Endoscopic biopsies in dogs

An invaluable tool in the diagnosis and management of chronic gastrointestinal disease

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In the past, histological assessment of endoscopic biopsies often proved an unrewarding experience for both clinicians and pathologists. One reason was a lack of correlation between histological changes and clinical signs. Furthermore, there appeared to be inconsistency among pathologists when reporting histopathologic changes in endoscopic biopsies. Significant progress has been made to standardize diagnostic reports and to better understand the correlation between histopathological changes and clinical disease.

This update covers the method by which pathologists assess, score and report endoscopic biopsies of the gastrointestinal tract and highlights practical considerations when sampling and submitting biopsies. Furthermore, current knowledge relating to the clinical relevance of these biopsies in dogs with chronic gastrointestinal (GI) disease is discussed. Clinical aspects such as a diagnostic approach, endoscopy procedures and treatment options are beyond the scope of this document.

Endoscopic or full-thickness biopsies?

Different clinical presentations dictate a preference for endoscopic versus full-thickness biopsy. Endoscopic biopsies are most commonly used, but full-thickness biopsies may be indicated if involvement of the submucosa or muscularis layer is suspected, e.g. with suspicion of neoplasia, to diagnose intestinal lipogranulomatous lymphangitis, or when endoscopic biopsy findings fail to correlate with clinical presentation. In veterinary medicine there is, however, a lack of studies documenting the superiority of one biopsy technique over the other. This diagnostic update focuses specifically on the submission and reporting of endoscopic biopsies.

Endoscopic biopsy:

Advantages	Disadvantages
Minimally invasive, fast recovery	Upper GI access only to stomach and duodenum; lower GI tract preparation and endoscopy is needed to assess and sample the ileum and colon. Lesions in jejunum may be missed* No sampling of liver, lymph nodes etc.
Allows inspection of mucosa and biopsy of focal lesions	Samples may be subject to artefacts (crushed and fragmented samples)
Permits multiple biopsies from different areas; at least 6 adequate individual tissue specimens from each location is recommended	Only small superficial biopsies; could miss deeper lesions e.g. lymphangiectasia, lymphoma, GIST, leiomyoma, and possibly carcinoma
Lower risk of complications (e.g. dehiscence, septic peritonitis)	Expertise performing endoscopy and specific equipment required

Full-thickness biopsy:

Advantages	Disadvantages
Permits large, full-thickness biopsies of all layers of GI tract	More invasive, slower recovery
All areas of GI tract can be sampled, less artefacts	Smaller number biopsies, inability to visualize mucosal lesions which prevents targeted biopsies
Inspection/sampling of other abdominal organs possible	Increased risk of complications, especially with PLE and large intestinal samples

* The jejunum and ileum are the most common segments of bowel affected in dogs with chronic GI disease. Biopsy of the ileum in addition to the duodenum is therefore recommended.

Submission and quality of endoscopic biopsies submitted for examination

To maximize the value of histopathologic examination as a diagnostic tool, it is essential to provide high quality endoscopic biopsies. In addition to operator experience and technique, there are several factors which may affect the quality of biopsies. For example, unnecessary handling of the biopsies may cause artifacts as they are easily damaged. It is essential to avoid crushing of the biopsies with forceps and avoid stretching of the tissue during sampling. Great care should also be taken to follow the specific laboratory's requirements with regards to sample submission. When possible, biopsies should be gently mounted and orientated on their side on a thin moistened synthetic sponge inlay and placed in a plastic cassette. Drying out of the samples should be avoided. Orientation of the biopsies, which is much more difficult when attempted in the laboratory, allows optimal visualization and assessment of the biopsies. Cassettes should be immersed in 10% neutral-buffered formalin before being dispatched to the laboratory (see publication by Ruiz et al, open access). If cassettes are not available, samples can be submitted floating in formalin in different containers for the different sites.

The need for standardization in reporting endoscopic biopsies

There has been long-standing controversy regarding the value of endoscopic biopsies in diagnosing GI disease in the dog. There are several possible reasons for this:

- The difficulty in defining 'normal'. Even within clinically normal dogs, the small and large intestinal mucosa contain variable numbers of leucocytes, especially lymphocytes and plasma cells. One of the greatest challenges is to differentiate between a sample which falls within the spectrum of 'normal' and one that is only 'mildly abnormal'
- Correlation of histopathologic findings with clinical findings often seems unclear
- Traditionally, a lack of a widely accepted scoring system for lesions in the GI tract led to a broad spectrum of variation among pathologists when reporting endoscopic biopsies

Figure 1. Opened cassettes with endoscopic biopsy samples of stomach, duodenum and colon; orientation of biopsies on a thin synthetic sponge inlay is advised (see figure 2).

