Haryana Vet. (June, 2023) **62(1),** 68-72 ISSN 0033-4359 (P) **Research Article**

HISTOCHEMICAL AND HISTOENZYMIC STUDIES ON LINGUAL TONSIL OF GOAT

DEVENDRA SARAN, OPINDER SINGH and DEVENDRA PATHAK
Department of Veterinary Anatomy, College of Veterinary Science,
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, India

Received: 12.07.2022; Accepted: 24.08.2022

ABSTRACT

The present study was undertaken on (n = 24) adult unidentified breed of goats (*Capra hircus*) to elucidate the histochemical and histoenzymic distribution in the lingual tonsil of goat. The surface epithelium of the circumvallate papillae of the lingual tonsil was weak to moderately positive for neutral mucopolysaccharides, however, weakly positive for acidic mucopolysaccharides. The aggregated lymphocytes were moderately positive for neutral mucopolysaccharides and weakly positive for sulfated acid mucopolysaccharides, carboxylated acid mucopolysaccharides, and glycoproteins. Protein content was observed throughout the lingual tonsil. Large lipid droplets were seen in the deepest region of the propria-submucosa. In the epithelium of the lingual tonsil, the activities of Alkaline phosphatase (AKPase), Glucose-6-phosphatase (G-6-Pase), Lactate dehydrogenase (LDH), Succinic dehydrogenase (SDH) and Malic dehydrogenase (MDH) were found to be weak and the activities of Glucose-6-phosphatase dehydrogenase, Nicotinamide adenine dinucleotide diaphorase (NADH-d), Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) and nonspecific esterase's (NSE) were found to be moderate. AKPase activity was weak in scattered lymphocytes, however, G-6-Pase and MDH activity was weak to moderate in scattered lymphocytes. Strong activity of LDH, G-6-PD, NADH, NADPH, and NSE were observed in lymphocytes present in the connective tissue core of the circumvallate papilla.

Keywords: Histochemical, Histoenzymic, Goat, Lingual Tonsil

How to cite: Saran, D., Singh, O. and Pathak, D. (2023). Histochemical and histoenzymic studies on lingual tonsil of goat. *Haryana Vet.* **62(1)**: 68-72.

The lingual tonsil is a significant constituent of the mucosa-associated lymphoid tissue (MALT) (Perry and Whyte, 1998). Lingual tonsil is the first line of defence, because of their close relationship with lymphoid tissue and their role in antigen processing (Indu *et al.*, 2018). Various enzymes are distributed variably within lymphocytes and the lingual tonsil epithelium. The ionic exchange that occurs across the membrane is associated to alkaline phosphatase (AKPase). It occurs frequently in cells that are specialised for endocytosis and pinocytosis as well as in the endothelium of smaller blood vessels. Glucose-6-Phosphatase (G-6-Pase) is associated with metabolism of carbohydrate. All aerobic cells contain succinate dehydrogenase (SDH), which is a crucial component of the Krebs cycle. The presence of diaphorases indicate both cytoplasmic and mitochondrial activity (Singh and Singh, 2014). These enzymes' tissue-specific localizations are also used as biochemical markers of tissue damage. The objective of the present study was to identify the specific distribution of different enzymes in the lingual tonsil of goat and to correlate it with functional importance.

MATERIALS AND METHODS

The present study was conducted on (n = 24) the lingual tonsil of goat dissected from oro-pharyngeal region of head. These samples were collected from the Slaughter-house in the periphery of the Ludhiana district of Punjab. After the localization of tonsil, the samples were fixed at 10% NBF (Neutral buffered formalin) and processed by acetone-benzene schedule (Luna, 1968) and sections of 5-6 m were obtained on glass slides with a rotary microtome. The paraffin sections were stained with PAS-AB pH 2.5 for neutral and carboxylated acidic mucopolysaccharides and glycoproteins, Alcian Blue at pH 1.0 and 2.5 for sulphated mucopolysaccharides and carboxylated acid mucopolysaccharides, and glycoproteins (Luna, 1968) and bromophenol blue for basic protein (Pearse, 1972).

