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The architecture of the lymph nodes in the abdominal and thoracic cavities of wild boar

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ABSTRACT

The distrubition of lymph nodes located in the abdominal and thoracic cavities of ten wild boars, and their structure were determined anatomically, histologically and immunohistochemically, to be the first detailed investigation on the wild boar. Though general localization and distribution were similar, the number of lymph nodes showed small differences from those of domestic pig. Histological investigations did not reveal a significant hilus. Besides, T lymphocytes with anti-CD3, CD4, CD8, B lymphocytes with anti-CD79a, macrophages with anti-macrophage monoclonal antibodies, and follicular dendritic cells using anti-S100 polyclonal antibody and their distrubution in the lymph nodes were detected. Many CD3 positive T lymphocytes were observed in the germinal center of the lymph follicles, in the cortical area and in the medulla. CD8 positive T lymphocytes were few, and CD4 positive T lymphocytes were not seen. CD79 positive cells were scanty.

Key words: Anatomy, Histology, Immunohistochemistry, Lymphatic system, Wild boar.

INTRODUCTION

Lymph nodes are organs comprised of lymphoid tissue, scattered along the lymphatic vessels. They are capsulated, annular or kidney-shaped (Saar and Getty, 1963; Tipirdamaz, 2000; Junqueira and Cameiro, 2006). The lymphatic drainage of the thoracic cavity is complicated and differs among species (Dyce *et al.*, 2002). Lymph nodes located in the abdominal cavity are comprised of two parts-those of the abdominal wall and of the organs located in the cavity proper. Lumbar lymph center and lateral and medial iliac lymph nodes are the lymph nodes of the abdominal wall (Sarsilmaz *et al.*, 1994; Dursun, 1999; Tipirdamaz, 2000; Dyce *et al.*, 2002). The lymph nodes of the organs located in the abdominal cavity are the celiac and, cranial and caudal mesenteric lymph centers. Lumbar lymph center comprises of renal and testicular lymph nodes, as described earlier only in pig (Dursun, 1999; Dyce *et al.*, 2002).

Medullary tissue was located in peripheral area of the lymph node of the pig. The cortical tissue with most of the lymphoid follicles along the trabecular sinuses was located at the center of the lymph node (Bacha and Bacha, 2000; Tingstedt *et al.*, 2003; Eurell and Frappier, 2006). Based on this different structure, lymph drainage is atypically reverse. For this reason, these lymph nodes are named "reverse type of lymph nodes" (Hoshi *et al.*, 1986; Bacha and Bacha, 2000). Earlier Reports of T lymphocytes and their subgroups, B lypmhocytes, macrophages, plasma cells in the lymph nodes of the domestic pigs were based on presence and distribution of the same (Jonjic *et al.*, 1987; Chianini *et al.*, 2001; Tingstedt *et al.*, 2003; Eurell and Frappier, 2006). S-100 protein is a calcium binding protein (Heizmann *et al.*, 2002) and S-100 positive cells were detected in domestic pigs (Sugimura *et al.*, 1990).

The wild boar is one of the wild animals, pervasively live in Turkey and hunted by persistence hunting. Detailed research articles on the anatomic, histologic and immunohistochemical examinations of the lymph nodes of the wild pig are scanty. In the study performed, normal distribution and architecture of lymph nodes of the wild boar which form an important component of the immune system were put forward, to contribute scientifically to the future studies.

MATERIALS AND METHODS

In the study carried out, lymph nodes of ten hunted wild boar, *viz.*, seven male and three female, were collected in the season when the hunting was allowed by Central Hunting Commision. The present study was approved by our local MKU Animal Experiment Local Ethical committee. Following dissection, the thoracic and abdominal cavities were examined for lymph nodes, as these are the reported areas of lymph node localization in domestic pig (Schummer *et al.*, 1981; Dyce *et al.*, 2002; NAV, 2012).

