Topics in Compan An Med xx (2012) xxx

Exocrine Pancreatic Insufficiency in the Dog: Historical Background, Diagnosis, and Treatment

Elias Westermarck, DVM, Dipl. ECVIM-CA, PhD, and Maria Wiberg, DVM, PhD

Keywords:
Exocrine pancreatic insufficiency
trypsin-like immunoreactivity
tylosin
pancreatic acinar atrophy
canine

Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Finland

Address reprint requests to: Elias Westermarck, DVM, Dipl. ECVIM-CA, PhD, Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, PO Box 57, 00014 University of Helsinki, Finland.

E-mail: elias.westermarck@helsinki.fi.

A B S T R A C T

This overview summarizes research performed during the last decades that has had an impact on the diagnosis and management of exocrine pancreatic insufficiency (EPI) in dogs. Pancreatic acinar atrophy is by far the most common cause for the maldigestion signs of canine EPI. The ability to diagnose pancreatic acinar atrophy in the subclinical phase before the development of total acinar atrophy and manifestation of clinical signs has offered new possibilities to study the pathogenesis of the disease. Diagnosis of exocrine pancreatic dysfunction is based on typical findings in clinical histories and clinical signs and is confirmed with pancreatic function tests. In recent years, the measurement of serum canine trypsin-like immunoreactivity has become the most commonly used pancreatic function test to diagnose canine EPI. Serum trypsin-like immunoreactivity measurement is species- and pancreas-specific. When clinical maldigestion signs of EPI appear, enzyme replacement therapy is indicated. Despite accurate enzyme supplementation, only a small portion of orally administered enzymes are delivered functionally intact into the small intestine. In dogs, the highest enzyme activity in the duodenum has been obtained with nonenteric-coated supplements: raw chopped pancreas or powdered enzymes. Aside from dietary enzyme supplements, dietary changes are often made to improve clinical response, but sometimes weight gain and stool quality remain suboptimal. Other medications for treatment of gastrointestinal tract signs are often used in such dogs with EPI. Antibiotics are the most common adjunctive medication. Of the antibiotics administered, tylosin is used in Finland almost exclusively.

Hardly any organ has been known for so long and yet remained so relatively poorly understood as the pancreas. This puzzling gland and its significance in digestion have concerned the most famous scientists throughout the ages. The one who finally made the breakthrough was Claude Bernard, who published a book in 1856 entitled *Memoir on the Pancreas and on the Role of Pancreatic Juice in Digestive Processes Particularly in the Digestion of Neutral Fat.*[1] Bernard made pancreatic fistulas in dogs, rabbits, and cats, and his experimental results confirmed that fats were emulsified and broken down into fatty acids and glycerol by pancreatic juice. He also concluded from his studies that pancreatic juice is absolutely essential to the absorption of fats, and therefore fatty stools were often a symptom of pancreatic disease. In 1859, Alexander Fles showed for the first time that a human patient with exocrine pancreatic insufficiency (EPI) could be treated by ingestion of raw calf pancreas with every meal.[2]

EPI in Dogs

Exocrine pancreatic function may be diminished by chronic diseases leading to inadequate production of digestive enzymes and classic signs of maldigestion. EPI is a functional diagnosis based on measuring decreased pancreatic secretion capacity by pancreatic function test. The exocrine pancreas has a large reserve secretory capacity, and maldigestion signs are usually not seen until 90% of the secretory capacity is lost. Exocrine pancreatic diseases in dogs that may result in clinical signs of EPI include pancreatic acinar atrophy (PAA), much more rarely by chronic pancreatitis, and very rarely by pancreatic neoplasia.[3-8]

Etiopathogenesis

EPI has been reported in many different breeds, but some breeds appear to be more predisposed than others. EPI is most commonly found in German Shepherds, followed by Rough-coated Collies, Chow Chows, and Cavalier King Charles Spaniels.[5,7,9-12] Female dogs are reported to be overly represented with EPI.[12] The prevalence of the various pancreatic diseases causing clinical signs of EPI is difficult to assess, because pancreatic morphologic examination is needed for the specific diagnosis. However, PAA is reported to be by far the most common cause of severe EPI in dogs. Of all dogs diagnosed with EPI, approximately 50% to 70% were German Shepherds, and in Finland 20% of the cases are found in Rough-coated Collies.[5,11,12] In German Shepherds and Rough-coated Collies, the underlying cause for EPI is essentially always PAA. The estimated prevalence of the disease within these 2 breeds is approximately 1%.[5,10] A similar etiopathogenesis to classical PAA is suspected in other breeds.[12,13]

