DEVELOPMENTAL FEATURES OF PORCINE HAEMAL NODES: A HISTOLOGICAL PERSPECTIVE

UDOUMOH, Anietie Francis and EZEASOR, Daniel Nwagbo

Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria.

Corresponding Author: Udoumoh, A. F. Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka 410001, Enugu State, Nigeria. **Email**: anietie.udoumoh@unn.edu.ng **Phone**: +234 8030996966

ABSTRACT

Histological techniques were employed to provide detailed information on the histological features of haemal nodes in piglets and adult pig. Ten pigs were used for this study. The result demonstrated progressive changes in the structure of porcine haemal nodes. The capsule and trabeculae of piglet haemal nodes exhibited dense irregular connective tissues with reticular cells and smooth muscle cells. The cortex was more central while the medulla was peripheral with poorly defined boundaries. However, the sinusoids contained less densely packed erythrocytes. In adult pigs, haemal nodes demonstrated capsules composed of loose irregular connective tissue, fat cells, and mostly reticular cells while the trabeculae showed dense irregular connective tissues with reticular cells and smooth muscle cells. The atypical cortex and medulla of adult pig haemal nodes were distinct. The paracortex of adult pig haemal node showed high endothelial venules and the sinusoids contained densely packed erythrocytes. Afferent lymphatics, efferent lymphatics, blood vessels and veins were observed in the haemal nodes of piglets and adult pigs. Finally, the present study has provided essential information on the structural features of haemal nodes in piglets and adult pigs, revealing its atypical nature, and probable roles of blood storage, erythrophagocytosis and immune functions.

Keywords: Piglets, Adult pigs, Haemal nodes, High endothelial venules, Histological features

INTRODUCTION

The immune system comprises specific cells and organs that are distributed throughout the mammalian body. The primary function of these organs and cells is to protect the organism against invasion and damages by microorganisms and foreign bodies. The functional morphology of the organs of the immune system has been largely elucidated, but one entity that has not received adequate attention is the haemal node. Initially, its existence and nature was in doubt hence the emergence of the nomenclature: haemal and haemolymph nodes, with the implication that haemal node, like the spleen filters blood while haemolymph node filters both blood and lymph. Pathologists initially regarded them as haemorrhagic lymph nodes (Meyer, 1917). There has been a recent upsurge of interest in their histological characterization (Casteleyn *et al*., 2008; Zidan and Pabst, 2010; Ozaydin *et al.,* 2012).

The haemal node, an organ recently considered as a haematopoietic and secondary lymphoid structure was initially believed to be peculiar to ruminants (Casteleyn *et al.,* 2008; Singh, 1959; Garguilo *et al.,* 1987; Ezeasor and Singh, 1988). However some reports have shown that haemal nodes are present in non-ruminant species (Oláh and Törö, 1970; Zidan and Pabst, 2004) including man (Jordan, 1934).

There has not been consensus among researchers on the occurrence of haemal nodes in pigs. Meyer (1918) reported the absence of haemal nodes in domestic pigs. Moreover, the histological features of haemal nodes reported so far in ruminants and some non-ruminant species have not established the exact structural and functional significance of the node. Rather, available information have raised questions on the structural features that make haemal node a peculiar organ.

Therefore, the present study is designed to investigate the developmental features of piglet and adult pig haemal nodes with emphasis on their structural and functional significance.

MATERIALS AND METHODS

Animals: Ten pigs comprising of 10 weeks old piglets and 2 – 3 years old adult pigs were used for this study. They were obtained from local markets in Nsukka local government area, Enugu State, Nigeria and sacrificed for human consumption. Apparently healthy animals were used. Following slaughter, haemal node samples were collected for histological investigations. The handling and welfare of the experimental animals was in accordance with the ethics and regulations prepared by INSA, Animal Welfare Divisions of the Ministry of Environment and Forest, Council of International Organization of Medical Sciences (WHO/UNESCO), NIH and PHS. The research protocol was approved by the University of Nigeria, Nsukka.