For histological examinations, the lymph nodes found in the thoracic and abdominal cavities were removed and fixed in 10% buffered formalin solution for 24 h.

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Following routine histological procedures, 5 µm serial sections were prepared. Crossman's modified triple staining method was applied to study the general structure of the lymph node (Denk *et al.,* 1989). Methyl green-pyronin stain was used to determine plasma cells (Bancroft and Cook, 1984). Reticular fibres and reticular cells were visualised by Gordon and Sweet's silver staining method (Bancroft and Cook, 1984).

For immunohistochemistry, standard avidin-biotin-peroxidase (ABC) technique was performed using 5 µm thick slides. Antigen retrival was performed by the use of sodium citrate buffer (pH: 6.0). Another antigen retrival method also performed for CD4 and CD8 antibodies (0.25% Triton X-100). In this text, CD is used for the abbreviation of cluster of differentiation. Endogen Peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. The working dilutions and the sources of antibodies used are listed in Table 1. UltraVision Large Volume Detection System (Lab Vision Corp; Thermo Fisher Scientific Inc., Fremont, CA) was applied to the sections for 10 minutes. AEC was used as chromogen. Mayer's heamatoxylin was used for counterstaining. All the washings were made with Phosphate buffer saline (PBS, 0.01 M, pH: 7.4). PBS was used instead of primary antibodies for negative controls. Olympus BX50 research microscope was used to evaluate the results.

RESULTS AND DISCUSSION

Thoracic cavity of wild boars exhibited, 2-5 thoracic aortic, 1-2 cranial sternal, 1-3 cranial mediastinal, 4-9 caudal mediastinal, 1-2 right tracheobronchial, 1-3 left tracheobronchial, 1-2 middle tracheobronchial and 1-2 cranial tracheobronchial lymph nodes. Lymph nodes of the thoracic cavity were round, plane or oval in shape. Their sizes changed between 3- 20 mm in length and 3-15 mm in width (Fig. 1A). In the study performed it is found out that the number of lymph nodes were a little different from the domestic pig, while general localization and distrubition were similar. It is also observed that lymph nodes were located close to each other in case they belong to neighbouring lymph centers (Schummer *et al.,* 1981; Dyce *et al.,* 2002). Numbers of the wild boar represents a little difference compared with the numbers given for domestic pigs (Schummer *et al.,* 1981; Dyce *et al.,* 2002). Schummer *et al.* (1981) observed 2-65 mm sized lymph nodes located in the thoracic cavity of the domestic pigs. Results of the present study revealed that size of the lymph nodes of the wild boar were 3-20 mm in length and 3-15 mm in width, much smaller than the domestic pigs.

Abdominal cavity of wild boars had 8-15 lumbar aortic, 3-5 renal, 1 phrenico-abdominal, 1-3 gastric, 1-2 hepatic, 3-5 pancreatico duodenal, 2-5 celiac, 1-4 splenic, 1-3 cranial mesenteric, 2-5 iliocolic, 5-20 colic, 6-12 caudal mesenteric, 2-5 medial iliac, 1-2 lateral iliac and 30-80 jejunal lymph nodes. Lateral iliac lymph nodes were not observed in five cadavers, whereas the phrenico-abdominal lymph nodes were absent in six. Of the seven male wild boars examined, only three presented the testicular lymph node, one each. No uterine lymph node was observed in any of the females. Jejunal lymph nodes were observed as 8-20 cm long chains of 10-30 nodules each, embedded in mesojejunum. Colic lymph node was located at the conical center of the helix, as a chain of 5-20 nodules. Lymph nodes of the abdominal cavity were varied in shape and in size. The size of the lymph nodes showed significant differences depending upon their localization. Nodules of the medial iliac lymph nodes had a maximum of 10-60 mm length and 10-30 mm width. The smallest nodules of 2-12 mm in length and 2-10 mm in width belonged to the caudal mesenteric lymph nodes. Size of the other lymph nodes varied in this scale (Fig. 1B,C,D,E,F). The data (Schummer *et al.,* 1981; Sarsilmaz *et al.,* 1994; Dursun, 1999; Tipirdamaz, 2000; Dyce *et al.,* 2002) indicated that lumbar lymph center, lateral and medial iliac lymph centers belonged to the wall of the abdominal cavity, whereas the celiac lymph center, and cranial and caudal mesenteric lymph centers were located close to the organs in the abdominal cavity and the abdominal aorta (Dursun, 1999; Dyce *et al.,* 2002). The present study revealed that the localization of the lymph nodes were similar to that in the domestic pig. All the known lymph nodes of the domestic pig were also seen in the wild boar except uterine lymph node. It is also stated that lateral iliac, phrenico-abdominal, testicular and uterine lymph nodes might not be seen always (Schummer *et al.,* 1981; Dyce *et al.,* 2002). With a little difference in the number, all the lymph nodes were seen in the wild boar except the uterine lymph node in females. Schummer *et al.* (1981) also indicated that there's been variation in the size of the lymph nodes. According to earlier reports (Schummer *et al.,* 1981), hepatic and colic