For localization of enzymes, lipids and phospholipids, fresh unfixed tissue samples were taken. Cryostat sections of 10 m thickness at -20º C were obtained on glass slides and incubated with different substrates to study distribution pattern of different enzymes *viz*; Phosphatases: Alkaline phosphatase (AKPase) by simultaneous coupling azo dye method (Barka and Anderon,1963) and Glucose-6-Phosphatase (G-6-Pase) by lead nitrate method (Barka and Anderon,1963), Non-specific esterase (NSE) by Naphthol acetate method (Thompson and Hunt, 1966) and Oxidoreductases *viz*; Succinic dehydrogenase (SDH), Glutamic dehydrogenase (GLD), Lactic dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G-6-PD), Malic dehydrogenase (MDH), Nicotinamide adenine dinucleotide diaphorase (NADH-d) and Nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) (Pearse, 1972). The lipids and phospholipids were localized using Sudan Black B for lipids and acid hematin for phospholipids.

RESULTS AND DISCUSSION

Mucopolysaccharides: The surface epithelium of the

*Corresponding: devvet16@gmail.com

circumvallate papillae of the lingual tonsil was weak to moderately positive for neutral mucopolysaccharides (Figs. 1 & 2, Table 1). Tonsillar epithelial cells were known to exhibit energy demanding absorptive and secretory functions, so the stored neutral mucopolysaccharides in the upper strata of the epithelium may form an energy source (Perry,1994). The mucous acini were strongly positive for neutral mucopolysaccharides (Fig. 1) and moderately positive for sulphated acid mucopolysaccharides (AB pH: 1.0), carboxylated acid mucopolysaccharides and glycoproteins (AB pH: 2.5). Similar observations were recorded in goat, horse, pig, and buffalo by Kumar and Kumar (2005), Kumar and Timoney (2005), Ranjit *et al.* (2015a) and Girgiri and Kumar (2019). Alcian blue reactivity was moderate and neutral mucopolysaccharides were more copious than acidic. The connective tissue of propria sub-mucosa was moderate to strongly positive for neutral mucopolysaccharides (Fig. 1). The aggregated lymphocytes were moderately positive for neutral mucopolysaccharides and weakly positive for sulfated acid mucopolysaccharides, carboxylated acid mucopolysaccharides and glycoproteins (Figs. 1, 2, 3 & 4; Table 1).

Proteins: Protein content was observed throughout the lingual tonsil. However, the content of protein varied in different structures of the tonsil. The epithelium of the lingual tonsil exhibited weak to moderate reactions for proteins. The glandular acini were weakly positive for proteins (Fig. 5, Table 1). Similar observations were made by Ranjit *et al.* (2015b) in tubal tonsil of pigs. The connective tissue in the propria submucosa of the lingual tonsil exhibited strong protein activity. The strong activity might be attributed to the presence of collagen and reticular fibers that contain glycoproteins.

The scattered lymphocyte exhibited moderate to strong activity of the protein (Fig. 5, Table 1). This strong reaction in the lymphocytes might be due to the presence of nucleoproteins in the nucleus and glycoprotein in the plasma membrane.

Lipids and Phospholipids: There was very less amount of lipid in the epithelium and subepithelial region of the propria-submucosa of lingual tonsils of goats. Large lipid droplets were seen in the deepest region of the propria-submucosa, between the muscle fasciculi and glands, in the lingual tonsils of goats (Fig. 6). Similar findings were recorded by Kumar and Kumar (2005) lingual tonsil of goats.

The acid hematin technique revealed scanty phospholipids in the epithelium and subepithelial region of the propria-submucosa of lingual tonsil of goats. The scattered lymphocytes were also weakly positive for phospholipids in the lingual tonsil of goats. There was no available literature for the comparison between the tonsils.

Table 1. Distribution pattern of histochemical moieties in lingual tonsil of goat of goat

S. No.	Group	Lingual Tonsil	
		Epithelium	Lymphoid follicles
1.	Neutral Mucopolysaccharides	+/++	++
2.	Acid Mucopolysaccharides	+	+
3.	Proteins	+/++	++/+++

0 Not observed; + Weak; ++ Moderate; +++ Strong

Table 2. Histoenzymic distribution lingual tonsil of goat

S. No.	Group	Lingual Tonsil	
		Epithelium	Lymphocyte
1.	AKPase	+	++
2.	G-6-Pase	+	+/++
3.	G-6-PD	++	+++
4.	LDH	+	+++
5.	SDH	+	+
6.	MDH	+	+/++
7.	NADH	+/++	+++
8.	NADPH	++	+++
9.	NSE	++	+++

