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Histomorphological study of the lymphoglandular complex in adult local breed dog (*Canis familiaris*)

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Abstract

The morphological and histological features of the lymphoglandular complex of ten adult local breed dogs were studied. The dogs were hunted in cages and put in the animal house of Veterinary Medicine College / University of Baghdad. The dogs that were hunted and kept under supervision were then anesthetized, and slaughter was done to collect the samples of caecum. For the histological study, the abdominal region opened, and the cecum was identified and extirpated out properly, then washed with normal saline and immersed inadequate 10% neutral buffered formalin fixative for 72 hours at room temperature. After fixation, the specimens were washed with tap water and processed using routine histological techniques. Morphologically, the mucosa of the cecum had openings of the lymphoglandular complex that were randomly distributed and appeared as a rounded elevation at the upper caecum folds of the mucosal surface and in all portions of the caecum. Histologically, the lymphoglandular complexes in the tunica submucosa appeared rounded or oval in shape, which is closely related to the profound aspect of the muscularis mucosa, and they scarcely conquered the whole thickness of the submucosal layer. In the studied dogs, there were no cortical and medullary zones of the lymphoid structure of the lymphoglandular complex, and they didn't have germinal centers. The conclusion of the current results represents that the lymphoglandular complex has a different structure from other lymphoid organs, which have a cortex and medulla in addition to germinal centers.

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Introduction

The *Canis familiaris* species was the domesticated carnivore type, a portion of the family Canidae, the genus Canis (1,2). In addition, the dog was the most popular domestic animal in the world. Knowledge of comparative anatomy is necessary for scientists whose research involves food-producing animals (3). The gastrointestinal system is one of the most metabolically active systems in the body, and it is essential during all stages of life because it provides energy to digest food, absorb nutrients, and remove waste substances. The cecum, colon, 'ascending colon, transverse colon and descending colon,' rectum, and anus, in that order, were parts of the large intestine that have different functions (4-7). The absorption of water, electrolytes, and minerals in the large intestine resulted in a concentration of ingesta in the feces. The large intestine in all species contains an enormous microbial population, which is especially important for hindgut fermenters (8-10). The caecum is the first portion of the large intestinal tract, which lies on the right side of the median plane and almost within the duodenal circuit (11,12). In dogs, the cecum is an irregularly twisted tube attached to the ileum and ascending colon by short peritoneal folds (13). Yildiz *et al.* (14) mentioned that the intestinal tract comprises the cecum, colon, and rectum. The apex of the caecum appeared as an irregular blunt and was closely attached at the end to the ileocolic sphincter. The last portion of the small intestine is communicated with the ascending colon and cecum. As well as, the ileum was attached to the colon only, and the cecum exists as an opening of the first portion of the

colon (15,16). Atkins and Schofield (17) observed the lymphoglandular complex in the caecum as lymphoid follicles in dogs. Its fine opening was observed in the tunica mucosa surface that lies near smooth rounded elevations, which measure about 3 mm in diameter and are uniformly distributed in all segments of the caecum, almost on and between the irregularly organized mucosal folds. The gut-associated lymphoid tissue is seen as solitary lymphoid follicles throughout a dog's large intestine. Lymphoid patches were also shown at the ileocecal entrance and proximal colon. In carnivores, the diameter of lymphatic nodules that appear rounded or oval was arranged from 0.1 to 0.4 cm. It is closely attached to the muscularis mucosa and rarely occupies all thicknesses of the tunica submucosa. The lymphoglandular complex mucous membrane has a pit, and mucosal glands were present in the pit's depth, which extended into the submucosal lymphoid nodule. The lining epithelial cells to these glandular extensions are arranged from squamous to columnar. The fundus of submucosal glands is characterized by the absence of goblet cells, and few are found in other parts. The epithelium of the lymphoglandular complexes had many lymphocytes, which were predominant cells, and phagocytes were identified by their acidophilic cytoplasm. The aggregation of lymphoid nodules in the submucosa was homogeneous in appearance in each part of the large intestine that was located in it. The lymphoglandular complex is characterized by not having division into cortical and medullary areas, and no germinal centers were present (17-19). In dogs and cats, the appendix of the caecum, a heavy infiltration of lymphoid tissues was seen in the mucosal and submucosal layers due to the bowel juices in their caecum, which led to the absorption of digested food taken by these animals (20-22).

This study aims to provide broader insight into the morphological and histological characteristic features of the lymphoglandular complex in dogs.

