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A. Kalita *

Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and A.H., Central Agricultural University, Selesih, Aizawl, Mizoram – 796 014, India

Email: arup.kalita@gmail.com



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Light Microscopic Study on the Peripheral Lymphnodes of Mizo Local Pig (Zo Vawk)

A. Kalita*, P.C. Kalita, P.J. Doley

Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and A.H., Central Agricultural University, Selesih, Aizawl, Mizoram – 796 014, India

Abstract

The present study provides a baseline data on histology of peripheral lymph nodes of Mizo local pig (Zo Vawk). The mean adult body weight was recorded 34.48 ± 2.18 Kg in male and 21.17 ± 2.29 Kg in female. The parenchyma of the peripheral lymph nodes revealed cortex like tissue, medulla like tissue and reticulum. Several lymphoid segments of various sizes in the cortex like tissues, referred to as “nodules”, were confirmed. Two types of hiluses were recorded viz. afferent type (A-type) and efferent type (E-type). The cortex like tissue contained primary and secondary types of nodules; however, most of the nodules were secondary. The cell population of the nodule was mainly lymphoblast cells, some with mitotic figures, lymphocytes, few macrophages along with reticular cells and plasma cells and occasional neutrophils. More concentration of immunogenic cells like macrophages was observed in the nodule which might be attributed to the better resistance to disease in Mizo local pig (Zo Vawk).

Keywords: Light Microscopy, Peripheral Lymph Node, Mizo local pig.

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INTRODUCTION

The indigenous pig of Mizoram is a non-descript breed, popularly known as Mizo local pig or Zo Vawk in Mizo language. It plays a vital role in the economic development of rural population of Mizoram. This variety of pig population is thriving under poor management condition at the hands of economically backward villagers. The rearing of this variety of pig is not a problem because of its high disease resistance capacity and adaptability to the geo climatic conditions of Mizoram and neighbouring North Eastern states of India. The pork from these animals is in high demand to the local people because of its proper meat-fat ratio. Despite its immense economic value, the population of this variety of pig is not enhanced because of less scientific approach. The anatomical and physiological status of secondary lymphoid organs is important to determine the quality of meat for human consumption. On the other hand, there is no literature available in regards to the anatomy of the peripheral lymph nodes of Zo Vawk. In view of these facts, the present investigation was undertaken to establish a baseline data of the normal histological aspects of these lymphoid organs for basic as well as applied purposes.

METHODS

The Mizo local pigs are reared in semi intensive system in the pig farm of the College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India, following the standard management procedures. Adequate feed, drinking water and health care are provided to the animals. Excess numbers of animals than the parent stock are slaughtered by using captive bolt pistol for commercial purpose. The present investigation was conducted on peripheral lymph nodes collected from twelve adult, apparently healthy Mizo local pigs of either sex, slaughtered in the college pig farm during the experimental period from 2009 to 2011. The external morphological data of the animals were recorded before slaughtering. The organs were then collected and washed properly as per the method of Habel [1] and were fixed in 10% neutral buffered formalin for histomorphology and histomorphometry. After fixation the tissues were processed for paraffin sectioning as per the method described by Luna [2]. The paraffin blocks were cut at 5 μ thickness by Leica Semimotorized Rotary Microtome and stained by Harris' haematoxylin and eosin for routine study. The special staining of the tissue was done for collagen fibres by Masson trichrome technique, for reticular fibres by Gomori's method and for elastic fibres by Verhoeff's method as described by Luna [2] and Bancroft and Stevens [3]. The histomorphological

characteristics of the lymph nodes were observed and interpreted for cellular details with the help of Olympus trinocular research microscope (BX-51) fitted with image analysis software and photomicrography attachment. The photomicrographs of selected slides were taken for typical demonstration.

The data were statistically analyzed as per the methods of Snedecor and Cochran [4] by using SYSTAT Version 6.0.1., 1996 SPSS INC software.

RESULTS

The Mizo local pig or Zo Vawk is normally small, timid and sensitive animal. However, the lactating mothers become very aggressive. External morphological observation revealed that they possessed small, straight, pointed head, and very thick and glossy hairs. They were almost similar to the "doom" variety pigs found in the neighboring North-East Indian state - Assam. However, the "pot belly" condition is more prominent in doom variety of Assam.