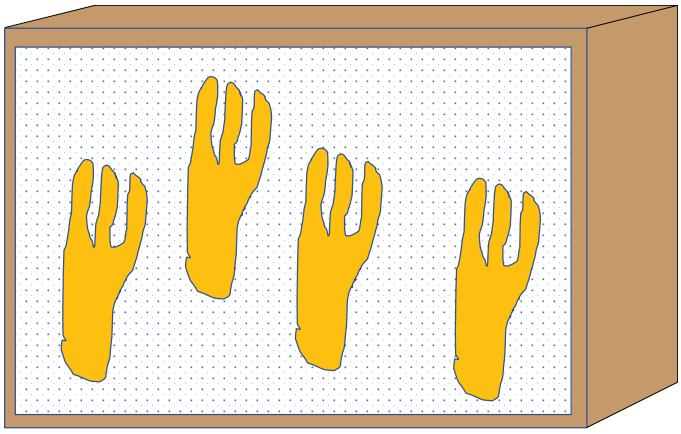

Figure 2. Endoscopic biopsies should preferably be orientated on an inlay in a plastic cassette (see figure 1) before immersing the cassette in formalin. The biopsies should be aligned with the villi parallel to the inlay (meaning horizontally).

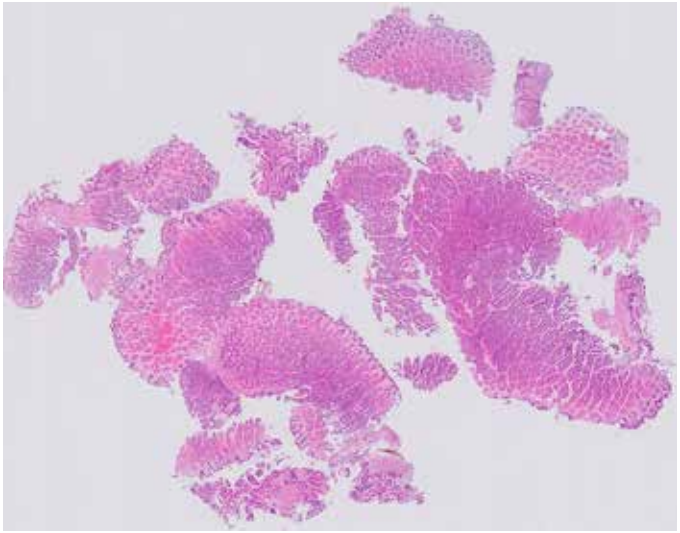

Figure 3. Good quality endoscopic biopsies from the stomach of a dog. Several biopsies are of optimal size and include a significant portion of the mucosa.

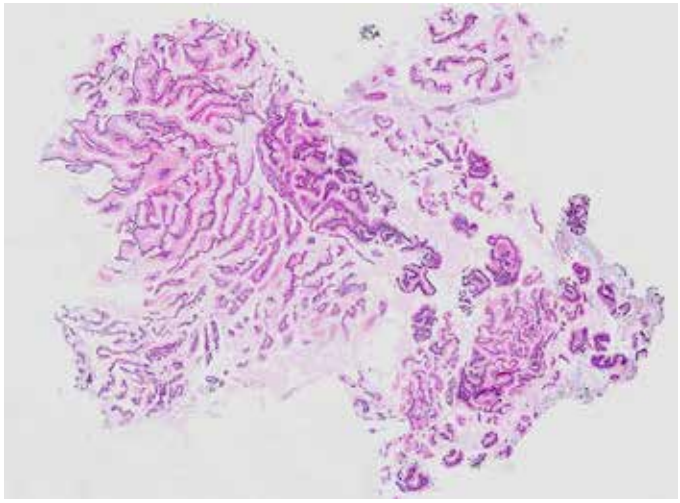

Figure 4. Fragments of superficial stomach mucosa in endoscopic biopsies. These are non-diagnostic samples and inadequate for interpretation since very little lamina propria and gastric glands are included.