Materials and methods

Ethical approval

The Scientific Ethical Committee for the research was obtained from the Research Ethics Committee at the University of Baghdad, College of Veterinary Medicine, Iraq, approved this study issued on 8- 7- 2024, numbered P.G/1284.

Animals' collection

Ten adult local breed dogs were used in the current study, and they were gathered by hunting and put in cages at the animal house of Veterinary Medicine College, University of Baghdad. The collection of these samples of dogs was conducted in the period extended between January 2024 and June 2024. Their ages range between 1 -2 years, and their body weight is 10- -25 kg.

Samples collection

The abdominal muscles were opened longitudinally in the midline from the pubic bone to the xiphoid cartilage, and the position of the cecum and large intestine in situ was immediately observed as the relationship of the cecum and large intestine with surrounding organs. A general description of the cecum with the adjacent organs achieved the gross aspect of the current study.

Morphological study

Five cocci were used for morphological study, including the characterization of the cecal folds and lymphoglandular complex openings. The morphometric studies were performed about the number of lymphoglandular complex openings and cecal folds.

Histological study

Five samples measuring 1-2 cm^2 were used for histological examination, obtained from the caecum after removal and subsequently fixed in 10% neutral buffered formalin (23-27). After fixation, the specimens were processed using a routine histological processing method (25). The samples were sectioned into five μm thicknesses using a rotary microtome, then stained with Hematoxylin and Eosin, Masson Trichrome, and Van Gieson stains (27-33).

Results

Morphological results

The current results revealed that the mucosa of the cecum had lymphoid follicles opening, forming a lymphoglandular complex. The openings of the lymphoglandular complexes appeared as rounded elevations. They were observed at the apical portion of the folds located in the mucosal layer for each section of the cecum (Figure 1). These openings were noted as pin-head grey submucosal structures that were indiscriminately distributed and slightly elevated from the surrounding mucosal surface (Figures 1 and 2). The current results revealed that the number of openings to the lymphoglandular complex in dogs ranged from 95 to 105 openings, with a mean of 99.8±3.701. The cecal folds were arranged in various patterns along the internal surface of the cecum, and their number ranged from 24 to 30 folds, with a mean of 27.00±2.55.

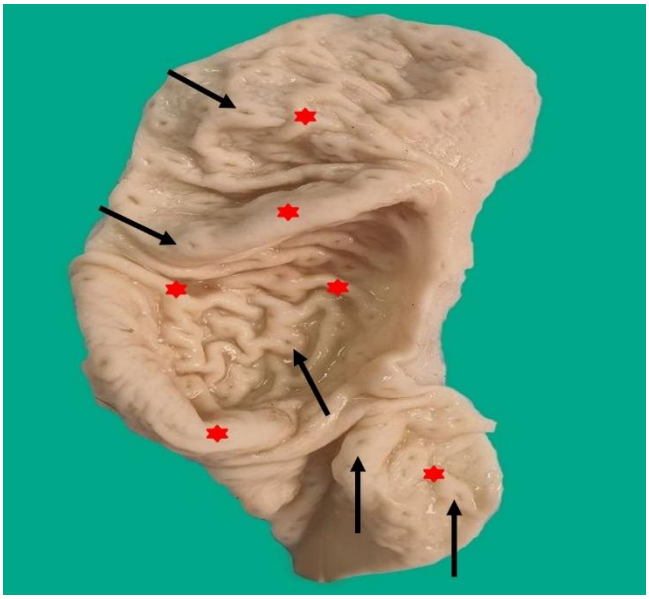

Figure 1: An anatomical section in the internal surface of the cecum in adult dogs shows lymphoid follicles (black arrow) and cecal folds (red star).

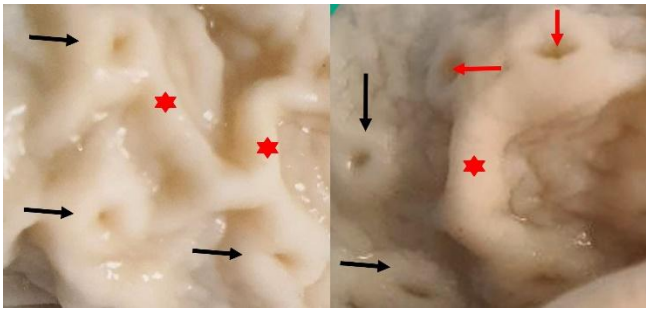

Figure 2: An anatomical section in the internal surface of the cecum in an adult dog shows lymphoid follicles (black arrow), lymphoglandular complexes opening (red arrow), and cecal folds (red star).

