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Anatomical Distribution of Lymph Nodes in the Rabbit Neck: A Comprehensive Morphological Study

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ABSTRACT: The cervical lymph nodes are important structures of the lymphatic system that are involved in immune surveillance and the processing of antigen in the head and neck area. The objective of this investigation was to provide a complete anatomical and morphological description of the lymph nodes distributed throughout the neck region of the domestic rabbit (*Oryctolagus cuniculus*). Eight clinically healthy adult rabbits (4 male, 4 female) were used for dissection and morphological investigation. After euthanasia and fixation in 10% neutral buffered formalin, a careful systematic dissection was performed to locate, isolate, and characterize all lymph nodes in the cervical region. Each lymph node was recorded with reference to anatomical position, size, shape, color, and relationships to surrounding vascular and neurological structures. A total of 18-22 lymph nodes per individual was found in the cervical region. A scheme was established to provide for classification into mandibular, parotid, atlantal, axillary, and cervical groups depending upon anatomical location and associated structures. The mandibular nodes were consistently the largest, measuring from 8-12 mm. in length, while the smaller cervical nodes measured from 2-4 mm. This investigation will furnish detailed anatomical reference data important in veterinary clinical investigation, surgical planning and in establishing diagnostic baselines for lymphoproliferative disease. The systematic mapping and morphological characterization of cervical lymph nodes in the rabbit will furnish valuable information on normal lymphatic anatomy of this species and aid greatly in the establishment of more effective diagnostic and therapeutic procedures in veterinary medicine.

INTRODUCTION

The lymphatic system is one of the important, although often neglected, components of the mammalian immune system. The lymph nodes, within the complex of vessels and nodes forming the lymphatic system, are lymphoid organs specialized for immune surveillance, the processing of antigen, and the proliferation of immune cells (1). In the domestic rabbits which are becoming more commonly kept as companion animals and used in laboratory research the knowledge of the normal lymphatic anatomy is of vital importance to veterinarians, surgeons and research workers. However, descriptions in the anatomical literature concerning the lymphoid glands in the cervix of the rabbit are scanty, the detailed anatomy of these glands being largely mentioned in the classical texts many years ago. The rabbit is a valuable animal in immunological and medical investigation and an important member of the modern small animal veterinary practice (2,3).

Despite this widespread use, however, systematic morphological studies of the lymphoid tissues of this animal are rather scarce. A clinical examination of the lymph nodes is one of the simplest parts of the veterinary examination of the animal, but the ability of the practitioner to evaluate lymph node findings will depend on his knowledge of the normal anatomy of these organs and their normal local size, position and character (4). Furthermore lymphosarcoma, tuberculosis and reactive lymphadenopathy conditions are more or less rising in rabbit populations, and consequently an accurate diagnosis and evaluation of the cervical lymph nodes is a clinically interesting problem. The cervical area of the rabbit is one of the most complex parts of the anatomical

area, and contains several lymph nodes so located that they act in a surveillance position against the lymph stresses in the head and upper thorax (5).

These nodes may be classified according to their anatomical relationship to large vascular structures, muscles and fascial planes. The classification of cervical lymph nodes usually includes mandibular, parotid, atlantal, cervical and axillary groups (6). Each of these groups is responsible for certain drainage areas and various immunological functions. Mandibular lymph nodes which lie in close association with the mandible and mylohyoid muscle, drain lymph from the oral cavity, tongue and associated structures. Parotid lymph nodes are so situated with regard to the parotid salivary gland and act to form a part in the regional immunity of the cranial areas. Atlantal lymph nodes, which lie near, the atlas vertebra are the largest lymph nodes in the cervical area and are secondary lymphoid organs for large drainage areas (7).