Figure 5. High quality endoscopic biopsy specimen from the small intestine. Note numerous villi as well as deeper mucosa with crypts.

Figure 6. Good quality full thickness biopsy from the small intestine. In comparison to an endoscopic biopsy (see figure 5), the sample shows all layers of the gastrointestinal wall.

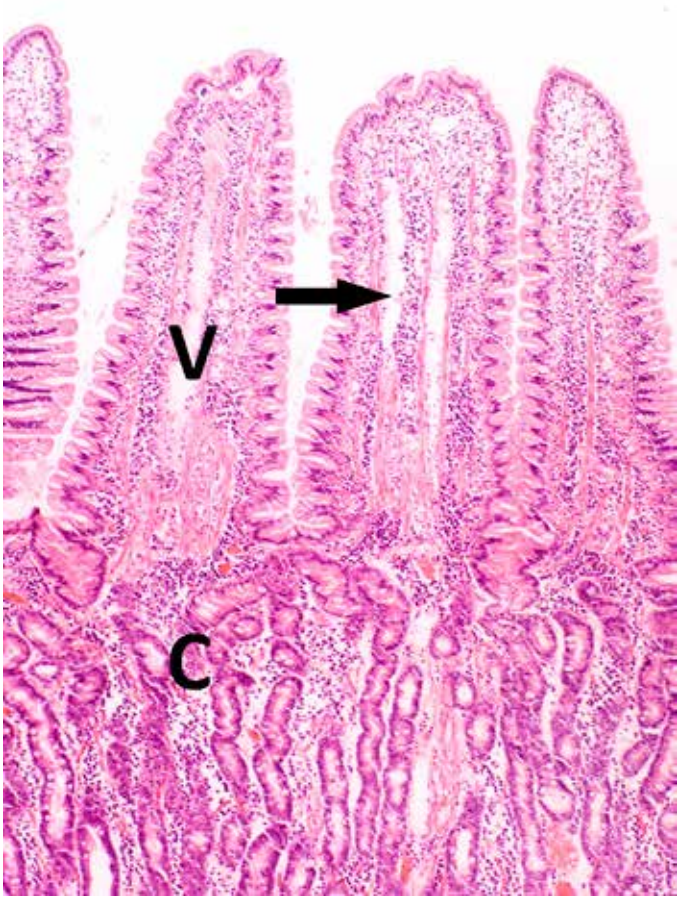

Figure 7. Higher magnification of the superficial mucosa of the small intestine. The fingerlike villi (V) consist of a layer of surface epithelium and lamina propria. The lamina propria contains a capillary network and lacteals (or lymphatic capillaries, arrow). Below the villi are the crypts (C).

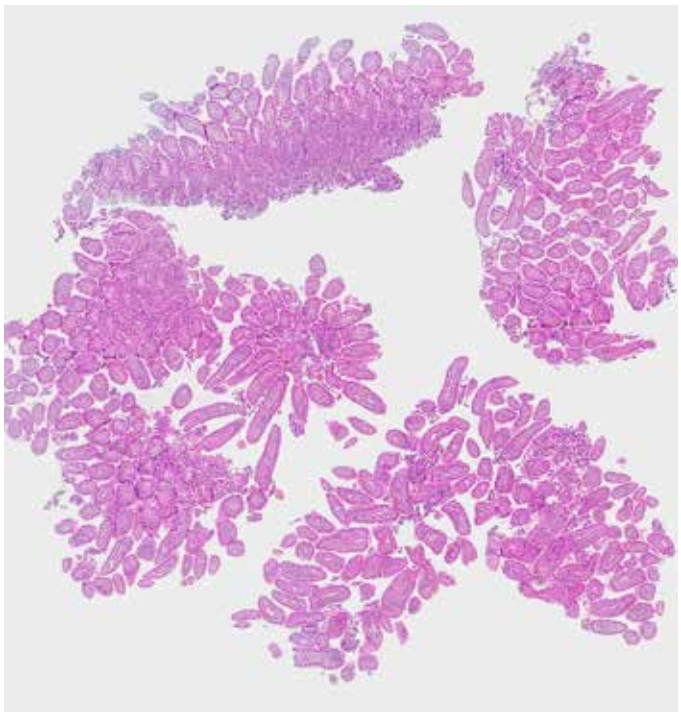

Figure 8. Endoscopic biopsies from the duodenum. Although the quality of the biopsies seems adequate at low power, most samples in the histological slide only show villus tips (see also figure 9) and are considered inadequate for interpretation.

