HISTOMORPHOLOGY OF HAEMOLYMPH NODES OF WATER DEER (*HYDROPOTES INERMIS ARGYROPUS)*: NOVEL STUDY

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ABSTRACT

The objective of the present work was to investigate the anatomical and histological structures of haemolymph nodes in the adult water deer *(Hydropotes inermis argyropus)*. Methodology: the morphology of haemolymph nodes from 5 adult water deer was studied by gross inspection and by histological methods. Results: the haemolymph nodes were determined for the first time in water deer *(Hydropotes inermis argyropus)*. The nodes located in the neck on the trachea, thoracic and abdominal cavities in water deer. Dark red in color and oval- or round shaped haemolymph nodes in water deer were observed particularly along the abdominal aorta and caudal vena cava; as well as on the trachea along the jugular vein, on the internal carotid artery and internal jugular vein and on the aortic arch. These nodes had a hilus with hilar vessels, which entered the haemolymph nodes through the trabeculae as trabecular artery. The parenchyma was organized in the form of cortex and medulla. Lymphoid rim was located between subcapsular sinus and cortex. The cortex was composed of a many secondary lymphoid follicles and diffuses interfollicular lymphoid tissue, and medulla contained medullary cords and medullary sinuses. A reticular meshwork extended throughout the haemolymph nodes formed the structure backbone. The interstices of the reticular meshwork were filled with free blood cells, many macrophages, lymphocytes, and plasma cells. Conclusion: the dark red haemolymph nodes of water deer examined were seen along the course of large blood vessels in the neck on the trachea, thoracic and abdominal cavities. This is first study has demonstrated the presence of haemolymph

nodes in water deer (Hydropotes inermis argyropus) and provided essential information on their anatomical and histological structures. The data obtained in the present study may be used for further research in this field.

INTRODUCTION

Haemolymph nodes are unique lymphoid organs which have connection with the blood circulation (6). They were reported along large blood vessels of thoracic, abdominal, and pelvic cavities of ruminants (9,12). Although, their functional role is yet to be elucidated, some reports provided evidence that haemolymph nodes seem to participate a role in storage and filtration of blood, erythropoiesis, erythrophagocytosis, platelet formation, and defense of the body (5,9,19, 23). Haemolymph nodes were found in various ruminant species such as sheep, goats, cattle, water buffalo, and Iberian red deer (4,7,12,14,24). The results of these reports showed that number and histological characteristics vary considerably in various ruminant species. Several authors stated that haemolymph nodes consist of a cortex and medulla in sheep and dromedary camel (12,22), whereas other investigators haven't differentiated these regions in nodes of roe deer and water buffalo (1,23). Until the present study there was no information about the presence of haemolymph nodes in water deer. Therefore, the objective of the present study was to investigate the anatomical and histological structures of haemolymph nodes in the adult water deer *(Hydropotes inermis argyropus)*.

MATERIALS AND METHODS

Five adult water deer *(Hydropotes inermis argyropus)* with weight 10-11 kg, which were wounded due to various causes and died despite all interventions after being referred to the wild animal center in Jeonju (South Korea), were used in present study. The gross examination and collection of samples were conducted in Department of Veterinary Clinical Pathology College of Veterinary Medicine, Chonbuk National University. The haemolymph nodes were harvested from the neck, thoracic and abdominal cavities from each animal. The samples were then

fixed in 10% neutral buffered formaldehyde until observation. Further studies were conducted in Research Institute of Veterinary Science of Eastern Siberia-branch SFRCA. Following routine histological procedures, serial sections (4 µm) were prepared and stained. Mayer's hematoxylin and eosin (H&E) stain was applied to study the general structure of the nodes (16). Crossman trichrome stain method was used to differentiate smooth muscle cells from connective tissue and to demonstrate the presence of fibroblasts (8). Gomori's silver impregnation stain was used to differentiate reticular fibers and reticular cells (13). Giemsa (Bancroft and Cook, 1984) and toluidine blue (Kiernan, 1990) stains (16) were used to differentiate the blood cells.

