Nodular hyperplasia of lymphoglandular complexes in dogs—a potential diagnostic pitfall for rectal masses

Veterinary Pathology

Nodular hyperplasia of lymphoglandular complexes in dogs—a potential diagnostic pitfall for rectal masses

Journal:	*Veterinary Pathology*
Manuscript ID	VET-23-CR-0021.R2
Manuscript Type:	Case Report
Date Submitted by the Author:	02-Jul-2023
Complete List of Authors:	STENT, ANDREW; Gribbles Veterinary Pathology Australia, Kiupel, Matti; Michigan State University Veterinary Diagnostic Laboratory, Veterinary Diagnostic Laboratory Dandrieux, Julien; The University of Edinburgh Royal Dick School of Veterinary Studies, Hospital for Small Animals Liffman, Rebekah; ASAP Laboratory Bera, Monali; Asia Veterinary Diagnostics
Keywords:	Dog < Domestic Mammals < Species, Gastrointestinal < Digestive Tract < Tissue, Lymphoreticular < Tissue, Immunohistochemistry < Technology, Hyperplasia, Histology, Lymphoglandular complex
Abstract:	Lymphoglandular complexes are components of the gut-associated lymphoid tissue that are characterized by submucosal lymphoid aggregates invested by projections of mucosal epithelium. Reports of pathology involving these structures are rare in both human and veterinary literature. Here we report two cases of rectal masses excised from dogs following a period of tenesmus and haematochezia. In both animals the masses were composed of lymphoid tissue closely encompassing tubuloacinar structures. Immunohistochemistry and PARR testing demonstrated that the lymphoid population was polyclonal, comprising T and B cells arranged in loosely follicular aggregates centered on the epithelial foci. In light of these findings, a diagnosis of lymphoglandular complex nodular hyperplasia was reported. To the author's knowledge, this is the first report of this condition in dogs.

Nodular hyperplasia of lymphoglandular complexes in dogs—a potential diagnostic pitfall for rectal masses

Andrew W Stent, Matti Kiupel, Julien R S Dandrieux, Rebekah Liffman, Monali M Bera

Institutions:

Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Australia (AS, JD, RL)

Veterinary Diagnostic Laboratory, Michigan State University, USA (MK)

Asia Veterinary Diagnostics, Singapore (MB)

Corresponding author:

Andrew Stent

Gribbles Veterinary Pathology

1868 Dandenong Road

Clayton, Vic, Australia 3168

P: (+613) 9538 6740

E: andrew.stent@gribbles.com.au

Abstract

Lymphoglandular complexes are components of the gut-associated lymphoid tissue that are characterized by submucosal lymphoid aggregates invested by projections of mucosal epithelium. Reports of pathology involving these structures are rare in both human and veterinary literature. Here, we report two cases of rectal masses excised from dogs following a period of tenesmus and haematochezia. In both animals, the masses were composed of lymphoid tissue closely encompassing tubuloacinar structures. Immunohistochemistry and polymerase chain reaction antigen receptor rearrangement testing demonstrated that the lymphoid population was polyclonal, comprising T and B cells arranged in loosely follicular aggregates centered on the epithelial foci. In light of these findings, a diagnosis of lymphoglandular complex nodular hyperplasia was reported. To the author's knowledge, this is the first report of this condition in dogs.

Keywords

Dog, gut-associated lymphoid tissue, histology, immunohistochemistry, lymphoglandular complex, nodular hyperplasia, rectum

---

Lymphoglandular complexes are components of the gut-associated lymphoid tissue, formed from a close association between lymphoid follicular tissue and epithelial diverticula projecting from the overlying mucosa. They are predominantly located within the distal small intestine, cecum, and colon and have been described in a wide range of mammalian species,[6] including humans,[14] dogs,[2] cattle,[10] horses,[11] pigs,[13] dolphins,[5] and echidnas.[16] While undoubtedly involved in immune surveillance of the gastrointestinal tract, the specific role of these structures in mucosal immunity is

unclear. Some researchers have speculated that they may be mammalian analogues of the bursa of Fabricius that act as secondary lymphoid organs.[16] The clearest indication of their function is derived from studies in pigs, where they are the predominant site of IgA production during *Campylobacter jejuni* infection.[12]

