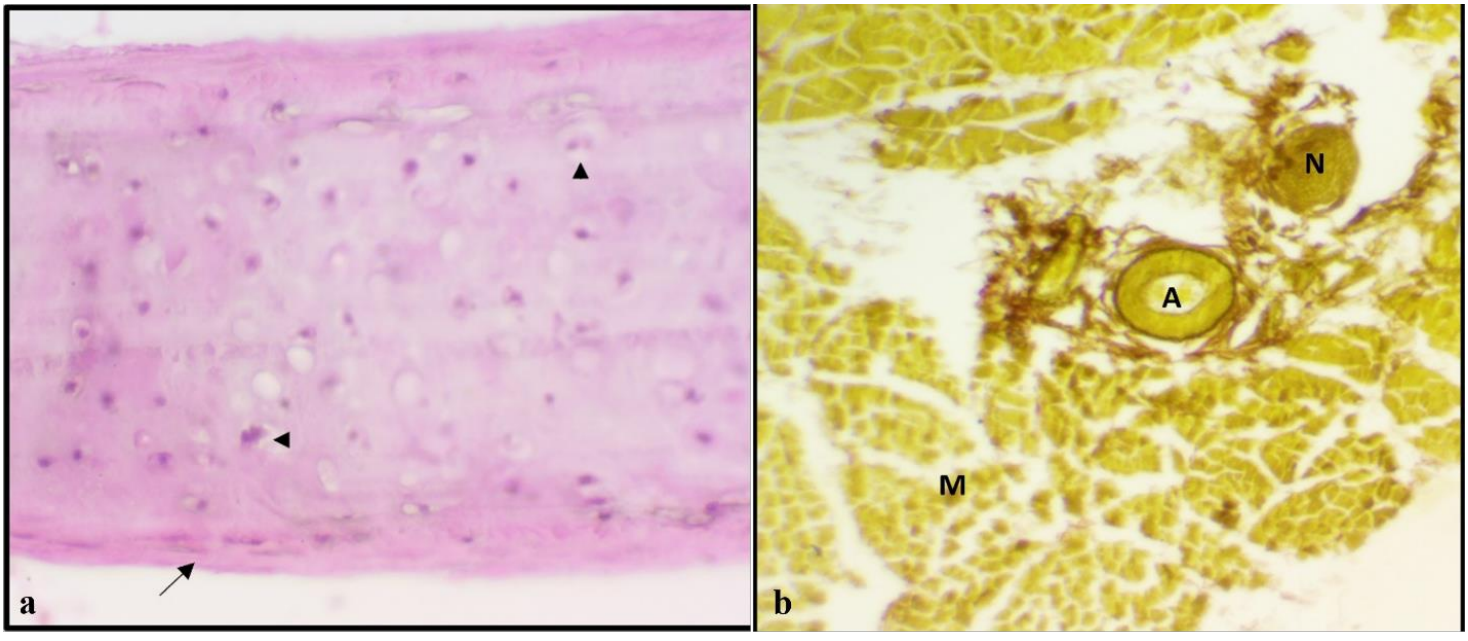

Fig. 3 **(a)** Photomicrograph of tracheal cartilage showing perichondrium (arrow) and lacunae (arrow head). x400 H&E stain. **(b)** Trachea showing artery (A) and nerve (N) in tunica adventitia. Striated muscles (M) are also scattered. x100 VG & Verhoeff's stain

