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## Caprine haemolymph nodes: Its structure and functions

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### Abstract

caprine haemal nodes were located in the thoracic, abdominal, costocervical vessels and pelvic retroperitoneal cavities. The shape and size of the haemal node was pea sheaped and 5mm in average diameter. The histological structure of haemolymph node was consisted of a capsule, subcapsular, sinuses, differentiated cortex and medulla with hilus. The two layers thick capsule was found around the parenchyma. Besides connective tissue fibres smooth muscle and blood capillaries were predominant in the innermost layer. CD4 and CD8 positive cells were mostly distributed in the subcapsular, inter follicular and within the outer dome shaped part of the haemal lymph node. B cells were found from date of birth. At birth the B cells were found in the medullary zone and from 2<sup>nd</sup> months the B cells were mostly found in the germinal center of the secondary follicles. Caprine haemal node, the distribution of lymph vessels and the finding of only efferent lymph channels were related to the roles of haemal nodes in blood storage by hemoconcentration and in immune defense mechanisms.

**Keywords:** Caprine, Haemolymph nodes, Immune system

### Introduction

Goat rearing popularized in India among poor and marginal farmers for livelihood. Today goat husbandry contributes a notable share in National GDP. Low cost goatery is not developed because Kid mortality is a biggest threat to the goat farmers. In Eastern and north eastern part of India is proud of prevailing very good quality goat breeds like Black Bengal goat.

General health and disease susceptibility is directly related with the immune status of the individual. Secondary lymphoid organs are rich in macrophages and dendritic cells that trap and process antigens and in T and B lymphocytes, which mediate the immune responses. Secondary lymphoid organs include the lymph node, spleen and haemolymph node.

The structure, fine structure and blood supply of haemal nodes are currently uncontroversial. In particular, current anatomical knowledge provides a basis for distinguishing between haemolymph nodes, which are endowed only with blood vessels. This structure is named haemolymph node (Robertson, 1890) [16]. But their development is still unanswered. Haemal nodes are independent lymphoid organs located within the blood vascular system between arterioles and venules of different mammalian species and also in some birds (Vincent and Harrison, 1897) [19]. True haemal lymph nodes occur in the sheep (Copenhaver, 1964) [5]. The haemolymph nodes of sheep and goats contain myeloid cells and erythroblast, and suggested that they act in the same way as bone marrow. Haemolymph nodes are very similar to lymph nodes except that afferent and efferent lymphatic vessels are absent and the sinuses contain blood instead of lymph. True haemal related to the spleen, because they undertake blood filtration, and to the bone marrow because erythropoiesis and myelopoiesis takes place in them (Erencin, 1948) [6]. Antibodies are produced in haemal nodes and thus they clearly belong to the lymphatic system (Nickel *et al.*, 1976) [14]. By contrast with their anatomy, the function of haemal nodes has not been fully elucidated: postulated functions include erythrophagocytosis, erythropoiesis, and platelet formation and, most recently, immune functions. Hence the present study has been undertaken to elucidate the structure and function of caprine haemal nodes.

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## Results and Discussion

### Occurrences of the haemal nodes

Haemal nodes were mainly located in the thoracic, abdominal and pelvic retroperitoneal cavities of sheep and goats (Gargiulo *et al.*, 1987; Ezeasor and Singh, 1988) [11, 8]. The haemal nodes were present along the ventral border of the molar part of the mandible at the junction of the lingual and facial veins. Small haemal nodes lie at the dorsal side of the cranial part of the neck, ventral to the wing of the atlas, usually embedded in fat. Between the costocervical vessels small haemal nodes were seen in its vicinity (Choudhary *et al.*, 2011) [4].

### Shape, size and colour of the haemal nodes

The haemal nodes were deep red or dark brown in colour and were usually not large than a pea and their size varies from 1 to 20 mm in diameter (Getty, 1975) [12]. In small ruminants they rarely exceed 5mm (Nickel *et al.*, 1976) [14]. The haemal nodes were generally small, brown to dark red organs, but their size and number varied within wide limits (Eurell and Frappier, 2006; Zidan and Pabst, 2004) [7, 20]. The sizes of the nodes were 3-4mm upto 2<sup>nd</sup> months of age and there after the size increased 5-6mm from 3<sup>rd</sup> months onwards (Choudhary *et al.*, 2011) [4].