Nine types of peripheral lymph nodes were observed in the present study. They were: 1) Parotid, 2) Lateral Retropharyngeal, 3) Mandibular, 4) Accessory Mandibular, 5) Dorsal Superficial Cervical, 6) Ventral Superficial Cervical, 7) Prefemoral, 8) Superficial Popliteal and 9) Superficial Inguinal lymph node.

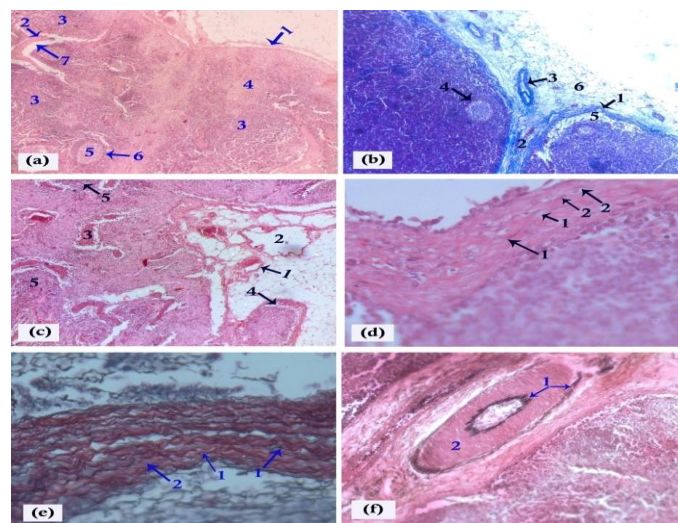


Fig.1. Histology of the peripheral lymph node of Zo Vawk. (a) Superficial inguinal LN: 1 capsule, 2 trabeculae, 3 cortex like tissue, 4 medulla like tissue, 5 germinal center, 6 secondary nodule and 7 sinuses, H&E, X50. (b) A-type hilus of mandibular LN: 1 capsule, 2 trabeculae, 3 lymph vessel, 4 nodule, 5 subcapsular sinus and 6 pericapsular fat, Masson trichrome technique, X100. (c) E-type hilus of superficial LN: 1 blood vessel, 2 lymph vessel, 3 trabeculae, 4 subcapsular sinus and 5 nodule, H&E, X50. (d) Trabeculae of ventral superficial cervical LN: 1 smooth muscle cells and 2 connective tissue cells and fibres, H&E, X400. (e) Trabeculae of ventral superficial cervical LN: 1 reticular fibres and 2 collagen fibres, Gomori's method, X400. (f) Trabeculae of superficial inguinal LN: 1 elastic fibres and 2 blood vessels. Verhoeff's method. X100.

By examining the stained paraffin sections, it was possible to identify the stroma, parenchyma and sinuses in the peripheral lymph nodes of adult Mizo local pigs (Fig. 1a). The stroma consisted of capsule, trabeculae and reticulum; whereas parenchyma was clearly divided into "cortex like tissue" and "medulla like tissue". The cortex like tissue contained several lymphoid segments of various sizes which may be referred to as "nodules". The sinuses were of subcapsular and trabecular types (Fig. 2b).

On the external surface of the lymph nodes two types of hiluses were recorded. They were afferent type (A-type hilus) and efferent type (E-type hilus). In afferent type, the lymph vessels entered the node and were associated with each nodule (Fig. 1b), whereas in efferent type, the lymph vessels exited the node and located between the nodules (Fig. 1c). The blood vessels entered with the afferent lymph vessels and exited with the efferent vessels.

Capsule and trabeculae:

The capsule of the peripheral lymph nodes was found to be surrounded by connective tissue and pericapsular fat (Fig. 1b). Both the capsule and trabeculae were made up of dense white fibrous connective tissue (Fig. 1b, 1d). The trabeculae were found to be more prominent in superficial inguinal lymph nodes compared to the other lymph nodes. Some of the trabeculae were originated from the capsule, and terminated without much penetration into the deeper part of the parenchyma; whereas the others were small and dispersedly located in the parenchyma in between the nodules (Fig. 1c). The capsule contained blood vessels with lymphatics (Fig. 1b) which were lined by loosely arranged endothelial cells.