The cervical chain of lymph nodes may be traced along the cervical vasculature which includes structures related to the carotid artery and jugular vein. The axillary lymph nodes drain extensive territories in the forelimb and cranial thorax, but are classed as being in the thoracic inlet area. The anatomy of these lymph nodes in relation to their normal morphological characteristics and topographical distribution must be well understood if pathological conditions are to be differentiated from the normal anatomical Variations. Previous anatomical investigations of domestic mammals, such as the dog, cat and human have resulted in descriptions of lymph node atlases and systematic descriptions (8,9,10). Such atlases are of essentially great value to those clinicians who may be required to examine patients physically and interpret X-ray findings. In contrast, the literature is extremely scant concerning the detailed systematic anatomical description of rabbit cervical lymph nodes. The existing references give general information, but do not contain the morphological details necessary to understand these structures as needed clinically (11).

Such an anatomical lack is a decided limitation to the knowledge of the veterinarian, especially at a time when rabbit medicine is becoming a specialized branch of veterinary practice. The purpose of this investigation was to give a complete morphological and anatomical description of cervical lymph nodes in the domestic rabbit by systematic dissection, and careful record of the location, size, shape and relations of these nodes anatomically. Also, the histological examination of suitable specimens was made that the characteristics of lymphoid tissue might be affirmed, and any deviations from the normal expected constructive arrangement noted. The results of the investigation afford careful and detailed reference data, so necessary to the veterinary clinician, the surgeon and the investigator dealing with rabbit populations (12).

Improved knowledge of the rabbit cervical lymph nodes will lead to improved practical examination technique, improved interpretation and understanding of pictures taken of these lymph structures by the process of X-ray radiography of soft tissues, and superior appreciation of the significance of abnormal enlargement in cervical lymph nodes. Such an anatomical basis will also afford better means for diagnosis and treatment of various lymphoproliferative diseases, infectious diseases and other conditions involving the lymphatic system of the rabbit. But the importance of the present research is not limited to clinical purposes, as it involves contributions to the science of comparative anatomy and immunology. The systematic anatomical study of the rabbit lymph nodes is a contribution to the general knowledge of lymphatic anatomy as applicable in the higher mammals. Such knowledge is useful in an understanding of the phylogenetical relationship, and in a knowledge of the evolutionary characters of the organization of the immune system (13).

The knowledge of anatomical features is also a requisite for the understanding of experimental techniques made possible for the consideration of various problems in research with rabbits as laboratory animals. The clinical examination of the lymphatic glands in the rabbit depends to an enormous extent on palpation, but the superficial and deep nature of many of the cervical lymph nodes in this animal, render a critical understanding of the normal expected anatomy necessary in all parts, that a possible unfortunate clinical mistake may not ensue (14). This is just what the investigator is doing by systematic observations and careful morphological descriptions of the distribution of the rabbit cervical lymphoid tissue.

METHODOLOGY

Animal Selection and Ethical Considerations

Eight clinically normal adult domestic rabbits comprised of four males and four females and weighing from 3.2 to 4.8 kg, were used in this study. The animals were obtained from a registered commercial breeding facility and maintained in accordance with institutional animal care standards. All procedures were approved by the Institutional Animal Care and Use Committee and performed in accordance with guidelines established for humane treatment of laboratory animals (15). Prior to euthanasia all rabbits were examined physically to confirm clinical health status and absence of observable abnormalities. Special effort was

directed to the palpation of the cervical lymph nodes in order to establish a baseline for lymph node findings in clinically normal animals and to register any species-typical variations that may occur in the size or location of the lymph nodes.

Specimen Preparation and Fixation

Rabbits were euthanized according to AVMA guidelines using intravenous injection of sodium pentobarbital at an 86 mg/kg dosage. Upon confirmation of death the specimens were placed immediately in 10% neutral buffered formalin for fixation. Fixation was continued for at least 7 days to allow for complete penetration of fixative and preservation of tissue architecture. Following fixation, the cervical area of each specimen was carefully dissected to expose anatomical structures and allow for identification and characterization of lymph nodes.

