

## Light Microscopic Studies on the Palatine Tonsil of the Buffalo (*Bubalus bubalis*)

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### Abstract

The present study examined palatine tonsils of 6 adult buffaloes of local mixed breed of by light microscopy to elucidate the histomorphological and histochemical characteristics. The palatine tonsil was lined by stratified squamous keratinized epithelium which modified into non-keratinized type towards the crypt where it was associated with the lymphoid tissue and also called as lymphoepithelium. The lymphoepithelium further modified into reticular epithelium because of infiltration of the lymphoid tissue present underneath in the propria submucosa. The lymphoid tissue of propria submucosa was distributed in the form of scattered lymphoid cells, isolated aggregations, diffuse arrangement and the lymphoid follicles. The mucous glandular acini demonstrated strong PAS positive reaction for glycogen, acidic mucopolysaccharides, weakly sulfated mucosubstances, hyaluronic acid and sialomucins. The acini also showed a strong reaction for performic acid-Alcian blue, indicating the presence of more than 4% cysteine content in the secretions.

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### 1. Introduction

The palatine tonsils are located at the crossing of the digestive and respiratory tracts where vast amount of foreign antigens enter the body during feeding and breathing and thus play key role in immunity (Bernstein *et al.*, 2005). They are lymphoepithelial tissue and constitute part of the integrated MALT (Ogra, 2000) which shared morphological and functional characteristics with secondary lymphoid organs such as Peyer's patches and lymph nodes (Liebler-Tenorio and Pabst, 2006) and may in addition participate as effector organs of local type as well as mucosal-type of adaptive immunity (Brandtzaeg, 2003). However unlike lymph nodes, they do not possess afferent lymphatic vessels and immune stimulation requires direct foreign antigens interaction with the mucosal surface structure without prior processing (Palmer *et al.*, 2011). Crypts formed by invaginations of the tonsillar epithelium serve as an entrance of exogenous antigens (Jovic *et al.*, 2015), whereas lymphoepithelium (LE), lymphoid follicles (LF), interfollicular regions (IF), connective tissue, lymphoid cells (B- and T-lymphocytes), dendritic cells and macrophages provide innate, cellular and humoral immunity at the local and systemic levels (Kumar and Timoney, 2005a). The

structures of the palatine tonsil have been described in horse (Kumar and Timoney, 2005b), small ruminants (Kumar *et al.*, 2006, 2008), Egyptian buffalo (Zidan and Pabst, 2011), camels (Zidan and Pabst, 2009; Achaaban *et al.*, 2016), and recently yaks (Sun *et al.*, 2018). The present study was aimed at identifying basic histomorphological features of palatine tonsils which may play major role in the induction of immune responses in the buffaloes.

### 2. Material and Methods

The heads of 6 adult buffaloes of local mixed breed were procured from slaughter house Ghazipur, New Delhi after routine decapitation. The tonsils were collected from the oropharyngeal region and fixed in 10% neutral buffered formalin solution for 48 hours. The fixed tissues were processed by routine paraffin technique and sections of 5-6  $\mu$  were cut and stained with Routine Harris' hematoxylin and eosin stain, Gomori's method for reticulum, Weigert's method for elastic fibres, (Luna, 1968), Crossman's trichrome stain for collagen fibres (Crossman, 1937), McManus' method for glycogen (PAS), Alcian blue method for muco-substances (pH 2.5), PAS-Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Meyer's mucicarmine method for mucin, colloidal iron

method for acid mucopolysaccharides (Luna, 1968), and Performic acid-Alcian blue method for proteins (Pearse, 1968).

### 3. Results and Discussion

In the present study, the outer mucosal surface of the palatine tonsil was lined by stratified squamous keratinized epithelium (Figs 1, 2, 3) as observed in the sheep (Casteleyn *et al.*, 2007; Kumar *et al.*, 2008) however, a non-keratinized type has been reported in Egyptian buffalo (Zidan and Pabst, 2011) and sheep (Casteleyn *et al.*, 2011; Raju *et al.*, 2012). The stratified squamous keratinized epithelium was comprised of strata basale, spinosum, granulosum and corneum (Fig 3) as reported in sheep (Kumar *et al.*, 2008). The stratum basale presented a single row of high cuboidal to columnar cells having round to oval nuclei with finely granular and eosinophilic cytoplasm. The stratum spinosum had varying number of rows of cuboidal cells of different size. Their nuclei were structurally similar to those of the stratum basale cells except their size which increased towards the surface of the epithelium (Fig 3).