The capsule and trabeculae contained variable amounts of smooth muscle cells, which were oriented parallelly with the connective tissue fibres (Fig. 1d); however, it was not consistent in all the lymph nodes under study. Higher concentration of smooth muscle cells were observed in the trabeculae than in the capsule. The capsule and the trabeculae of both cortical and medullary regions exhibited predominantly collagenous fibres (Fig. 1b) and in certain areas, some reticular fibres were also evident (Fig. 1e) in the inner part of the capsule forming an inner lamina. These fibres were observed in very less amount in the middle portion of the capsules. Less amount of elastic fibres were also found both in the capsules as well as trabeculae. Blood vessels present in the capsules and trabeculae contained large amount of elastic fibres (Fig. 1f).

The quantitative parameters recorded in the present histological work included thickness of the capsule and trabeculae of individual peripheral lymph nodes

(Table 1). The thickness of the capsule varied amongst the peripheral lymph nodes under study. It was found to be more at the hilus, where many blood vessels and lymphatics were present (Fig. 1b). None of the peripheral lymph nodes under study showed significant differences in regards to the mean capsular and trabecular thickness between the right and left nodes. Mean capsular thickness was found to be maximum in case of prefemoral lymph node and the minimum was found in the superficial popliteal lymph node. The mean values of trabecular thickness in all the peripheral lymph nodes (irrespective of right and left) were found to be more than those of the capsule. Mean thickness of the trabeculae was found to be maximum in the superficial inguinal lymph node and minimum in the superficial popliteal lymph node (Table 1).

Cortex like tissue:

The cortex like tissue of the peripheral lymph nodes were diffusely distributed in the central area of the lymph nodes (Fig. 1a) and in the subcapsular area of the A-type hilus (Fig. 1b). Most of the nodules occupied a deep position along with trabecular sinuses (Fig. 1a, 1c). However, nodules were also observed near the subcapsular area (Fig. 1b). These nodules were of primary and secondary types and they were mostly secondary in nature. The secondary nodules had a centrally located pale area (germinal center) containing large lymphocytes. The germinal centers were surrounded by a layer of densely packed small lymphocytes (corona) (Fig. 1a, 2a).

The primary lymphatic nodules (Fig. 2b) revealed mostly small lymphocytes with very few macrophages and plasma cells. The cell population of the germinal centre of the secondary lymphatic nodules was mainly composed of lymphoblast cells, some with mitotic figures, few macrophages along with reticular cells and plasma cells (Fig. 2c). The population of the lymphoblast cells was found to be reducing towards the periphery of the germinal center, where small lymphocytes were predominant. Occasionally neutrophils were also observed in the germinal center. The corona layer consisted mainly of small lymphocytes. The post capillary venules were observed in the deep cortex like tissues near the group of nodules. They had a swollen endothelium with few lymphocytes and red blood cells in the lumen (Fig. 2d).

The quantitative parameters recorded in the cortex like tissues of the peripheral lymph nodes of adult Mizo local pig included diameter of the lymphatic nodules and their germinal centers (Table 1).