Pancreatic Acinar Atrophy

The characteristic finding in dogs with PAA is a selective destruction of the digestive enzyme producing acinar cells. Loss of acinar tissue leads to inadequate secretion of pancreatic enzymes and to signs of maldigestion typical of EPI. The endocrine function of the pancreas is usually spared in this process.[3,4,7,14] Canine PAA is a unique disease compared with that in other species. In humans, PAA has been reported but in association with multiorgan diseases such as Sjögren's and Shwachman-Diamond syndromes, associations not recognized in veterinary medicine.[15] Congenital isolated deficiencies in pancreatic enzymes are reported in humans[15] but not in dogs. Experimental studies show that acinar atrophy can be a result of multiple pathogenetic processes involving the exocrine pancreas, such as pancreatic duct obstruction, ischemia, toxicity, nutritional deficiencies or imbalances, and defective secretory and/or trophic stimuli.[8] That said, there is no evidence to support the involvement of these factors in naturally occurring PAA in dogs.[8,16] Congenital exocrine or compound exocrine and endocrine pancreatic hypoplasia in young puppies has been reported.[17-19] Westermarck et al[20] followed the morphologic

0958-3947/$ – see front matter © 2012 Topics in Companion Animal Medicine. Published by Elsevier Inc.
http://dx.doi.org/10.1053/j.tcam.2012.05.002

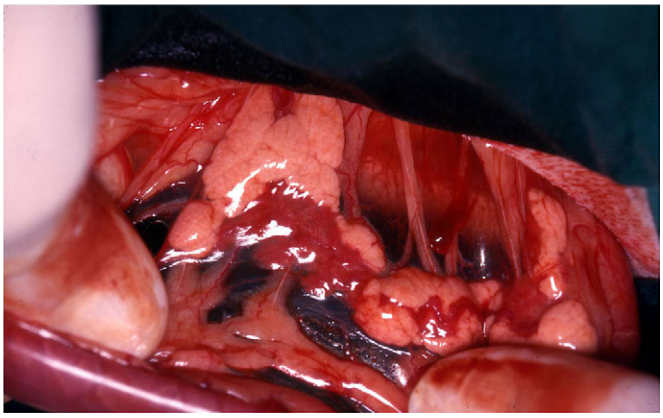

Fig. 1. Dog with subclinical EPI showing a markedly diminished pancreatic mass, including areas of normal glandular structure and areas of tissue having lost its glandular appearance.

changes in the pancreas of a German Shepherd puppy bred from parents with PAA. The puppy was born with a grossly and histologically normal pancreas but developed EPI later in life. This finding supports the hypothesis that PAA in this breed is neither hypoplastic nor congenital, but rather an acquired progressive disease process.

The clinical signs of EPI caused by PAA are usually seen in young adults 1 to 4 years of age, although sometimes the clinical disease may develop later in life.[21] The hereditary nature of PAA has been demonstrated in German Shepherds, Rough-coated Collies, and recently in Eurasian dogs. Pedigree analyses suggest that the disease in these 3 breeds is heritable by an autosomal recessive trait.[9,10,13,22,23] Results of a test mating between 2 German Shepherds with PAA showed that only 2 of the 6 offspring were affected, thus suggesting that EPI is not a single-gene disease but rather a polygenic disease.[24]

Recent etiopathogenetic studies showed that PAA in German Shepherds and Rough-coated Collies has some features of autoimmune disease.[25,26] These features include genetic susceptibility to disease and characteristic morphologic and immunologic findings during progression of disease. The ability to diagnose PAA by assay of serum trypsin-like immunoreactivity (TLI) before development of total acinar atrophy and manifestation of clinical maldigestion signs permits the progression to atrophy to be closely monitored.[11] The progression of PAA was divided into a subclinical phase characterized by partial acinar atrophy and a clinical phase with severe end-stage atrophy. In the subclinical phase, both atrophied and normal acinar

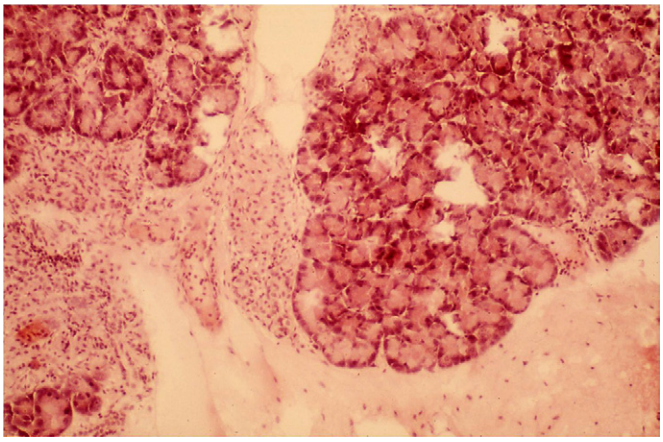

Fig. 2. Exocrine pancreas of a dog with subclinical EPI. Severe mononuclear cell inflammatory reaction is associated with gradual destruction of acinar architecture (border zone active).