Fig 1: Photomicrograph of PT showing non-keratinized epithelium (E) towards the crypt (C) (H. and E. x 40).

The stratum granulosum showed variation in number of compactly arranged rows of cells, possessing round to oval nuclei with eosinophilic and finely granular cytoplasm as observed in the sheep (Kumar *et al.*, 2008). The strongly basophilic flat rod shaped nuclei of more superficially placed cells of the stratum corneum were drastically reduced in size and some showed degenerative changes as reported in sheep (Kumar *et al.*, 2008) and the horse (Kumar and Timoney, 2005b). The free epithelial surface presented the keratinized layer which was desquamating at different places as described in sheep (Kumar *et al.*, 2008).



Fig 2: Photomicrograph of PT showing stratified squamous keratinized epithelium (E), propria submucosa (P), and lymphoid tissue (L) (H. and E. x 100).

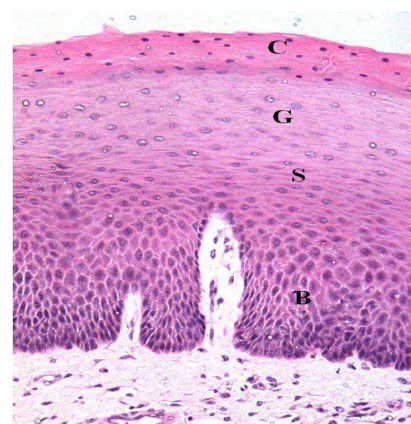


Fig 3: Photomicrograph of PT showing stratum basale (B), stratum spinosum (S), stratum granulosum (G) and stratum corneum (C) (H. and E. x 200).

A small amount of glycogen and acidic mucopolysaccharides was observed between the epithelial cells of the outer surface and those of the crypts in the horse (Kumar and Timoney, 2005b). Tonsillar epithelial cells were known to exhibit energy demanding absorptive and secretory functions and so the stored glycogen in the upper strata of the epithelium may form an energy source (Perry, 1994). The crypts were well developed (Figs 1, 4) as reported in the goat (Kumar *et al.*, 2006), more numerous and often branched in the camel (Yang *et al.*, 2011; Achaaban *et al.*, 2016), blind-ended tubular with a few lateral ramifications in humans (Perry, 1994) and were lacking in dog (Belz and Heath, 1995). In cattle, the palatine tonsillar crypts opened into a larger tonsillar sinus that communicated with the oral cavity (Palmer *et al.*, 2009) while in the buffalo (Zidan and Pabst, 2011)

and yaks (Sun *et al.*, 2018) it presented a double crypt system which greatly expanded the epithelial surface area to antigen (Casteleyn *et al.*, 2007) signifying a major characteristic immunological role in these species. In the sheep, the crypts appeared as single opening towards the free surface but divided underneath (Kumar *et al.*, 2008) which resulted in the formation of primary and secondary crypts within the tonsil substance (Raju *et al.*, 2012).

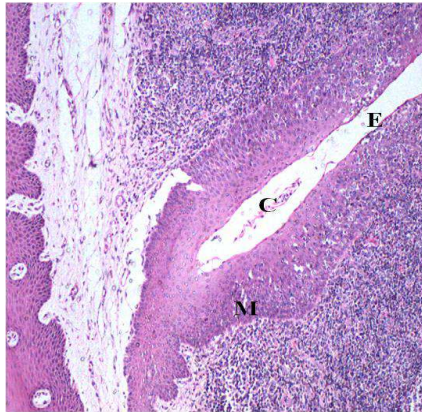


Fig 4: Photomicrograph of PT showing crypt (C), lymphoepithelium (M) and non-keratinized epithelium modified into reticular epithelium (E) (H. and E. x 100).