Histological Procedures: Segments of haemal nodes were fixed by immersion in Bouin's fluid for 48 hrs. These were later dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. Following mounting on wooden blocks, 5µm thick sections were obtained with a rotary microtome. These were mounted on glass slides and stained with haematoxylin and eosin for light microscopy. Photomicrographs were captured using a Moticam® digital camera (Motic China Group Co., Ltd, Xiamen, China).

RESULTS

Histology of Piglet Haemal Node: The haemal nodes of piglets were composed of the capsule, cortex, medulla and hilum. Each haemal node was surrounded by a thin capsule of dense irregular connective tissue with evenly distributed smooth cells and reticular cells (Figure 1). Circumferential lymphatic vessels which were observed within the capsule converged and exited the capsule as a large lymphatic vessel. The trabeculae extended from the capsule through the subcapsular region and terminated in the periphery of the cortex. Similar to the capsule, the trabeculae was composed of dense irregular connective tissue, reticular cells and smooth muscle cells. The trabeculae contained radial lymphatic vessels and arterioles, and their lateral boundaries showed erythrocyte-filled sinusoids (Figures 1, 2 and 3).

Furthermore, the cortex of the piglet haemal node was more central and the medulla more peripheral (Figure 1). The cortex and medulla showed sinusoids which were lined by endothelium. These sinusoids contained numerous erythrocytes, isolated lymphocytes and macrophages that lined the sinusoids. Although the cortex and medulla of piglet haemal nodes were observed, the boundary between the cortex and medulla were not distinct (Figure 2). The cortex was less compact and contained diffuse infiltration of small lymphocytes with 3 to 15 primary lymphoid follicles. The primary lymphoid follicles were randomly distributed within the cortex while the paracortex was characterized by diffuse lymphocytic infiltration. The medulla of piglet haemal node was composed of medullary cords and medullary sinusoids. The medullary sinusoids showed dilated erythrocytes-filled spaces with isolated lymphocytes interspersed between the erythrocytes. The subcapsular, trabeculae and medullary sinusoids showed dark-brown pigment of haemosiderin.

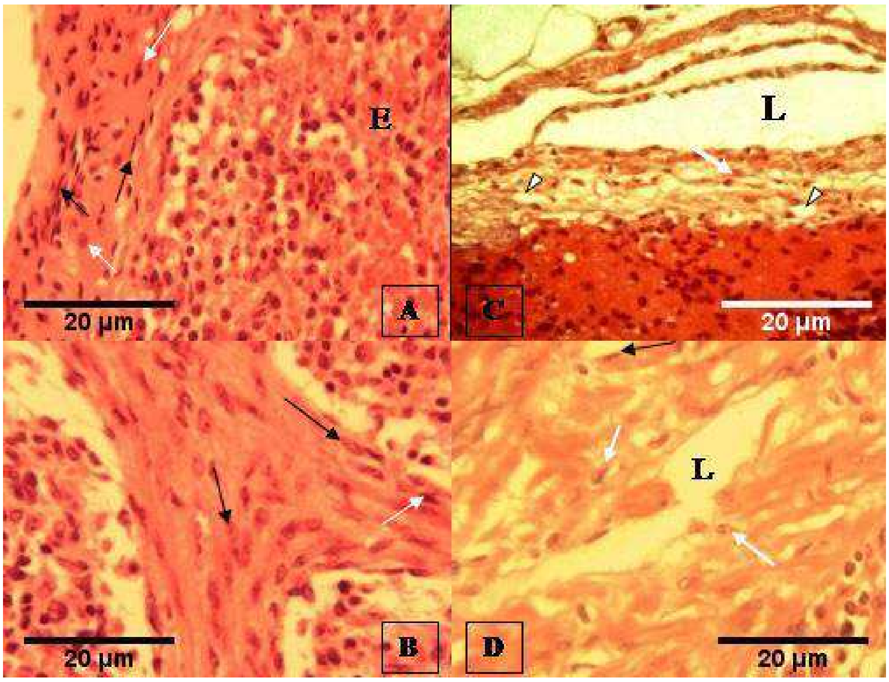

Figure 1: The capsules of piglet (A) and adult (C) pig haemal nodes; and the trabeculae of piglets (B) and adult pig (D) haemal nodes showing smooth muscle cells (black arrow), reticular cells (white arrow), fat cells (arrow head) and lymphatic vessels (L), H&E stain, X400

