

Original Research Article

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Histological and Immunohistochemical Observations of Supramammary Lymph Node in Sheep and Goat

S. Senthilkumar¹, T.A. Kannan^{1*}, Geetha Ramesh¹ and D. Sumathi²

¹Department of Veterinary Anatomy, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-07, Tamil Nadu, India

²Department of Veterinary Clinical Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-07, Tamil Nadu, India

*Corresponding author

ABSTRACT

Lymph node involved in activation and maturation of lymphocytes and stimulates antigenic response against invading foreign substances. Supramammary lymph nodes had high significance in mastitis and other udder infections in small ruminants. For the present study, six supramammary lymph nodes of Madras Red ewe and Boer local she-goat were collected from Chennai corporation slaughter house. Histological and immunohistochemical observations were carried out. In both the species, supramammary lymph node was composed of two parts viz., parenchyma and connective tissue stroma. The lymph node was covered by a fibroelastic capsule composed of collagen, elastic and reticular fibres. Trabeculae originated from the capsule and divided the lymph node into many lobules. Parenchyma composed of an outer cortex and inner medulla. Cortex region contained several lymphoid follicles, which was made by outer mantle zone and inner germinal centre. Statistically no significant difference was observed in micrometric parameters such as capsule thickness, trabeculae thickness, lymphoid follicle diameter, germinal centre diameter and number of follicles per field (50x) between ewe and she-goat. Immunohistochemical staining for CD3⁺ 'T' lymphocytes revealed their localization was found to be in interfollicular region and less in germinal centre. Medullary region showed sparsely located 'T' lymphocytes.

Keywords

Madras Red sheep,
Boer local goat,
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Introduction

Lymph nodes are the secondary lymphoid organs situated along the course of lymph vessels and only lymphoid organ in the body with afferent and efferent vessels (Dellman and Brown, 1987). Histological organization

and cell population of each lymph node reflect both the background activity of the immune system and local response of the node to small amounts of antigen and shows marked differences depending on their location in the body (Fawcett and Raviola, 1994). In ewes, One to three mammary lymph nodes are

situated along the postero dorsal aspect of each mammary gland. These nodes varies in size, large node is kidney shape or crescentic shape (Lee and Lascelles, 1969). In she-goats two to three mammary lymph nodes are situated caudodorsal to the udder. They related medially to the external pudental vessels (Getty, 2012). The nodes served as filters to remove or destroy the foreign substances and also provides a source of lymphocytes to fight infection affecting the mammary gland (Nickerson and Akers, 2011). Madras Red sheep is a medium-sized breed present in the northern regions of Tamil Nadu (Balasubramanyam *et al.*, 2012). Boer local is a crossbreed goat evolved by crossing Boer and Local goats at Post Graduate Research Institute in Animal Sciences unit of Tamil Nadu Veterinary and Animal Sciences University (Palanivel *et al.*, 2012). In general, the histology of various lymph nodes of ruminants is explored by various authors. But, no much work was carried out on the comparative histomorphometry and immunohistochemistry of supramammary lymph node of Madras Red ewes and Boer local she-goats. Hence, the present study was conducted to establish a basic data about histomorphometry and immunohistochemistry of supramammary lymph node of Madras Red ewes and Boer local she-goats.

Materials and Methods

The present study was conducted at the Department of Veterinary Anatomy, Madras Veterinary College. For histological studies, six supramammary lymph nodes from adult healthy Madras Red ewes and Boer local she-goats were collected from the corporation slaughterhouse, Chennai. After collection, the tissues were fixed in 10% NBF (Neutral buffered formalin) and Bouin's fluid. For immunohistochemical staining six supramammary lymph nodes were used. Sections were stained by standard

Haematoxylin and Eosin (H&E) method (Bancroft and Gamble, 2003), Masson's trichrome method for collagen and muscle fibres, Verhoeff's method for elastic fibres, Gomori's sliver method for reticulum (Luna, 1968) for the histological study.

