Buffalo (Bubalus bubalis) Histological and Histochemical Studies on the Lingual Tonsil of the \*Department of Veterinary Anatomy, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004, India

INTRODUCTION

The tonsils consist of accumulation of lymphocytes and lymphoid tissue that are surrounded by extracellular matrix. They constitute part of the integrated pharyngeal mucosal immune system which initiates an immune response to specific antigens. The mucosal immune system is important for the first line of defence against ingested or inhaled pathogens entering through the mouth cavity. The lingual tonsil at the root of the tongue is a component of Waldeyer's ring and an important site for invasion of microbial pathogens and immune surveillance. This location involves an important role for tonsils in the development of new strategies for oral vaccines.

RESULTS

The lingual tonsil was lined by stratified squamous non-keratinized epithelium especially toward the deeper portion and was characterized by a reduced number of epithelial nucleoli. These HEVs are involved in trafficking of lymphocytes by transendothelial and interendothelial migration. The follicles of varying shapes and size showed darkly stained corona and lightly stained germinal centre were surrounded by pilar epithelium along with glanular acini presented strong reactions for glycogen, acidic mucopolysaccharides, weakly sulfated mucosubstances (pH 2.5), PAS (PAS), Alcian blue method for acidic and neutral mucosubstances (pH 2.5), Meyer's mucicarmine, modified reticular fibres, diastase digestion method, Gomori's method for reticulum, Weigert's method for collagen fibres, Alcian blue method for proteins, elastic fibres, and performic acid for collagen. The presence of modified reticular fibres involved in sampling of oral secretions of acini also showed the presence of more than 4% content of cysteine. The presence of modified reticular fibres, mucosubstances, hyaluronic acid, sialomucins and mucins as demonstrated by different histochemical techniques. The study was conducted on lingual tonsil of six adult buffaloes (5-6 years of age) of the local mixed breed to the present study was envisaged to explore modifications and distribution of lymphoid tissue with emphasis on mucosal epithelium along with its secretions.

Abstract:

The tonsils consist of accumulation of lymphocytes as secondary lymph nodules that are surrounded by extracellular matrix which is usually concentrated in the nodular zones and covered by reticular epithelium. The parafollicular areas possessed high endothelial venule (HEV) with large sized endothelial cells having round to oval nuclei with distinctly visible centric or eccentric nucleoli. These HEVs are involved in trafficking of lymphocytes as secondary lymphoid tissue. They constitute part of the integrated pharyngeal mucosal immune system which initiates an immune response to specific antigens. The mucosal immune system is important for the first line of defence against ingested or inhaled pathogens entering through the mouth cavity. The lingual tonsil at the root of the tongue is a component of Waldeyer's ring and an important site for invasion of microbial pathogens and immune surveillance. This location involves an important role for tonsils in the development of new strategies for oral vaccines.

Keywords: Lingual tonsil, Reticular epithelium, High endothelial venule, Mucopolysaccharides, Buffalo.
Surface of the epithelium was uneven, irregular and uniform, whereas the deeper surface presented the papillary pegs (Figures 1, 2). The epithelium was comprised of the different strata having a varying number of rows. The stratum basale, present towards the basement membrane, was having oval to elongated strongly basophilic nuclei which were vertically oriented (Figure 2). These strongly basophilic nuclei contained chromatin material which was aggregated into smaller clumps irregularly throughout the nucleoplasm. These nuclei towards the papillary pegs became narrow cylindrical like structure and were comparatively more basophilic, because of uniform distribution of the chromatin material that masked the presence of the nucleoli. The cytoplasm of these cells was eosinophilic and finely granular (Figure 2).

Figure 1: Photomicrograph of LT showing stratified squamous keratinised epithelium, lamellated structure (L), lymphoid follicle (F) and diffused lymphoid tissue (D). H & E x 100; (bar 200 µm).

The stratum spinosum was having a varying number of cell layers (16-20) depending on the thickness of the epithelium. The nuclei of these cells, towards the deeper part close to the stratum basale, were round to oval to elongate in shape (Figure 2). The basophilia of these nuclei was similar to that of stratum basale cells. These nuclei contained the chromatin material which was aggregated into smaller clumps especially toward the outer nuclear membrane. These nuclei were oriented vertically, but towards the superficial layers, these were larger and oriented obliquely or horizontally (Figure 2). These less basophilic nuclei had a fine dusting of chromatin and contained 1-2 centric/eccentric nucleoli. The cytoplasm of these cells was eosinophilic and finely granular and the eosinophilia accentuated as progressed towards the surface of the epithelium. These cells presented spicules or spike-like arrangement because of the tapering ends of the adjacent cells and shrinkage of the cytoplasm (Figure 2).