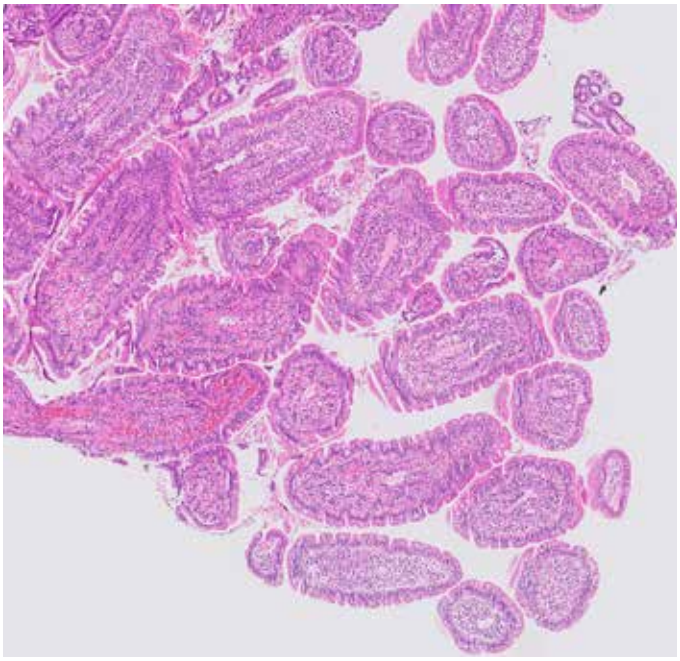

Figure 9. Higher magnification of a biopsy shown in figure 8 (lower right). The sample consists entirely of villus tips therefore considered non-diagnostic.

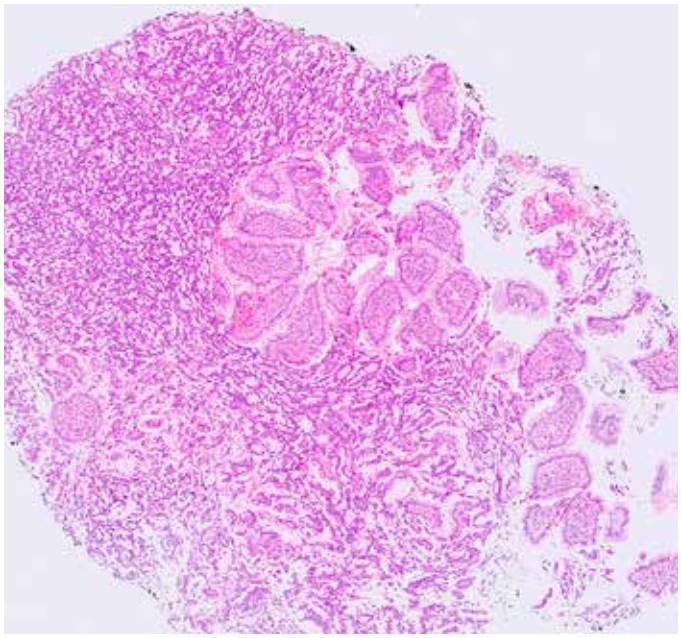

Figure 10. Poor quality non-diagnostic biopsy from the duodenum. The sample only shows tips of villi (at right) and crush artefact evidenced by loss of architecture (at left).

Scoring system used when reporting endoscopic biopsies

The World Small Animal Veterinary Association (WSAVA) International Gastrointestinal (GI) Standardization Group defined a **scoring system** of morphological and inflammatory lesions in GI biopsies from dogs. Subsequently, this scoring system was simplified and showed improved utility in correlating histopathologic features of endoscopic biopsies with clinical activity in dogs with inflammatory bowel disease (IBD). The simplified WSAVA scoring system is now globally applied within IDEXX when assessing, scoring, and reporting gastric, duodenal, and colonic endoscopic biopsies.

The scoring system adapted from the WSAVA scheme uses a combination of architectural and inflammatory changes to assign a score of normal or mildly / moderately / markedly altered basing on objective cut-off values. Different cellular infiltrates (e.g., lymphoplasmacytic, eosinophilic, and granulomatous) are also recognized.