Immunohistochemistry staining method

3 µm paraffin sections were cut and mounted on charged slides and incubated at 60-70 °C for 30 minutes. The sections were deparaffinized by two changes in xylene, dehydrated in absolute alcohol (two changes) and washed twice in distilled water. Heat mediated antigen retrieval was done using TRIS-EDTA buffer (pH 8.5 – 9.0). Then the sections were washed twice in distilled water for two minutes. Blocking of endogenous peroxidase was done with 3 per cent hydrogen peroxide for ten minutes. Then the sections were incubated in CD3 (ready to use) primary antibody in a moist chamber for one hour. Polyexcel HRP (ready to use) secondary antibody was added and incubated for 12 minutes and sections were washed three times in PBS. Diaminobenzidine (DAB) chromogen solution (1ml DAB buffer + 1 drop DAB chromogen) was added and kept for two to five minutes and washed in distilled water. Gill's haematoxylin was used to counter stained the sections for one minute. Bluing the sections was done with running tap water for five minutes. Finally, sections were dehydrated through graded series of alcohol, cleared in xylene and mounted in synthetic mountant (Kannan *et al.*, 2019).

Microscopic images were captured using the Leica microscope (CH9345 Heer brugg). Micrometry was done using the Leica application suite (LAS V4.4). The following parameters were measured in supramammary lymph node of ewe and she-goats *viz.*, Capsule thickness, trabeculae thickness, diameter of lymphatic nodule and diameter of germinal

center, number of nodules per field (50x). The data were subjected for statistical analysis. Independent 't' sample test was used to test the significant difference in micrometric parameters between adult ewe and she-goat. SPSS® 26.0 for Windows was used for statistical analysis of data.

Results and Discussion

Histology

In the present study, the histoarchitecture of supramammary lymph node in both Madras Red ewes (Fig. 1) and Boer local she-goats were found to be similar and resembled that of lymph node of other region.

The parenchyma was covered by a connective tissue capsule constituted of collagen (Fig. 2), elastic and smooth muscle fibres. Capsule was made of fibro muscular tissue. Thickness of capsule was $112.54 \pm 28.38 \mu\text{m}$ in ewe and $71.23 \pm 9.68 \mu\text{m}$ in she-goat.

Trabeculae from the capsule passed into the parenchyma divided the node into many lobules. The fibrous trabeculae passed in

between the lymphoid follicles, containing collagen, elastic (Fig. 3) and reticular fibres. Similar observations were made by Ganga Naik (2015) in Malnad Gidda cow's Supramammary lymph node. Thickness of the trabeculae was found to be $139.76 \pm 34.95 \mu\text{m}$, $179.71 \pm 36.80 \mu\text{m}$ in ewe and she-goat respectively. In both ewes and she-goats trabeculae thickness was found to be more than the capsule thickness. A similar observation was made by Sarma *et al.*, (2008) in Kagani goats. Reece reported that this trabeculae provided support for the entire lymph node and carried blood vessels along its path.

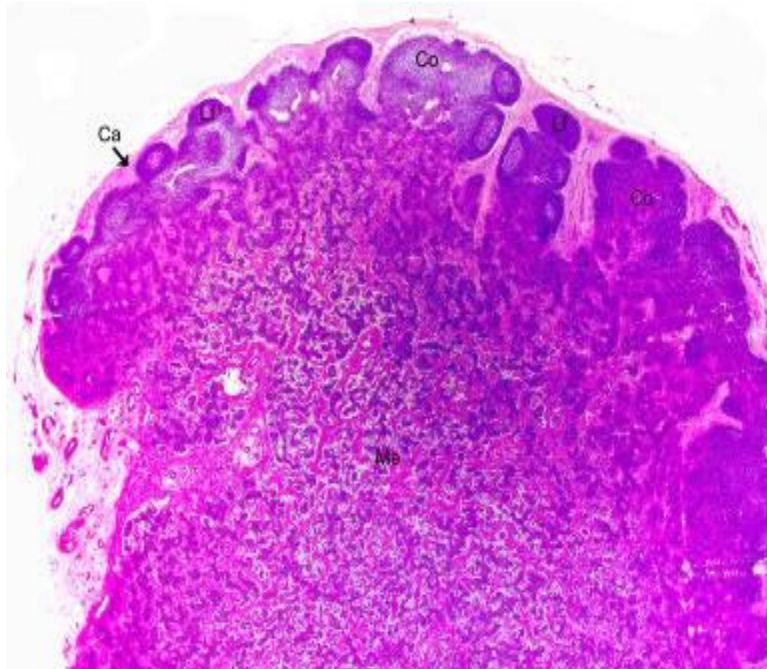
Parenchyma was made of an outer cortex and inner medulla. The cortex consisted of lymphoid follicles, each follicle showed the presence of the germinal centre made of lymphoblasts and large-sized lymphocytes and an outer mantle zone consisted of numerous small and medium sized lymphocytes (Fig. 4). In the present study, lymphoid nodule diameter in ewe was $414.56 \pm 28.92 \mu\text{m}$ and $378.87 \pm 44.32 \mu\text{m}$ in she-goat. In ewe and she-goat number of nodules per field (50x) was 3.16 ± 0.70 and 5.33 ± 0.76 respectively.

