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Varieties of atypical lymph nodes of water deer (*Hydropotes inermis argyropus*)

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Abstract. The morphology and topography of atypical lymph nodes of Korean water deer (*Hydropotes inermis argyropus*) is described for the first time. The aim of this work was to study the structural organization of atypical lymph nodes in water deer. Fresh organ samples were taken from 5 adult water deer. Samples are fixed in formalin and embedded in paraffin wax (Hystomix). Stain serial sections, 4-5 µm thick, are stained with hematoxylin-eosin, Masson's trichrome stain, Gomori's silver impregnation stain. Atypical lymph nodes in Korean water deer are located on the trachea along the jugular vein and under the trachea on the internal carotid artery and internal jugular vein. The nodes are localized on the adipose tissue of the pericardium in the thoracic cavity, as well as directly on the aortic arch. In the abdominal cavity, nodes are located along the caudal cava and abdominal aorta, as well as in the connective tissue layers of the pancreas. Organs have a dark red color, oval or round shape, its size - from millet grain to peas. Atypical lymph nodes are heterogeneous organs that differ not only from the usual lymph nodes, but also from each other, both in the type of bringing vessels and some features of the structure. Based on a detailed morphological study, atypical lymph nodes in the abdominal cavity were subdivided into 3 types: 1) hemolymphatic nodes; 2) venous hemal nodes and 3) arterial hemal nodes.

1. Introduction

The mammalian immune system is the fundamental system of the body, which is responsible for the survival of the animal in nature. The immune system includes both central (red bone marrow and thymus) and peripheral organs (spleen, lymph nodes, lymphoid formations and atypical lymph nodes). Despite the good knowledge of the morphology of these organs by this day remain an open question concerning the functional significance of atypical lymph nodes in mammals. For a long time, there were conflicting opinions about the nature of these structures in the body. For a long time, atypical lymph nodes were considered nothing but hemorrhagic lymph nodes, as a result of which nodes were not given special attention. Only in recent years, these concerns have been debunked and are available in literature providing brief reports on the existence of atypical lymph nodes (hemal and hemolymphatic) as independent permanent structures. It is now established that atypical lymph nodes are involved in erythrophagocytosis, erythropoiesis, thrombocytopoiesis, blood filtration and storage, as well as in the immune defense of the body [5, 6, 9, 13, 18].

There are only single research works in the Russian literature devoted to the peculiarities of the structure of these nodes in humans and water deer [1, 2]. These organs in foreign literature are



described in more detail in rats, some carnivorous animals [13, 17], ruminants: cattle [5, 21], one-humped camel [11, 23], goats [6, 9], water buffalo [24, 25], roe deer [3], Iberian red deer [10]. However, the Korean water deer (*Hydropotes inermis argyropus*) is a rare poorly understood species of ruminants inhabited in East Asia, atypical lymph nodes remains poorly studied bodies.

The purpose of these studies was to study the specifics of structural organization of atypical lymph nodes in the Korean water deer (*Hydropotes inermis argyropus*).

2. Material and Methods

The material for the study was atypical lymph nodes taken from 5 adults Korean water deer with the body weight is 8-9 kg. The collection of material was carried out in the period from June to August 2014 in a diagnostic laboratory of Chonbuk National University, Jeonju, South Korea. The samples were fixed in 10% neutral buffered formalin. Further work was done on the basis of Research Institute Veterinary of Eastern Siberia. Tissue processing of the material was carried out according to the standard procedure. After embedding in paraffin wax (Hystomix), the sections (4-5 μ m thick) were prepared on a rotary microtome HM 340E Electronic Rotary Microtome (USA). The sections were stained with hematoxylin-eosin (H&E), Gomori's silver impregnation stain and Masson's trichrome stain.