Table 1: Details of the primary antibodies used in the immunohistochemistry in wild boar.

Specificity	pAb/mAb (clone) *	Reactivity	Source	Dilution
CD3	mAb (SP7)	T lymphocyte	Abcam	1:100, (overnight/ 4° C)
CD8	mAb (CC63)	cytotoxic T lymphocyte	Serotec	1:10, (overnight/ room temperature)
CD4	mAb (CC30)	helper T lymphocyte	Serotec	1:10, (overnight/ room temperature)
CD4	mAb (74-12-4)	helper T lymphocyte	Abcam	1:10, (overnight/ room temperature)
CD79a	mAb (HM57)	B lymphocyte	Abcam	1:25, (overnight/ 4° C)
L1	mAb (MAC387)	granulocyte,monocyte, macrophage	Abcam	1:100 (2 h/ room temperature)
S100	pAb	Interdigitating reticular cells	Abcam	1µg/ml (15min/ room temperature)

*mAb: monoclonal Antibody, pAb: polyclonal Antibody.

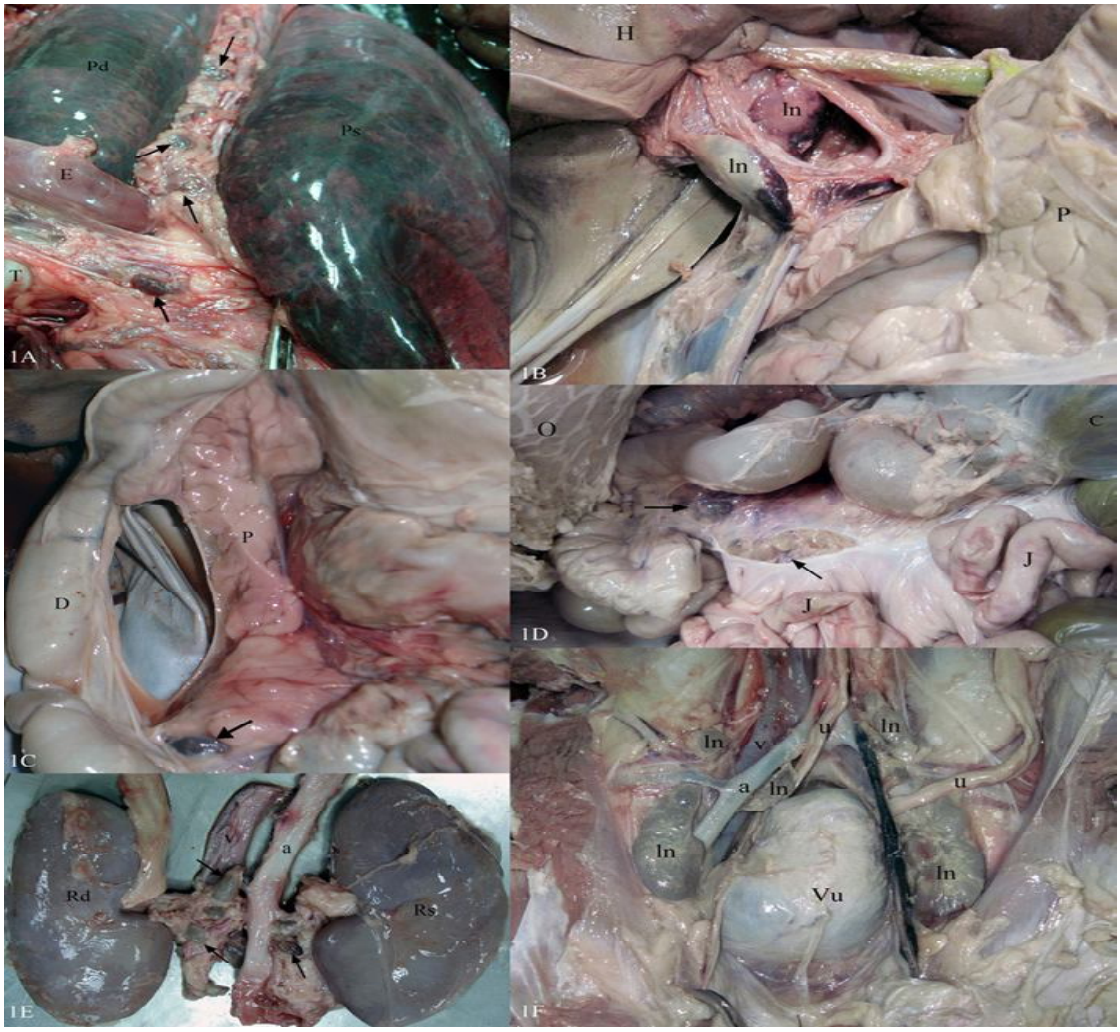


Fig 1: The anatomical view of the lymph nodes in wild boar. A) Pd: pulmo dexter (right lung), Ps: pulmo sinister (left lung), E: esophagus, T: trachea, Arrows: mediastinal lymph nodes. 1B) H: hepar (liver), P:pancreas, ln: hepatic lymph nodes. 1C) D: doudenum, P: pancreas, arrow: pancreaticodoudenal lymph node. 1D) C: colon, J: jejenum, O: omentum, arrows: cranial mesenteric lymph nodes. 1E) a: abdominal aorta, v: caudal vena cava, Rd: ren dexter (right kidney), Rs: ren sinister (left kidney), arrows: renal lymph nodes. 1F) Vu: urinary vesicle, u: ureter, ln: medial iliac lymph nodes, u: ureter, a: external iliac artery, v: external iliac ven.

lymph nodes grow up to 88 mm and 90 mm in length respectively, while the length of the others remained between 2 - 60 mm. In the present study, the biggest lymph node was medial iliac lymph node with 10 - 60 mm length and 10 - 30 mm width; with the smallest being caudal mesenteric lymph nodes with 2 – 12 mm length and 2 - 10 mm width.

The lymph nodes were encapsulated by irregular compact connective tissue (Fig 2A). Capsule was continuous with trabeculae that extended through the organ. Trabeculae branched through the cortex-like tissue (Fig 2 A and B). Both the capsule and the trabeculae had collagen and elastic fibers. Smooth muscle cells and fibroblasts were also seen in the capsule (Fig 2C). It is stated that the capsule and the trabeculae of the lymph node of the wild boar were similar to the domestic pig, comprising of nodular units and medulla located at the center of the lymph node and the cortex at the peripheral of the organ (Hoshi *et al.,* 1986; Ramos *et al.,* 1990; Bacha and Bacha, 2000; Eurell and Frappier, 2006).