Histological results

The current histological results indicated that lymphoglandular complexes, which were observed as lymphoid follicles, were present in the tunica submucosa of the dog's cecum. At the mucosal surface, an opening was present that was orderly distributed throughout the caecum (Figures 3 and 4). At the tunica submucosa of the caecum, the lymphoglandular complexes appear as oval or round shapes closely attached to the muscularis mucosa and do not fill the entire thickness of the tunica submucosa. A depression, or pits, was observed in the mucous membrane of the lymphoglandular structure that opens into the mucosal glands; these openings are characterized by a paucity of muscularis mucosa (Figures 5 and 6). The macroscopic observation of the lymphoid structure revealed that the lymphoglandular complex lacks cortical and medullary zones, and germinal centers are absent. Its structure contains a high number of predominant lymphocytes, while other cells, such as phagocytes, are present in fewer numbers. The predominant lymphocytes support the mentioned research, which plays an important role in immunity (Figures 7 and 8). The lymphoglandular complex is surrounded by mucous membranes, which exhibit pit-like depressions that appear saucer-shaped. In each instance, these depressions typically occupy the full thickness of the mucous membrane and are invariably lined by columnar epithelial cells (Figures 9 and 10).

Figure 3: Micrograph of the lymphoglandular complex in the dog (black rectangle), encircling by the muscularis mucosa (Mm), lymphatic tissue (L), opening of the lymphoglandular complex (O), tunica mucosa (TM), crypt of Lieberkühn (C) and collagen fiber (black arrow). X40.H&E stain.

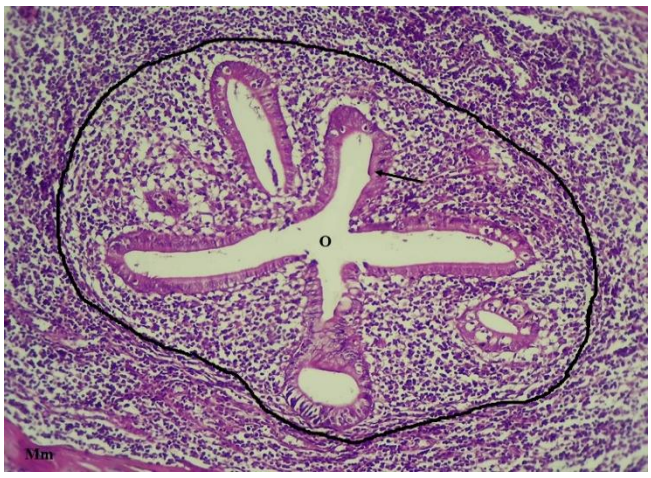

Figure 4: Micrograph to the lymphoglandular complexes (black circle drawn line) muscularis mucosa (Mm), lined by simple columnar epithelium (black arrow), opening of the lymphoglandular complex (O). X100.H&E stain.

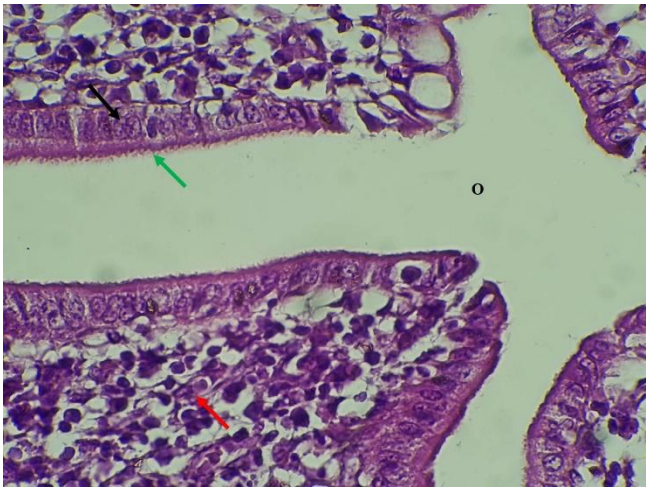

Figure 5: Micrograph of the opening of lymphoglandular complexes (O), the simple columnar epithelium (black arrow), cilia (green arrow), and interstitial tissue (red arrow). X400.H&E stain.