3. Results

By using the classical anatomical methods of the research, atypical lymph nodes have been found in Korean water deer. These structures were rounded or oval-shaped formations, the color of which varies from pale red to dark brown. The number of nodes was different in each animal, so some individuals had only single nodes, while others - 20-24 pieces. Single atypical lymph nodes of dark red color were detected directly on the trachea along the jugular vein, as well as under the trachea on the internal carotid artery and internal jugular vein (Figure 1a). Slightly smaller than pea nodes were found on the adipose tissue of the pericardium, as well as directly on the aortic arch.

In the abdominal cavity, the nodes are surrounded by mesenteric fat and are most often located along the course of large blood vessels: along the abdominal aorta and caudal cava. Nodes are filled with blood, resulting in nodes color varies from rich red to black. Nodes sizes - from millet to pea (Figure 1b).

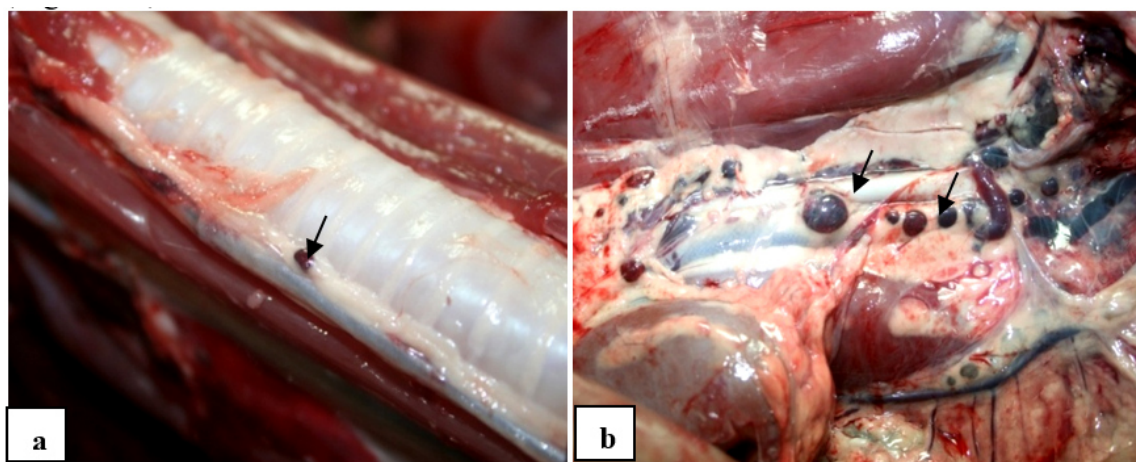


Figure 1. Atypical lymph nodes of water deer. a - node on the trachea along the jugular vein; b - numerous nodes in the abdominal cavity along the caudal cava and abdominal aorta.

Occasionally, atypical lymph nodes were found in the connective tissue layers of the pancreas.

Our histological studies have shown that atypical lymph nodes have significant differences not only in structure, but also in the type of bringing vessels, through which blood flows into the sinuses.

Resulting in atypical lymph nodes were differentiated into the following types: hemolymphatic nodes, venous hemal and arterial hemal nodes.

The hemolymph enters the node by bringing vessels with mixed content and by lymphatic vessels in hemolymphatic nodes of water deer. The collagen fibers are intertwined with reticular and smooth muscle fibers in the capsule of the node, fibroblasts and fibrocytes are present (Figure 2c, d). The vein of non-muscular type has been identified in the capsule of some nodes. The short thin trabeculae were separated from the inner surface of the capsule. On the side of the entering the node, there are massive trabeculae containing blood vessels – arteries that penetrate into the inner part of the node.