No significant hilus was observed, except where afferent lymph vessels were approaching the organ (Fig 2D). Trabeculae were seen at the center of the nodular units and the trabecular lymph vessels were surrounded by peri-trabecular sinuses (Fig 2B). Many efferent lymph vessels left the surface of the lymph node (Fig 2A). In the hilus, where afferent lymph vessels entered the organ, the cortical tissue was denser, while medulla was a little denser where efferent lymph vessels left the organ (Fig 2A, D). Afferent lymph vessels enters the capsule from one or more points via large trabeculae and invades to the organ by the lymph nodes connecting to the trabecular sinuses (Spalding and Heath, 1989; Eurell and Frappier, 2006). A typical structure might not be seen for the hilus, instead microscopic hilus-

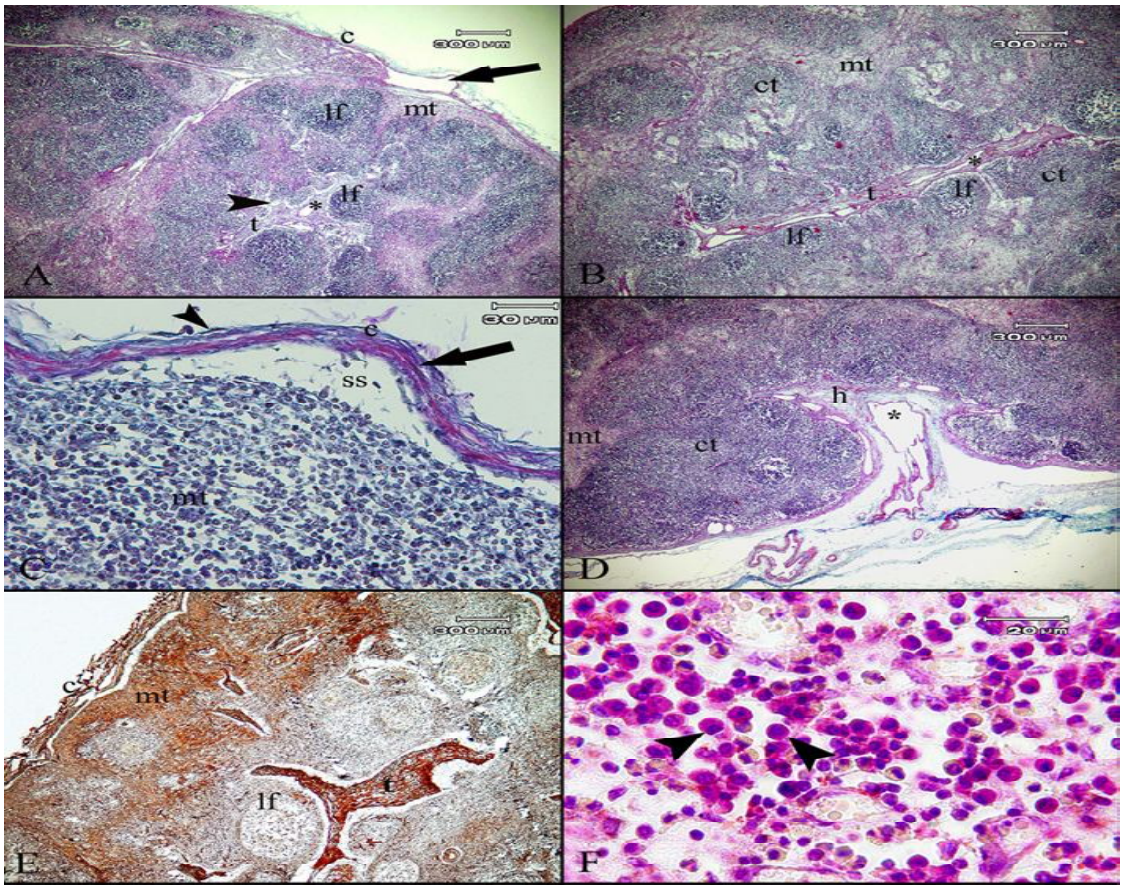


Fig 2: The histological view of the lymph node of the wild boar. A, B) c: capsule, ct: cortical tissue, lf: lymph follicle, mt: medulla-like tissue, t: trabecula, *: lymph vessel inside the trabeculae, arrow: efferent lymph vessel, arrow head: trabecular sinus. C) c: capsule, ss: subcapsular sinus, arrow: smooth muscle cells, arrow head: fibroblast. D) h: hilus-like area, ct: cortical tissue, mt: medulla-like tissue, arrow: afferent lymph vessel (A,B,C,D Triple stain). E) Reticular fibers in lymph node of the wild boar. c, capsule, lf: lymph follicle, mt: medulla-like tissue, t: trabecula, Gordon and Sweet's silver stain. F) arrow heads: Plasma cells. Methyl green-pyronin.

like indents can be seen at the places where the afferent lymph vessels enters the organ. Since a few small lymph nodes might have formed a large node stack, it is hard to determine the localization of the hilus in the pig lymph nodes (Eurell and Frappier, 2006). Of the two types of hilus reported in the pig, it is stated that in the mizo local pig, one of these hiluses was the afferent type located where the afferent lymph vessels enters the organ and related with each follicle; and the other one was the efferent hilus located among the lymph follicles with the efferent vessels leaving the organ both from this point and from the convex surface of the organ (Hoshi *et al.