

Update on the non-invasive monitoring of intestinal disease in dogs and cats

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SUMMARY

Diagnosis and management of intestinal disease in the dog and cat can present a considerable challenge to the clinician because of the many potential causes and the relative inaccessibility of the small intestine. Intestinal disease is typically characterised on the basis of histopathologic criteria. However, this approach provides little information on the underlying cause of damage and many cases may be overlooked by reliance on these morphologic criteria alone. In addition, it is impractical to take serial samples of the intestine for the objective assessment of response to treatment. Assessment of intestinal disorders in dogs and cats by non-invasive procedures has clear advantages, particularly for monitoring of progress during treatment. Current tests available to practitioners include assays of serum folate and cobalamin which provide indirect evidence of intestinal disease but have limited sensitivity and specificity. Measurement of intestinal permeability using dual sugar absorption tests has recently been validated as a sensitive test in dogs, not only for the detection of mucosal damage but also to monitor response to treatment. This test has been combined with the hydrogen breath test to allow the simultaneous detection of small intestinal bacterial overgrowth in dogs. New tests that are currently being developed are designed to detect protein-losing enteropathies (faecal alpha-1-protease inhibitor) and small intestinal bacterial overgrowth (serum deconjugated bile acids).

KEY-WORDS : - canine intestinal disease - feline intestinal disease - folate and cobalamin assay - intestinal permeability - hydrogen breath test.

RÉSUMÉ

Actualités sur la surveillance des affections intestinales du chien et du chat. Par R. BATT.

Le diagnostic et la surveillance des affections intestinales du chien et du chat peut constituer un véritable défi pour le clinicien en raison des nombreuses causes potentielles et de la relative inaccessibilité de l'intestin grêle. Les affections intestinales sont classiquement caractérisées en fonction de critères histopathologiques. Cependant, cette approche n'apporte que peu d'informations quant à la cause sous-jacente et de nombreux cas peuvent ne pas être détectés en se basant sur ces seuls critères morphologiques. De plus, il est impossible d'effectuer des prélèvements répétés de l'intestin dans le but de suivre objectivement la réponse à un traitement. L'évaluation des affections intestinales par des techniques non-invasives a de nets avantages, notamment pour surveiller l'évolution pendant un traitement. Les tests actuellement disponibles pour les praticiens comprennent le dosage des folates et de la cobalamine sériques, qui donnent une preuve indirecte d'affection intestinale mais manquent de sensibilité et de spécificité. La mesure de l'absorption intestinale fondée sur la double absorption de sucres a été récemment validée comme un test sensible chez le chien, non seulement pour détecter des lésions de la muqueuse mais aussi pour suivre la réponse à un traitement. Ce test a été utilisé en association avec le test de l'hydrogène respiratoire pour permettre la détection simultanée d'une surcharge bactérienne du grêle chez le chien. Les nouveaux tests actuellement développés visent à détecter les entéropathies (inhibiteur de l'alpha-1-protéase fécal) et la surpopulation bactérienne du grêle (acides biliaires déconjugés dans le sérum).

MOTS-CLÉS : maladie intestinale - folates - cobalamine - perméabilité intestinale - test de l'hydrogène respiratoire - chien - chat.

Introduction

Diagnosis and management of intestinal disease in the dog and cat can present a considerable challenge to the clinician because of the many potential causes and the relative inaccessibility of the small intestine. Characterisation of intestinal disease usually occurs on the basis of histopathologic criteria, but these provide little information on the underlying

cause of the damage whereas many cases may be overlooked by reliance on these morphologic criteria alone. These tests are also ill suited for serial monitoring of response to treatment. There are clearly advantages to the assessment of intestinal disorders in dogs and cats by the use of indirect non-invasive procedures. These can assist not only the diagnosis but also the management of intestinal damage, allowing response to treatment to be monitored without the need serial

intestinal biopsies. This overview summarises how alternative, non-invasive approaches have facilitated advances in the diagnosis and management of chronic intestinal disease in dogs and cats.