The stratum granulosum also showed a varying number of rows of nuclei of smaller dimensions. The nuclei were comparatively less basophilic and were oriented horizontally (Figure 2). The cytoplasm of these cells was finely granular and strongly eosinophilic. The cell layers towards the free surface presented a few large-sized nuclei with a vacuolated appearance due to less chromatin material. These cells with fine granular and less eosinophilic cytoplasm presented a ground-glass appearance similar to that of stratum lucidum (Figure 2). The stratum corneum was having small-sized, deeply basophilic round to oval to narrow elongated nuclei, some of which presented pyknotic appearance (Figure 2). These strongly basophilic nuclei masked the appearance of nucleoli. The cytoplasm of these cells was also finely granular and comparatively more eosinophilic than that of the deeper cells. The keratinized layer present towards the free surface varied in thickness at different places and it was peeling off at some places. A few concentric whorl-like arrangements were also observed towards the superficial portion of the epithelium (Figure 2). The deeper surface presented the papillary pegs which...
Lymphocytes were infiltrated in between the cells of the stratum basale especially in the region of underlying lymphoid tissue. The stratified squamous non-keratinized epithelium abruptly changed into the reticular epithelium (Figures 3, 4) towards the deeper part of the folds/crypts because of heavy infiltration in the regions of underlying lymphoid tissue. The different strata of the reticular epithelium were not distinctively visible depending upon infiltration of the lymphoid tissue (Figure 4). At these places, only a few surface epithelial cell layers were present. In the rest of the portion of these patches of the modified epithelium, the lymphoid cells were predominant. At some places, the lymphoid infiltration was so much extensive that it reached to the surface of the epithium and even the lymphoid cells were also observed towards the groove or the crypt. At some places, the epithelial layer of stratified squamous epithelium presented a spongy appearance, because of large infiltration of lymphoid tissue especially in the region of lymphoid follicles just adjacent to the epithelium. At some places, it was difficult to discern the epithelium and the lymphoid cells.

Figure 3: Photomicrograph of LT showing the stratified squamous non-keratinised and reticular epithelium (R) toward crypt and lymphoid follicles (F). H & E x 40; (bar 450 μm).

The propria-submucosa was having loose irregular connective tissue comprising of collagen and reticular fibres. The reticular fibres formed the basement membrane which was interrupted in the region of the reticular epithelium. The concentration of the reticular fibres increased towards the periphery of the lymphoid follicles and the interfollicular areas. The collagen fibres were observed intermingled with a fine meshwork of reticular fibers and fine blood capillaries in the subepithelial portion, although the number of collagen fibres was drastically reduced.

The lymphoid aggregations were present in the form of lymphoid follicles or the diffused form (Figures 1, 3-5). The lymphoid follicles of varying shapes and size generally showed darkly stained corona and lightly stained central portion (Figures 3, 5). The lymphoid follicles were surrounded by parafollicular areas, which were separated from those of the adjacent ones by interfollicular areas. The parafollicular areas showed the presence of the high endothelial venules (Figure 6). These venules presented the endothelial cells, having round to oval nuclei with distinctly visible centric or eccentric nucleoli. The cytoplasm of these endothelial cells was finely granular and eosinophilic. In most of the cases, the lymphocytes were observed in these venules. The diffuse arrangement of the lymphoid tissue was also present in the subepithelial portion of the propria-submucosa which was separated from the deeper part by the dense arrangement of the collagen bundles, reticular fibres and glandular tissue. The mucous glands and striated muscles were observed towards the deeper part. The mucous glands were also observed in the superficial part where the lymphoid tissue was absent. These mucous acini presented vacuolated appearance because of washing of the mucous during the processing of the tissues.

Figure 4: Photomicrograph of LT showing reticular epithelium at higher magnification. H & E x 100; (bar 200 μm).
Histological and Histochemical Studies on the Lingual Tonsil of the Buffalo

Figure 5: Photomicrograph of LT showing lymphoid follicle and high endothelial venules (H) in the parafollicular area. H & E x 200; (bar 100 µm).

Figure 6: Photomicrograph of LT showing the presence of high endothelial venules (H) in the parafollicular area. H & E x 400; (bar 50 µm).

The glandular ducts with varying types of epithelia coursed towards the surface of the epithelium. In the deeper part, the fatty tissue was also observed in between the fasciculi of the muscles and in between the clusters of the glandular tissue. Very fine elastic fibres were present in the subepithelial portion of propria and in between clusters of the glandular acini. These were well demonstrated in the tunica intima of the blood vessels.