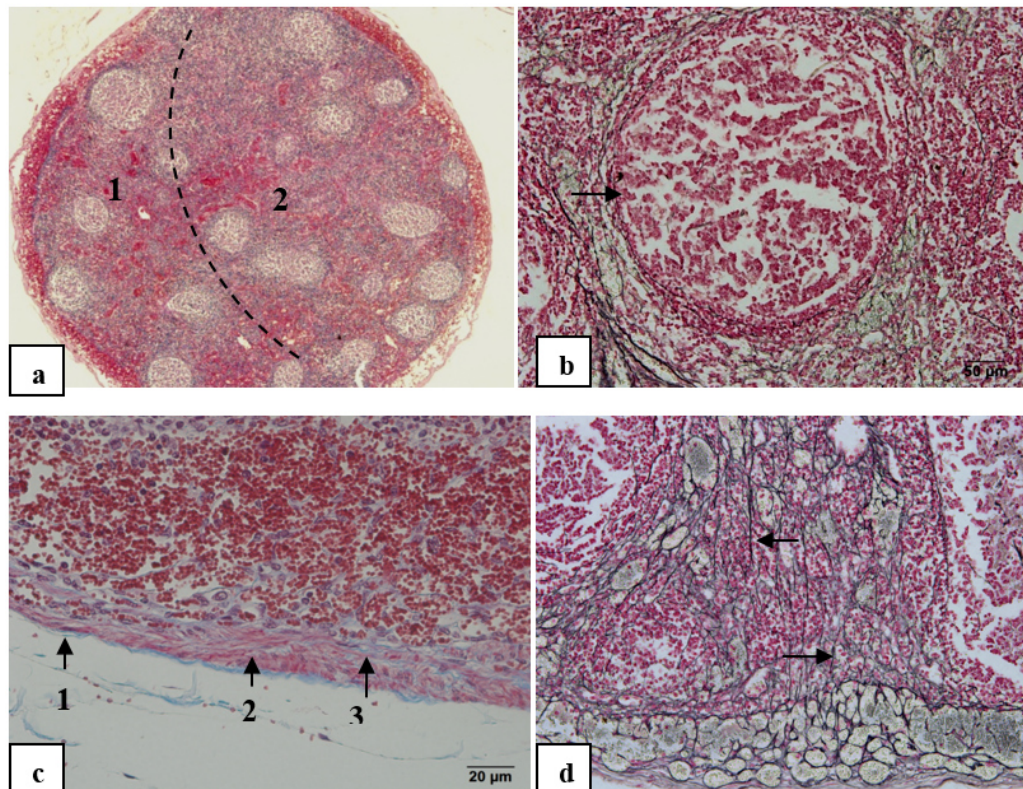


Figure 2. Hemolymphatic node of water deer. a – parenchyma of node: 1– cortex; 2 – medulla. Formalin. H&E. 5x10; b-secondary lymphatic follicle with a germinative center, surrounded by a well-defined reticular network. Formalin. Gomori's silver impregnation stain. 5x10; c – capsule of node consisting of collagen fibers (1), myocytes (2), fibroblasts and fibrocytes (3). Formalin. Masson's trichrome stain. 20x10; d – well-defined reticular tissue permeating the entire parenchyma of the node. Formalin. Gomori's silver impregnation stain. 10x10.

Trabeculae contain collagen fibers and myocytes. Under the capsule is a subcapsular sinus filled with hemolymph. The internal walls of the blood sinus are represented by a layer of endothelial cells. Between the subcapsular sinus and cortical substance is lymphoid rim, contents: lymphocytes, basophilic and orthochromatically and erythroblasts, and plasma cells. The parenchyma of the node is divided into cortex and medulla (Figure 2a). In hemolymphatic nodes of water deer cortical substance contains a large amount of secondary lymphatic follicles with a distinct germ center, the periphery of which is mantle zone (Figure 2b). The network of reticular fibers located on the mantle zone serves as a backbone for follicles.

Postcapillary venules with a high cubic endothelium are located in the surface area of the follicles, near the sinuses on the border with the medulla. The medulla contains lymph cords and is located between cords the blood sinuses are rarely visible lymph follicles. Lymphatic cords consist of lymphoid tissue and erythrocytes. Between the cords are blood sinuses, which are lined with

endothelial cells and contain only lymph. In the medulla there are broad sinusoids lined with flat endothelial cells. The reticular network permeates the entire parenchyma of the node, its cells contain a large number of free blood cells, many macrophages, lymphocytes and plasmocytes (Figure 2d). In addition to hemolymphatic nodes, water deer have oval venous hemal nodes covered with a thin connective tissue capsule consisting of smooth muscle and reticular cells. The capsule contains small vessels. Hemolymph (an admixture of blood and lymph) fills the subcapsular sinus unevenly. Trabeculae containing trabecular veins depart from the capsule (Figure 3a).