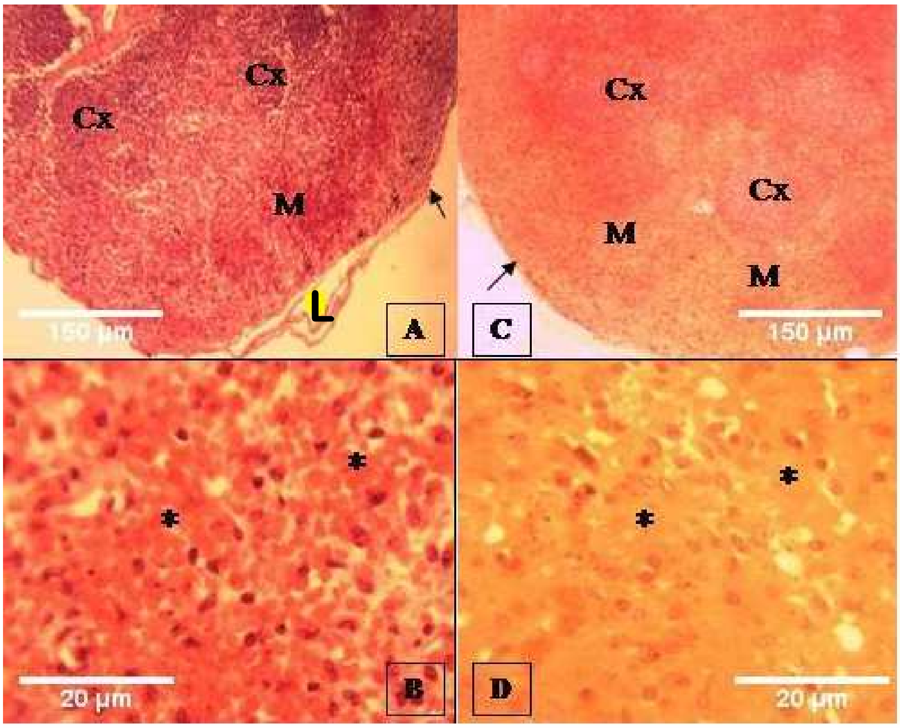

Figure 2: Light micrograph of piglet (A) and adult pig (C) haemal nodes demonstrating the capsule (arrows), lymphatic vessel (L), cortex (Cx) and medulla (M). Note the concentration of erythrocytes in the medullary sinusoids (asterisks) of piglets (B) and adult pig (D) haemal nodes, H&E stain, X400