RESULTS

Macroscopic appearance the haemolymph nodes of water deer were dark red in color and their size varied between a millet seed and a pea. Oval- or round- shaped haemolymph nodes were varied between the individuals of water deer in the location and number. Single small nodes were rarely seen in the neck on the trachea along the jugular vein, under the trachea on the internal carotid artery and internal jugular vein. In the thoracic cavity pea-sized nodes were lined on the aortic arch and on the adipose tissue of the pericardium (Fig. 1). Small reddish haemolymph nodes were sometimes present between the tracheal bifurcation and pulmonary trunk. Haemolymph nodes in the abdominal cavity were located along the course of large blood vessels, particularly along and between the abdominal aorta and caudal vena cava (Fig. 2). Usually, haemolymph nodes were surrounded by fat tissue and had communication with large blood vessels.

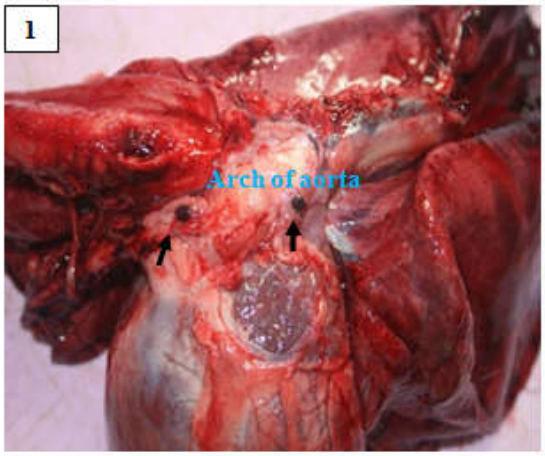

Fig.1; Photograph of macroscopic appearance of haemolymph nodes located in thoracic cavity on the arch of aorta (arrows).

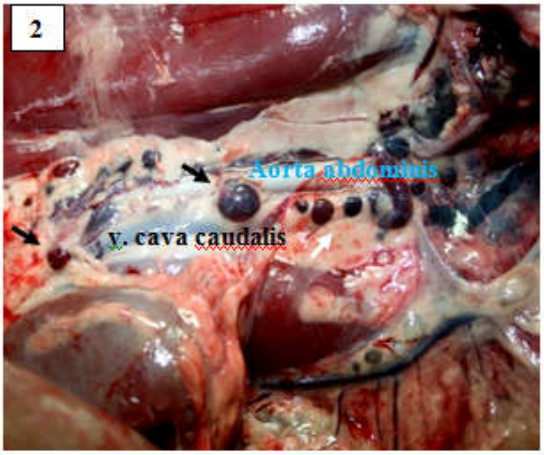

Fig. 2.; Photograph of macroscopic appearance of haemolymph nodes located in the abdominal cavity along aorta abdomininis and vena cava caudalis (arrows).

Microscopic appearance, the haemolymph nodes of water deer were consisted of a capsule, lymphoid rim, hilus, blood filled subcapsular and central sinuses, lymphoid follicles and lymphatic cords, sinusoids with arteries. A cortex and medulla were differentiated in haemolymph nodes of water deer. Each node was surrounded by a thin capsule that consists from connective tissue containing collagen fibers and strengthened by reticular cells, reticular fibers, and smooth muscle cells, rarely fibrocytes and fibroblasts. The capsule of some haemolymph nodes contains blood vessels, particular atypical (nonmuscular) veins (Fig. 3). The wall of such veins was lined with endothelial cells resting on the basement membrane and connective tissue.

A few short trabeculae were extended from the inner part of the capsule. The trabeculae were composed of mainly collagen fibers, smooth muscles, and rarely reticular cell and reticular fibers. Trabeculae were surrounded by trabecular sinuses. The latest were lined with endothelial cells.

The haemolymph nodes had a hilus containing blood vessels. The hilar artery entered the haemolymph node through the trabeculae as trabecular artery (Fig. 4).

The subcapsular sinus extended beneath the capsule and was always engorged with mixture of blood and lymph. Fine developed meshwork of reticular cells and reticular fibers extended through the lumen of the subcapsular sinus, that had

connection with the cortex and medulla sinuses (Fig. 7). These sinuses were lined with endothelial cells.