The pathologist's report: what to expect

The pathologist assessing the endoscopic biopsies will use a uniform and systematic approach to describe, score, and interpret morphological and inflammatory abnormalities. The report generally includes:

- Abbreviated history and mention of (suspected) clinical diagnosis
- Number and size of biopsies from each anatomic location in the GI tract
- Artefacts and biopsy size/**quality**; report should clearly indicate where suboptimal samples (insufficient material, mechanical injury/artefacts) has limited the ability to assess the biopsies
- **Histopathological description** of morphological and inflammatory abnormalities and scoring of these findings according to the simplified WSAVA scoring system.

Histological parameters of the quantitative scoring system

Stomach

Morphological parameters
- Surface epithelial injury
- Fibrosis

Inflammatory parameters
- Intraepithelial lymphocytes
- Lamina propria infiltrates: lymphocytes/plasma cells, neutrophils and/or eosinophils

Small intestine: duodenum and ileum

Morphological parameters
- Surface epithelial injury
- Villus stunting
- Crypt dilation
- Lacteal dilation

Inflammatory parameters
- Intraepithelial lymphocytes
- Lamina propria infiltrates: lymphocytes/plasma cells, neutrophils and/or eosinophils

Colon

Morphological parameters
- Surface epithelial injury
- Crypt dilation
- Fibrosis
- Goblet cell numbers

Inflammatory parameters
- Lamina propria infiltrates: lymphocytes/plasma cells, eosinophils, neutrophils and/or macrophages

Other pathological changes such as ulceration and infectious agents will also be described

- Morphological **diagnosis** which reflects the most significant morphological and inflammatory changes in the biopsies as per location; for example: duodenum: moderate chronic lymphoplasmacytic enteritis with marked villous stunting
- **Comments** which may include possible pathogenesis, etiology, differential diagnoses and/or recommendations for further tests, if applicable. If neoplasia is suspected, immunohistochemical staining or clonality testing (PARR test) may be advised
- **Name** of the reporting pathologist at the end of the report, including contact details if further discussion on the case is needed

Terminology has clinical significance: idiopathic inflammatory bowel disease (IBD) or chronic inflammatory enteropathy (CIE)

Assessment of endoscopic biopsies from dogs with primary chronic GI disease often reveals a lymphocytic / lymphoplasmacytic infiltrate in the gastrointestinal mucosa. In the past, this has frequently been reported as "compatible with IBD".

The term IBD became embedded in veterinary terminology as the mucosal inflammation in dogs resembles IBD in humans. IBD in humans is an umbrella term denoting chronic inflammation of the gastrointestinal tract and encompasses two main entities: Crohn's disease and ulcerative colitis. In humans, drug therapy (anti-inflammatory drugs and immune system suppressors) are often the first step in the treatment of IBD. In contrast, most dogs with chronic GI disease and confirmed mucosal inflammation will not require immunosuppressant treatment and therefore it can be misleading to use the term IBD in these cases. Instead, the term chronic inflammatory enteropathy (CIE) is now preferred to encompass this chronic disorder in dogs, once other extra-intestinal and intestinal causes have been ruled out.

Other conditions of the gastrointestinal system besides CIE that can be diagnosed by endoscopic biopsies are e.g. lymphangiectasia, intestinal lipogranulomatous lymphangitis, granulomatous colitis, and neoplasia.

Protein-losing enteropathy

Endoscopic biopsies are used to identify the causes of protein-losing enteropathy (PLE) and optimize treatment. PLE is a syndrome characterized by an abnormal loss of protein-rich fluid into the gastrointestinal lumen. Histologically, PLE caused by GI disease in dogs is associated with lymphoplasmacytic enteritis in approximately 66% of cases, lymphangiectasia in around 50% of cases (both lesions may occur in the same patient), and lymphangitis and intestinal crypt lesions each in <10% of cases (Craven et al, 2019). Alimentary lymphoma and other types of enteritis, namely granulomatous and eosinophilic enteritis, are also causes of PLE.

It has been debated whether endoscopic biopsies are adequate for the diagnosis of intestinal lymphangiectasia. In a recent publication, the ability to diagnose lymphangiectasia in endoscopic (44/83; 53%) and full-thickness biopsies (38/64; 59%) was equivalent (Craven et al, 2019). However, it has been shown that lymphangiectasia may especially occur in the ileum and may thus be missed when only duodenum is sampled.