### Histomorphology of the haemal nodes

The capsule of the haemal node was composed of fibrous connective tissue and contains few smooth muscle cells. Hemal nodes had a well-developed peripheral sinus which contains blood from this; secondary sinuses extend into the interior of the node and from an intercommunicated system of blood spaces. There was no clear division into cortical and medullary parenchyma of the lymphatic tissue. The lymphatic tissue in the haemal node consisted of lymph nodules with reaction centers (Getty, 1975) [12].

In the ox the capsule was thin, but in the small ruminants it was thick and composed of two distinct layers. The outer layer was fibrous and the inner layer was characterized by a dense network of blood capillaries. In sheep especially the capsule and trabeculae contain collagen, elastic fibres and also smooth muscle cells. The parenchyma was made up of a three dimensional reticular mesh work. In the sheep but not the goat, a cortex and a medulla can be differentiated. Secondary nodules occur in the sheep in the cortical substance only while in the diffuse lymphoreticular tissue of the medulla, erythrocytes, erthroblasts and gaint cells were encountered. In the haemolymph nodes of the goat secondary nodules were more-eventy distributed in the parenchyma. Between the capsule and the cortex there was a marginal sinus which was broad in the sheep but narrower in the other ruminants (Nickel *et al.*, 1976) [14].

The caprine haemal node consisted of a capsule, subcapsular and other sinuses, cortex, medulla and hilus. Elements of circulating blood filled the interstices of the reticular meshwork and associated macrophages which traversed the lumina of subcapsular and medullary sinuses. The latter were rare in 1 month old goats, progressively increased in number and size in 2 to 4 month-old goats and coalesced with each other and the subcapsular sinus in adult animals. The cortical tissue appeared as lymphoid nodules. Circumferential lymphatic vessels abutted on outer margins of the nodules and gave origin to several radial lymphatics which branched and anastomosed between the medullary blood sinuses. Medullary cords were organized around the radial lymphatics. A single efferent lymphatic was formed at the hilum by confluence of

the radial lymphatic. The lymphoid tissue contains plasma cells and mast cells in goats of different age (Ezeasor and Singh, 1988) [8].

Caprine hemal node, the lymph vessels were characterized by thin walls and wide lumens containing inspissated lymph in which a few cells were suspended. The lymph vessels contrasted sharply with adjacent blood sinuses that were filled with elements of circulating blood. A circumferential lymph vessel in the cortex joined radial branches in the medulla that met at the hilum to drain through a large efferent lymph channel (Ezeasor and Singh, 1990) [10].

The bovine haemal nodes had a capsule of connective fibrocollagenous tissue, strengthened by reticular fibres and smooth muscle cells that send trabeculae to their interior divided the parenchyma partially. The latter was represented by lymphatic tissue which is distributed in two well defined regions: a cortical one and a medullary one. In the cortical zone, primary and secondary lymphatic nodes were described (Flemming's reactional centre) and in the medullary zone the lymphatic tissue adopts a cord like shape, limiting the central sinus. These structures were set on reticular fibres. The sinus wall was composed of endothelial cells, basal membrane and reticular cells (Cerutti *et al.*, 1998) [3].

The haemal nodes of the camel had one or two hili and a capsule and trabeculae of connective tissue and smooth muscles. The main parenchyma was composed of a cortex and a medulla. The cortex was formed from lymphoid follicles and diffuses interfollicular lymphocytes. The medulla consisted of lymphoid cords separated by medullary sinuses. There were also subcapsular, peritrabecular and medullary blood sinuses (Zidan and Pabst, 2004) [20].

Roe deer haemal nodes, had connection only with blood vessels, were covered with a thin or thick capsule of connective tissue in the foetus and the adult respectively. Trabeculae were not observed in foetal nodes. In adults, the capsule, trabeculae and, in particular, the sinuses were strongly supported by reticular fibres and cells. A cortex and medulla could not be differentiated either in foetal or in adult haemal nodes. The haemal nodes had no distinct hilus. There were no mast cells in the lymphoid follicles and lymphatic cords of adult and foetal haemal nodes. The morphology of these nodes displayed similarity to that of other ruminant species (Bozkurt *et al.*, 2010) [2].

The lymph nodes were encapsulated with two distinct layers. The outer layer was made up of connective tissue and inner layer was made up of smooth muscles fibers. Capsule tightly packed the parenchyma and was absent at the point of hilus. Collagen fibers were predominantly found around the capsule and spindle shaped nucleus denoted the presence of smooth muscle fiber component of the capsule. The inner layer of capsule was characterized by dense network of blood capillaries (Choudhary *et al.*, 2011) [4].