The dynamic nature of the tonsillar lymphoid tissue, such as changes in number and size of follicles, may account for the varied shapes and size of these crypts (Perry, 1994). The outer surface epithelium modified into non-keratinization towards the crypts. It has been stated that crypt epithelium of the palatine tonsil was not uniform but contained patches of stratified squamous non-keratinized epithelium and patches of reticulated sponge-like epithelium (Brandtzaeg, 2015). In this study, the non-keratinized epithelium towards the crypts was associated with lymphoid tissue which formed the lymphoepithelium (Figs 1, 4) as described in the horse (Kumar and Timoney, 2005b), sheep (Kumar *et al.*, 2008), and the Egyptian buffalo (Zidan and Pabst, 2011). Recently, Sun *et al.* (2018) in yaks identified the distribution of both IgA and IgG antibody secreting cells in the subepithelial areas of the non-reticular crypt epithelium, and the reticular crypt epithelium which increased in density and expression with age, signifying the immunologic effector functions of the palatine tonsil in these species. The lymphoepithelium provided the framework in which lymphoid and other mononuclear cells transformed the squamous epithelium into reticular network of intercellular passage way (Perry, 1994). It formed a barrier that sampled and translocated antigen to the underlined

lymphoid tissue (Perry and Whyte, 1998) having clinical significance in pathogenesis of several ruminant diseases (Palmer *et al.*, 2009; Bellworthy *et al.*, 2005; Thuring *et al.*, 2005). It was shown that crypt epithelium was highly modified form of stratified squamous epithelium that covered the oropharyngeal surface of the tonsil (Perry, 1994). Furthermore, the estimated crypt epithelial surface area was measured as 23.5 cm<sup>2</sup> (Casteleyn *et al.*, 2007) in the sheep, whereas in human was 295 cm<sup>2</sup> (Perry and Whyte, 1998). Altogether, the crypt epithelium represented a specialized compartment of potential importance for several immunological functions (Brandtzaeg, 2015). In the present histological study, the crypt surface and the basale surface were slightly irregular and the latter lacked the interpapillary pegs. This epithelium was having the stratum basale, stratum spinosum and stratum superficiale as reported in the sheep (Kumar *et al.*, 2008). The stratum basale had single row of cells having nuclear details almost similar to those of the keratinized surface epithelium. The stratum spinosum was having the varying number of rows with round to oval nuclei with slightly eosinophilic and finely granular cytoplasm. Their characteristic spiny appearance observed in the keratinized epithelium was absent. The stratum superficiale layer also had varying number of cell layers. The nuclei of the deeper cells were having features similar to those of stratum spinosum. The lymphoepithelium at some places was further modified into reticular epithelium because of infiltration of the lymphoid tissue present underneath the propria submucosa (Fig 4). The modification was comparable to follicle associated epithelium reported in the nasopharyngeal tonsil (Kumar and Nagpal, 2007). Reticulated epithelium played a significant role in the initiation of immune responses in the palatine tonsils (Belz and Heath, 1995) by providing a favorable environment for intimate contact between various cells of the immune system (Brandtzaeg, 2015). In the present study, the reticular epithelium presented only few cell layers as observed in the horse (Kumar and Timoney, 2005b), goat (Kumar *et al.*, 2006), and the sheep (Kumar *et al.*, 2008). At some places, it was difficult to differentiate between the epithelial and the lymphoid cells with the lymphoid tissue reaching the surface of the epithelium, leading to reduction of the number of epithelial cell layers as reported by Zidan and Pabst (2011) in Egyptian buffalo. A characteristic of reticulated epithelium was the coexistence of epithelial and non-epithelial cells and at places, the predominance of the latter with disruptions of the basement membrane (Kumar and Timoney, 2005a, b). This could be attributed to antigen stimulation where the less strongly stimulated area may have retained the non-reticular epithelial characteristics (Zidan and



Pabst, 2011). In a related study, reticular epithelium not associated with lymphoid tissue has been documented in the sheep (Kumar *et al.*, 2008). Similarly, lamellated structures of unknown function associated with the surface epithelium were observed in the crypt epithelium of lingual as well as palatine tonsils in the horse (Kumar and Timoney, 2005a, b) and the goat (Kumar *et al.*, 2006) however these structures have not been observed in the palatine tonsil of the present study. The propria submucosa was having loose irregular connective tissue, with varying concentration of collagen, reticular and elastic fibres, small blood capillaries, a few glandular acini and their ducts (Figs 5, 6, 7) as reported in the sheep (Kumar *et al.*, 2008). However in the Egyptian buffalo, the propria was comprised of dense irregular connective tissue (Zidan and Pabst, 2011).