0 Not observed; + Weak; ++ Moderate; +++ Strong

Alkaline Phosphatase (AKPase): In the present study, alkaline phosphatase activity was constantly weak in the epithelium of lingual tonsils. However, the aggregated lymphocyte within the core of connective tissue of circumvallate papilla and endothelium of blood vessels exhibited moderate reaction of AKPase (Fig. 7, Table 2). These findings confirm with Smith *et al.* (1958) who reported endothelium of arteries and capillaries as the most evident location of AKPase enzyme in the mice lymph node and correlated activity with a transfer of electrolytes across the wall of blood vessels. The enhanced AKPase activity in the capillary walls indicated greater electrolyte transfer across the wall, suggesting high activity in the lymphocyte throughout fetal life (Kapoor and Singh, 2016). The presence of AKPase in lymphocytes might be involved in inducing in situ transformation of lymphocytes and providing a suitable environment for lymphopoiesis.

Glucose-6-Phosphatase (G-6-Pase): The epithelium of the lingual tonsil was weakly positive for glucose-6-phosphatase, however, the aggregated lymphocyte and muscle bundles in propria submucosa exhibited weak to the moderate activity of G-6-Pase (Fig. 8; Table 2). Glucose - 6-phosphatase is an amino acid-based enzyme that is

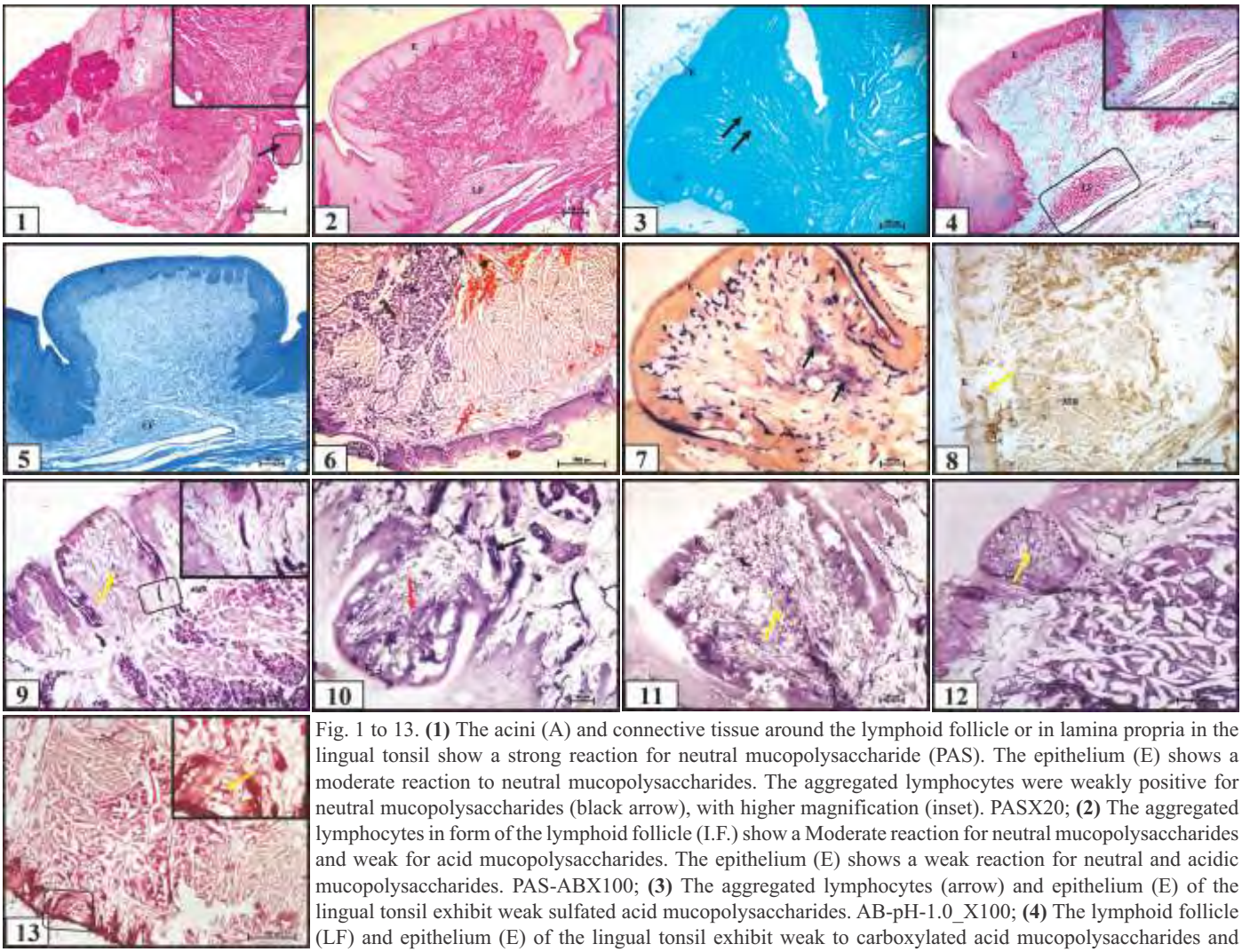

Fig. 1 to 13. **(1)** The acini (A) and connective tissue around the lymphoid follicle or in lamina propria in the lingual tonsil show a strong reaction for neutral mucopolysaccharide (PAS). The epithelium (E) shows a moderate reaction to neutral mucopolysaccharides. The aggregated lymphocytes were weakly positive for neutral mucopolysaccharides (black arrow), with higher magnification (inset). PASX20; **(2)** The aggregated lymphocytes in form of the lymphoid follicle (I.F.) show a Moderate reaction for neutral mucopolysaccharides and weak for acid mucopolysaccharides. The epithelium (E) shows a weak reaction for neutral and acidic mucopolysaccharides. PAS-ABX100; **(3)** The aggregated lymphocytes (arrow) and epithelium (E) of the lingual tonsil exhibit weak sulfated acid mucopolysaccharides. AB-pH-1.0_X100; **(4)** The lymphoid follicle (LF) and epithelium (E) of the lingual tonsil exhibit weak to carboxylated acid mucopolysaccharides and glycoprotein. AB-pH-2.5 X100; **(5)** The weak to moderate deposition of protein content in Epithelium (E). The high amount deposition of protein content in the lymphoid follicle (LF) in the lingual tonsil. Bromophenol Blue X100; **(6)** A few depositions of lipid droplets (arrow) in propria submucosa of lingual tonsil. No deposition of any lipid droplets around the epithelium (E) and connective tissue core of the circumvallate papilla. Oil Red O X20; **(7)** The epithelium (E) of lingual tonsil exhibits a weak reaction of AKPase and a strong reaction aggregated lymphocyte (arrow) in connective tissue core of circumvallate papilla. Azodye method X100. **(8)** The epithelium (E) of the lingual tonsil doesn't exhibit any reaction of G-6-Pase and a weak to moderate reaction in muscle bundles (MB) in propria submucosa and aggregated lymphocyte (arrow) in connective tissue core of circumvallate papilla. Lead nitrate X20; **(9)** The epithelium (E) of the lingual tonsil exhibits moderate activity of G-6-PD. The blood vessels (inset) and aggregated lymphocyte (arrow) in the connective tissue core of the circumvallate papilla exhibit the strong activity of G-6-PD. Nitro BT method X40; **(10)** The epithelium (E) of the lingual tonsil doesn't exhibit activity of LDH. The blood vessels (black arrow) and aggregated lymphocytes (red arrow) in the connective tissue core of the circumvallate papilla exhibit the strong activity of LDH. Nitro BT method X100; **(11)** The epithelium (E) of the lingual tonsil exhibits weak to moderate reaction of NADH. The aggregated lymphocytes (arrow) in the connective tissue core of the circumvallate papilla exhibit moderate to strong activity of NADH. Nitro BT method X100; **(12)** The epithelium (E) of the lingual tonsil exhibits moderate activity of NADPH. The aggregated lymphocytes (arrow) in the connective tissue core of the circumvallate papilla exhibit moderate to the strong activity of NADPH. Nitro BT method X40; **(13)** The epithelium (E) of the lingual tonsil exhibits moderate activity of NSE. The aggregated lymphocytes (arrow, inset) in the connective tissue core of the circumvallate papilla exhibit the strong activity of NSE. Naphthol Acetate method X20.

attached to the endoplasmic reticulum (ER) and helps to release glucose into the circulation (Kaur *et al.*, 2018).

Glucose-6-Phosphate Dehydrogenase (G-6-PD): The epithelium of the lingual tonsil exhibited moderate activity of Glucose-6-phosphate dehydrogenase (G-6-PD). The blood vessels and scattered lymphocytes within the connective tissue core of the circumvallate papilla exhibited strong activity of G-6-PD. The connective tissue in propria submucosa also exhibited weak to moderate activity of G-6-PD (Fig. 9, Table 2). The pentose phosphate shunt is linked to glucose 6 phosphate dehydrogenase enzyme activity (Fennell and Pearse, 1961). During development, these pentose phosphates might be used to synthesize nucleic acids. Turkoglu *et al.* (2003) also reported G-6-PD activity in all mammalian tissues, particularly in the cytosol and mitochondria.