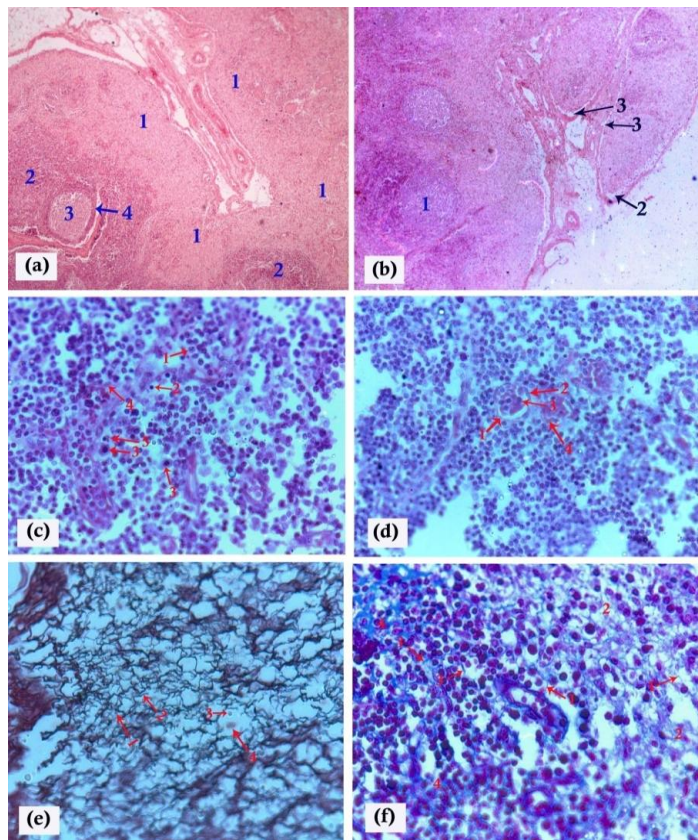


Fig.2. Histology of the peripheral lymph node of Zo Vawk. (a) Parotid LN: 1 medulla like tissue, 2 cortex like tissue, 3 germinal center, 4 nodule, H&E, X100. (b) Parotid LN: 1 primary nodule, 2 subcapsular sinus and 3 trabecular sinus, H&E, X50. (c) Germinal center of Sup. inguinal LN: 1 lymphoblast cell, 2 small lymphocyte, 3 macrophage, 4 reticular cell and 5 plasma cell, H&E, X400. (d) Post capillary venules of Sup. inguinal LN: 1 post capillary venule, 2 swollen endothelium, 3 lymphocyte and 4 red blood cell, H&E, X400. (e) Medullary tissue of sup. inguinal LN: 1 reticular cell, 2 reticular fibre, 3 lymphocyte and 4 macrophage, Gomori's method, X400. (f) Sup. inguinal LN: 1 fine collagen fibres, 2 medulla like tissue, 3 coarse collagen fibres and 4 cortex like tissue, Masson trichrome technique, X400.

Presently, the mean values of the diameters of lymphatic nodules and their germinal centers in both right and left sides were found to be maximum in mandibular lymph node. However, minimum mean values were recorded in superficial popliteal lymph node (Table 1).

Medulla like tissue:

The medulla like tissues of peripheral lymph nodes of adult Mizo local pigs were observed both in the peripheral regions (Fig. 2a) of E-type hilus and in the internal regions. They consisted of many reticular cells with reticular fibres in which lymphocytes, few plasma cells and macrophages were suspended (Fig. 2e). The sinuses were narrow and the typical medullary cords were absent. The medullary trabeculae were composed predominantly of collagen fibres with very few

reticular cells. The collagen fibres present in the medulla like tissue were finer than that observed in the cortex like tissue (Fig. 2f). The elastic fibres were also very scanty in the medullary trabeculae, but were distinctly present within and around the blood vessels (Fig. 1f).

Reticulum:

The reticulum of the peripheral lymph nodes was composed of reticular fibres along with large, stellate reticular cells. Fine reticular fibres were uniformly distributed in the primary lymphatic nodules, while, thicker reticular fibres were observed at the periphery of the secondary lymphatic nodules.

Sinuses:

The sub capsular sinuses of the peripheral lymph nodes were observed in between the capsule and the parenchyma of the lymph nodes. They were criss-crossed by a meshwork of reticular fibers. The meshwork lodged lymphocytes, free macrophages and plasma cells. The trabecular sinuses originated from the sub capsular sinuses. They were narrower than the sub capsular sinuses (Fig. 2b). Both the sub capsular and trabecular sinuses were lined by flat endothelial cells.

DISCUSSION

The topographical locations of peripheral lymph nodes observed in the present study were similar to that of the common large breed of pigs [5] [6] [7], dog [8] and buffalo [9].

In the present investigation, most of the peripheral lymph nodes were single on each side. This finding was contrary to the findings in common large breed pigs [6] [7] where these nodes were found in groups. However, the mandibular lymph nodes were found in groups and occasionally double nodes were recorded in case of lateral retropharyngeal, accessory mandibular and mandibular lymph nodes. These variations in the number of lymph nodes might be considered as the characteristic of Mizo local pig. The biometrical values in regards to length, width, thickness and weight (Table 2) were lesser as compared to other common large breed pigs [6] [7] might be due to the size of the animal.

Histologically, the peripheral lymph nodes of adult Mizo local pigs were found to be composed of stroma, parenchyma and sinuses. The hilus were of two types. In afferent type, the lymph vessels entered the node and were associated with each nodule, whereas in efferent type, the lymph vessels existed the node and located between the nodules as reported in pigs [10].

The capsule and trabeculae were made up of dense white fibrous connective tissue as reported in ruminants [11] and in buffalo [12]. The trabeculae were more prominent in superficial lymph nodes.