A range of proliferative lesions involving the gastrointestinal lymphoid tissue are recognized in humans and animals. Lymphoid follicular hyperplasia is a common endoscopic and histological observation in many chronic inflammatory enteropathies, reflecting expansion of the mucosa-associated lymphoid tissue (MALT) in response to persistent antigenic stimulation. While this is a non-specific finding seen with a variety of infectious and hypersensitivity diseases,[1] gastrointestinal MALT hyperplasia is also closely associated with several immunodeficiency syndromes in humans,[8] indicating that genetic predisposition may be a factor in some cases. Though hyperplastic foci are typically small, they can sometimes form larger masses up to several centimeters in size, and distinguishing these lesions from MALT lymphoma can be challenging. Indeed, progression from hyperplasia to neoplasia has been noted to occur in humans.[7] Hyperplastic lesions of the lymphoglandular complexes are rarer still,[19] and the incorporation of an epithelial component into these lesions introduces the risk of a misdiagnosis of both carcinoma and lymphoma. The following report describes nodular hyperplasia of lymphoglandular complexes within the rectum of two dogs.

In the first case, a 12-year-old, male Chinese crested dog was presented for acute large bowel diarrhea and tenesmus after 3 weeks of boarding. Similar episodes of diarrhea occurred over the previous two years after antibiotic or non-steroidal anti-

Commented [JW1]: Reviewer comment:
L51/52: if LGC are mammalian analogues of BF which is a primary lymphoid organ, should it read ... that act as primary lymphoid organ... ???

Commented [AS2R2]: My intent with this statement was to indicate that in contrast to the BF, LGC are more likely to be secondary lymphoid organs in mammals. Upon review this adjunct statement adds little except confusion, and so I have removed it.

inflammatory treatment, with one episode of hematochezia. The dog was regularly treated with anthelminthics.

Rectal endoscopic examination identified two large masses (each over 1 cm in diameter) and three small masses (less than 5 mm in diameter), arising from the dorsal and ventral rectal walls, 3–7 cm cranial to the anus. The masses were broad-based with a sunken center and intact overlying epithelium (both with white light and narrow-band imaging). The colon and distal ileum were assessed and no other masses were noted. Needle aspirates were collected from one of the masses for cytology. The samples contained cuboidal epithelial cells displaying mild to moderate pleomorphism and arranged in tubular-type structures within a background of lymphocytes and plasma cells. An epithelial malignancy was considered unlikely but could not be entirely excluded.

In the second case, a 9-year-old, male neutered cross-breed dog presented with frequent bleeding during defecation and tenesmus. The dog was originally treated with antibiotics for a suspected anal gland infection, but the problem persisted. Further endoscopic investigation of the rectum revealed a small pale raised polypoid lesion arising from the rectal mucosa.

Masses from both cases were excised for histological examination. The lesions were well-circumscribed and largely confined to the submucosa (Fig. 1a, b). Histologically, the masses were essentially identical in composition, containing irregularly shaped tubuloacinar structures lined by tall columnar epithelium. The epithelium was crowded and sometimes displayed loss of polarity (Fig. 1c, d). The epithelial cells

displayed mild anisokaryosis, sometimes with hyperchromatic nuclei and prominent nucleoli, and frequent mitoses were present. Surrounding these glands, there were lymphocytes arranged in loosely follicular architecture with occasional germinal centers intermixed with plasma cells and histiocytes. The lymphoid cell population displayed a high mitotic rate. Immunohistochemical phenotyping ~~(Fig. 2a, b)~~ of the lymphocytes was performed as previously described,[3,17] using normal canine lymph node as control tissue. T~~identified t~~he lymphocytes immediately surrounding and adjacent to the glandular structures ~~as~~ were predominantly CD20 and Pax-5 positive, consistent with a B-cell phenotype, and; these foci were encircled by a population of CD3-positive cells, consistent with T-cells (Fig. 2a, b). Low numbers of both T- and B-cells infiltrated the epithelium of the glandular structures, and occasional intraepithelial clusters of B-cells were identified. In some areas, the epithelial lining of the tubuloacinar structures was discontinuous, with apparent protrusion of the lymphoid tissue into the lumen. In the first case, the overlying mucosa was inflamed and mildly eroded, while the mucosa in the second case was unremarkable. No infectious agents were identified in the tissues histologically, and bacterial culture of the tissue in the first case yielded only light growth of mixed gastrointestinal flora.