Table.1 Mean \pm SE of various micrometrical parameters in supramammary lymph node of Madras red ewes and Boer local she-goats

Parameters	Mean \pm SE		t value
	Sheep (N=6)	Goat (N=6)	
Capsule thickness (μm)	112.54 ± 28.38	71.23 ± 9.68	1.37 ^{NS}
Trabecular thickness (μm)	139.76 ± 34.95	179.71 ± 36.80	0.78 ^{NS}
Lymphoid nodule diameter (μm)	414.56 ± 28.92	378.87 ± 44.32	0.67 ^{NS}
Germinal centre diameter (μm)	214.98 ± 27.09	166.34 ± 17.01	1.52 ^{NS}
Number of follicles per field (50x)	3.16 ± 0.70	5.33 ± 0.76	0.06 ^{NS}

^{NS} - No significant difference between sheep and goat ($P \geq 0.05$)

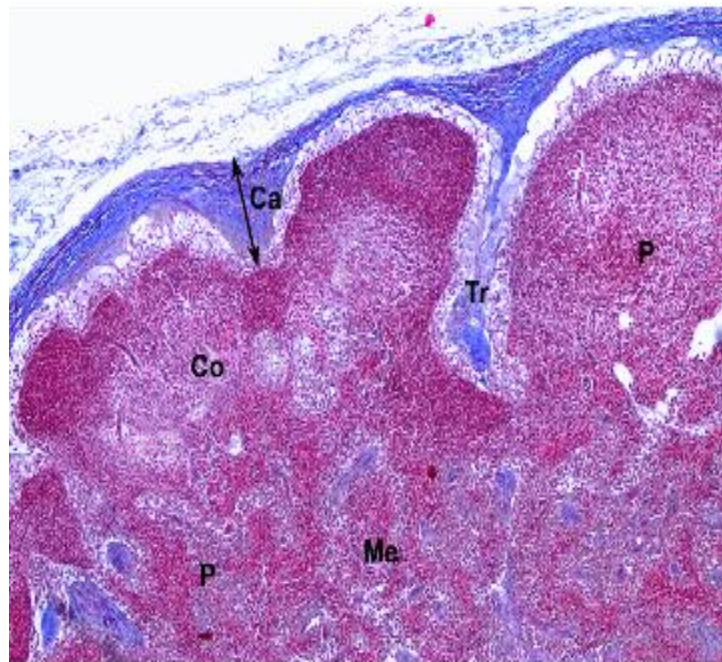
Fig.1 Photomicrograph of the supramammary lymph node of adult ewe