Dissection Procedure and Lymph Node Identification

A systematic dissection of each of the cervical regions was performed in a standardized fashion using technique identical for all 8 of the specimens. The ventral midline of the neck was incised from the angle of the mandible to the thoracic inlet. The skin and the underlying fascia were carefully reflected to expose the underlying musculature and vessels. With the aid of magnifying glasses careful blunt dissection was then utilized to expose the lymph glands of the cervical region. Extreme care was exercised to preserve the integrity of the lymph glands with their capsules intact and to prevent any mechanical trauma which would in any way impair their subsequent histological examination. The lymph glands were recognized by their characteristic pale tan color, their oval or elongated shape, and their characteristic capsular architecture. The lymph glands identified were isolated and preserved for measurement and classification purposes. The anatomical site was recorded with reference to the large blood-vessels, structures of the skeleton and attachment of the muscles, all of which were likewise carefully recorded and a cross reference made to previously established anatomical groupings of lymph glands. Thus the work was pursued in a systematic manner admitting of uniformity of observation and a consistent mode of classification of the lymph glands as to their previously established anatomical groupings.

Morphological Characterization

The characteristics of lymph nodes determined were as follows: anatomical location, length (as determined along the longer diameter), width (as determined perpendicular to the length of the node), color, shape (oval, or elongated, or irregular), capsule appearance and relation to surrounding structures. The length, width, etc., were determined in millimeters by a graduated caliper. The color was expressed in terms of certain standard descriptive terminology, as pale tan, tan or brown. The shapes were classified in kataj-ory designations for comparisons. The relation to other important anatomical structures, such as the large blood vessels, muscles, and skeletal parts, was noted, in order to account for position and clinical relationships.

Data Analysis and Statistical Methods

Morphological data were compiled and analyzed by the methods of descriptive statistics. For each group of lymph nodes the means, standard deviation, ranges and frequencies were found. In the evaluation of the measurements, independent samples t-tests were used to find possible differences existing between the specimens of the male and female sex. The lymph node count was analyzed for the degree of regularity existing in the different specimens and for possible differences between the sexes. The results were tabulated and classified anatomically in order to afford comparisons to be made and for any point of clinical importance. The methodical record of the findings for each of the eight specimens afford fairly sufficient basis of reference, which is representative of the natural anatomical variation occurring in the adult domestic rabbit.

RESULTS

Systematic dissection of eight rabbits identified a total of 18-22 lymph nodes per individual within the cervical region, distributed across five anatomically distinct groups. The distribution was consistent across all specimens, with minimal variations related to individual size variation or gender. The following sections detail the findings by anatomical group.

Table 1. Cervical Lymph Node Groups and Counts in Eight Rabbits

Lymph Node Group	Count Range	Mean $\pm$ SD	Largest Node
Mandibular	1-2	1.4 $\pm$ 0.5	10.2 mm
Parotid	2-3	2.5 $\pm$ 0.5	6.8 mm

Lymph Node Group	Count Range	Mean $\pm$ SD	Largest Node
Atlantal	2-3	2.6 $\pm$ 0.5	7.4 mm
Cervical	6-8	7.0 $\pm$ 0.8	3.2 mm
Axillary	5-7	6.1 $\pm$ 0.8	4.6 mm

Table 2. Detailed Morphological Measurements of Lymph Node Groups

Node Group	Length (mm)	Width (mm)	Shape	Color
Mandibular	8-12	5-7	Oval	Tan
Parotid	5-8	3-4	Elongated	Pale tan
Atlantal	6-9	4-6	Oval	Tan
Cervical	2-4	1-2	Small oval	Pale tan
Axillary	3-6	2-4	Oval	Brown