The parenchyma was organized in the form of cortex and medulla. Lymphoid rim was located between subcapsular sinus and cortex (Fig. 5). This rim contained lymphocytes and plasmocytes.

The cortex was composed of a many secondary lymphoid follicles and diffuse interfollicular lymphoid tissue, the latest was formed by large number of lymphocytes, erythrocytes, macrophages. Secondary lymphoid follicles were with clear germinal center and corona (Fig. 6). Germinal center contained macrophages, basophilic erythroblasts and erythroblasts in different stages of differentiation, small, medium and large lymphocytes, monocytes, plasmocytes, and eosinophils. A fine network of reticular cells and reticular fibers was observed around the lymphoid follicles (Fig. 7). High endothelial venules were clearly seen on the border with the medulla and were lined with a cubic endothelium.

The medulla was formed by medullary cords and medullary sinuses, which were supported by reticular cells and fibers. The sinus wall were formed by endotelial cells. The medullary cords in the haemolymph nodes contained many lymphocytes, plasmocytes, macrophages and erythrocytes. The medullary sinuses rich in lymphocytes. Moreover, the medulla was composed of the wide sinusoid containing erythrocytes. The arteries are located in the lumen of the sinusoids (Fig. 8).

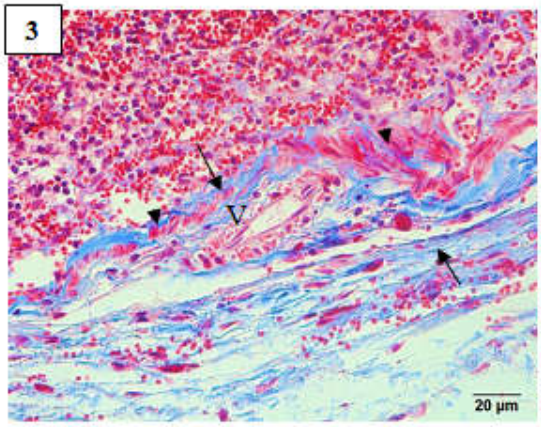

Fig.3; Microphotograph of haemolymph node showing a thin capsule (c) is formed by collagen fibers (arrows) and smooth muscles (arrowheads). Atypical (nonmuscular) vein (V).Crossman trichrome. X 200.

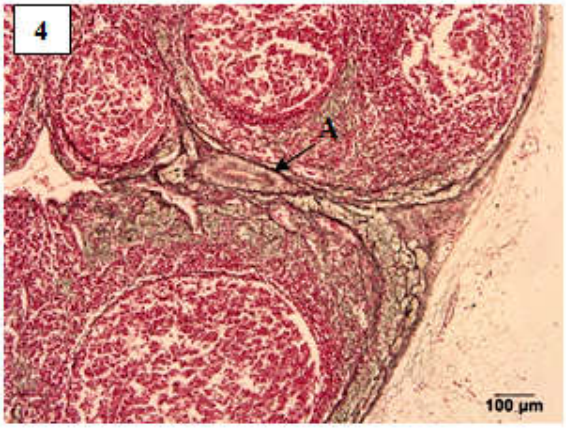

Fig. 4. Microphotograph of haemolymph node showing hilus with hilar artery (A) Gomori's silver impregnation. X 100.

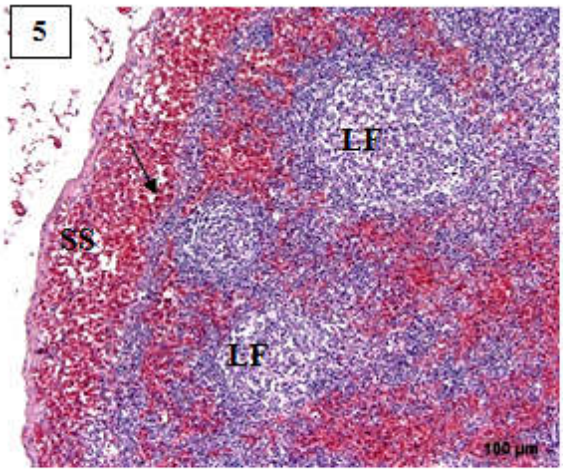

Fig.5; Microphotograph of haemolymph node showing lymphoid rim (arrow), which separates the subcapsular sinus (SS) from the lymphoid follicles (LF). H&E. X 100.

