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Histomorphometric and immunohistochemical details of hemal nodes in Indian buffalo

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Abstract
Histological and histometric details of hemal nodes in Indian buffalo were carried out. Tissue samples for the study were collected from five adult apparently healthy buffaloes slaughtered in the Corporation slaughter house, Chennai. About 4-6 hemal nodes were collected in and around the iliac arteries of the abdominal aorta. Tissue samples were fixed in 10% neural buffered formalin, sections were made and stained for histological observations and CD3 and CD79a antibodies were used for Immunohistochemical observations of T and B lymphocytes. Size of the hemal node varied from pin head to peanut size. It was surrounded by a connective tissue capsule made up predominantly of collagen fibres with few smooth muscle and reticular fibres. Subcapsular sinuses were lined by endothelium was observed. The parenchyma was not clearly divided into cortex and medulla as in the lymph nodes. It was composed of lymphoid follicles with germinal centre, interfollicular region was made up of lymphoid cords. Large, irregular sinusoid were noticed in between the cords. Immunohistochemical staining revealed the distribution of more number of T lymphocytes in the interfollicular and at the periphery of lymphoid follicles. Whereas, few T lymphocytes were also observed within the lymphoid follicle. Few B-cells were found at the mantle zone of the lymphoid follicle.

Keywords: Hemal node, Indian buffalo, histology, micrometry, immunohistochemistry

1. Introduction
Spleen and lymph nodes are major part of secondary immune organs in mammals and constitutes the major components of peripheral lymphoid tissue [1].
Hemal nodes are lymphoid organs that constitute part of the immunologic defense system in ruminants and rats [2]. These are commonly found in the dorsal wall of thoracic and abdominal cavities, particularly around the caudal vena cava and abdominal aorta in the sublumbar region [3, 4].
These are independent lymphoid organs situated along the course of the blood circulation of various mammalian species and also in some birds [5]. These nodes are considered to play a role in erythropoiesis, platelet formation, erythrophagocytosis and also immunological in function [2].
Hemal nodes in man are described as a structure resembling lymph nodes but the sinusoids were filled with blood instead of lymph [6] and were described as hemolymph node [7].
Though, a lot of histological work has been carried out in sheep [8, 9], in bovines [10, 11]; saanen goat [12] and in roe deer [13]. However, Dellmann and Brown [14] reported that in all mammals, the hemal nodes found to have a general structure with some species variations. Hence, the present work aims to describe the histological, histometric and immunohistochemical details of hemal nodes of Indian buffaloe by light microscopy, as a basis for understanding the structure of hemal nodes.

2. Materials and Methods
Fresh specimen of hemal nodes were collected from five apparently healthy adult buffaloes (n=5). The animals were slaughtered for human consumption in a Corporation slaughter house, Chennai. About 4-6 hemal nodes were collected from each animal around the iliac arteries of the abdominal aorta.
Samples were fixed in 10% neutral buffered formalin and processed for paraffin sectioning. The sections were stained with H&E, Masson's trichrome and Gomori's method for reticular fibres [15].
For Immunohistochemical localization of T lymphocytes, the sections were processed through xylol and alcohol solution.

Heat mediated antigen retrieval was done using TRIS-ED buffer (pH 8.5 to 9.0). Blocking of endogenous peroxide was done with 3% hydrogen peroxide stained with CD3 ready to use primary antibody (Pathn Situ Co.) for 30 to 45 minutes in a moist chamber. Then the section were incubated with ready to use polyexcel HRP (Pathn Situ Co.) for 12 min. DAB chromogen (1 ml DAV buffer + One drop DAB chromogen) for 2 to 5 minutes was used to make antigen-antibody reaction visible. Gill's hematoxylin was used for counterstaining. For B-Lymphocytes, CD79a (Pathn Situ co.) monoclonal antibodies were used.