*, 1986; Kalita *et al.*, 2014). In the present study, a hilus-like indent was only located where the afferent lymph vessels enters the organ, with the structure of hilus lacking at the location of the efferent lymph vessels. Hence it can also be concluded that the afferent type hilus-like areas were present in the lymph node of the wild boar while efferent type are not.

The lymph nodes were made of nodular units. The subcapsular sinus was located just under the capsule mostly (Fig 2C), but rarely medulla-like tissue was seen to replace the former under the capsule (Fig 2A). The cortex and medulla were easily identified in the parenchyma (Fig. 2A). The cortex-like tissue was composed of nodules located at the center of the lymph nodes (Fig 2B). The medulla-like tissue located in the periphery under the capsule, varied in thickness between different parts of the organ and was made of a lot of reticular cells and fibers (Fig 2E). The cortex-like tissue comprised of two parts- follicular and dense cortex (Fig 2A, B). The primary and the secondary lymph follicles were observed in the cortex-like tissue. A pale-stained germinal center was seen in the middle of each secondary lymph follicle. The mantle zone stained darker at the periphery of the germinal center where lymphocytes are denser and huddled. The cortex-like tissue had less reticular cells and fibers compared to the medulla-like tissue (Fig 2E). Plasma cells were seen in the medulla-like tissue and at the border of dense cortex with the latter. The dense cortex had very few plasma cells. The plasma cells were also randomly seen in the trabecular connective tissue (Fig 2F). The cortex-like structure had less reticular cells and fibers compared with the medulla-like structure. The plasma cells

were fewer in the dense-cortex; and less in the medulla-like structure confirming the earlier reports (Spalding and Heath, 1989; Kalita *et al.*, 2014). Besides, there has not been any other information in the literature that the plasma cells were a little more in number where the medulla-like tissue meets with the border areas of the dense cortex, with very few plasma cells found in the trabecular connective tissue.