Overall approach to diagnosis

Initial investigations should include detailed history, physical examination, and a minimum database of haematology, serum biochemistry, urinalysis and faecal examination in order to determine whether disease of the large bowel, intestinal pathogens or parasites, systemic diseases or metabolic disorders may be responsible for the clinical signs [21]. Diagnostic imaging may be helpful to detect partial obstruction and neoplasia, and abdominal ultrasound is particularly useful to assess intestinal wall thickness, preservation of layering, and size of mesenteric lymph nodes. Furthermore, serologic tests performed in cats can include a FeLV and FIV test, toxoplasma titre, and in older cats an assay of serum T4.

Diagnosis of exocrine pancreatic insufficiency (EPI)

The next step is to rule-out EPI by the assay of serum trypsin-like immunoreactivity (TLI). This test is available as a single blood test and has shown good sensitivity and specificity in the diagnosis of EPI in dogs [19, 21]. Serum TLI is species-specific and was until recently only available for use in the dog, but assay of feline serum trypsin-like immunoreactivity (fTLI) has now also been established as a specific and more convenient test for feline EPI [16]. Severely decreased TLI concentrations ($< 2.5 \mu\text{g/L}$ in the dog, $< 8 \mu\text{g/L}$ in the cat) are diagnostic for EPI.

Non-invasive assessment and monitoring of intestinal disease

Assays of serum folate and cobalamin are widely available and provide indirect evidence of intestinal disease but have limited sensitivity and specificity. Recently, two tests that have been established in human medicine (intestinal permeability test and hydrogen breath test) have become available for routine use in dogs, but there are limitations to their application for cats. New tests in dogs that hold promise for the future include assessment of protein-losing enteropathy (alpha-1-protease inhibitor) and bacterial overgrowth (serum unconjugated bile acids).

SERUM FOLATE AND COBALAMIN

The assay of serum folate and cobalamin (vitamin B12) concentrations represents a helpful initial diagnostic test that can assist in the detection and characterisation of small intestinal disease, particularly in dogs [3, 11, 21]. Folate absorption may be reduced in proximal small intestinal disease. Low serum folate concentrations particularly draw attention to the possibility of dietary hypersensitivity since the proximal small intestine is exposed to the highest concentrations

of food antigens. A hypersensitivity reaction causing mucosal damage is therefore most likely to result in folate malabsorption. High serum folate may be a consequence of small intestinal bacterial overgrowth (SIBO), in which bacteria synthesise folate which is subsequently absorbed by the small intestine. Cobalamin is normally absorbed in the distal small intestine, and low serum levels may reflect distal small intestinal disease, or, more commonly, SIBO. However, normal serum vitamin concentrations do not exclude the possibility of intestinal disease, because alterations depend on the type and numbers of organisms present, the severity of any secondary mucosal damage which may interfere with folate absorption despite a high intraluminal concentration, and depletion of body stores. High serum folate may also be a consequence of high folate intake, such as a high-folate diet or coprophagia. Both parameters have fair specificity but poor sensitivity in diagnosing SIBO in the dog [11]. Demonstration of low serum cobalamin is the more useful finding, since it is less influenced by diet and coprophagia and appears to relate more to the severity of clinical disease. Finding concurrent increases in serum folate but decreases in cobalamin concentration has very high specificity for SIBO but very low sensitivity. Measurement of serum folate and cobalamin concentrations is therefore a convenient test, readily available to practitioners, which may assist in the diagnosis of SIBO, although normal results do not rule it out. It is also important to be aware that dogs with EPI may also have alterations in serum folate and cobalamin levels due to concurrent SIBO and/or cobalamin malabsorption, and therefore EPI must always be ruled out before interpreting the results of these vitamin assays [20, 21].