Polymerase chain reaction for antigen receptor rearrangement (PARR) testing was performed for both cases at the Michigan State University Veterinary Diagnostic Laboratory by extracting DNA from 5, serial 5-µm-thick sections (25 µm thickness total) from formalin-fixed, paraffin-embedded tissues. Rearrangements of the T-cell receptor gamma and the immunoglobulin heavy chain gene variable regions were assessed by amplifying the *complementary determining region 3* (*CDR3*), as previously described.[4,18] Clonality was not detected for either receptor and based on

Commented [JW3]: Immunohistochemistry methods, including controls, should be included by either referencing the methods are including the methods in the supplemental materials.

Commented [AS4R4]: Methodology now referenced

this result and the histological appearance, the masses were~~the~~ diagnos~~is~~ed ~~was~~as nodular~~reported as inverted~~ hyperplasia of lymphoglandular complex ~~polyposis~~es (Fig. 2c).

In both cases the tenesmus resolved soon after surgery, with only a single small nodule ~~of stable~~2–3 millimeters in size palpated on rectal exam 4.5 months after surgery in the first dog and no further progression over a 4-year follow-up, while the second dog was clinically unremarkable through to 6-month follow-up.

Commented [JW5]: What is the approximate size of the nodule?

Commented [AS6R6]: Added

Lymphoglandular complexes have been found throughout the intestinal tract of dogs, but occur with particular abundance in the duodenum, colon, and rectum.[6] Two architectural forms of lymphoglandular complex have been described: *Strauchdrüsen* (shrub glands), which form from branching projections of a single epithelial tubule, and *Büscheldrüsen* (tufted glands), in which multiple adjacent epithelial tubules independently invest the lymphoid tissue.[6] *Büscheldrüsen* were reported as most prevalent form in dogs, but it is uncertain which form of lymphoglandular complex these cases most closely resemble, as the communication between the epithelial structures and the overlying mucosa was unclear from the histological sections. Nevertheless, the masses described in this report displayed features broadly consistent with lymphoglandular complexes reported in dogs and other species—albeit of greater size and complexity—and are considered to represent hyperplastic enlargement of these structures. Lymphoid proliferation with prominent follicle formation has been reported in porcine lymphoglandular complexes in association *C. jejuni* infection,[12] but no evidence of campylobacteriosis or any other infectious disease was identified histologically or by bacterial culture in the present cases.

Based on the history of previous episodes of acute diarrhea after medical treatment, the neutrophilic inflammatory component found on endoscopic biopsy and resolution without specific treatment, an episode of acute colitis was suspected to be the trigger for hyperplasia in the first case. No further information about the cause was determined as the dog was boarded when the tenesmus and diarrhea initially developed. However, the presence of multiple concurrent and persistent masses in the first case suggests this dog may have had an underlying predisposition to development of such lesions.

Similar masses containing varying proportions of epithelial and lymphoid components have been reported rarely in the human literature,[9,15,19] though some of these cases are more consistent with inverted adenomatous polyps or submucosal herniation of colonic glands than primary proliferation of the lymphoglandular complexes. The case documented by Zhou et. al., designated an inverted lymphoglandular polyp, bears closest resemblance to the lesions described here, but the arrangement of the epithelial component was slightly different, comprising sparse, irregular ectatic glandular structures rather than the small, contorted tubuloacinar foci observed in these dogs. This discrepancy may reflect anatomical differences in human lymphoglandular complexes, which typically contain relatively few, superficially distributed epithelial projections.[14]