Sl No	Lymph Node	Side	Capsular Thickness (μm) Mean (\pm S.E.)	Trabecular Thickness (μm) Mean (\pm S.E.)	Diameter of Lymphatic Nodule (μm) Mean (\pm S.E.)	Diameter of Germinal Centre (μm) Mean (\pm S.E.)
1	Parotid	Right	19.890 \pm 1.58	59.932 \pm 9.42	365.080 \pm 53.49	279.140 \pm 42.30
		left	19.670 \pm 1.38	61.062 \pm 9.22	375.074 \pm 53.48	278.766 \pm 42.06
		Overall	19.780 \pm 0.97 (15.60 - 4.36)	60.497 \pm 6.22 (24.84 - 79.18)	370.077 \pm 35.70 (192.67 - 526.76)	278.953 \pm 28.12 (140.73 - 371.30)
2	Lateral Retropharyngeal	Right	13.165 \pm 1.97	58.206 \pm 10.33	140.084 \pm 15.87	111.456 \pm 11.70
		left	14.580 \pm 1.83	67.634 \pm 8.46	138.012 \pm 14.72	102.676 \pm 13.01
		Overall	13.873 \pm 1.29 (8.32 - 19.93)	62.920 \pm 6.49 (32.04 - 85.46)	139.048 \pm 10.21 (84.46 - 176.40)	107.066 \pm 8.38 (72.59 - 138.10)
3	Dorsal Superficial Cervical	Right	15.898 \pm 1.76	50.316 \pm 15.92	250.820 \pm 39.44	147.984 \pm 29.13
		left	15.378 \pm 1.27	49.748 \pm 16.89	250.100 \pm 38.89	148.060 \pm 28.80
		Overall	15.638 \pm 1.03 (11.26 - 20.20)	50.032 \pm 10.94 (17.2 - 115.20)	250.460 \pm 26.11 (182.60 - 404.50)	148.022 \pm 19.31 (96.12 - 261.10)
4	Ventral Superficial Cervical	Right	25.014 \pm 8.13	60.986 \pm 9.11	312.420 \pm 8.38	219.180 \pm 10.69
		left	24.822 \pm 7.59	60.816 \pm 9.06	311.520 \pm 8.45	218.600 \pm 10.39
		Overall	24.918 \pm 5.24 (10.47 - 55.64)	60.901 \pm 6.06 (35.68 - 90.53)	311.970 \pm 5.61 (280.60 - 329.90)	218.890 \pm 7.03 (189.10 - 247.80)
5	Prefemoral	Right	32.084 \pm 4.69	51.760 \pm 8.91	323.080 \pm 48.62	224.366 \pm 46.08
		left	30.802 \pm 4.67	58.650 \pm 5.91	285.440 \pm 55.04	200.760 \pm 41.69
		Overall	31.443 \pm 3.13 (19.08 - 6.13)	55.205 \pm 5.17 (34.88 - 84.45)	304.260 \pm 35.18 (165.60 - 480.80)	212.563 \pm 29.56 (65.93 - 351.50)
6	Superficial Inguinal	Right	21.566 \pm 1.88	64.284 \pm 11.48	313.960 \pm 41.53	215.880 \pm 39.13
		left	20.966 \pm 2.45	63.644 \pm 12.03	312.840 \pm 40.24	214.080 \pm 37.86
		Overall	21.263 \pm 1.46 (13.18 - 7.49)	63.964 \pm 7.84 (38.13 - 99.03)	313.400 \pm 27.26 (213.30 - 410.30)	214.980 \pm 25.67 (107.20 - 310.50)
7	Mandibular	Right	20.232 \pm 3.00	61.960 \pm 10.20	453.340 \pm 101.48	374.840 \pm 96.49
		left	19.660 \pm 3.04	47.754 \pm 3.73	312.360 \pm 41.80	226.420 \pm 38.46
		Overall	19.946 \pm 2.01 (14.59 - 1.72)	54.857 \pm 5.64 (36.34 - 98.43)	382.850 \pm 56.82 (206.60 - 840.80)	300.630 \pm 54.86 (141.00 - 732.70)
8	Accessory Mandibular	Right	15.784 \pm 2.23	47.154 \pm 3.19	302.840 \pm 30.09	191.600 \pm 20.20
		left	14.062 \pm 1.30	46.128 \pm 5.37	289.740 \pm 25.50	230.460 \pm 27.92
		Overall	14.923 \pm 1.25 (10.29 - 21.54)	46.641 \pm 2.95 (31.20 - 63.73)	296.290 \pm 18.72 (224.50 - 381.90)	211.030 \pm 17.49 (142.30 - 335.50)
9	Superficial Popliteal	Right	10.851 \pm 0.77	19.152 \pm 1.08	121.668 \pm 12.73	90.584 \pm 11.62
		left	9.155 \pm 0.76	18.962 \pm 1.46	132.200 \pm 6.97	99.990 \pm 9.71
		Overall	10.003 \pm 0.58 (7.592 - 12.28)	19.057 \pm 0.86 (15.37 - 23.58)	126.934 \pm 7.06 (76.54 - 152.30)	95.287 \pm 7.31 (53.68 - 125.30)