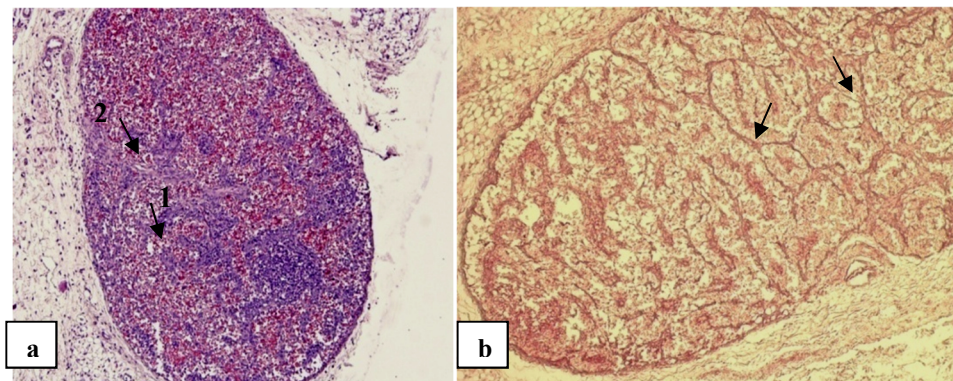


Figure 3. Venous hemal node in adults water deer. a – node with well-defined lymphatic cords (1) and blood sinuses (2). Formalin. H&E. 10x10. b – venous hemal node with well-developed thin-sinuous reticular network inside the sinuses. Formalin. Gomori's silver impregnation stain. 10x10.

The parenchyma is dominated by blood sinuses, between which there are lymphatic cords. Inside the blood sinuses is a well-developed thin-sinuous reticular network (Figure 3b). Blood sinuses contain erythrocytes with a large number of macrophages. Small sinusoide fall in cerebral sinuses. The lymphatic cords contain macrophages, plasma cells and lymphocytes.

Arterial hemal nodes have a capsule that consists of smooth muscle cells, reticular fibers and cells, as well as fibroblasts and fibrocytes. Short trabeculae extend from the capsule of the node. Blood flow in the sinuses occurs through the arteries flowing into the portal sinus and lymphatic vessels, the latter flow into the subcapsular sinus of the node (Figure 4a). The subcapsular sinus was not clearly seen. Reticular tissue is poorly developed in the blood sinuses (Figure 4b). From the subcapsular sinus, cortical sinuses extend into the node, passing in turn into cerebral sinuses. The blood sinuses are lined with endothelial cells, contain many lymphocytes and a small number of erythrocytes, basophilic erythroblasts, plasmocytes and macrophages with captured erythrocytes. The cerebral sinuses are filled with lymphocytes. Between the blood sinuses are lymphatic cords containing medium and large lymphocytes. In the lymphoid tissue of the arterial hemal node the somatic capillaries are lined with continuous endothelium. Parenchyma of node is not divided into cortical and cerebral zones, that contains a large amount of lymphatic tissue with a small admixture of diffuse erythrocytes.