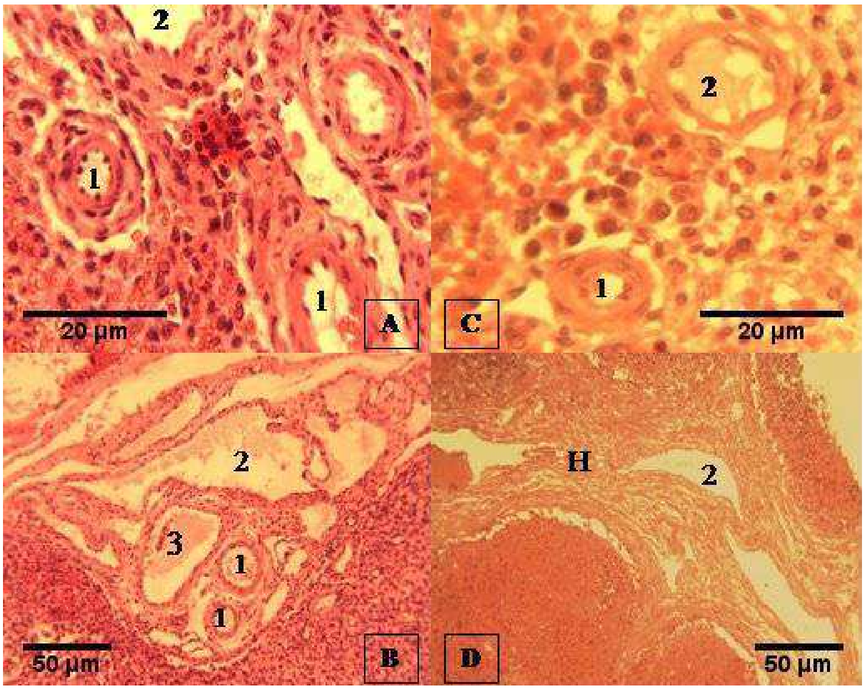

Figure 3: The trabeculae of piglet (A) and adult pig (C) haemal nodes containing blood vessels (1), and lymphatic vessels (2). Note the hilum of piglet (B)and adult pig (D) haemal nodes demonstrating blood vessels (1), lymphatics (2), vein (3) and a connective tissue framework (H). H & E stain, X400

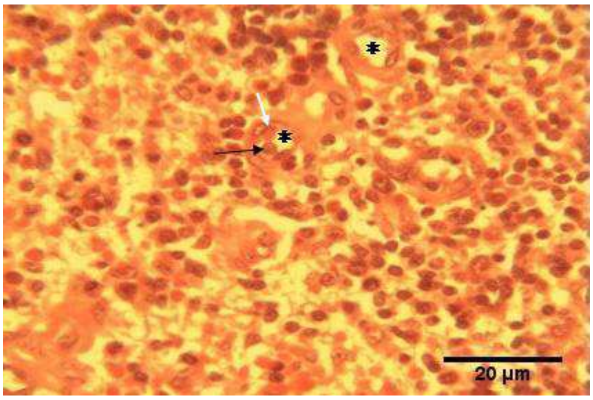

Figure 4: The paracortex of adult pig haemal node exhibiting high endothelial venules (asterisks). Note the lymphocyte (black arrow) and erythrocyte (white arrow) within the lumen of high endothelial venules. H&E stain, X400

The piglet haemal nodes exhibited a single hilum which was located in a thickened and indented area on the capsule. The hilum contained an artery, a vein and a large lymphatic vessel (Figure 3).

Histology of Adult Pigs Haemal Nodes: The haemal nodes of adult pigs exhibited a connective tissue capsule made up of a loose connective tissue, reticular cells and few smooth muscle cells which were distributed along points of the circumferential lymphatic vessels. The loose connective tissues of the capsules were infiltrated by adipose cells which gave the capsule of adult pig a unique appearance (Figure 1). The capsule extended as the trabeculae deep into the haemal node tissues. The trabecula was composed of dense irregular connective tissue, evenly distributed reticular cells and smooth muscle cells (Figure 1). Radial lymphatic vessels, arterioles and sinusoids were observed within the trabeculae. In addition, adult pig haemal node showed the subcapsular sinusoid which was delimited by endothelial lining cells and was continuous with the medullary sinusoid (Figure 2). Blood capillaries were observed within the subcapsular sinusoid.

Furthermore, the haemal node parenchyma of adult pigs was characterized by a central cortex and a more peripheral medulla (Figure 1). These two regions were well defined (Figure 2). The cortex was compact and exhibited outer and inner cortical regions. The outer cortex was composed of randomly distributed primary and secondary lymphoid follicles while the inner cortex showed diffuse infiltration of lymphocytes. Inter-follicular areas of the outer cortex and inner cortical regions contained diffuse infiltration of small lymphocytes and blood-filled sinusoids. Adult pig haemal node also exhibited a paracortex characterized by intense infiltration of lymphocytes, isolated plasma cells and reticular cells. It also exhibited numerous high endothelial venules (HEVs). These HEVs were lined by simple squamous to simple cuboidal endothelium and each lumen contained occasional isolated red blood cell (Figure 4).

The medulla of adult pig haemal nodes showed extensive areas of blood sinusoids, the medullary sinusoid. It was characterized by medullary cords and medullary sinusoids. Each cord was composed of connective tissue, reticular cells, lymphocytes, plasma cells and also exhibited arterioles and capillaries. The medullary sinusoids exhibited numerous red blood cells and isolated lymphocytes. These sinusoids appeared as spaces which coalesced establishing continuity with the subcapsular and trabeculae sinusosoids. The sinusoids of adult pig haemal node exhibited dark brown haemosiderin pigments (Figures 1 and 2).