The parenchyma was incompletely divided by the trabeculae which was distinct from 2<sup>nd</sup> months onward. From the date of birth cortex, medulla, subcapsular and medullary sinuses were observed. Megakaryocytes, proerythroblasts and erythroblasts were detected from the day old goat and size increase according to the advancement of age. Efferent lymph vessel was found but there was no trace of afferent lymph vessel. The lymph vessels were present around the border of the secondary lymph nodules (Choudhary *et al.*, 2011) [4].

Grossly the color of the haemal nodes appeared brownish in some of the goats of 4<sup>th</sup> months of age and histologically the eosinophilic pink appeared yellowish brown. Under microscopical observation the phagocytic / macrophage cells

were observed. It resembled the presence of haemocederin in the haemal node sinuses and in the cytoplasm of some macrophage cells (Choudhary *et al.*, 2011) [4].

#### Distribution of lymphocyte subsets in the haemal nodes

In case of sheep that marked differences were found in the percentage and distribution of lymphocyte subsets (CD5, CD4, CD8, T19, CD45, and CD45R cells) in hemal nodes compared to those in mesenteric lymph nodes and blood. There were a greater number of T19 (gamma/delta)+ lymphocytes and fewer CD8+ lymphocytes in hemal nodes than in mesenteric nodes and a higher percentage of CD4+ in hemal and mesenteric lymph nodes than in blood. T19 (gamma/delta)+ lymphocytes were found throughout hemal nodes but were absent from the follicles. Of special interest was the finding that 20% of hemal node lymphocytes appeared not to express CD45 (leucocyte common antigen) (Thorp *et al.*, 1991) [17].

The haemal nodes of the camel the interfollicular lymphocytes and those in the medullary cord were CD3-positive. The lymphoid follicles showed CD22-positive cells. MHC class II/DR was expressed by most cells of the parenchyma (Zidan and Pabst, 2004) [20].

CD4 positive cells were mostly distributed in the subcapsular, inter follicular and within the outer dome shaped part of the haemal lymph node. The number of CD4 positive cells were more in the-inter follicular area in all experimental age groups. The number of CD4 positive cells increased with age. In the medullary sinuses the CD4 positive cells were scatteredly distributed. The CD8 positive cells were found in the same manner like that of CD4 positive cells. The number increased with age. In all the experimental groups the ratio of CD4 and CD8 positive cells were same. CD8 positive cells were also noticed around the lymphatic vessels. B cells were found from date of birth. At birth the B cells were found in the medullary zone and from 2<sup>nd</sup> months the B cells were mostly found in the germinal center of the secondary follicles. The numbers of cells were found more from 2<sup>nd</sup> months onwards. Beside this area B cells were also located scatteredly in the-inter follicular area (Choudhary *et al.*, 2011) [4].

#### Ultra structural morphology of the haemal nodes

##### Scanning electron microscopy

In bovine haemal nodes the capsule was made up of dense fibrous connective tissue, where it was possible to observed fibres of a different diameter, collagen fibres of a greater thickness and reticular fibres. Blood vessels go towards the organ interior through the hilus. Underneath this structure it was found that the blood vessels flow into the marginal sinus, which communicates with the medullar sinus in the centre of the centre of the organ through the peritrabeculum sinus. The lymphocytes were organized in nodes and lymphatic cords, above a reticular fibril skeleton, which was externally related to collagen fibres that belong to the capsule walls, where the entrance to the peritrabeculum sinus was observed (Cerutti *et al.*, 1998) [3].

The gross surface area of a caprine haemal lymph node collected at birth. It was covered by a dense connective tissue covering. The blood vessels were found entering the node through the hilus. In higher magnification section it was revealed that there were huge network of collagen and reticular fibre over the outer most capsule of the gland (Choudhary *et al.*, 2011) [4].