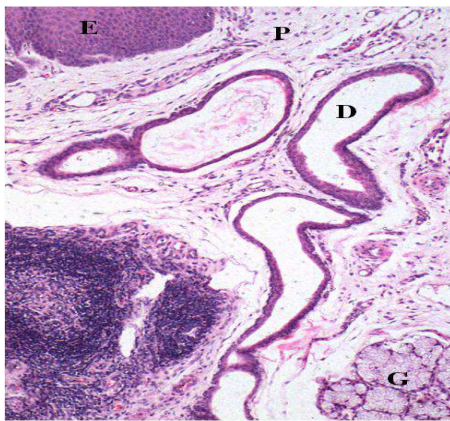


Fig 5: Photomicrograph of PT showing surface epithelium (E), propria submucosa (P), inter-glandular ducts (D), blood vessel, glandular acini (G), and lymphoid follicle (H. and E. x 100).

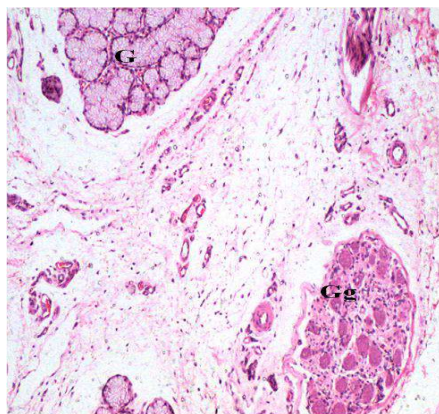


Fig 6: Photomicrograph of PT showing glandular acini (G), connective tissue and ganglion-like structures (Gg) in the deeper part of propria submucosa. (H. and E. x 100).

In the deeper part, the propria submucosa had clusters of mucous glands separated from each other by loose irregular connective tissue along with fine blood capillaries, few serous demilunes-like structures, nerve bundles and ganglion like structures (Fig 6) as observed in the goat (Kumar *et al.*, 2006). The reticular fibres formed the basement membrane which was interrupted in the region of the reticular epithelium. The concentration of reticular fibres increased in the region of lymphoid tissue, especially towards the periphery of the lymphoid follicles and inter-glandular regions. The collagen fibres were densely arranged in the subepithelial portion of the propria submucosa, along with fine meshwork of reticular fibres as reported in the horse (Kumar and Timoney, 2005b).

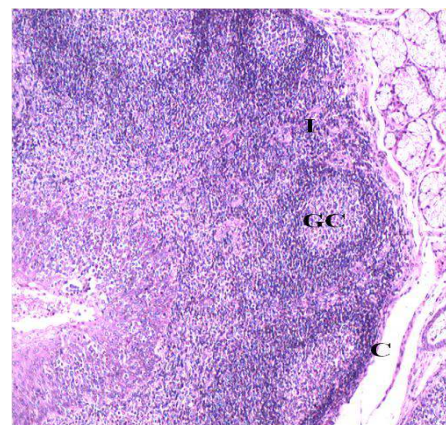


Fig 7: Photomicrograph of PT showing darkly stained corona (C), germinal centre (GC) and interfollicular area (I). (H. and E. x 100).