A single hilum was observed in the core of the organ. It contained a lymphatic vessel whose lumen was distended and the wall reinforced by the increased deposition of connective tissue fibres. The connective tissue framework was composed of dense irregular connective tissue, and reticular cells (Figure 3).

DISCUSSION

In the present study, changes in the connective tissue profile of the capsules of piglets and adult pig haemal nodes from dense irregular connective tissue to loose irregular connective tissue type were age related. It is most probable that at pre-pubertal age, both the capsule and trabeculae were composed of dense irregular connective tissue, reticular cells and smooth muscle cells, typical of the capsules and trabeculae of most lymphoid organs. However, as the animal attained adulthood, the tissues of the capsule may have been replaced by adipose tissues. This was evidence in the present study as the capsule of adult pig haemal node was composed of loose connective tissue type, adipose tissues, reticular cells and scanty smooth muscle cells (present only at points of the capsular lymphatic vessels) while the trabeculae exhibited similar histology as the trabeculae of the piglet haemal node. Previous studies have shown that the histology of organ could be influenced by age, nutritional and physiologic states of the animal (Palmer, 2011; Zidan *et al.,* 2012). The smooth muscle cells together with the intrinsic nerves could modulate the contractile activity of the capsule and trabeculae, thus, concentrating the red blood cells within the blood sinusoids. Moreover, recent findings have shown that dense irregular connective tissue fibres together with reticular cells and fibroblast may contract and relax in a smooth muscle-like manner and may influence biomechanical behavior of the capsule and trabeculae (Tomasek *et al.,* 2002; Schleip, 2003). Therefore, apart from the role of providing a framework for the lymphatics and blood vessels (Ezeasor and Singh, 1988; Zidan and Pabst, 2004), the dense irregular connective tissue of the capsules and trabeculae together with their cells could play a role in the contraction of the capsule and trabecula.

Furthermore, the poorly defined cortico-medullary boundary of the parenchyma of piglet haemal node and the well defined boundaries of the cortex and medulla of adult pig haemal node is a function of age. It proves that the partitioning of the parenchyma of organs may be progressive, becoming clearly defined as the animal progresses in age. Moreover, the atypical parenchyma which was similar to that reported in the lymph nodes of pigs (Bacha and Bacha, 2000) showed that both the lymph nodes and haemal nodes of pigs may be of similar embryonic origin and development. The parenchyma of the cortex of piglet haemal nodes had more of lymphocytic infiltration than lymphoid follicles. The presence of lymphoid follicles often suggests the animal's exposure to certain degrees of pathogenic challenges (Allen and Cyster, 2008; Zidan and Pabst, 2012). According to Casteleyn *et al.* (2008) the number of lymphoid follicles in lymphoid tissues may increase with increasing age. Thus, the presence of lymphoid tissues in the cortex of porcine haemal nodes reveals the role of the organs in lymphocyte and anitibody production.

One characteristic feature of adult pig haemal node in the present study which was absent in the piglet haemal node was the present of HEVs in the paracortex. The presence of high endothelial venules in the paracortex of adult pig haemal node together with the luminal content of isolated lymphocyte is very significant, as it may play a role in lymphocyte recirculation (Zidan and Pabst, 2004; Cupedo *et al.,* 2004). HEVs have been reported in the paracortex of dromedary camel haemal nodes and in lymph node paracortex (Zidan and Pabst, 2012; Cupedo *et al.,* 2004).