The distinctive histological features of this condition were rectal submucosal masses that were well-circumscribed and displayed integration of glandular structures into lymphoid tissue. Lymphoid follicles were centered on these glands and there was extension of the lymphocytic population into the gland lumina. Several notable

differentials were considered for this case. Submucosal herniation of crypts as seen in focal colitis cystica profunda could have a similar appearance, but while there may be secondary inflammation with this condition due to compression or rupture of crypts, it lacks the intimate association between lymphoid and glandular tissues seen in these cases. Furthermore, goblet cells typical of crypt mucosa were absent from the epithelium in the lymphoglandular complex. Lymphoma arising within a lymphoglandular complex was a concern due to the nests of lymphocytes in the glandular epithelium. However, the mixed population of inflammatory cells throughout the lymphoid tissue, the presence of recognizable follicular architecture, the lack of clinical progression, and the absence of detectable clonality with PARR testing makes this diagnosis highly improbable. An invasive adenocarcinoma was a further consideration, but the dispersed distribution of glands within the lymphoid tissue, close association between lymphoid and epithelial components, lack of stromal reaction or invasion outside of the mass, retention of basement membrane surrounding glands, absence of marked anaplasia within the masses or the overlying mucosa, and the lack of progression all refute a diagnosis of invasive adenocarcinoma. Failure to account for these factors engenders considerable risk of a misdiagnosis of malignancy, particularly as the submucosal location could make the procurement of truly representative samples problematic.

These two cases of lymphoglandular complex nodular hyperplasia demonstrate a non-neoplastic disease in the rectum that must be considered as a potential diagnosis for any submucosal mass containing both lymphoid and epithelial components. Considering the prognostic implications of possible differentials, such

as adenocarcinoma or lymphoma, it is imperative that biopsy samples are of sufficient depth and quality to allow careful scrutiny and recognition of this condition.

Acknowledgements

The authors would like to thank Annie M Zimmerman for her contribution to pathological assessment of these cases.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Authors' Contributions

AWS, MK, RL, and MMB performed pathological evaluations; MK and MMB performed PARR analysis; JRSD performed clinical investigations; the manuscript was written by AWS with contribution from the other authors.

References

1 Albuquerque A: Nodular lymphoid hyperplasia in the gastrointestinal tract in adult patients: A review. *World J Gastrointest Endosc.* 2014;6:534-540.
2 Atkins AM, Schofield GC: Lymphoglandular complexes in the large intestine of the dog. *J Anat.* 1972;113:169-178.
3 Bacci B, Stent AW, Walmsley EA: Equine intestinal lymphoma: clinical-pathological features, immunophenotype, and survival. *Vet Pathol.* 2020;57:369-376.
4 Carrasco, Carrasco V, Rodríguez Bertos A, et al.: Distinguishing Intestinal Lymphoma From Inflammatory Bowel Disease in Canine Duodenal Endoscopic Biopsy Samples. *Vet Pathol.* 2015;52:668-675.
5 Cowan, Cowan D, Smith TL: Morphology of complex lymphoepithelial organs of the anal canal ("anal tonsil") in the bottlenose dolphin, *Tursiops truncatus*. *J Morphol.* 1995;223:263-268.
6 Hebel R: Untersuchungen uber das Vorkommen von lymphatischen Darmkrypten in der Tunica submucosa des Darmes von Schwein, Rind, Schaf, Hund und Katze. 1960.
7 Hong J, Kim E, Park S-W, et al.: Long-term Prognosis of Localized Lymphoid Hyperplasia of the Rectum. *Gut Liver.* 2021;15:77-84.
8 Hornick J: Tumors of the Small and Large Intestines, Including Anal Canal. In: Fletcher C, ed. *Diagnostic Histopathology of Tumors*. 5th ed. Elsevier Health Sciences UK; 2019.
9 Lee HE, Wu T-T, Chandan V, Torbenson M, Mounajjed T: Colonic Adenomatous Polyps Involving Submucosal Lymphoglandular Complexes. *Am J Surg Pathol.* 2018;42:1083-1089.