Serum folate and cobalamin have very broad control ranges in cats and these determinations appear to provide different information than in dogs [16]. Both folate and cobalamin are plentiful in feline diets, and dietary deficiencies are unlikely. Healthy cats have markedly higher serum folate concentrations than healthy dogs, which may reflect the higher numbers of bacteria that have been reported to be normal inhabitants of the upper small intestine of cats. Very few cats have increased serum folate, and the significance of this finding in the cat is unclear, especially since it is uncertain whether SIBO occurs in the cat. In cats, a subnormal serum folate concentration is always associated with unequivocal morphologic abnormalities in the small intestine, particularly villous atrophy and/or inflammatory cell infiltration. Cats with EPI often have low serum folate concentration, suggesting that concurrent small intestinal disease is a far more common complication of EPI in cats than in dogs. Serum cobalamin concentrations are often very low in cats with EPI, indicative of severe and prolonged cobalamin malabsorption and depletion of body stores. Such patients should be treated with cobalamin parenterally.

INTESTINAL PERMEABILITY

Permeability of the small intestine to macromolecules represents a potential route for the mucosal passage of ingested antigens, bacterial products and endogenous proteins normally present within the lumen. This may be of great impor-

tance in the aetiopathogenesis of local and systemic disease states including inflammatory bowel disease and autoimmune disease. Measurement of permeability to a single probe will potentially be affected by errors due to rates of gastric emptying and intestinal transit, renal excretion, and completeness of urine collection. Calculation of the ratio of the urinary excretion or blood levels of a mixture of two probes of different sizes minimises such problems. In the dog, this test has been performed by determination of 5h urinary excretion or 2h blood levels of lactulose and rhamnose following oral administration [10, 13, 14, 15]. Lactulose, a disaccharide, is the larger molecule and is normally poorly absorbed. Intestinal damage is thought to allow increased passage of this probe across the mucosa by a paracellular route due to interference with the integrity of tight junctions between enterocytes. Rhamnose is a monosaccharide that is normally well absorbed by passive diffusion by a transcellular route. Intestinal damage causing a decrease in surface area results in decreased rhamnose absorption. The ratio of lactulose to rhamnose is therefore a sensitive indicator of intestinal damage and this dual sugar absorption (DSA) test overcomes problems such as delayed stomach emptying. The test has also been performed with cellobiose in place of lactulose and mannitol substituted for rhamnose, but has limitations due to degradation of cellobiose by intestinal cellobiase and urinary excretion of endogenous mannitol [4]. Intestinal function can be assessed simultaneously by determining the urinary excretion ratio [14] or blood ratio [15] of xylose and 3-O-methylglucose.

The DSA test has been shown to provide useful information for the detection and management of clinical disease in dogs, while contributing to the understanding of the pathogenesis of intestinal damage [4, 5, 10, 13]. Intestinal permeability testing has been shown to be particularly useful for the diagnosis of dietary sensitivity in dogs, providing an objective parameter to assess the response of the intestine to exclusion diet and subsequent challenge [4, 10]. Repeated intestinal biopsy before, during and after dietary exclusion and challenge is the only other objective method to document this response, but this is clearly not practical in most clinical patients [2]. The value of this approach has been well documented in studies in Irish setters with gluten-sensitive enteropathy [2, 4]. In a study of 15 dogs with diet-responsive diarrhoea, five dogs (all retrievers) showed normalisation of permeability with clinical remission, whereas permeability remained high in the other 10 dogs, suggesting that the DSA can help distinguish between food allergy and intolerance [10].

Intestinal permeability has also been shown to be increased in 50-60 % of dogs with SIBO [13], indicative of intestinal damage even when there are no obvious histologic abnormalities [1]. Furthermore, the DSA test has proved to be particularly useful in the long-term management of SIBO [13]. Normalization of intestinal permeability following 4 weeks of antibiotic therapy indicates successful repair of mucosal damage and antibiotics should be discontinued. In contrast, dogs with high permeability at that time are between 2 and 3 times more likely to relapse despite an apparent clinical response to treatment, and continuation of antibiotic therapy is

recommended. A persistent high permeability in dogs with a poor clinical response should prompt further investigation of underlying disease, such as a primary inflammatory bowel disease.

Abnormal permeability in intestinal disease in dogs makes this a useful method for the detection of intestinal damage and the monitoring of response to treatment. Unfortunately, permeability tests appear to be of little value in the cat, since normal cats have very high permeability compared to dogs, and cats with gastrointestinal disease typically have results that fall within the control range [6, 7, 8].