The clusters of the mucous type glandular acini were surrounded by fine collagen and reticular fibres. The lymphoid tissue was lacking in the subepithelial region. The intra-glandular ducts were lined by simple cuboidal type of epithelium, whereas the inter-glandular ducts were lined by varying types of epithelia ranging from simple to stratified cuboidal type and coursed toward the surface epithelium. According to Zidan and Pabst (2011) a mucous glandular discharge mixed with cellular contents of the crypt passed into the lumen, forming the white discharge was observed in palatine tonsils of the buffalo. The lymphoid tissue was organized in the form of scattered lymphoid cells, isolated aggregations, diffuse arrangement and the lymphoid follicles. However, in the horse (Kumar and Timoney, 2005b) there was no isolated aggregation of lymphoid tissue. The lymphoid follicles were arranged generally along the length of the epithelium towards the crypt and had well developed germinal centres, parafollicular area and darkly stained corona (Figs 1, 7) as reported in other domestic species (Kumar and

Timoney, 2005b; Zidan and Pabst, 2011; Sun *et al.*, 2018). In a related study, Breugelmans *et al.* (2011) calculated the cellular density of interfollicular region which was significantly lower 37.8 cells/2500  $\mu\text{m}^2$  than 44.4 cells/2500  $\mu\text{m}^2$  of the follicular region of the ovine palatine tonsil. The cellular profiles of the germinal centre were identified as lymphocytes, plasma cells, follicular dendritic cells (FDC) and few macrophages (Kumar and Timoney, 2005a, b). The FDC acted as antigen-presenting cells that offered an appropriate environment for the proliferation and differentiation of germinal centre B-cells (Brandtzaeg, 1996), and their plasma membrane can retained high amounts of immune complexes for extended period of time (Noble *et al.*, 1996; Dieu *et al.*, 1998). The parafollicular and interfollicular regions were richly populated with lymphocytes, plasma cells, macrophages, interdigitating cells, blood capillaries, venules and high endothelial venules (HEV's) (Fig 8) along with a meshwork of fine reticular fibers as reported in small ruminants (Kumar *et al.*, 2006; 2008), horse (Kumar and Timoney, 2005b), Egyptian buffalo (Zidan and Pabst, 2011) as well as yak (Sun *et al.*, 2018). The greater number of HEV's observed in the horse was related with possible heavy concentration of lymphoid cells (Kumar and Timoney, 2005b).

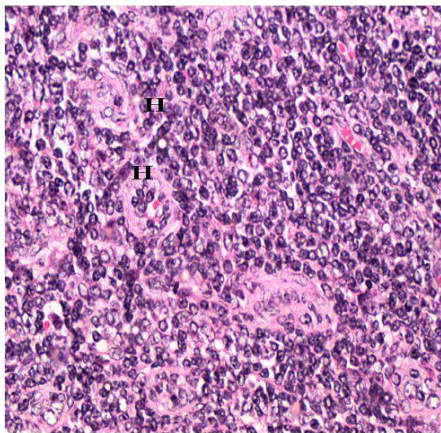


Fig 8: Photomicrograph of PT showing the presence of high endothelial venules (H) in the interfollicular area (H. and E. x 400).

The high endothelial venules as mentioned earlier were specialized vessels that supported active lymphocyte transmigration from peripheral blood to secondary lymphoid organs (Kumar and Timoney, 2005a) depending on molecules on the lymphocytes and corresponding receptors on the endothelial cells (Zidan and Pabst, 2009) regulated by similar molecular principles in the systemic and the mucosal immune system (Brandtzaeg, 2015). The entire process of lymphocyte migration was consisted of rolling,

adhesion, arrest or activation and transmigration (Indrasingh *et al.*, 2002). The keratinized layer was weakly positive for glycogen demonstrated by McManus' PAS, and the tunica media of the blood vessel was mildly positive. Small amounts of glycogen and acidic mucopolysaccharides were present between the epithelial cells of the outer surface and those of the crypt in the horse (Kumar and Timoney, 2005c). The glandular acini showed strongly positive reaction for glycogen (Fig 9), weakly sulfated mucopolysaccharides, hyaluronic acid and sialomucins as demonstrated by Alcian blue (Fig 10), but were moderate in the goat (Kumar *et al.*, 2006) and weak in the horse (Kumar and Timoney, 2005b).



Fig 9: Photomicrograph of PT showing strong PAS positive activity of the mucous glandular acini for glycogen whereas negative in inter-glandular ducts (McManus' PAS x 100).