Chronic inflammatory enteropathy (CIE) in the dog

Dogs with CIE present with chronic clinical signs (> 3 weeks) such as vomiting, diarrhoea, borborygmus, hyporexia, abdominal pain, nausea and/or weight loss. CIE is diagnosed **after exclusion** of extra-intestinal (such as hepatic, pancreatic, renal disease, and hypoadreno-corticism), infectious or parasitic diseases and intestinal disease of other aetiology (examples include mechanical obstruction from intussusception, foreign body, or neoplasia). Minimum Database (routine haematology, clinical chemistry, urinalysis) sample collection, faecal sample analysis and performing a fasting serum GI profile panel (IDEXX code PROFPH for dogs) are important steps in the workup of chronic GI disease and should be performed before starting treatment and before histology is considered. Imaging also provides useful information to rule out obstructive disorders, extra-GI tract changes and to help localizing changes and choosing appropriate sampling technique.

CIE is used as an umbrella term and can be subdivided into 4 entities based on response to treatment into:

- food-responsive enteropathy
- antimicrobial-responsive enteropathy*
- immunosuppressant-responsive enteropathy
- non-responsive enteropathy

Clinical signs overlap, and histopathological assessment of GI biopsies **cannot distinguish** between these four entities. In cases with mild/moderate signs, endoscopic biopsy collection is usually postponed and supportive therapy with trial therapy for food-responsive enteropathy performed first.

*antimicrobial therapy should be reserved to cases with adherent-invasive bacteria (e.g. granulomatous colitis) and cases with evidence of severe bacterial enteritis, signs of systemic inflammatory response syndrome (SIRS), immunodeficiency-, and extra-GI infections. Recommended treatment of intestinal dysbiosis currently consists of dietary therapy with pro-/pre- or synbiotics. The canine microbiota dysbiosis index can be performed for an accurate assessment of dysbiosis (DYSBIND code).

Intestinal lipogranulomatous lymphangitis

Full-thickness biopsies are preferred over endoscopic biopsies in the diagnosis of intestinal lipogranulomatous lymphangitis, a rare condition with PLE and chronic diarrhoea and vomiting the predominant clinical signs. Laparotomy will allow visual inspection and sampling of abdominal organs whereby the typical small firm nodules (lipogranulomas) along the serosal and mesenteric lymphatics can be visualized. This condition is histologically characterised by transmural granulomatous inflammation mainly involving the muscularis and serosa and extending into the mesentery. The overlying mucosa may be spared which implies that in superficial endoscopic biopsies this diagnosis may be missed.

Granulomatous colitis (histiocytic ulcerative colitis)

In most cases of CIE in dogs, a lymphocytic/lymphoplasmacytic infiltrate in the intestinal mucosa is reported on histology. In rare cases, a granulomatous infiltrate is observed, suggesting that infectious (fungal or bacterial) aetiologies should be considered. In cases with signs of colitis, colonoscopy is required to diagnose granulomatous colitis or 'histiocytic ulcerative colitis' associated with adherent-invasive *E. coli* (AIEC) infection. In this condition, macrophages with periodic acid–Schiff (PAS) positive cytoplasm infiltrate the intestinal wall. This is an antibiotic-responsive disorder most commonly described in young Boxers and related breeds such as French Bulldogs.

Key Points

- sample quality is essential
- with chronic inflammatory enteropathy (CIE) suspicion, upper and lower GI endoscopy is generally recommended, with sampling of stomach, duodenum, ileum and colon
- at least 6-8 biopsies per location
- submit biopsies preferably in cassettes
- provide clinical history, past treatments, and clinical diagnosis
- biopsies are assessed, scored, and interpreted by pathologists according to a simplified WSAVA scoring system for endoscopic GI biopsies
- a histological diagnosis of lymphocytic / lymphoplasmacytic gastritis, enteritis and/or colitis is not a specific disease, only implies an inflammatory process within the gastrointestinal tract and should be related to clinical signs and other diagnostic test results
- CIE is used as an umbrella term
- histopathology cannot distinguish between different forms of CIE but is essential to investigate the type of inflammatory infiltrate, presence of neoplasia, lymphangiectasia / lymphangitis, granulomatous inflammation etc. to confirm the final diagnosis and optimal therapy

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