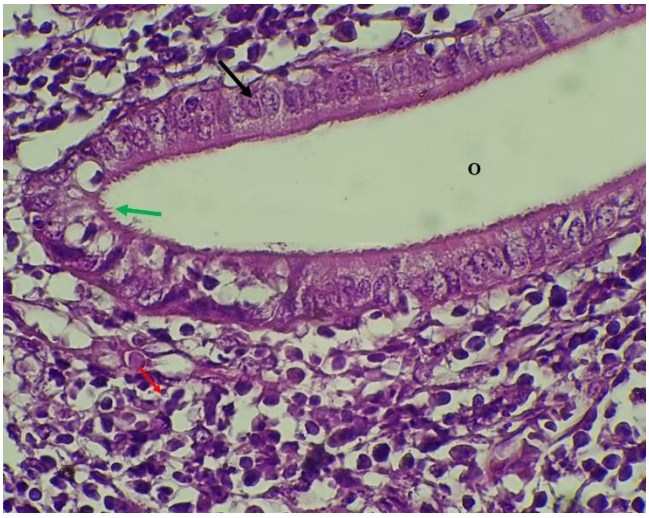

Figure 6: Micrograph of the lymphoglandular complexes shows the opening of lymphoglandular complexes (O), simple columnar epithelium (black arrow), cilia (green arrow), and interstitial tissue (red arrow). (A) and (B). X400.H&E stain.

Figure 7: Micrograph of the lymphoglandular complexes shows the Masson's trichrome stain presented that the lymphoglandular complexes (black rectangle) muscularis mucosa (Mm) and dense irregular collagenous connective tissue (red arrow), tunica mucosa (TM), lymphatic tissue (L) and opening of the lymphoglandular complex (O). X40 Masson's trichrome stain.

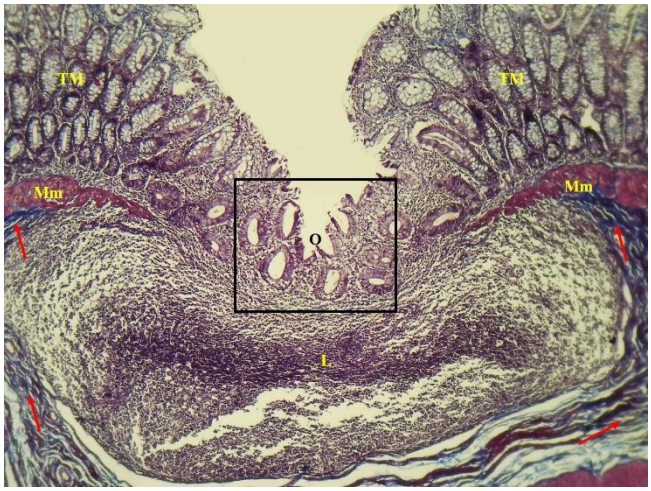

Figure 8: Micrograph of the lymphoglandular complexes shows the Masson's trichrome stain presented that the lymphoglandular complexes (black rectangle) muscularis mucosa (Mm) and dense irregular collagenous connective tissue (red arrow), lymphatic tissue (L), tunica mucosa (TM) and opening of the lymphoglandular complex (O). X40 Masson's trichrome stain.

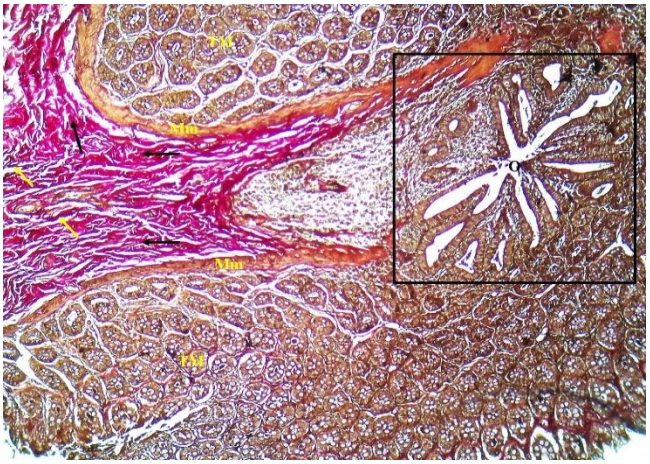

Figure 9: The transverse section shows the lymphoglandular complexes (black rectangle), the Verhoeff stain present that the lymphoglandular complexes (black rectangle) muscularis mucosa (Mm), collagen fiber-stained pink (black arrows), and the elastic fibers stained black (yellow arrows), tunica mucosa (TM) and opening of the lymphoglandular complex (O). X40 Verhoeff stain.