10 Liebler EM, Pohlenz JF, Woode GN: Gut-associated Lymphoid Tissue in the Large Intestine of Calves. I. Distribution and Histology. *Vet Pathol.* 1988;25:503-508.
11 Lowden S, Heath T: Lymphoid tissues of the ileum in young horses: distribution, structure, and epithelium. *Anat embryol.* 1995;192:171-179.
12 Mansfield LS: Lymphoglandular complexes are important colonic sites for immunoglobulin A induction against Campylobacter jejuni in a swine disease model. *Comp Med.* 2004;54:514.
13 Morfitt DC, Pohlenz JF: Porcine colonic lymphoglandular complex: distribution, structure, and epithelium. *Am J Anat.* 1989;184:41-51.
14 O'Leary AD, Sweeney EC: Lymphoglandular complexes of the colon: structure and distribution. *Histopathology.* 1986;10:267-283.
15 Patel S, Desai J, Desai Z, et al.: A Rare Case of Inverted Lymphoglandular Colonic Polyp: 1604. *Am J Gastroenterol.* 2017;112:S874-S875.
16 Schofield GC, Cahill RN: Intestinal and cloacal lymphoepithelial glands in the Australian echidna: a possible homologue of the bursa of Fabricius. *J Anat.* 1969;105:447-456.
17 Stein L, Bacmeister C, Kiupel M: Immunophenotypic Characterization of Canine Nodal T-Zone Lymphoma. *Vet Pathol.* 2021;58:288-292.
18 Valli VE, Kiupel M, Bienzle D: Hematopoietic system. In: Maxie G, ed. *Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*. 6th ed. St. Louis, MO Elsevier; 2016: 232-234.
19 Zhou S, Ma Y, Chandrasoma P: Inverted lymphoglandular polyp in descending colon. *Case Reports in Pathology.* 2015;2015:1-3.

Figure legends

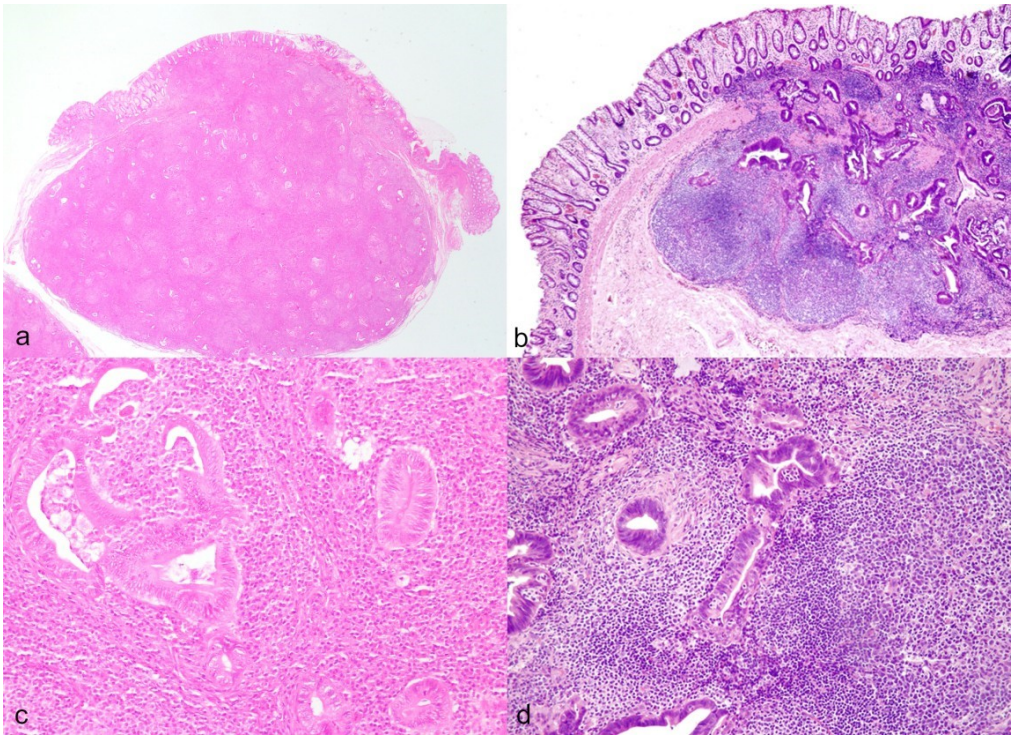
Figure 1. Lymphoglandular complex nodular hyperplasia, rectum, dog. Excisional rectal biopsies from case 1 (**a** and **c**) and 2 (**b** and **d**), both demonstrating well-demarcated submucosal masses containing a dual population of lymphoid and epithelial tissue. Hematoxylin and eosin.