Lactate Dehydrogenase (LDH): The epithelium of the lingual tonsil was weakly positive for Lactate Dehydrogenase (LDH). The scattered lymphocyte in the connective tissue core of the circumvallate papilla and tunica intima of blood vessels exhibited strong activity of LDH. The connective tissue in propria submucosa also exhibited weak to moderate activity of LDH (Fig. 10; Table 2). LDH is a NAD-dependent enzyme present in cells that are using the glycolytic pathway. It catalyzes the synthesis of lactate and pyruvate in anaerobic glycolysis and aerobic respiration, respectively (Kaur *et al.*, 2019). The presence of the LDH enzyme in tonsils indicated the existence of a glycolytic pathway in cellular components, particularly the epithelium and lymphoid follicles.

Succinic Dehydrogenase (SDH): The epithelium of the lingual tonsil exhibited weak activity of succinic Dehydrogenase (SDH). The scattered lymphocyte within the connective tissue core of the circumvallate papilla exhibited weak of SDH, however, tunica intima of blood vessels and connective tissue in propria submucosa and muscle bundles exhibited strong activity of SDH (Table 2). SDH is a mitochondrial enzyme involved in the oxidation-reduction pathway, which the cell uses to generate energy (Smith, 1969). This enzyme is involved in the oxidation of fatty acids, which results in the synthesis of lipid pigment granules (Smith, 1969).

Malic Dehydrogenase (MDH): The epithelium of the lingual tonsil exhibited weak activity of malic dehydrogenase (MDH). The scattered lymphocyte within the connective tissue core of the circumvallate papilla also exhibited weak to moderate activity of MDH. The connective tissue in propria submucosa and tunica intima of blood vessels exhibited weak activity of MDH (Table 2). The MDH enzyme is linked to a high level of cell physiological activity. Because this enzyme was found in epithelial reticular cells and lymphoid follicles or dispersed lymphocytes, it was assumed that this enzyme is involved in lymphocyte proliferation and maturation. It catalyzes the conversion of oxaloacetate and malate which is important reaction cellular metabolism (Musrati *et al.*, 1998).

Nicotinamide Adenine Dinucleotide Diaphorase (NADH-d): The epithelium of the lingual tonsil was weak to moderately positive for NADH-diaphorase. The scattered lymphocyte within the connective tissue core of the circumvallate papilla exhibited moderate to the strong activity of NADH-diaphorase. The tunica intima of blood vessels and connective tissue in propria submucosa exhibited strong activity of NADH (Fig. 11; Table 2). NADH-diaphorase is a mitochondrial enzyme and coenzyme dehydrogenase that is involved in the Krebs cycle and intense enzyme activity may indicate lymphocyte differentiation and maturation (Fennell and Pearse, 1961).

Nicotinamide Adenine Dinucleotide Phosphate Diaphorase (NADPH-d): The epithelium of the lingual tonsil exhibited moderate activity of NADPH-diaphorase. The scattered lymphocyte within the connective tissue core of the circumvallate papilla exhibited moderate to the strong activity of NADPH. The tunica intima of blood vessels and connective tissue in propria submucosa and muscle bundles also exhibited strong activity of NADPH (Fig. 12, Table 2). NADPH diaphorase is co-enzyme dehydrogenase that is involved in the hydrogen transport chain in the cell. The cell's metabolic activity was represented by the enzyme intensity (Kaur et al., 2019).

Non-Specific Esterase's (NSE): The epithelium of the lingual tonsil exhibited moderate activity of NSE. The scattered lymphocyte within the connective tissue core of the circumvallate papilla exhibited moderate to the strong activity of NSE (Fig. 13, Table 2). The connective tissue in propria submucosa also exhibited strong activity of NSE. Non-specific esterase's are a group of enzymes that help in lipid metabolism (Smith, 1969). The presence of high NSE activity in lymphocytes and epithelial reticular cells suggests that these cells are involved in the synthesis of lipids and phospholipids, which are needed for cell membrane construction during lymphopoiesis.