* Figures in parentheses indicate the range of the data

Table 1: MEAN \pm SE for the Micrometry of various histomorphological structures of peripheral lymph nodes of adult Mizo local pig (Zo Vawk)

Bloom and Fawcett [13] also stated that the trabeculae were prominent in large sized peripheral lymph nodes in ruminants. The capsule contained blood vessels with lymphatics. The thickness of the capsule was found to be more at the hilus, where many blood vessels and lymphatics were present. These findings could not be compared due to lack of available literature. High concentration of smooth muscle cells were observed in the trabeculae than the capsule. Similar types of findings were also reported in bovine [12] [14] [15] [16] [17] and in fallow deer [18]. The capsule and trabeculae of both cortical and medullary regions were found to be composed predominantly of collagenous fibres with reticular and scanty elastic fibers. The present findings were in accordance with the findings of Hosletler [19], Folse *et al.* [20], Copenhagen *et al.* [21], Dellmann and

Eurell [22] and Singh *et al.* [12]. Blood vessels present in the capsule and trabeculae also contained large amount of elastic fibres.

The parenchyma of peripheral lymph nodes of Mizo local pigs was clearly divided into "cortex-like tissue" and "medulla-like tissue". The cortex like tissues were located in the central area of the lymph nodes as also reported in pigs [23] and in the subcapsular area around the A-type hilus. They were composed of both primary and secondary lymphatic nodules, which was in agreement with the findings in buffalo [12]. Most of the nodules observed in the present study were secondary in nature, contained a centrally located pale area (germinal center). The cell population of the germinal center of the secondary lymphatic nodules was mainly composed of lymphoblast cells, some with mitotic

figures, few macrophages along with reticular cells and plasma cells as reported in the lymph nodes in buffalo [12]. The medulla like tissues were distributed both in the peripheral regions around the E-type hilus and in the internal regions. Similar findings were also recorded in pigs [10] [24]. The medulla like tissues were composed of meshworks of reticular and collagen fibres. The collagen fibres present in this region were finer and denser than those of the cortex like tissues as also found in pig [10]. The medullary sinuses were narrow and the typical medullary cords were absent as also observed in pigs [23]. The post capillary venules were observed in the deep cortex like tissues near the group of nodules as also recorded in pigs [23]. The reticulum of the peripheral lymph nodes was composed of reticular fibres along with large, stellate reticular cells. Dellmann and Brown [25] also mentioned that the reticulum of the lymph nodes in ruminants was made up of the reticular cells and reticular fibres. The subcapsular sinuses were criss-crossed by a meshwork of reticular fibers. The meshwork lodged lymphocytes, free macrophages and plasma cells. Similar findings were also reported in buffalo [12].

Histomorphometrical observations revealed no significant differences in regards to the mean capsular and trabecular thickness between the right and left nodes. Mean capsular and trabecular thickness were found minimum in superficial popliteal lymph nodes; whereas the maximum value was recorded in prefemoral and superficial inguinal lymph node, respectively. These values could not be compared because of non availability of literature in pig. However, highest capsular and trabecular thickness were found in the prefemoral lymph node in adult buffalo [9]. In regards to the mean values of the diameters of lymphatic nodules and their germinal centers were found to be maximum in mandibular lymph node and minimum in superficial popliteal lymph node. No literature in regards to the histomorphometry of the lymphatic nodules and the germinal centers was available in pig to compare to the present findings. However, Bagi *et al.* [9] reported that, among the superficial lymph nodes of adult buffalo, lymphatic nodules with the largest diameter was found in the parotid lymph node, while the lowest value was recorded in the mandibular lymph node. Similarly, germinal centers with highest diameter were found in the prefemoral lymph node and that with the lowest diameter in case of the mandibular lymph node in buffalo. The findings of Bagi *et al.* [9] were not similar with the present study, might be due to the species variation.

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