Figure 2. Lymphoglandular complex nodular hyperplasia, rectum, dog. **a)** Photomicrograph of Pax-5 immunohistochemistry within the rectal mass, displaying aggregates of strongly Pax-5-positive cells surrounding tubuloacinar structures. Case 1. DAB chromogen, hematoxylin counterstain. **b)** Photomicrograph of CD3 immunohistochemistry within the rectal mass, demonstrating a cuff of CD3-positive cells that surround the central foci of B cells and tubuloacinar structures. Case 1. DAB chromogen, hematoxylin counterstain. **c)** Polymerase chain reaction (PCR) to

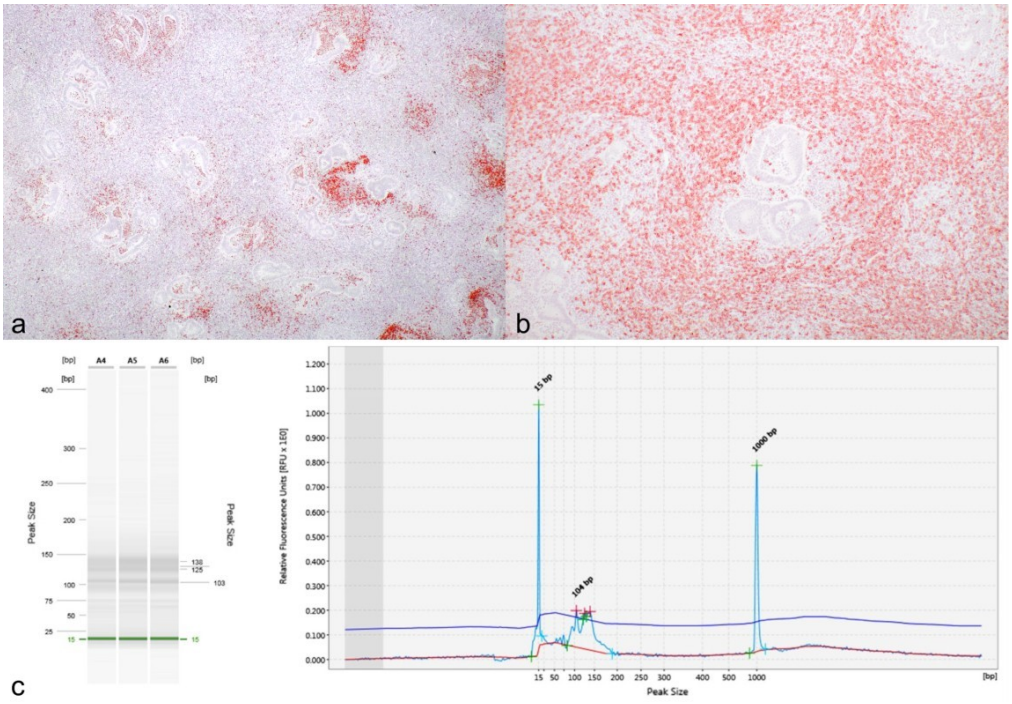
Commented [JW7]: Please ensure the images are directly apposed with no white space between the figures.

Commented [AS8R8]: Corrected

detect antigen receptor rearrangement (PARR) in the T-cell receptor gamma gene for case 1. The non-neoplastic lymphocytic (polyclonal) population presents as a smear (A4-A6) between 125 to 138 bp in the PCR gel and in the electropherogram produce multiple small, broad peaks rather than a single tall peak that is observed in clonal populations. Size markers are represented as peaks at 15 bp and 1000 bp in the electropherogram and green lines in the PCR gel.



179x130mm (300 x 300 DPI)



180x124mm (300 x 300 DPI)