Ca - Capsule Co – Cortex Lf – Lymphoid follicle Me – Medulla

H&E x 12.5

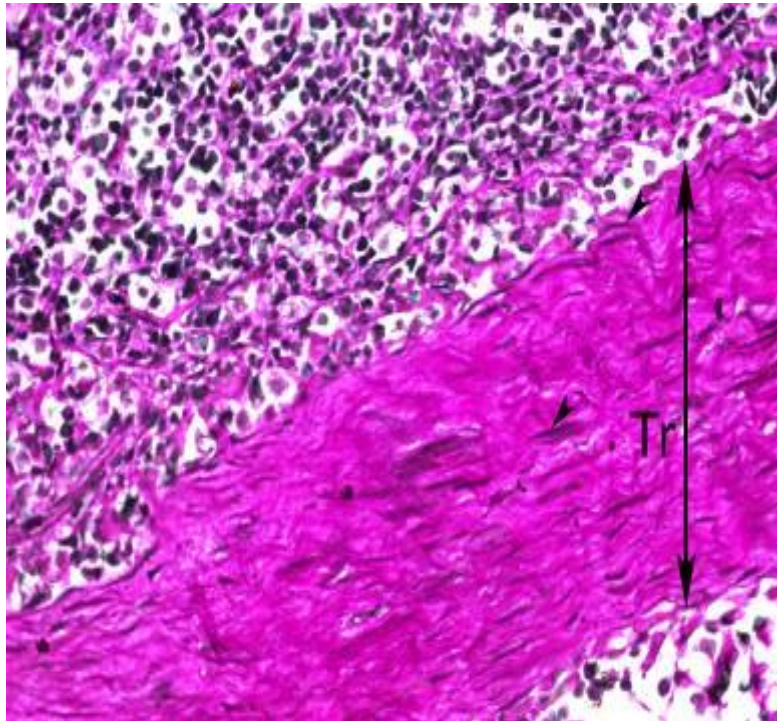
Fig.2 Photomicrograph of the supramammary lymph node of adult ewe showing connective tissue capsule and parenchyma



Ca - Capsule Co -Cortex Me -Medulla P - Parenchyma Tr - Trabeculae

Masson's Trichrome x 100

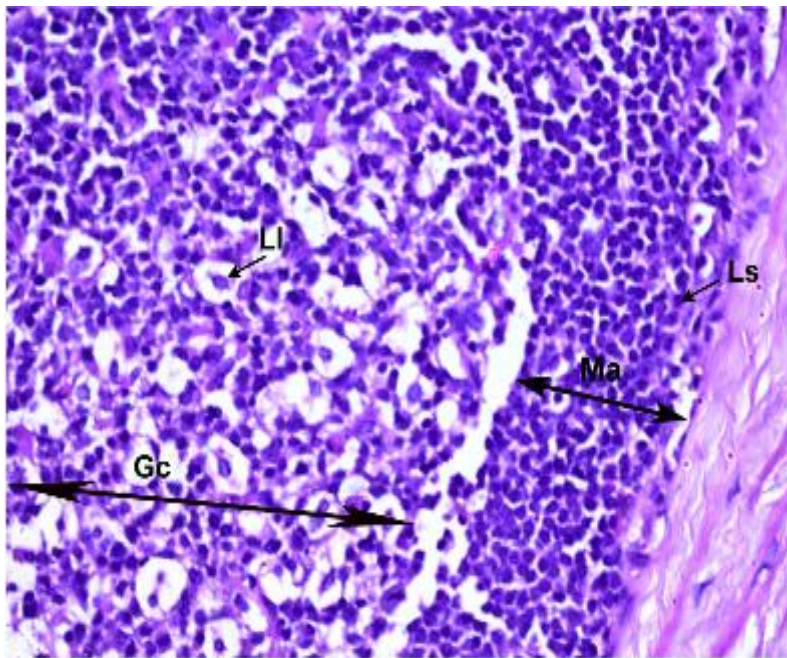
Fig.3 Photomicrograph of the supramammary lymph node of adult she-goat showing the distribution of elastic fibres (arrows) in trabeculae