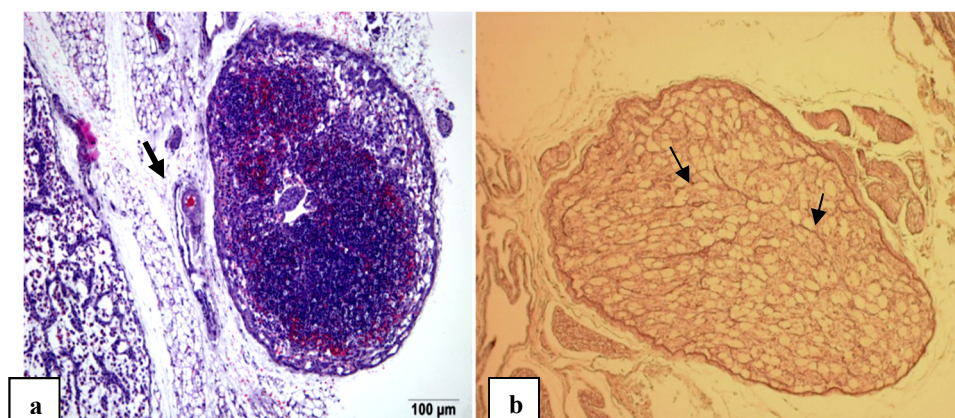


Figure 4. Blood hemal node of water deer. a - portal sinus with clusters of arterioles in the arterial hemal node in water deer. Formalin. H&E. 10x10; b - an underdeveloped reticular tissue of arterial hemal node. Formalin. Gomori's silver impregnation stain. 10x10.

4. Discussion

Our studies have shown that the atypical lymph nodes of the Korean water deer are formations from rich red to brown, small in size, oval or round in shape. Similar results were obtained by authors studying the data nodes in other ruminants [5,8,9,16].

With the help of classical anatomical research methods, we found that in water deer these structures are located in the cervical part of the trachea and under the trachea, in the thoracic and abdominal cavities. A similar arrangement of atypical lymph nodes was revealed in such ruminants as camel, sheep, water buffalo, roe deer [3, 18, 23, 24].

Most often, the nodes are localized among large blood vessels and surrounded by adipose tissue, a similar arrangement was observed in goats and cattle [6,22]. We found that the number of nodes varied in animals of one species, which also confirms the previously stated conclusion that the number of nodes can be varied in animals of one species and in animals of different species [8,23].

In the course of a detailed study of the peculiarities of histostructure we differentiated atypical lymph nodes into several types: hemolymphatic, venous hemal and arterial hemal nodes, which is consistent with the results Pototskaya O. Y. and A.S. Lapsar (2016) studying atypical lymph nodes in humans [2]. A number of authors noted that the structure of atypical lymph nodes in sheep and cattle is also heterogeneous [4, 18]. However, M. Zidan and R. Pabst (2010), R. K. Choudhary and co-authors (2011) there was no difference in the structure of nodes located in one or different locations. Our studies have shown that even in animals of the same species, atypical lymph nodes differ not only in histological structure, but also in the type of bringing vessels.

During the histological study, we revealed the presence of bringing the vessel with mixed content and lymphatic vessels in hemolymphatic nodes of water deer. Y-S. Yoon with co-authors (1999) and D. N. Ezeasor and A. Singh (1989) observed afferent and efferent lymph vessels in hemolymphatic nodes in Korean goats [19, 20]. However, some authors pointed to the absence of lymphatic vessels in hemolymphatic nodes in goats and sheep [14, 16, 19]. D. N. Ezeasor and A. Singh (1989, 1990) wrote that the difficulty in identifying lymphatic vessels may be due to the fact that with the maturation of the animal there is a histological alteration: the development of blood sinuses at the expense of the rest of the parenchyma node [19, 20]. The presence of collagen fibers with reticular and smooth muscle fibers in the node capsule, as well as the presence of fibroblasts and fibrocytes promotes blood concentration in the node sinuses by relaxing and reducing these structures in the node [15, 23]. We found that the parenchyma in hemolymphatic nodes consists of cortical and cerebral matter, which is consistent with the studies of other authors Y-S Yoon and co-authors (1999), studying these nodes in Korean goats [20].

In the hemolymphatic nodes of water deer in the cortex, there is a large number of secondary lymphatic follicles with a pronounced germinative center, on the periphery of which is the mantle zone. Similar findings were made by C. R. Casteleyn (2008) in cattle. These authors indicated that the presence of secondary lymph follicles in the node indicates the participation of these organs in the production of lymphocytes and antibodies [4].