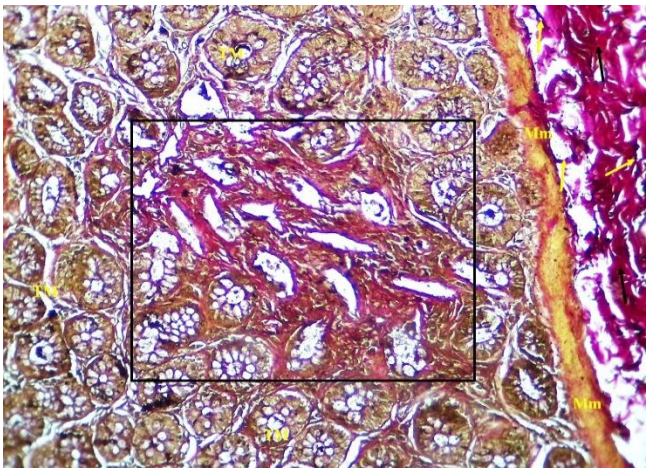

Figure 10: The longitudinal section of lymphoglandular complexes shows lymphoglandular complexes (black rectangle) muscularis mucosa (Mm), collagen fiber-stained pink (black arrows), and the elastic fibers stained black (yellow arrows), and tunica mucosa (TM). X100 Verhoeff stain.

Discussions

The current results regarding the number of openings and cecal folds are consistent with previous literature by Atkins and Schofield (17) and Abd-El-Hady *et al.* (18), who illustrated that the cecal folds in mongrel dogs are aligned vertically along the entire length of the caecum, with a mean number of approximately 28±4, ranging from 22 to 33 folds. Meanwhile, the mean number of openings of lymphoglandular complexes located at the apical portion of the folds is about 30±8, ranging from 21 to 40. Atkins and Schofield (17) indicated that the openings of lymphoglandular complexes were orderly distributed within the caecum, while the mucosal folds were atypically disposed. At the center of smooth, rounded elevations, a fine opening about 3 mm in diameter was present. In the colon, similar structures were also found in smaller numbers adjacent to the caecal opening. The lymphoglandular complexes are located only in the caecum and the immediately adjacent area of the colon, comprising submucosal extensions of intestinal glands that branch within submucosal lymphoid nodules. The structure and development of these complexes suggest that their lymphoid component acts as a secondary lymphoid organ. In dogs, it appears likely that the development of humoral capacity is neither associated with nor dependent on the presence of lymphoglandular complexes.

In the large intestine, the Peyer patches (PPs) were observed in various forms: fusiform, cup-shaped, and scrotiform. At the ileocecal orifice, only the scrotiform type was present in high density. The Peyer patches are believed to be the site of immune induction in the gastrointestinal tract, as they are responsible for antigen uptake, processing, and presentation on the mucosal surface (34,35). On the mucous membrane surface, a scrotiform form developed and was observed as a pit with a large cystic body and a small cystic mouth. Its height is approximately 0.7–1.3 cm, and its size is about 1.3, 3, 1.5 cm^2. The cyst was coated with foreign substances like manure balls and sand particles. According to Zidan and Pabst (36), the cup-shaped variety had a hollow center and an elevated edge surrounding it. The cup featured a honeycombed bottom and a smooth wall. The fusiform patches appeared oval or round in shape, with a honeycombed surface; the mean area, height, and size were approximately 1.3, 3, 1.3 cm^2, 0.5–0.8 cm, and 0.9, 3, 1.1 cm^2, respectively. Every Bactrian camel had all three types of Peyer's patches, but at the ileocecal orifice, the Peyer's patches were distributed.

In Bactrian camels, the mucosa-associated lymphoid tissue is distributed across the mucosal lining of the large intestine. At the ileo-cecocolic opening, many scrotiform forms were observed on the mucosal lining of the caecum, whereas the mucosa in the residual parts of the cecum had few of them, despite the fact that folds were much more commonly visible in the cecum than in the ileocecal orifice (37,38).

The mucosa-associated lymphoid tissue was observed on the mucosal surface of the entire ileo-cecocolic opening of a single-humped camel. Its number in the ileocecal orifice ranged from 22 to 27, while in the apex and body of the caecum, the numbers were between 5 and 9 and 4 and 7, respectively. In contrast, the count in the first portion of the colon was about 16 to 19 (34).