CD3 positive T-lymphocytes were seen in the germinal center of the lymph follicles, dense cortical area and medulla-like tissue (Fig 3A, B). Positive results are achieved using pAb (polyclonal antibody) Anti-CD3 primary antibody in the interfollicular areas of the domestic pigs (Chianini *et al.*, 2001). A variable number of positive cells were also mentioned at the subcapsular sinuses and at the germinal centers. It was also noted that pAb Anti-CD3 antibody stained both the surface and the cytoplasm. Using mAb PPT3 for CD3, positive T-cells were demonstrated at the T-cell areas of the lymph node of the pig; scattered immunopositive cells were seen at the medullary area of the lymph node with few positive cells located at the lymph follicle. The staining was specific to the membrane of the immunopositive T-cells (Tingstedt *et al.*, 2003). Staining criteria of positive cells are based on the localization of the stain, since it is located both in the surface and in the cytoplasm compatible with the results of Chianini *et al.* (2001). Localization of the positive cells are in accordance with these observations. In the antigen retrival trial made with citrate buffer solution and in microwave oven, no positive results were gained for CD8. Using Triton X-100 for antigen retrival, few CD8 positive T-lymphocytes were seen (Fig 3C), while CD4 was tried with different antigen retrival methods and different dilutions and gained no specific and significant positivity. A study performed on the the lymph nodes of the pig (Tingstedt *et al.*, 2003) using anti-CD8 (76-2-11) and anti-CD4 (74-12-4) primary antibodies at +4°C, positive results were achieved at the T-cell areas, while few cells reacted positively in the medullary area and inside the follicles. Jonjic *et al.* (1987), said that anti-T8 and anti-T4 positive stained cells were found in the perifollicular area. Besides, scattered $T8^+$ ve $T4^+$ cells were also present in the medullary area. In the lymph nodes of the calves, different antigen retrival methods were tried for CD4 (CC30) and CD8 (CC63) (Gutierrez *et al.*, 1999). Positive results were achieved when the sections were kept at room temperature for 30 minutes in TBS containing 0.25% Triton X-100. In the present study, microwave treatment was used