Hydrogen breath test

Hydrogen breath tests have been used in both human and veterinary medicine for a variety of reasons, including diagnosis of SIBO, detection of carbohydrate malabsorption and measurement of oro-caecal transit time [12, 17]. Hydrogen is produced in the intestine, absorbed and excreted in breath when carbohydrates come into contact with gut bacteria. In dogs, an early peak is indicative of SIBO, whereas a later peak is suggestive of malabsorption of carbohydrate when it comes into contact with the large bowel flora [12]. Interpretation of this test in the cat is more difficult, since clinically healthy cats can show a pattern that might be expected in SIBO, probably due to the high numbers of bacteria in their proximal small intestine [6, 7]. This may also interfere with interpretation of the lactulose breath hydrogen test, which has been used in cats to determine oro-caecal transit time [9].

A hydrogen breath test for the detection of SIBO in dogs has been established by combining it with the DSA test for concurrent assessment of intestinal permeability and function [12]. Following oro-gastric administration of a multiple sugar solution (lactulose 25 g/l, rhamnose 10 g/l, xylose 25 g/l, and 3-O-methylglucose 10 g/l) at a dose of 100 ml (< 20 kg bodyweight) or 200 ml (> 20 kg bodyweight), breath hydrogen was measured at 30 minute intervals for 2 hours. This is a simple procedure involving relatively inexpensive equipment. Based on mean breath hydrogen excretion in control dogs, an abnormal result has been defined as either a baseline breath hydrogen concentration ≥ 5 ppm, or two consecutive breath hydrogen concentrations ≥ 6 ppm within the first 2 hours. Beyond this time, there can be confusion with a colonic peak due to hydrogen production by the colonic flora. This test has proved more sensitive than the assay of serum folate and cobalamin concentrations. A positive result is very suggestive of SIBO, and there is no need to culture duodenal juice. However, a negative test does not rule out SIBO, and in these cases culture of duodenal juice is indicated.

MISCELLANEOUS NON-INVASIVE TESTS

The classical gold standard for diagnosis of protein-losing enteropathy is to quantitate loss of radioactive (^{51}Cr -labeled) albumin into the gastrointestinal tract. Obvious technical constraints, safety concerns and expense have all limited the application of this approach. Assay of alpha- $_1$ -protease inhibitor (α_1 -PI) has been shown to be a reliable method to detect

protein-losing enteropathy in human patients. It has a molecular weight similar to that of albumin and is lost in the gut when there is leakage from the vascular bed, intercellular spaces and/or lymph. Unlike albumin, α_1 -PI is passed into the faeces essentially intact and can then be detected using species-specific immunologic assay methods. Preliminary investigations suggest that the method has promise as a new test for the diagnosis of protein-losing enteropathy in dogs and for indirect quantitation of enteric protein loss [18].

Deconjugation of bile salts by bacteria in SIBO may result in increased serum concentrations of unconjugated bile acids. Recent developments have overcome difficulties with analysis, and preliminary findings suggest that measurement of unconjugated bile acids in serum may be a useful approach to the diagnosis of SIBO in dogs [21].

Direct assessment of intestinal disease

Endoscopy and histologic examination of intestinal biopsies allows the direct assessment of intestinal disease. Multiple biopsies should be taken from the duodenum, jejunum and ileum, and in vomiting animals also from the stomach. When endoscopy is not available, and for localised rather than diffuse, it may be necessary to take full-thickness biopsies at laparotomy. This approach lends itself to the classification of intestinal diseases by histologic appearance rather than cause, and this obviously has limitations for effective management. In dogs, concurrent quantitative aerobic and anaerobic culture of duodenal juice may assist in the diagnosis of SIBO ($> 10^5$ colony-forming organisms (cfu)/ml or $> 10^4$ anaerobic bacterial cfu/ml) anaerobes, that would be considered to be SIBO in other species [1, 11]. Furthermore, as might be anticipated, these counts do not normally appear to be increased in cats with signs of intestinal disease, suggesting that SIBO does not occur as a clinical syndrome in cats [6, 7].

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