Microscopic observation was done with a Leica microscope (CH9435 Heer brugg) under different magnifications. Micrometric observations such as capsule thickness, diameter of lymphatic nodule, diameter of germinal centre and number of nodules per field (10x) were measured using Leica Application Suite V4.4 software [16]. The data were analysed using SPSS software to calculate mean and SE [17].

3. Results and Discussion

In the present study, size of the hemal nodes varied from pin head to peanut size. Larger nodes were found to be fewer in number than smaller one as reported by Bozkurt *at al*. [12]. The nodes were brown in colour embedded in the adipose tissue.

The hemal nodes were covered by connective tissue capsule which was mainly composed of collagen fibres and few smooth muscle fibres (Fig 1). Smooth muscle fibres were parallel to the connective tissue fibres. A few trabeculae extended from the capsule and divided the parenchyma. Reticular fibres were also observed in both capsule (Fig 2) and trabeculae. Subcapsular sinuses were observed and lined with endothelial cells (Fig 3). Trabecular sinuses were also observed and were continuous with the subcapsular sinus as per Zidan and Pabst [18].

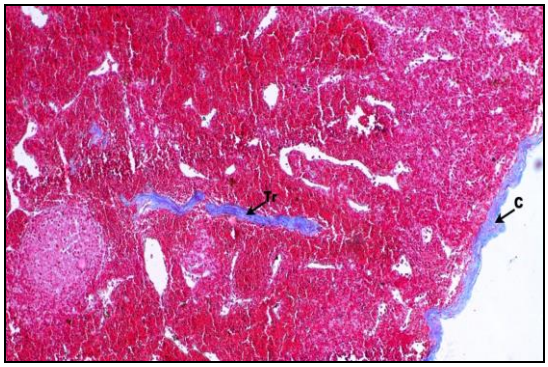

Fig 1: Photomicrograph of the capsule (C) and trabecula (Tr) showing the presence of collagen and smooth muscle fibres Masson's trichrome x 100

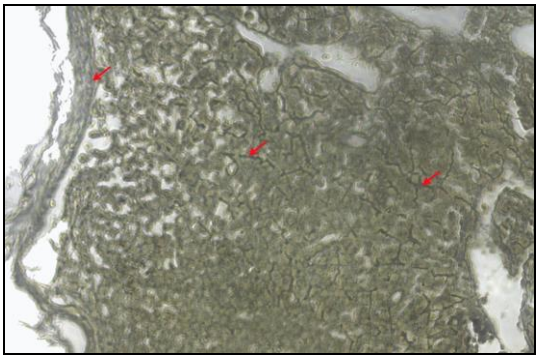

Fig 2: Photomicrograph showing the distribution of reticular fibres (arrows) in capsule and parenchymaGomori's method x 400

The parenchyma was supported by a fine network of reticular fibres (Fig 2) and reticular cells which formed a structural backbone. This is similar to the findings of Gargiulo *et al.*, [9] who reported that the reticular meshwork aid in migration of large numbers of free blood cells to collect in the subcapsular sinus.

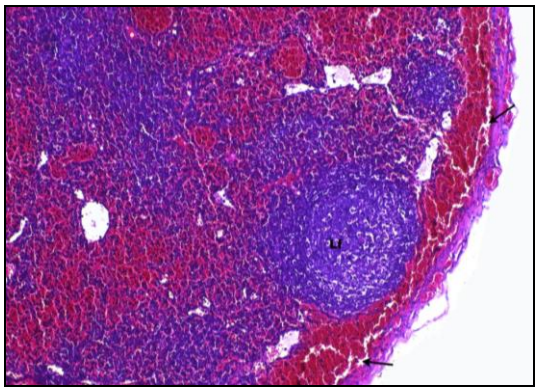

Fig 3: Photomicrograph showing subcapsular sinus (arrow) and lymphoid follicle (Lf) H & E x 100

There was no clear demarcation of parenchyma into cortex and medulla as observed in lymph node. However, small primary lymphoid follicles were observed with a clear germinal centre (Fig 4). Germinal centre was consisted of lymphoblasts, lymphocytes and plasma cells (Fig 5). No afferent lymphatics were observed. The presence of primary and secondary follicles indicates the role of the hemal node in antibody production as per Ceccarelli *et al*., [19] in ovine and bovine. Mantle zone of the lymphoid follicle was made of numerous small sized lymphocytes.