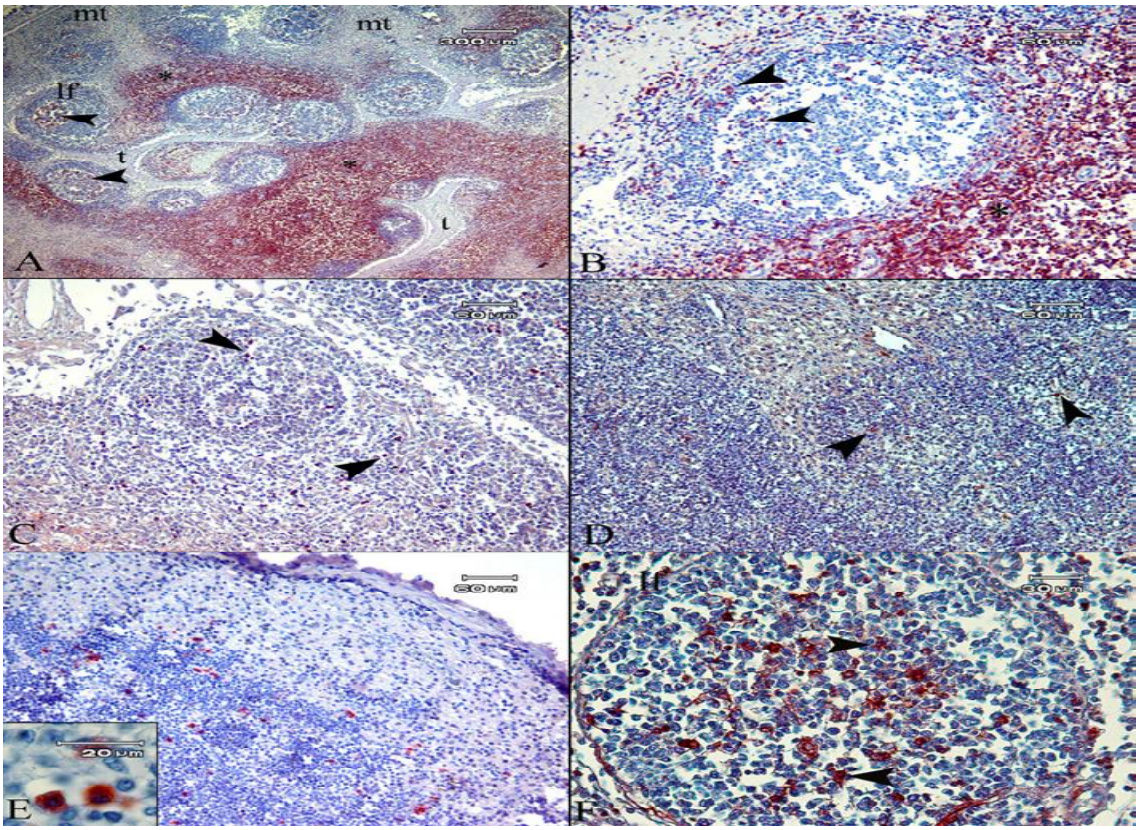


Fig 3: Immunohistochemical results of the lymph nodes in wild boar. A) The distribution of the CD3 positive cells in the dense cortical tissue (*), in medulla-like tissue (mt) inside the lymph follicle (lf) (arrow heads) t: trabeculae. B) Distribution of the CD 3 positive cells (arrow heads) inside the lymph follicle and in the dense cortical tissue (*). CD 8 positive cells (C, arrow heads),CD 79a positive cells (D, arrow heads) in the lymph node of the wild boar. E) MAC387 positive cells. Inset: MAC387 positive macrophages. F) S-100 positive dendritic positive cells within the lymph follicle (arrow heads).

with citrate buffer and Triton X-100 as antigen retrival method and no positive results were obtained for CD4 (74-12-4) and CD4 (CC30). Using Triton X-100 for antigen retrival, positive results were observed for mouse anti bovine CD8 (CC63) mAb. In the present study, positive cells were not met in the medullary area, few $CD8^+$ T lymphocytes were found in the lymph follicles and in the dense-cortical area, agreeing with the literatue. Very few CD79a positive B-lymphocytes were observed in the lymph follicles and in the dense cortical area (Fig 3D). Positivity of mAb Anti-CD79a [HM57] primary antibody in the primary follicles and in the mantle zone area of the secondary follicle resembled that in the lymph nodes of the pig (Chianini *et al.*, 2001). Earlier reports (Jonjic *et al.*, 1987) also stated that B-cells located in the lymph follicles of the lymph nodes of the pig were stained with biotin-labelled goat anti-pig IgG, while the cells located in the mantle zone of the secondary follicles presented weak membrane staining. In the present study, however, less positive staining results were achieved in the same areas.

MAC387 positive macrophages were mostly located at the dense cortical area but also seen few in the medulla-like tissue. Macrophages were seen remarkably at the border areas of the cortical tissue to the medullar tissue (Fig 3E), in addition to the mAb (monoclonal antibody) stained granulocytes. Using MAC 387 primer antibody on the lymph node of the domestic pig, it is stated that polymorphnuclear granulocytes were stained, while monocyte-macrophage original cells were not (Chianini *et al.*, 2001). The conducted study, both polymorphnuclear granulocytes and the macrophages reacted positive with MAC 387 mAb.

The follicular dendritic cells positive of S-100 protein were observed at the germinal center of the lymph follicles (Fig 3F). S-100 immunreactivity was detected in the arterial endothelium and in the some lymphocytes located in the dense cortex. Sugimura *et al.* (1990) stated that the follicular dendritic cells located in the germinal center of the lymph node of the pig, and arterial endothelial cells and most of the small type lymphocytes located in the paracortex-like area reacted positive for S-100 protein; confirming the results achieved.

There has been different studies performed on the lymph nodes of both wild and domestic pigs. In order to elaborate the lymph nodes of the wild boar in anatomic, histologic and immunohistochemical aspects, the study carried out is initial. Results of the study performed are thought to help fulfill the missings in the literature.

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