Tr – Trabeculae

Verhoeff's method x 400

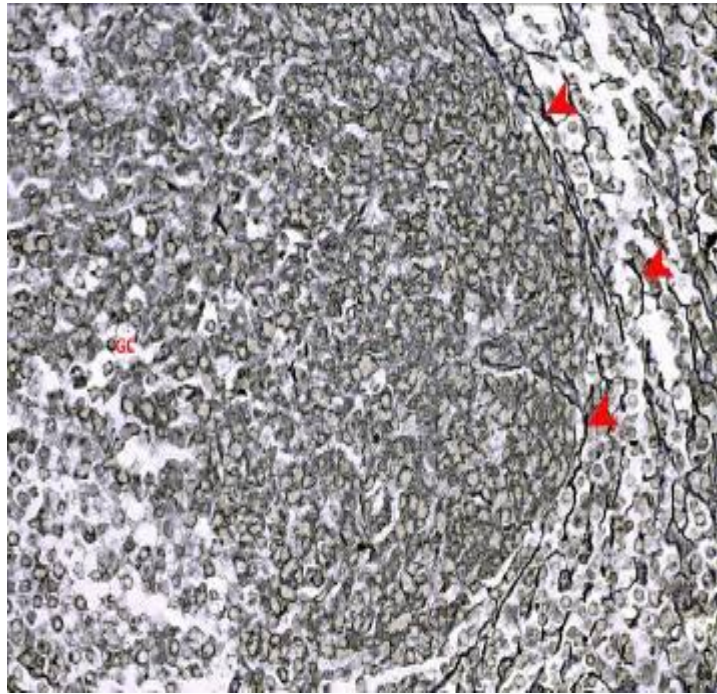
Fig.4 Photomicrograph of the supramammary lymph node of adult ewe showing germinal center



Gc - Germinal center Ll - Large lymphocytes Ls - Small lymphocytes Ma - Mantle zone

H&E x 400

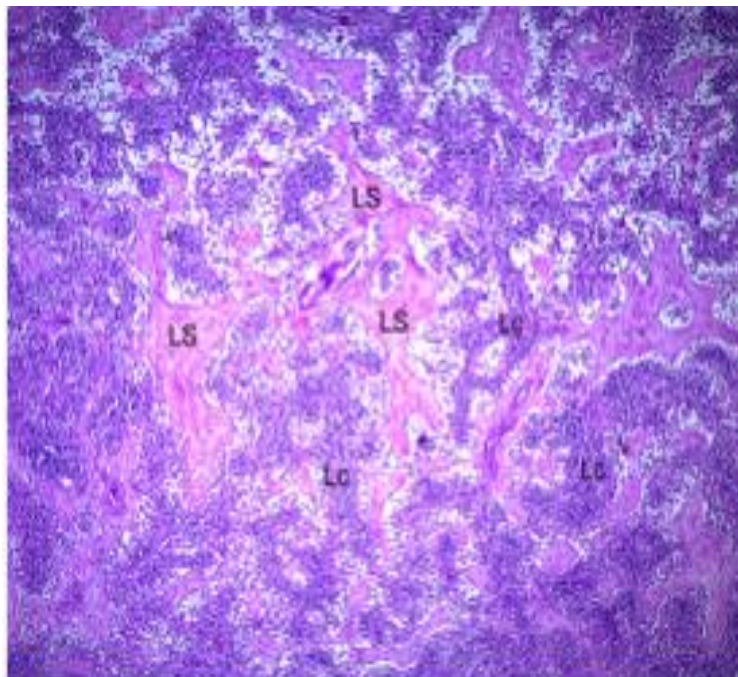
Fig.5 Photomicrograph of the supramammary lymph node of adult she-goat showing the distribution of reticular fibres (arrow heads) in lymphoid follicle



Gc – Germinal center

Gomori's method x 400

Fig.6 Photomicrograph of the supramammary lymph node of adult ewe showing medulla