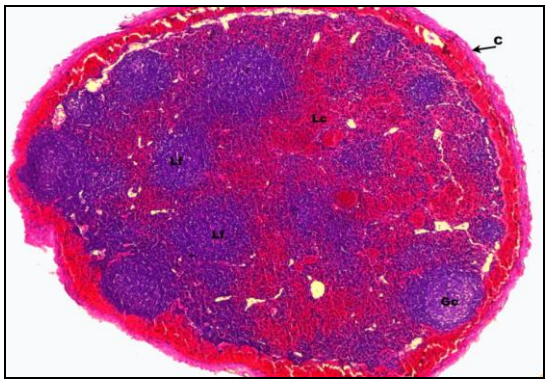

Fig 4: Photomicrograph of hemal node parenchyma showing lymphoid follicles (Lf), germinal centre (Gc) surrounded by connective tissue capsule (C) H & E x 50

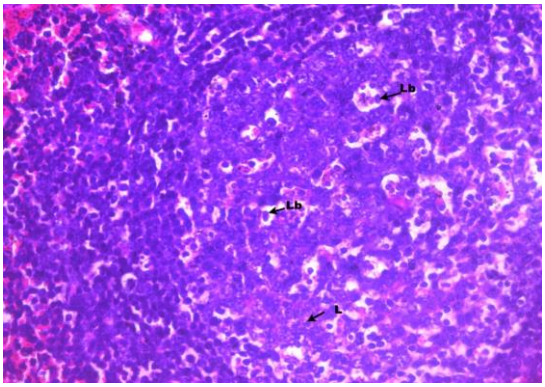

Fig 5: Photomicrograph of lymphoid follicles showing cellular population - Lymphoblast (Lb), Lymphocytes (L) H & E x 400

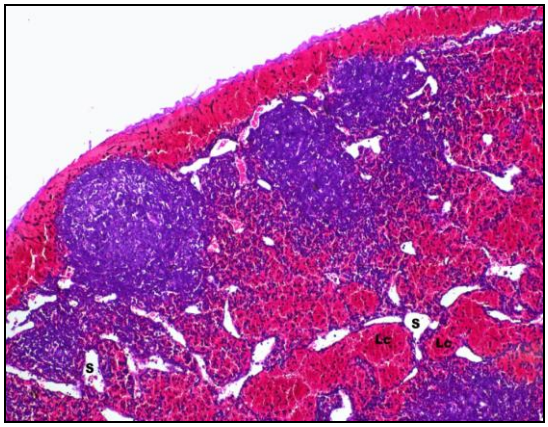

Fig 6: Photomicrograph of hemal node showing lymph cords (Lc) with large, irregular sinusoids (S) in between. H & E x 100

Remaining proportion of the parenchyma in the present study was made of irregular cords made of chiefly lymphocytes (Fig 6). Other cells like erythrocytes, macrophages, plasma cells and mast cells were also noticed. The presence of macrophages indicated that hemal nodes in buffaloe are entertained in phagocytosis of old or defective blood cells as described by Ezeasor and Singh [2] in goat. Another function of macrophages might be the filtration of particles.

In the present study, the lymphatic cords were separated by large, irregular sinusoids (Fig 6). Size of the sinusoids was found to be variable. Though, the sinusoids were found to be larger, storage function of blood in hemal node might not be possible due to the small size of the hemal node relative to the body weight of Buffalo as per Zidan and Pabst [18]. Micrometric observations of various parameters were presented in Table 1.