According to the classification of Weller (1938) that there are two distinct types of nodes: haemal nodes and haemolymph nodes, the nodes observed in this study represents haemal node. This is true because the parenchyma of the studied haemal nodes showed erythrocyte-filled sinusoids. Whereas, the sinusoids of adult pig haemal node contained densely packed erythrocytes, the nascent sinusoids of piglet haemal nodes showed less densely packed erythrocytes. More so, the population of red blood cells in the sinusoids of porcine haemal nodes was similar to the concentration of red blood cells demonstrated in the splenic sinusoids (Suttie, 2006), strongly suggesting that porcine haemal nodes may serve to store red blood cells and may also play a compensatory role of blood storage after splenectomy. The report of erythrocytes in the sinusoids of porcine haemal node is collaborated by the reports of erythrocyte-filled sinusoids of haemal nodes of ruminants by several previous authors (Bassan *et al.,* 1999; Casteleyn *et al.,* 2008; Ozaydin *et al.,* 2012), and dromedary camel (Zidan and Pabst, 2004). In addition, there has been controversy among researchers regarding the mechanism of by which these erythrocytes entered the sinusoids. Different authors hypothesized that erythrocytes entered haemal node sinusoids through afferent lymphatics (Job, 1918), reflux via lymphaticovenous communications (Andreasen and Gottlieb, 1946), communication between cortical capillaries and sinusoids (Zhang *et al.,* 2013) and erythropoiesis within the haemal node tissues (Cerutti and Guerrero, 2008). The capillaries, arterioles, small blood vessels, and the HEVs observed in the present study may be the source of the red blood cells.

The occurrence of lymphatic vessels in haemal node is another subject of controversy among authors. In the present study, the lymphatic vessel which entered through the hilum and the one which exited through the capsule may be afferent and efferent lymphatic vessels respectively. The report of afferent and efferent lymphatics in piglet and adult pig haemal nodes is not strange, as the afferent lymphatic is necessary for selective conveyance of lymphocytes and antigen presenting cells into the cortical tissues of the haemal nodes, and the efferent lymphatic is also required to carry lymphocytes and plasma out of the haemal nodes (Haig *et al.,* 1999). This observation is supported by the reports of both afferent and efferent lymphatic vessels in the haemal nodes of sheep (Dellman and Brown, 1987), dromedary carmel (Zidan and Pabst, 2004) and Buffalo (Zidan and Pabst, 2010). However other authors reported the absence of both afferent and efferent lymphatic vessels in the haemal nodes of sheep (Thorp *et al.,* 1991; Dassanayake *et al.,* 2013), hair goat (Ozaydin *et al.,* 2012), while Ezeasor and Singh (1988) observed only efferent lymphatics in West African Dwarf goat haemal nodes.

In conclusion, the present study has provided essential information on the structural features of haemal nodes in piglets and adult pigs, revealing its atypical nature, and probable roles of blood storage, erythrophagocytosis and immune functions.

REFERENCES

ALLEN, C. D. C. and CYSTER, J. G. (2008). Follicular dendritic cell networks of primary follicles and germinal centres: phenotype and function. *Seminars in Immunology*, 20: 14 – 25.

ANDREASEN, E. and GOTTLIEB, O. (1946). Haemolymph nodes of the rat**.** *Biology and Medicine (Paris),* 19: 3 – 27.

BACHA, W. J. and BACHA, L. M. (2000). *Color Atlas of Veterinary Histology.* Second

Edition, Lippincott Williams and Wilkins, London.

BASSAN, N., VASQUEZ, F., VINUESA, M., CERRUTTI, P. and BERNARDI, S. (1999). Morphological alterations in hemal nodes in splenectomized cattle. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* 51: 1 – 6.

CASTELEYN, C. R., BREUGELMANS, S., SIMEONS, P. and VAN den BROECK, W. (2008). Morphological and immunological characteristics of the bovine temporal lymph node and haemal node. *Veterinary Immunology and Immunopathology*, 126: 339 – 350.

CERUTTI, P. and GUERRERO, F. (2008). Erythropoiesis and erythrophagocytosis in bovine haemal nodes. *International Journal of Morphology,* 26: 557 – 562.

CUPEDO, T., LUND, F. E., NGO, V. N., RANDALL, T. D., JANSEN, W., GREUTER, M. J., WAAL-MALEFYT, R., KRAAL, G., CYSTER, J. G. and MEBIUS, R. E. (2004). Initiation of cellular organization in lymph nodes is regulated by non-B cell-derived signals and is not dependent on CXC chemokine ligand 13. *Journal of Immunology,* 15: 4889 – 4896.

DASSANAYAKE, R. P., TRUSCOTT, T. C., OZYIRIT, M. O., ZHUANG, D., SCHNEIDER, D. A. and O'ROURKE, K. I. (2013). Accumulation profiles of PrPSc in hemal nodes of naturally and experimentally scrapie-infected sheep. *BMC Veterinary Research*, 9: 82.