The surface and reticular epithelial cells were devoid of any PAS activity, whereas the basement membrane showed slight PAS-positive reaction. The glandular acini showed a strong positive reaction for acidic mucopolysaccharides and glycogen as demonstrated by PAS-AB and McManus' PAS methods, respectively (Figures 7-8). The neutral mucopolysaccharides were negligible. The glandular

Figure 7: Photomicrograph of LT showing PAS positive activity of the mucous glandular acini for acidic mucopolysaccharides (blue colour). PAS-AB x 100; (bar 200 µm).

Figure 8: Photomicrograph of LT showing PAS positive activity of mucous glandular acini for glycogen (magenta color). McManus' PAS x 100; (bar 200 µm).
Girgiri and Kumar

Acini also showed strong positive reaction for Alcian blue indicating the presence of weakly sulfated mucosubstances, sialomucins and hyaluronic acid (Figure 9). The glandular acini were also positive for the presence of mucin as well as acidic mucopolysaccharides, as demonstrated by Meyer's mucicarmine and colloidal iron method, respectively (Figures 10-11). The intra and interglandular ducts except at few places did not exhibit any PAS-positive reaction by any of the technique employed during the present study. Fine blood capillaries present in the lymphoid tissue showed a mild positive reaction. The glandular acini exhibited a strong reaction for performic acid-Alcian blue indicating the presence of more than 4% cysteine. The deepest part of the glandular tissue exhibited comparatively lower concentration of the cysteine as compared to that of the superficial part (Figure 12).

Figure 9: Photomicrograph of LT showing positive activity of the mucous glandular acini for weakly sulfated mucosubstances, sialomucins and hyaluronic acid (violet colour). Alcian blue x 100; (bar 200 µm).

Figure 10: Photomicrograph of LT showing distribution of mucin (rose colour) in the glandular acini. Meyer's mucicarmine x 100; (bar 200 µm).

Figure 11: Photomicrograph of LT showing positive activity of the mucous glandular acini for acidic mucopolysaccharides (Persian blue colour). Colloidal iron method x 100; (bar 200 µm).

Figure 12: Photomicrograph of LT showing positive activity of glandular acini for more than 4% cysteine (blue colour). Performic acid Alcian blue x 100; (bar 200 µm).
The strata had a varying number of cell layers. The mucosal surface of the lingual tonsil was lined by a stratified squamous epithelium and the outer stratified portion in sheep and goat was keratinized as reported in the buffalo [14], and the goat [15] in contrast to those of horse [4], goat [17], and the non-sinusoid mucosa of the bovine lingual tonsil [9, 23] were 16, however, the reaction was comparatively less in the smaller clumps as reported in the buffalo [25].

The epithelial lining of the lingual tonsil in the camel [14], and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

Tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

In the present study, the underlined lymphoid tissue was comprised of basale, spinosum and superficial in the buffalo [14]. The epithelial lining of the lingual tonsil in the camel [14] and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

DISCUSSION

Histologically, the strata had a varying number of cell layers. The mucosal surface of the lingual tonsil was lined by a stratified squamous epithelium and the outer stratified portion in sheep and goat was keratinized as reported in the buffalo [14], and the goat [15] in contrast to those of horse [4], goat [17], and the non-sinusoid mucosa of the bovine lingual tonsil [9, 23] were 16, however, the reaction was comparatively less in the smaller clumps as reported in the buffalo [25].

The epithelial lining of the lingual tonsil in the camel [14], and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

In the present study, the underlined lymphoid tissue was comprised of basale, spinosum and superficial in the buffalo [14]. The epithelial lining of the lingual tonsil in the camel [14] and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

Histologically, the strata had a varying number of cell layers. The mucosal surface of the lingual tonsil was lined by a stratified squamous epithelium and the outer stratified portion in sheep and goat was keratinized as reported in the buffalo [14], and the goat [15] in contrast to those of horse [4], goat [17], and the non-sinusoid mucosa of the bovine lingual tonsil [9, 23] were 16, however, the reaction was comparatively less in the smaller clumps as reported in the buffalo [25].

The epithelial lining of the lingual tonsil in the camel [14], and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

In the present study, the underlined lymphoid tissue was comprised of basale, spinosum and superficial in the buffalo [14]. The epithelial lining of the lingual tonsil in the camel [14] and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

Histologically, the strata had a varying number of cell layers. The mucosal surface of the lingual tonsil was lined by a stratified squamous epithelium and the outer stratified portion in sheep and goat was keratinized as reported in the buffalo [14], and the goat [15] in contrast to those of horse [4], goat [17], and the non-sinusoid mucosa of the bovine lingual tonsil [9, 23] were 16, however, the reaction was comparatively less in the smaller clumps as reported in the buffalo [25].