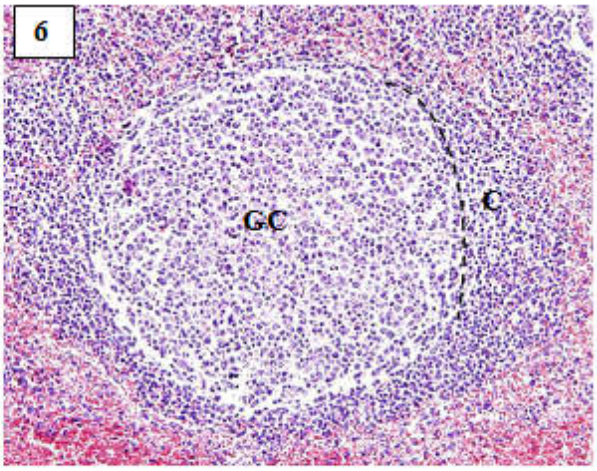

Fig. 6. Microphotograph of haemolymph node showing a lymphoid follicle with a clear germinal center and corona (C). H&E. X 400.

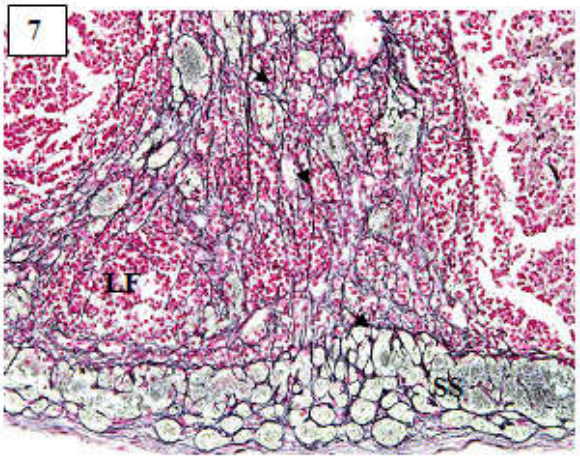

Fig. 7. Microphotograph of haemolymph node showing good developed reticular meshwork (arrows) around the lymphoid follicle and in the subcapsular sinus (SS) Gomori's silver impregnation. X 200.

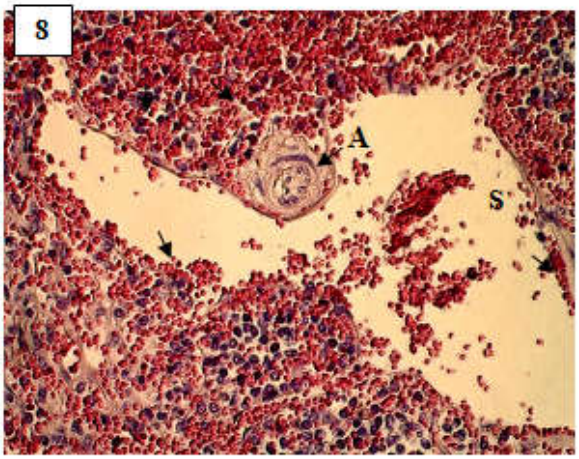

Fig. 8. Microphotograph of haemolymph node showing artery (A) and sinusoid (S) with large number erythrocytes (arrows). H&E. X 400.

DISCUSSION

In current study, the morphological structures of haemolymph nodes in water deer were demonstrated for the first time. The haemolymph nodes were dark red, oval- or round shaped with variable size that was in agreement with previous studies in other ruminant species (6,10,12,18). The nodes were embedded in fat tissue and located in the neighborhood to the blood vessels as reported by some investigators in roe deer and cattle (1,21).

The haemolymph nodes in water deer were located along and between the large blood vessels, such as the abdominal aorta and caudal vena cava, similar findings were demonstrated by (9,10,12). The nodes were also observed in the neck on the trachea as reported previously in goats and elephants (7,11).