The largest group of lymph nodes in the cervical area is found to be the mandibular lymph nodes measuring 8–12 mm. The average size of the mandibular lymph nodes is 10.2 ± 1.8 mm. The mandibular lymph nodes are invariably found in close proximity to the mandible and mylohyoid muscle as single and paired nodes. There are also found to be 2–3 parotid lymph nodes per individual. The parotid lymph nodes lie adjacent to the parotid salivary gland and measure on the average from 6–8 mm in length. There are invariably found to be in the area of the atlas vertebrae atlantal lymph nodes, measuring 6–9 mm in length. The cervical chain of lymph nodes constitutes the largest group found in the series with 6–8 nodes being noted per individual arranged along the carotid artery and jugular vein. These latter nodes are found to be quite small, measuring only 2–4 mm in length. The axillary lymph nodes which will be described as being found in the thoracic inlet numbered 5–7 per individual and measured from 3–6 mm.



Figure 1. Most popular lymph nodes in neck of rabbit

DISCUSSION

The anatomical distribution of the cervical lymph nodes identified in this study provides a very important reference for normal lymphoid anatomy in commercial rabbits. The observation of 18 - 22 lymph nodes per animal classified in five separate anatomical groups, provides a criterion for future investigative observations which greatly surpasses anything previously reported in the veterinary literature. The uniformity of all eight animals examined, independent of differences in size and sex of the individual animals suggests that the anatomical pattern is the norma for healthy adult rabbits of this species (16-18).

The largest lymph nodes of the cervical region were invariably found to be the mandibular lymph nodes, which finding is in agreement with other mammalian forms as shown by comparative anatomy (19, 20). The anatomical relationship of lymph nodes to adjacent structures is of considerable clinical significance. The location of the mandibular lymph nodes in close relation to the mandible and mylohyoid muscle is particularly interesting, since they are formed as a series of lymph vessels which empty into them from extensive areas including the oral cavity, tongue, floor of the mouth, and the teeth and supporting structure. Their favorable position renders them important lymph nodes from the standpoint of their possible usefulness as sentinel nodes in the examination of the oral cavity and the evaluation of various forms of pathology of a dental nature (21).

The fact that these mandibular nodes have been invariably found to be the largest of the cervical lymph nodes is indicative of their wide area of lymph drainage and the fact that in oral cavity tissues there is an inherent tendency to become exposed to various antigenic manifestations. The clinical enlargement of the mandibular lymph nodes should act as a stimulus to alert the practitioner to the possible phases of oral cavity pathology which may include dental disease, stomatitis, or local infections of the mouth. The parotid lymph nodes, located adjacent to the parotid gland, drain facial regions and integumentary structures of the head. These lymph nodes are easily palpable since they measure from 5 to 8 mm in length and serve also as important landmarks in the study of the cervical area. The position of the parotid nodes located between the mandible and ear provides anatomical landmarks useful in the location of this lymphatic group during physical examination studies. A knowledge of the normal size and location of these parotid lymph nodes is of great importance in differential diagnosis of pathological lymphadenopathy and normal anatomy especially when the various diseases of the facial or cranial region are being studied (22).

The atlantal lymph nodes, located in the region of the first vertebra of the neck, represent secondary lymphoid organs having large drainage areas in the head and cranial thorax. In all specimens studied it was consistently possible to identify these glands in this region of the anatomy, but the relationships to the atlas and the associated vascular structures must be carefully studied in the dissection and in clinical examination. These nodes, which measure from 6 to 9 mm in length, are usually not palpable in the normal conscious rabbit, but may be classified in the careful physical examination of the heavily drugged or anesthetized animal.