The epithelial lining of the lingual tonsil in the camel [14], and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].

In the present study, the underlined lymphoid tissue was comprised of basale, spinosum and superficial in the buffalo [14]. The epithelial lining of the lingual tonsil in the camel [14] and the goat [15] in contrast to the sheep [24, 28] and camel [14] where such infiltrations have not been observed. In the horse, some places in the present study. The tonsillar crypts were narrow epithelial diverticula which greatly increased the available surface area for intercellular immunoglobulins and antigenic stimulation [26].
The increased demand of reticulated and infiltrative vessels in the deeper part of the propria submucosa are the only trafficking routes of the lymphocytes related to stratum corneum. Desquamation of the corneum were observed in the present study. Only a few surface epithelial cells related to stratum corneum were observed as described in the goat [17]. The lymphoid tissue was separated from the other mucosal sites [38]. The lymphoid follicles and diffused forms in the interfollicular areas and high endothelial venules [33, 34] was a usual developmental process. T and B cells associated with bacterial activity [35]. These patches of the modified follicles showed darkly stained corona and lightly stained central portion, surrounded by a few IgA+, B cells and macrophages [7]. These HEVs presented the endothelial cells, having round to oval nuclei with distinctly visible nucleoli. In the continuity of the underlying basement membrane which was interrupted in the deeper part of the propria submucosa by the dense irregular connective, lymphoid, glandular, muscular, adipose tissue as reported in the horse [4], sheep [9, 10], goat [17] and the pig [18]. The reticular fibres formed the basement membrane which was interrupted in the deeper part of the propria submucosa. The lymphocytes of varying size as reported in the horse [4, 7]. Some of these follicles were either oriented towards the groove or the crypts as observed in the horse [4], buffalo calf [10] or not observed in the bovine [8]. These follicles showed darkly stained corona and lightly stained central portion, surrounded by lymphocytes of varying shapes and size as reported in the horse [4, 7]. The lymphoid cells in the vicinity of blood vessel reported in the horse [4] could not be observed during the present study. The HEVs were first identified in lymph nodes and tonsils [41]. B cells must migrate through areas rich in lymphocytes, macrophages, plasma cells, dendritic lymphoid organs [7]. The interfollicular zone was rich in lymphocytes, mature plasma cells, macrophages, lymphocytes, high endothelial venules (HEV) [33, 35]. The presence of these cells a...
The authors do not have any conflict of interest.
2009; 214(4): 516-59.  
https://doi.org/10.1111/1469-7580.2009.01066.

2007; 70(3): 251-54.  
https://doi.org/10.1292/00213751

2007; 46: 75-8.  
https://doi.org/10.1292/00213751

(Camelus dromedarius). 2016; 48: 1653-59.  
https://doi.org/10.1007/11250-016-1139-

1993; 3: 165-8.  
https://doi.org/10.1159/000024354

2008; 40(7): 637-42.  
https://doi.org/10.2746/042516408_322120

2007; 120: 124-35.  
https://doi.org/10.1016/S0167-8726(07)00100-0

2015; 2: 1985-2016.  
https://doi.org/10.1016/978-8-12-415847-4.00103-8

1991; 99: 905-15.  
https://doi.org/10.1111/1699-0463.1991_01278.

1988; 45: 83-95.  
https://doi.org/10.3109/00016488809125010

1998; 19: 414-21.  
https://doi.org/10.1016/0167-5699(88)01307-3

1982; 149: 485-90.  
https://doi.org/10.1007/978-1-4684-9066-4_68

1982; 224: 579-600.  
https://doi.org/10.1007/00213754

1975; 18: 53-62.  

1984; 237: 619-27.  
https://doi.org/10.1007/00228447

( ) 999/2001

2005; 52: 102-04.  
https://doi.org/10.1111/1439-0450.2005.00826.

2000; 122: 8-19.  
https://doi.org/10.1159/000024354

2011; 40: 426-32.  
https://doi.org/10.1111/1439-0264.2011.01088-

2006; 37: 257-80.  
https://doi.org/10.1051/2006001

2002; 184: 77-84.  
https://doi.org/10.1016/0940-9602(02)80040-1

1997; 21-37.

https://doi.org/10.6000/1927-520.2019.08.03.3

2019

( )