The haemolymph nodes in water deer were rarely seen in the thoracic cavity on the aortic arch and the pericardium in this study, while Choudhary *et al.* (7) observed in the neighborhood to the base of the heart in goats. Single nodes were located between the tracheal bifurcation and pulmonary trunk as in water buffalo (24). Number of haemolymph nodes in in each individuals of water deer varied between 8 and 26, that was agreement with several authors, who have observed these node in goats and water buffalo (9,23).

We revealed that although the histological structure of haemolymph nodes in water deer is similar to that of other ruminants, it has specific characteristics which

differ from those of other species. Lymphoid tissue in nodes was organized in the form of cortex and medulla that fact shows their similarities to structure of typical lymph nodes. Although, haemolymph nodes in water deer had also features of splenic morphology, such as presence of medullary cords separated by medullary sinuses, sinusoids with arteries and atypical (nonmuscular) veins. Several authors also noted that haemolymph nodes share morphological characteristics of spleens and lymph nodes (9,20,21).

The haemolymph nodes in water deer had a thin capsule and trabeculae which were composed of mainly collagen fibers, smooth muscle cells, reticular cells and reticular fibers as reported in roe deer, cattle, sheep, goats and camel (1,4,17,22). Some studies have shown that these muscles may play a role in the contraction the capsule and trabeculae to provide the concentration of red cells in the sinuses, such mechanism similar to that reported in the spleen (3).

Our study observed the presence of the hilus with artery and vein which were pass in and out node. Similar findings were obtained in sheep and dromedary camel nodes (12,22).

The water deer haemolymph nodes had a wide subcapsular sinus and blood sinuses. The latest were surrounded by medullary cords, as reported in roe deer, cattle, and goats (1,6,20,21).

In the present study, the observation of lymphoid rim which was located between subcapsular sinus and cortex. This rim contained lymphocytes and plasmocytes, however, there are no data found about existence such structure of haemolymph nodes in other ruminants.

In agreement with previous literature reports in sheep and camel (12,22), the parenchyma of haemolymph nodes in water deer was organized in cortex and medulla. Nonetheless, other authors haven't observed these regions in other ruminants (17,23). The cortex of water deer haemolymph nodes was composed of many secondary lymphoid follicles that were consisted of a clear germinal center. The latest composed of large number of lymphoid cells, macrophages, basophilic erythroblasts, erythroblasts in different stages of differentiation, monocytes,

plasmocytes and eosinophils, surrounded by a darker corona of small lymphocytes a reported in roe deer and water buffalo (1,24). Strong reticular network was located

around the lymphoid follicles. The presence of secondary follicles in haemolymph nodes is an indicator of a role of nodes in antibody production (5,22).

The medulla of nodes was composed of the medullary cords and medullary sinus. The medullary cords contained many lymphocytes, plasmocytes, macrophages and erythrocytes. Sinuses contained many lymphocytes, macrophages, and macrophages with captured red blood cells. Reticular cells and fibers supported the sinuses, lymphoid follicles and lymphatic cords as reported in goats (16). The presence of macrophages involved in erythrophagocytosis clear indicated that water deer haemolymph nodes were involved in phagocytosis of old and defective blood cells from the circulation, as was reported for goat and water buffalo nodes (9,23).

The high endothelial venules were clearly seen in medulla of haemolymph nodes in water deer, as reported in dromedary camel (22). Several authors suggested that high endothelial venules in haemolymph nodes help the exit of erythrocytes directly into the node (19). This may suggest a role for the high endothelial venules in blood recirculation.

A reticular meshwork extended throughout the haemolymph nodes formed the structure backbone. In agreement with several investigators the interstices of the reticular meshwork was filled with free blood cells, many macrophages, lymphocytes and plasma cells, as reported in sheep (12).

In conclusion, the dark red haemolymph nodes of water deer examined were seen along the course of large blood vessels in the neck on the trachea, thoracic and abdominal cavities. This is first study has demonstrated the presence of haemolymph nodes in water deer (Hydropotes inermis argyropus) and provided essential information on their anatomical and histological structures. The data obtained in the present study may be used for further research in this field.

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