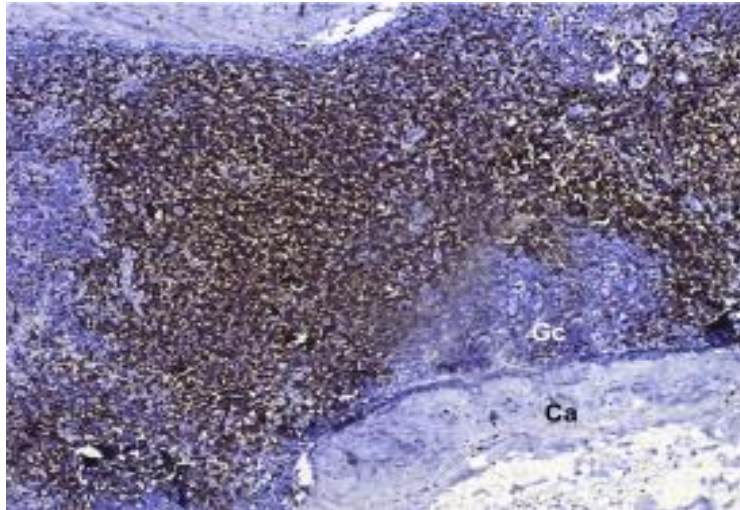


LS – Lymphatic sinuses

Lc – Lymphatic cords

H&E x 100

Fig.7 Photomicrograph of the supramammary lymph node of adult she-goat showing the distribution of 'T' lymphocytes (brown coloured cells) in lymphoid follicle

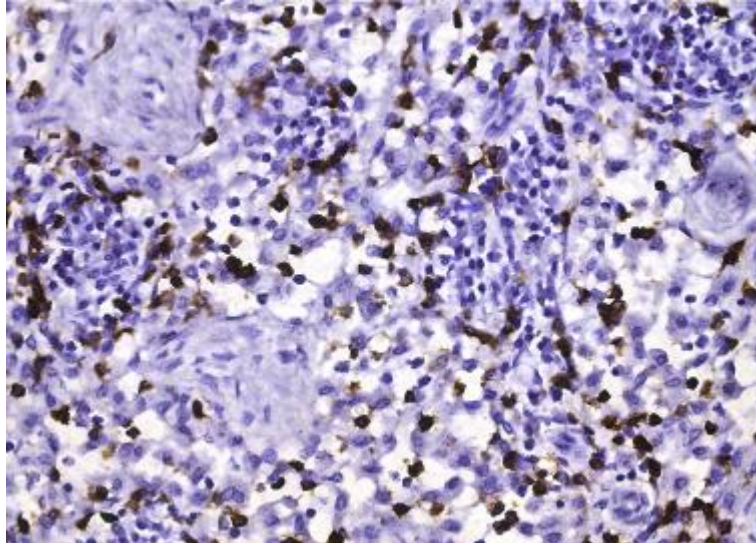


Gc – Germinal center

Ca – Capsule

IHC (DAB) x 400

Fig.8 Photomicrograph of the supramammary lymph node of adult she-goat showing the distribution of 'T' lymphocytes (brown coloured cells) in lymphoid follicle



IHC (DAB) x 400

The nucleus of cells in the germinal centre was euchromatic, whereas it was heterochromatic in mantle zone. Germinal centre diameter was $214.98 \pm 27.09 \mu\text{m}$, $166.34 \pm 17.01 \mu\text{m}$ in ewe and she-goat respectively. In the lymphatic follicle,

distribution of reticular fibres was more in the periphery and less in germinal centre (Fig. 5). The medulla of supra mammary lymph node was made of lymphatic cords separated by the lymph sinuses (Fig. 6). Similar observation was made by Sarma *et al.*, (2008) in

supramammary lymph node of Kagani goats. Various micrometric parameters *viz.*, capsule thickness, trabecular thickness, lymphoid follicle diameter, germinal center diameter and a number of follicles per field (50x) were measured and given in Table 1. It was found that statistically, no significant difference exist between supramammary lymph node of Madras Red ewe and Boer local she-goat.

Immunohistochemistry

Immunohistochemical staining for CD3⁺ 'T' lymphocytes revealed the presence of more number of CD3⁺ 'T' cells in the interfollicular region of cortex (Fig. 7) and mantle zone of the lymphoid follicle. Germinal centre of the follicle showed negative reaction, which indicated that the germinal centre consisted predominantly of 'B' lymphocytes. In medulla CD3⁺ 'T' cells were sparsely located (Fig. 8). These results were in concurrence with the findings of Christophe *et al.*, (2008) in bovine temporal and parotid lymph node and Huang *et al.*, (2018) in mandibular, bronchial and mesenteric lymph nodes in yaks and Kannan *et al.*, (2019) in hemal nodes of Indian buffalo. Soltys and Quinn, (1999) explained that, these lymphocytes got activated and migrate to the site of infection and fight against the bacterial pathogens during occurrence of mastitis in udder of cows.

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