Table 1: Micrometric observations of hemal node

S. No	Parameters	Mean±SE
1.	Capsule thickness (µm)	23.3±2.13
2.	Number of nodules (100x)	2.615±0.35
3.	Diameter of lymphatic nodules	252±13.8
4.	Diameter of germinal center	134±19.7
5.	Total diameter of haemal node	2193±185.04

Micrometric observations of hemal nodes in the present study was found to be lesser than the values of superficial palpable lymph node *viz.*, parotid, mandibular, popliteal etc. in goat as reported by Sarma *et al*., [20]. This indicated the smaller size of the hemal node when compared to superficial lymph nodes.

Immunohistochemical studies revealed that CD3 positive T-lymphocytes were identified as brown coloured cells. They were found to be more in the inter-follicular region (Fig 7). A similar observation was noticed by Bozkurt *et al.*, [13] in hemal nodes of deer and Ceccarelli *et al*., [19] in sheep and cow. Very few T-lymphocytes were observed within the lymphoid follicle and found to be more around the margin of lymphoid follicles (Fig 7) as per Zidan and Pabst [21] in dromedary camel. However, Casteleyn *et al.* [22] contradicted the present findings that he observed the existence of more number of T-lymphocytes in the germinal centre of lymphoid follicles. It was observed that CD79a positive B-cells were found to be more at the mantle zone of lymphoid follicles. A similar observation was reported by Yoon *et al.*, [23] and Bozkurt *at al.* [12] in goat.

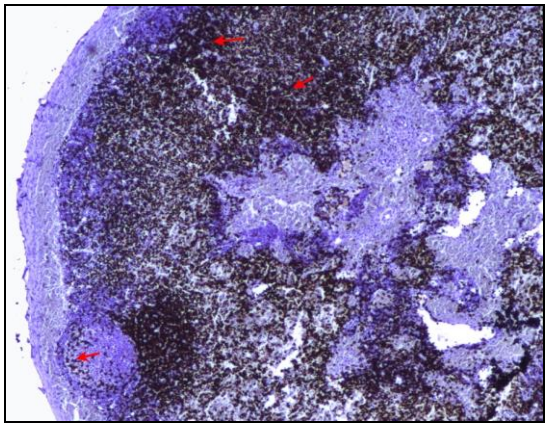

Fig 7: Photomicrograph showing CD3 positive T lymphocytes (brown colour cells -arrows) distributed in interfollicular region IHC (DAB) x 100

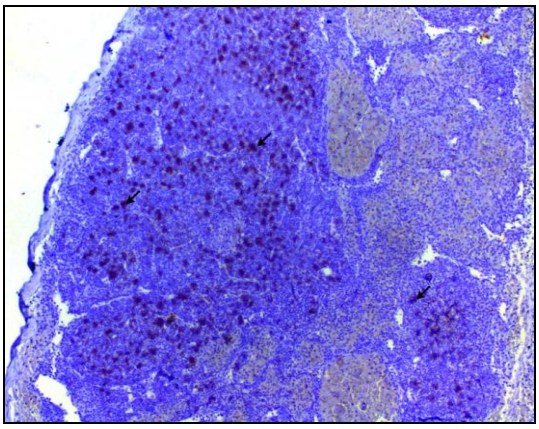

Fig 8: Photomicrograph showing CD79a positive B lymphocytes (arrows) distributed in inter follicular region IHC (DAB) x 100

4. Conclusion

Based on the results of this study, it is concluded that hemal node in Indian buffaloe was covered by a connective tissue capsule. The parenchyma was made up of lymphoid follicles with a germinal centre. Rest of the parenchyma consisted of lymphoid cords separated by large sinusoids. Immunohistochemical observations indicated the presence of more number of T lymphocytes and few B lymphocytes. Hence, it is concluded that the histological and immunohistochemical results of hemal node in Indian buffaloe were similar to other ruminants.

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