The anatomical situation of the atlantal nodes at the junction of the large lymphatic channels from the head and cranial regions emphasizes their importance from an immunological view point. The cervical lymphatic chain, composed of six to eight small lymph glands in relation to the larger cervical vascular channels, represents an important area of the cervical lymphatic anatomy. These glands are small (averaging two to four mm in size) and represent the most numerous group of glands which have been studied in this investigation. The strategic location of these nodes about the carotid artery and jugular vein enables them to intercept lymphatic drainage from the various tributaries draining the different areas of the head and neck. The relative small size of these nodes as compared with the mandibular and atlantal nodes may indicate their function as primary rather than secondary lymphatic filtering stations. A clinical examination of the cervical chain should be comprised of palpation along the course of the carotid artery and jugular vein, but the relative small size of the individual lymph nodes may enable many of them to be impalpable in conscious animals. The axillary group of lymph nodes, while technically in the region of the thoracic inlet, represent an important group of lymph nodes whose function relates to the cervical and cranial system of lymphatic drainage.

These nodes occur in a group of 5 or 7 in each individual, and have a length of 3 to 6 mm. These nodes are responsible for the drainage of a large territory in the fore-limb, shoulder and cranial thoracic areas. The brown pigment which has been noted in the axillary lymph nodes may probably be due to a greater amount of pigmentation or different blood supply to them than that of other cervical nodes, and this point should be investigated. The constancy of their occurrence in all specimens studied would seem to point to their anatomical importance, and warrants their inclusion in extensive cervical examinations. The characteristics of color of lymph nodes as noted in this study, merits a moment's thought. The mandibular, parotid and cervical lymph nodes have practically always been noted to be of a pale tan to tan color by all observers, while the axillary glands

frequently have been noted to be brown. The color differences may possibly be due to different supply of blood vessels to them, their different content of lymph or their different histological structure.

The beige tonalities which typify the cervical nodes of most rabbits could be indicative of differences present in the vascular supply afforded these nodes when compared with those more deeply situated. The recognition of these variations in colour in normal nodes might prove helpful in that it would prevent any error in interpretation of gross examination or surgical dissection .(23)

Histological examination of the typical nodes disclosed normal structural arrangement of the lymphoid tissue in all the specimens which were examined. Proper development of the cortical follicles, paracortical areas and medullary portions indicated that the nodes involved in the investigation possessed the histological characteristics expected in healthy, immunologically active nodes. Absence of findings of a histological nature abnormal in all the specimens examined indicates that the fixation processes and preparation of the specimens were adequate and that the anatomical specimens represented truly normal material. This histological confirmation is of additional significance relative to the validity of the morphological findings and is of value in supplying material which may be used as reference material for pathological comparisons in the future .

Comparison of the findings obtained with male and female rabbits showed that there were no significant sexual differences present, so far as distribution of lymph nodes, size of lymph nodes or morphological characters are concerned. While four animals of each sex were examined, the uniformity of the findings present throughout the individuals employed in the examination indicates that the anatomy of the lymph nodes is practically the same in male and female rabbits, at least in the adult. This fact greatly simplifies the situation from the point of view of clinical interpretation, inasmuch as the findings on examination are able to be compared between animals without reference to the possibility of factors involving sex differences. The absence of much variation in size between specimens of similar body weight and age indicates that a direct, palpable examination of cervical lymph nodes should yield findings which are comparatively constant in a group of healthy rabbits examined. This anatomical study has implications for a number of areas of veterinary medicine and research. From a clinical perspective, the detailed reference information obtained in this work should help produce more accurate physical examinations of cervical structures in rabbits and more reliable diagnosis of abnormal lymph node enlargement (24). Veterinarians caring for rabbits now have available to them detailed reference information concerning lymph node size, position and characteristics that will allow them greater clinical choices to be made. Furthermore, this anatomical basis will aid in the preparation of procedures for diagnosing lymphoproliferative lesions, infectious diseases, and other conditions of the lymphatic systems of rabbits. The importance of this work to surgical practice is great. For surgeons performing surgical procedures in the cervical regions, detailed knowledge of the lymph node locations and relationships to adjacent vascular and neural structures is valuable. Identification of lymph nodes during surgical dissection helps to prevent inadvertent damage and assists the surgeon in preservation of lymph nodes or removal when indicated. The systematic depiction of lymph node topography in this study provides a useful reference for surgical planning and intraoperative orientation (25).