The histological results indicated that the mucosal surface of the caecum displayed lymphoglandular complex openings uniformly distributed throughout the cecum. This finding aligns with Abd-El-Hady *et al.* (18) in mongrel dogs but differs from the observations of Zhaxi *et al.* (37) in Bactrian camels, who noted that lymphatic nodules were predominantly located at the ileocecal orifice, the apex of the caecum, and the ascending colon, while being less prevalent in the body and base of the caecum, as well as in the transverse and descending colon.

Samuelson (39) notifies of the presence of lymphatic tissue, with the mucosa and submucosa housing numerous lymph nodules that vary in concentration depending on the animal. The nodules are most visible near the beginning of the cecum in dogs, pigs, and ruminants; they are more common toward the end of the cecum in cats and horses. The presence of lymphatic tissue is significant here because of the importance of immune function in the digestive system, including the caecum.

Nzalak (40) in giant rats, Bacha and Bacha (41), and Călămar *et al.* (42) in ruminants described the lamina propria as composed of loose connective tissues and collagen fibers, separated from the submucosa by the muscularis mucosa. It also contains numerous lymphatic vessels and lymphocytes.

Macroscopic examination of the lymphoglandular complex revealed no cortical and medullary zones in the lymphatic structure, as previously indicated by many authors mentioned the same observation and did not detect germinal centers (43-45).

The cecum of the Mongrel dog exhibited submucosal lymphoglandular complexes, with lymphocytes being highly distributed alongside a few phagocytes. The openings were distributed randomly across the mucosal folds throughout the caecum (18).

The spleen, which is not compatible with other lymphoid organs, contains a cortex and medulla in addition to germinal centers within its lymphoid structures (43-45). These differences may be due to the varying functions and histological structures depending on their locations. Atkins and Schofield (17) and Malla (22) agreed with the current results, observing high infiltrations of lymphoid tissues in the mucosa and sub-mucosa coats, specifically in the cecum apex portion. Also, a large number of lymphatic nodules were indicated scattered throughout the caecum length, especially around the ileal ostium (46-50).

Conclusion

In conclusion, the current results indicated that the lymphoglandular complex in dogs has a special lymphoid structure compared to other lymphoid organs in the body.

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Conflict of interest

I declare that I have no competing interests.

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دراسة شكليائية نسجية للمجمع الليمفاوي الغدي في الكلاب المحلية البالغة

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الخلاصة

تم دراسة الخصائص الشكليائية والنسجية للتركيب اللمفي الغدي في عشرة كلاب من السلالات المحلية البالغة. تم جمع الكلاب المدروسة عن طريق بأقفاص ووضعت في بيت الحيوانات في كلية الطب البيطري / جامعة بغداد. تم تخدير الكلاب التي تم صيدها وإبقائها تحت المراقبة بعد ذلك تم ذبحها لجمع عينات الأعور. للدراسة النسيجية، تم فتح منطقة البطن وتحديد الأعور واستئصالها بشكل صحيح ثم غسلها بمحلول ملحي طبيعي ثم غمرها في مثبت فورمالين محايد ١٠٪ مناسب لمدة ٧٢ ساعة في درجة حرارة الغرفة. بعد التثبيت، غسلت العينات بالماء الجاري وعولجت باستخدام تقنيات المعالجة النسيجية الروتينية. من الناحية الشكلية، كان الغشاء المخاطي للأعور يحتوي على فتحات مجمع الغدد اللمفاوية موزعة بشكل عشوائي وتظهر على شكل ارتفاعات مستديرة في أعلى طيات الأعور من سطح الغشاء المخاطي وفي جميع أجزاء الأعور. من الناحية النسجية، تظهر المجمعات اللمفاوية الغدية في الغلالة تحت المخاطية مستديرة أو بيضاوية الشكل والتي ترتبط ارتباطًا وثيقًا بالجانب العميق من الغشاء المخاطي العضلي وهي بالكاد تغزو كامل سمك الطبقة تحت المخاطية. في الكلاب المدروسة لا توجد مناطق قشرية ونخاعية للبنية اللمفاوية للمجمع اللمفي الغدي ولا تحتوي على مراكز جرثومية للعقية اللمفاوية. يظهر استنتاج النتائج الحالية بأن المجمع اللمفي الغدي يختلف في بنيته عن الأعضاء اللمفاوية الأخرى التي يحتوي على قشرة ونخاع بالإضافة إلى المراكز الجرثومية.