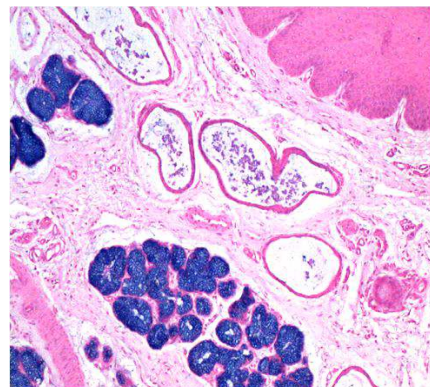


Fig 10: Photomicrograph of PT showing positive activity of the mucous glandular acini for weakly sulfated mucosubstances, sialomucins and hyaluronic acid whereas negative in inter-glandular ducts (Alcian blue x 100).

The glandular acini were also strongly positive for acidic mucopolysaccharides but negligible for neutral mucopolysaccharides (Fig 11) as observed in the goat (Kumar *et al.*, 2006). The intra and inter-



glandular ducts, did not exhibit any of the PAS reactions. Similarly, the glandular acini were strongly positive for acidic mucopolysaccharides as established by colloidal iron method (Fig 12) and mucin as shown by Meyer's mucicarmine method (Fig 13). The glandular acini showed a very strong reaction for performic acid-Alcian blue indicating the presence of more than 4% cysteine content in the secretions (Fig 14).

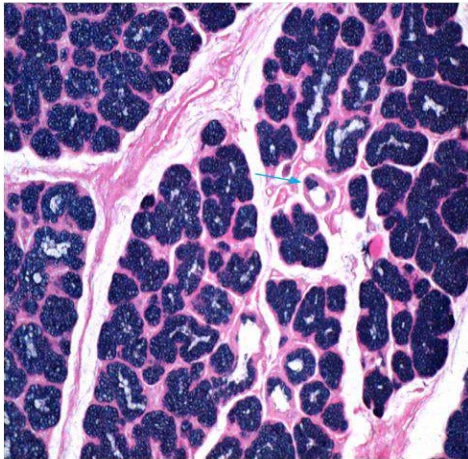


Fig 11: Photomicrograph of PT showing strong positive activity of the mucous glandular acini for acidic mucopolysaccharides. Note some PAS positive cells of intra-glandular ducts (arrow) (PAS-AB x 100).

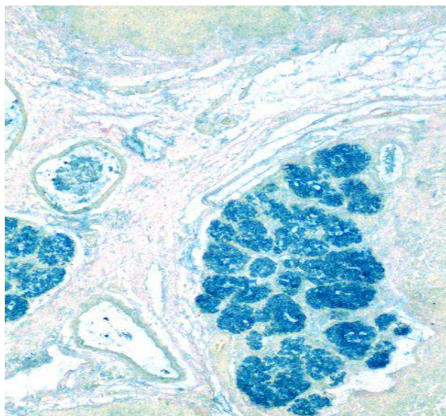


Fig 12: Photomicrograph of PT showing positive reaction of the mucous glandular acini for acidic mucopolysaccharides (Colloidal iron x 100).

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Fig 13: Photomicrograph of PT showing distribution of mucin in the glandular acini whereas absent in inter-glandular ducts (Meyers's mucicarmine x 100).

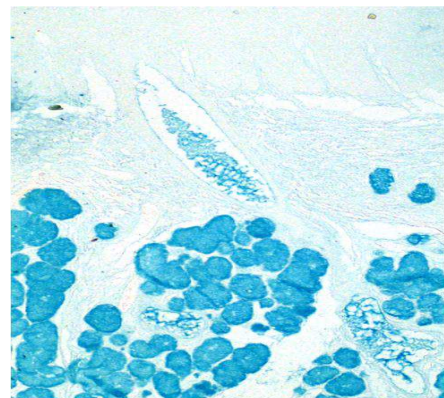


Fig 14: Photomicrograph of PT showing glandular acini with positive activity for more than 4% cysteine (Performic acid Alcian blue x 100).

## 4. Conclusion

The reticulated epithelium could provide a favorable environment for the intimate contact between the effector cells of immune responses and facilitate direct transport of antigens whereas, the crypts were well developed which could be provided for better trapping of antigen being ingested during feeding.

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