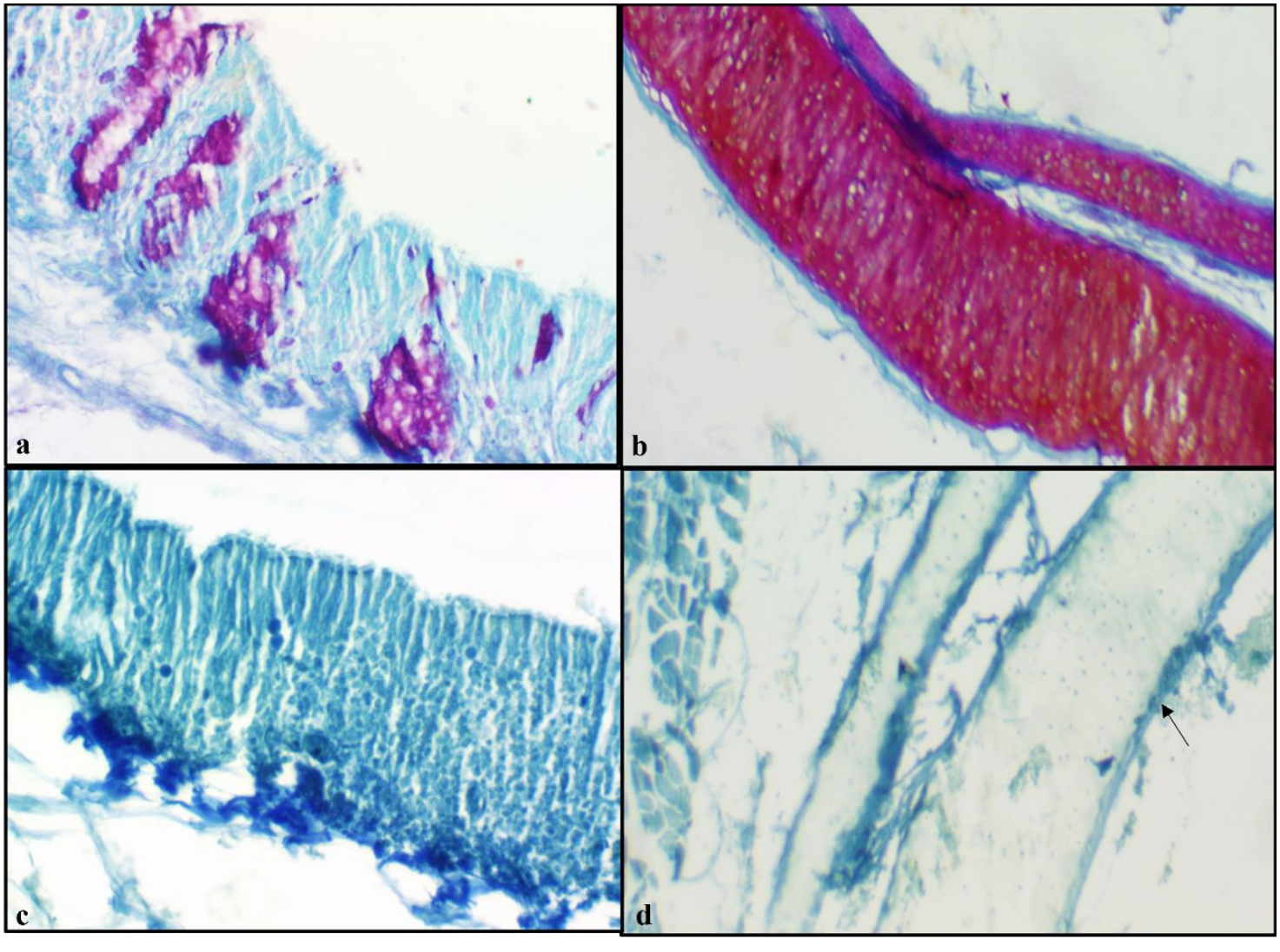

Fig. 4 **(a)** Photomicrograph of trachea showing strong PAS reaction of mucous glands. x400 PAS-AB stain. **(b)** Tracheal cartilage showing strong PAS reaction in ground substance and strong AB reaction in perichondrium. x100 PAS-AB stain. **(c)** Tracheal epithelium showing strong reaction for basic proteins. x400 Bromphenol B stain. **(d)** Perichondrium of trachea (arrow) showing moderate to strong reaction for basic proteins. x100 Bromphenol B stain

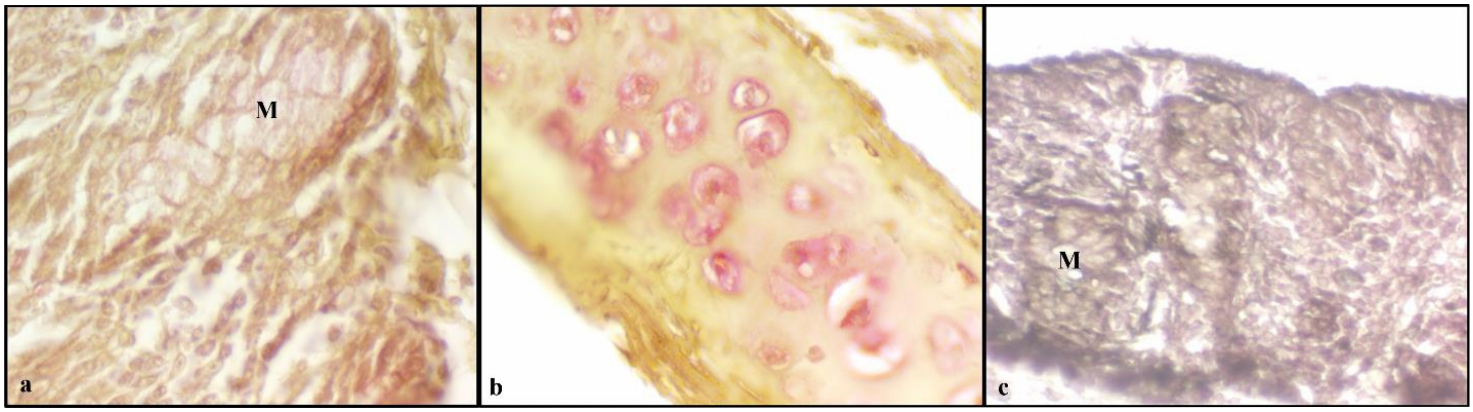

Fig. 5 (a) Photomicrograph of mucous glands (M) showing moderate reaction for glycogen. x1000 Best Carmine stain. **(b)** Photomicrograph of tracheal cartilage. Matrix surrounding the lacunae showing strong reaction for glycogen. x1000 Best Carmine stain. **(c)** Mucous glands (M) showing moderate to strong reaction for lipids. x400 Sudan Black B stain

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