DELLMAN, H. D. and BROWN, E. M. (1987). *Textbook of Veterinary Histology.* Lea and Febiger, Philadelphia.

EZEASOR, D. N. and SINGH, A. (1988). Histology of the caprine haemal node. *Acta Anatomica (Basel),* 133: 16 – 23.

GARGUILO, A. M., CECCARELLI, P. and PEDINI, V. (1987). Architecture of sheep haemal nodes. *Research in Veterinary Science,* 42: 280 – 286.

HAIG, D. M., HOPKINS, J. and MILLER, H. R. P. (1999). Local immune responses in afferent and efferent lymph. *Immunology,* 96: 155 – 163.

JOB, T. T. (1918). Lymphatico-venous communications in the common rat and their significance. *American Journal of Anatomy*, 24: 467 – 491.

JORDAN, H. E. (1934). Haemal nodes in man. *Anatomical Records,* 59: 297 – 310.

MEYER, A. W. (1917). Haemal nodes in bovines and goats. *American Journal of Anatomy*, 21: 359 – 373.

MEYER, A. W. (1918). Studies on hemal nodes. VIII The absence of hemal nodes in the domestic pig. *American Journal of Anatomy,* 24: 109 – 125.

OLÁH, I. and TÖRÖ, I. (1970). Fine structural investigation of the haemolymph gland in rat. *Cytobiologie*, 2: 376 – 386.

OZAYDIN, T., SUR, E., CELIK, I., OZNURLU, Y. and AYDIN, M. (2012). Histological and enzyme histochemical investigation of the haemal nodes of the hair goat. *Biotechnic and Histochemistry,* 87: 377 – 384.

PALMER, A. C. (2011). Nutritionally mediated programming of the developing immune system. *Advances in Nutrition*, 2: 377 – 395.

SCHLEIP, R. (2003). Fascial plasticity – a new neurobiological explanation: part I. *Journal of Bodywork and Movement Therapies*, 7: 11 – 19.

SINGH, A. (1959). On the microscopic structure of the haemal nodes of buffalo calves. *British Veterinary Journal*, 115: 271 – 275.

SUTTIE, A. W. (2006). Histopathology of the spleen. *Toxicologic Pathology*, 34: 466 – 503.

THORP, B. H., SENEQUE, S., STAUTE, K. and KIMPTON, W. G. (1991). Characterization and distribution of lymphocyte subsets in sheep hemal nodes. *Developmental and Comparative Immunology,* 15: 393 – 400.

TOMASEK, J. J., GABBIANI, G., HINZ, B., CHAPONNIER, C. and BROWN, R. A. (2002). Myofibroblasts and mechanoregulation of connective tissue remodeling. *Nature Reviews Molecular Cell Biology*, 3: 1349 – 1363.

WELLER, C. V. (1938). The hemolymph nodes. In: DOWNEY V. E. (Ed.) *Handbook of Hematology.* Harber, New York.

ZHANG, W., YASUDA, M., HOSAKA, Y. S. and NASU, T. (2013). Scanning electron microscopic study of the vascular system in the hemal node of the bovine cervical region. *Journal of Veterinary Medical Science,* 75: 79 – 83.

ZIDAN, M. and PABST, R. (2004). Histological, histochemical and immunohistochemical study of the hemal nodes of the dromedary camel. *Anatomia Histologia Embryologia,* 33: 284 – 289.

ZIDAN, M. and PABST, R. (2010). Histology of haemal nodes of the water buffalo (*Bos bubalus*). *Cell and Tissue Research*, 340: 491 – 496.

ZIDAN, M. and PABST, R. (2012). Histological, histochemical and immunohistochemical study of the lymph nodes of one humped Camel (*Camelus dromedaries*). *Veterinary Immunology and Immunopathology,* 145: 191 – 198.

ZIDAN, M., ZAGHLOUL, D., DERBALAH, A. and ELGHOUL, M. (2012). Age related morphological changes in Haemal nodes of the Egyptian water Buffalo (*Bos Bubalus*). *Alexandria Journal of Veterinary Science*, 37: 373 – 381.