#### Transmission electron microscopy

The plasma cells showed many cytoplasmic processes, indicating motility and phagocytic activity. They were randomly located in the medullary cords of the hemal lymph nodes (Bagdadi *et al.*, 1986) [1]. The caprine hemal node macrophages were observed to be engaged in Erythrophagocytosis. In the early stages of endocytosis, intact erythrocytes were contained in some of the heterophagic vacuoles of macrophages. Later, granular, electron-dense material appeared on erythrocytes, presumably as a result of lysosomal degradation of their matrices. Subsequently, the matrix fragmented and probably formed 'myelin-like figures' and residual bodies that dominated the macrophage cytoplasm. In addition, images of sinusoidal endothelium, reticular cells, lymphocytes and, rarely, eosinophils were observed that depicted structures resembling various stages of lysosomal digestion of erythrocyte matrix noted in macrophages (Ezeasor *et al.*, 1989) [9]. In caprine hemal node Ultra structurally, the lymph vessel wall comprised endothelial cells supported by a continuous basal lamina, collagen fibrils, and adventitial reticular cells. The cytoplasm of endothelial cells had fenestrations, plasmalemma-associated vesicles, vacuoles, and focal splits that enclosed large compartments (Ezeasor and Singh, 1990) [10]. The haemal lymph node revealed presence of macrophage. The macrophage was observed in normal stage without any noticeable phagocytic activity at birth. Besides the macrophage sinusoidal endothelium, erythroblast and lymphocytes were noticed. In one section myelin like figure was observed within the matrix of the macrophage. However from the other slide it was revealed that large macrophage was found with granular electron dense material on the erythrocytes (Choudhary *et al.*, 2011) [4].

The bovine haemal nodes sinuses wall ultrastructurally it could be described as a three stratified wall. This covered the lumen, endothelial cells, abasal membrane made up of an amorphous, electrondense material and collagen fibres and on the outer part there were reticular cells (Cerutti *et al.*, 1998) [3].

The medullary cords were separated by blood sinuses usually rich in erythrocytes. The sinus wall of the camel haemolymph node was formed from endothelial cells resting on a basement membrane supported by collagen fibres (Zidan and Pabst, 2004) [20].

#### Functions of the haemal nodes

The haemolymph node had same of the functions of the spleen and bone marrow. The reticular cells of haemal nodes were capable of phagocytosis and storage with the result the deposits of pigment can cause them to appear brown. The amount of blood and pigment presented in the node significantly effected its colour (Nickel *et al.*, 1976) [14]. The caprine hemal nodes were involved, in addition to classical functions, in blood storage by hemoconcentration (Ezeasor and Singh, 1988) [8]. The Erythrophagocytosis was currently defined as the process by which an organism removes old or degenerate erythrocytes from the circulation. It was performed primarily by macrophages, mainly in the spleen but also in liver, lung, bone marrow and, in certain species, in haemal or haemolymph nodes (Ezeasor *et al.*, 1989; Lorvik *et al.*, 1983; Oláh and Törö, 1970; Turner, 1971) [9, 13, 15, 18]. Caprine hemal node, the distribution of lymph vessels and the finding of only efferent lymph channels were related to the roles of hemal nodes in blood storage by hemoconcentration and in immune defense mechanisms (Ezeasor and Singh, 1990) [10]. The large quantities of haemosiderin pigment were

produced by degradation of haemoglobin, in both the sinuses and the cytoplasm of some macrophages (Nickel *et al.*, 1976; Choudhary *et al.*, 2011) <sup>[14, 4]</sup>. The presence of secondary follicles is indicative of a role for antibody production. The occurrence of both erythropoiesis and erythrophagocytosis in caprine haemal nodes started from the date of birth. Rate of destruction of erythrocytes was observed in high frequency from 4<sup>th</sup> month's onward (Choudhary *et al.*, 2011) <sup>[4]</sup>. The functional significance of hemal nodes was not clear, although it was probable that they respond to blood borne antigens (Eurell and Frappier, 2006) <sup>[7]</sup>.

The bovine haemal nodes were lymphatic organs with haemal circulation. The blood circulates inside of them through virtual cavities named sinuses. In these sinuses there was passage of cells and particulate materials from parenchyma to sinuses and vice versa (Cerutti *et al.*, 1998) <sup>[3]</sup>.

The camel haemolymph nodes perform similar functions in the spleen and lymph nodes in filtering the blood and lymph, respectively, in addition to their role as a source of antibodies in response to circulating antigens. The presence of secondary follicles was indicative of a role for haemal nodes in antibody production (Zidan and Pabst, 2004) <sup>[20]</sup>.

### Conclusion

Kids mortality more common in Black Bengal Goat, which is found in Eastern and North Eastern part of India. Antibodies are produced in haemal nodes and thus they clearly belong to the lymphatic system. Caprine hemal node, the distribution of lymph vessels and the finding of only efferent lymph channels were related to the roles of hemal nodes in blood storage by hemoconcentration and in immune defense mechanisms.

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