REFERENCES

Barka, T. and Anderson, P.J. (1963). Histochemistry: theory, practice, and bibliography. Hoeber Medical Division, Harper and Row Publishers, Inc., New York, pp. 237-316.

Fennell, R.A. and Pearse, A.G.E. (1961). Some histochemical observations on the bursa of fabricius and thymus of the chicken. *Anat. Rec.* **139(2)**: 93-103.

Girgiri, I.A. and Kumar, P. (2019). Histological and histochemical studies on the lingual tonsil of the buffalo (*Bubalus bubalis*). *J. Buffalo Sci.* **8(3)**: 68-76.

Indu, V.R., Lucy, K.M., Kumar, K.S.P., Ashok, N. and Maya, S. (2018). A comparative study on the lingual tonsil in goats and pigs. *Int. J. Curr. Micro. Appl. Sci.* **7(6)**: 3210-3216.

Kapoor, K. and Singh, O. (2016). Enzyme histochemistry of cecal lymphoid tissue during prenatal period of buffalo. *Vet. World.* **9(9)**: 1006-1011.

Kaur, H., Singh, O. and Pathak, D. (2019). Enzyme histochemistry of spleen of pig (*Sus scrofa*). *Int. J. Livest. Res.* **9(9)**: 137-142.

Kaur, J., Singh, O. and Pathak, D. (2018). Enzyme histochemistry of hemal nodes of Indian buffalo (*Bubalus bubalis*). *Int. J. Livest. Res.* **9(3)**: 249-55.

Kumar, P. and Kumar, P. (2005). Light and scanning electron microscopic studies on lingual tonsil of goat. *Haryana Vet.* **44**: 13-16.

Kumar, P. and Timoney, J. F. (2005). Histology and ultrastructure of the equine lingual tonsil. I. Crypt epithelium and associated structures. *Anat. Histol. Embryol.* **34(1)**: 27-33.

Luna, L.G. (1968). Manual of histological staining method of Armed Forces Institute of Pathology. (3rd Edn.), McGraw Hill Book Company, New York, USA. pp. 38-196.

Musrati, R.A., Kollarova, M., Mernik, N. and Mikulasova, D. (1998). Malate dehydrogenase: distribution, function and properties. *Gen. Physiol. Biophys.* **17**: 193-210.

Pearse, A.G.E. (1972). Histochemistry: Theoretical and Applied. (3rd Edn.), Vol II. Churchill Livingstone, London pp. 607 & 998.

Perry, M. (1994). The specialized structure of crypt epithelium in the human palatine tonsil and its functional significance. *J. Anat.* **185(1)**: 111-127.

Perry, M. and Whyte, A. (1998). Immunology of the tonsils. *Immunol. Today.* **19(9)**: 414-421.

Ranjit, Kumar, P. and Singh, G. (2015a). Histology, histochemistry and scanning electron microscopy of lingual tonsil of the young pigs. *Vet. Res. Int.* **3(1)**: 55-59.

Ranjit, Prakash, T., Kumar, P., Pal, R. and Singh, G. (2015b). Histology, histochemistry and scanning electron microscopy of tubal tonsil of the young pigs. *Vet. Res. Int.* **3(1)**: 1-6.

Singh, A.D. and Singh, O. (2014). Histoenzymatic studies on prenatal development of submandibular salivary gland in buffalo (*Bubalus bubalis*). *Vet. World.* **7(12)**: 1032-1036.

Smith, C. (1969). Studies on the thymus of the mammal. XVI. Lipopigment cells in the cortex of the thymus of the mouse. *Am. J. Anat.* **124(3)**: 389-409.

Smith, C., Wharton, T.J. and Gerhardt, A.M. (1958). Studies on the thymus of the mammal. XI. Histochemical studies of thymus, spleen and lymph node in normal and irradiated mice. *Anat. Rec.* **131(3)**: 369-387.

Thompson, S.W. and Hunt, R.D. (1966). Selected histochemical and histopathological methods. Springfield, IL.

Turkoglu, V., Aldemir, S. and Ciftci, M. (2003). Purification and characterization of glucose 6-phosphate dehydrogenase from sheep liver. *Turk. J. Chem.* **27(3)**: 395-402.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary Profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/submission.
- Please pay processing fee of Rs. 1000/- online in the account of Dean, College of Veterinary Sciences, along with each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondence should be addressed to 'The Editor', Haryana Veterinarian, Department of Veterinary Parasitology, College of Veterinary Sciences, LUVAS, Hisar-125004.

Editors