The presence of postcapillary venules located in the surface area of the follicles indicates that according to the venules, the selective passage of lymphocytes from the venule cavity into the lymphatic tissue and then into the sinuses is carried out. The presence of high-endothelial venules in the parenchyma of the node confirms participation of hemolymphatic nodes in the recirculation of lymphocytes, which is consistent with the results of Zidan M. and Pabst R. (2004), and T. Cupedo and others (2004) [7, 23]. We observed a well-developed reticular network in the parenchyma of a node with a large number of free blood cells (macrophages, lymphocytes and plasmocytes). With the help of thick reticular tissue entwining the capsule, subcapsular sinus, trabeculae and follicles, the blood is not only concentrated in the node, but also purified by macrophages.

Venous hemal nodes of water deer are covered with a thin connective tissue capsule, which consists of smooth muscle and reticular cells, similar components of the capsule were detected in hemal nodes in piglets [17]. Blood flow to the venous hemal nodes is carried out by veins and lymphatic vessels.

Trabeculae of nodes contained trabecular veins, which is consistent with the data of other authors noting the presence of small vessels in trabeculae of hemal nodes in cattle [22]. In many works, the authors indicate the presence of cortex and medulla substances in hemal nodes [7, 6, 8, 14, 19, 20], however parenchyma in hemal node of water deer has not been divided into zones. The parenchyma is dominated by blood sinuses, between which there are lymphatic cords, which is consistent with the studies of other authors [17]. In the blood sinuses were identified erythrocytes with a large number of macrophages, which is consistent with the studies of A. F. Udoumoh and D. N. Ezeasor (2015), who noted a high content of erythrocytes in the sinuses of hemal nodes in piglets [17]. A similar phenomenon is observed in the sinuses of the spleen, which indicates that the hemal nodes can play a compensatory role in blood storage, but because of their small size, their role as a blood storage is insignificant for the body [23, 24]. Macrophages, plasmocytes and lymphocytes were detected in lymphatic cords. T. Ozaydin and co-authors (2012) noted that monocytes, mast cells and neutrophil granulocytes can be observed in the hemal nodes of goats in lymphatic cords in addition to these cells [12], however, we did not find the above cells.

In arterial hemal nodes, the capsule consists of smooth muscle cells, reticular fibers and cells, as well as fibroblasts and fibrocytes. The structure of the capsule not only plays a role of a skeleton node, but also helps to reduce the capsule, as a result the blood is concentrated in the sinus node. Blood sinuses of arterial hemal nodes contain many lymphocytes and a small number of erythrocytes, basophilic erythroblasts, plasmocytes and macrophages with captured erythrocytes. According to A. Y Bozkurt and M. Kabak (2010) only blood flows in the sinuses of the hemal nodes [3], although some authors have indicated a small amount of additional lymph inflow [24].

5. Conclusion

Our results showed that atypical lymph nodes of water deer are formations from rich red to brown color, small size, oval or round shape. They are heterogeneous organs and differ not only from the usual lymph nodes, but also from each other in the type of bringing vessels, as well as some features of the morphological structure. The specific structure and cellular composition of atypical lymph nodes contribute to blood filtration and removal of old erythrocytes from the blood circulation. Macrophages located most often in the blood sinuses, are involved in erythrophagocytosis of old and damaged erythrocytes. In addition, plasmocytes are often found in atypical lymph nodes in water deer, which also serves as morphological evidence of the occurring erythrophagocytosis in the nodes. The presence of erythroblasts of different stages of development (polychromatophilic and orthochromatophilic erythroblasts) is a sign of erythropoiesis occurring in these nodes.

The results obtained on the structural and functional features of the atypical lymph nodes of the Korean water deer are a significant contribution to the development of theoretical foundations on the histology, physiology and species morphology of mammals and are of scientific interest for further research in this field.

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