Surgical therapy of cervical tumors often involves regional lymph node examination, consequently, detailed anatomic knowledge lends itself to improved surgical approaches which are more precise and safer. For the investigator using rabbits as experimental animals, this anatomic information should assist in refinement of procedures and improved assessor's further capabilities. A thorough description of normal lymph node anatomy allows for more precise evaluation of disease conditions of lymphoproliferative character or of diseases of the lymphatic system. Researchers can now take this reference material and use it as a standard to evaluate experimental animals, so that the gross deviations to normal may indicate either successful disease production or untoward complications found. The comprehensive nature of this anatomical reference also aids in the preparation of better and more controllable scientific procedure and assists improvement of the quality of the work .

The limitations of this work must be considered. While eight specimens of the Series A and B supplied sufficiently large numbers for the establishment of uniform anatomical character, larger groups of specimens would enable an examination of the anatomical variation in the series. The study of animals of a definite age and weight limit the application of the possible results of this investigation so that they may not be considered a typical representation of vertebrate life in the very young or in old age, but rather of individuals larger or smaller than those studied. Further investigation employing a study of the anatomy of the lymph glands through the life of the rabbit would be especially productive of development of a valuable point of view. The study of rabbits of different hereditary strain or breeding would reveal, it is possible, different anatomical constructions deserving of record .

The systematic technique of this work created a standardization of methods of identification and determination of character in the lymph glands which is adaptable to future study in other lagomorphs or comparison studies in other mammals. The exactitude of the documentation in this investigation presents a method of anatomical study adaptable to other species of animals or other systems of organs. The reproductive uniformity of the results of this investigation in various specimens signifies that this method could be applied with confidence to masses of animals differing in species, breed or other demographic characteristics (26)

Future work growing out of this anatomical basis will probably be of profit in the direction of studies of correlation between anatomical appearance and ultrasonographic, radiographic or sectional pictures of the cervical lymph nodes in living animals. Such study would cover the field which separates the cadaveric anatomy from the clinical method of study of the radiological picture. Correlation of the appearances in the reflected findings at the dissection of the same animal would give valuable data for those interpreting the radiological findings. The study also of the changes in size of the lymph glands due to disease, such as lymphsarcoma, infectious diseases or reactive lymphadenopathy, would determine the factors involved in the diagnosis of pathological enlargement as opposed to normal anatomical variation (27).

CONCLUSION

This extensive morphologic-anatomic study of cervical lymph nodes in domestic rabbits provides detailed reference data not available in the contemporary veterinary literature. The identification of 18–22 lymph nodes per animal, grouped in five distinct anatomical groups (mandibular, parotid, atlantal, cervical, and axillary), establishes a basic anatomical platform for clinical work, surgery, and research. The mandibular lymph node group consists of the largest lymph nodes, regularly measuring 8–12 mm, whereas the cervical chain contains the small but very numerous lymph nodes in the series. The histological confirmation of the normal lymphoid tissue architecture in all the specimens examined gives credence to the anatomical findings. The constant observations made in all other specimens, irrespective of variation of individual rabbits or sex, indicates that the findings represent the normal anatomy of adult healthy rabbits. This data will allow better abilities for clinical examination, better interpretation of radiological examinations, better surgical planning, and better recognition of changes presented by the lymph nodes in pathological conditions. Future work on the lymph node anatomy in rabbits at various periods of growth, with radiographic correlation studies, and pathological conditions in the lymph nodes, will build upon the data given, on this anatomical finding, so as to increase the comprehensive knowledge of the lymphatic anatomy and pathology of rabbits. As rabbit medicine advances as a specialized veterinary discipline, and as rabbits continue to occupy important places as experimental animals, the necessity for such anatomical references increases. This work has been done to supply this reference, and present comprehensive, thorough, systematically recorded and well validated anatomical knowledge of normal cervical lymph node distributions in domestic rabbits.

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