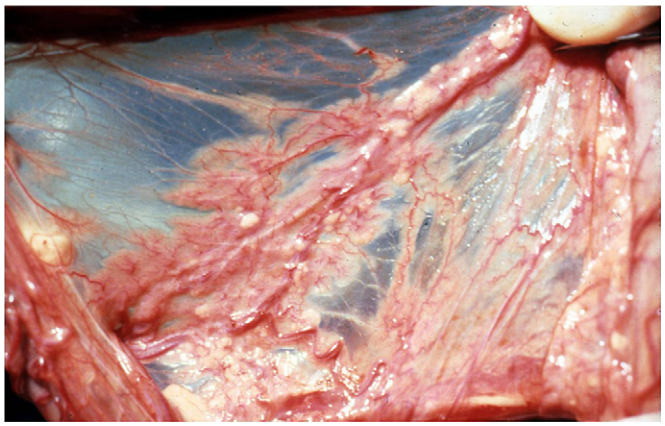

Fig. 3. The duodenal limb of the pancreas of a dog with pancreatic acinar atrophy.

parenchyma were found. Grossly, the normal pancreatic mass was diminished, and scattered areas of atrophied tissue were found among the normal tissue (Figs. 1 and 2) No hemorrhagic or fibrotic tissue was observed. The histologic findings during the progression of atrophy were typical for an autoimmune disease showing marked lymphocytic inflammation into the partially atrophied acinar parenchyma. Gradual destruction of acinar structure was found in association with the inflammatory reaction. Lymphocytic inflammation was most extensive in the border zones of the normal and affected acinar parenchyma, and lymphocytes spread into the normal acinar parenchyma and intra-acinar areas. As tissue destruction progressed, the findings became more typical of end-stage PAA.[25]

Clinical signs appear only in the end stages of PAA. The gross pathologic findings are typical, showing thin and transparent pancreas with no signs of fibrosis. The normal glandular structure is hardly recognizable and the pancreatic ducts are clearly visible. Histologically, no normal acinar tissue is left in the end stages, or if normal tissue is present, it is found in small, isolated lobuli. The normal acinar parenchyma is replaced by atypical tissue, and ductal structures are prominent. Fibrous tissue is not generally increased, and in some cases the normal tissue is replaced by adipose tissue. Inflammatory cells, lymphocytes, and plasma cells may be found, but in general inflammation is less prominent than in the subclinical phase (Figs. 3 and 4). The endocrine part of the pancreas in dogs with PAA is usually well preserved.[3,7,14,27]

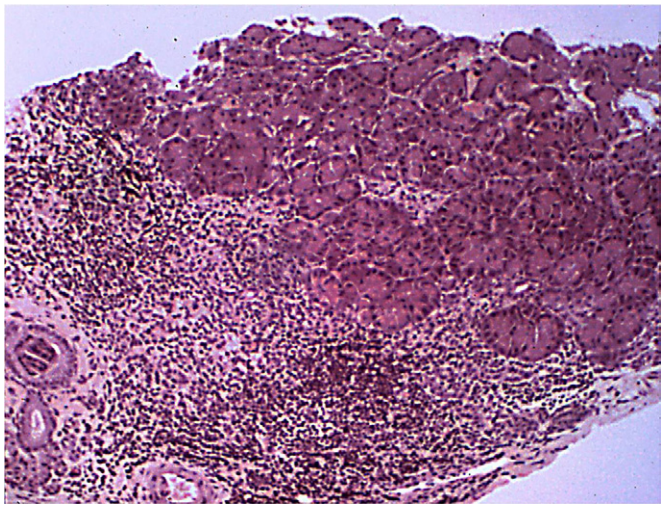

Fig. 4. Pancreas of a dog with clinical EPI, showing typical changes of pancreatic acinar atrophy. Note severe atrophied parenchyma consisting of ductal structures and disorganized cells.

Further immunologic studies with dogs with partial PAA have suggested that both cellular and humoral immune responses play a role in the pathogenesis of acinar atrophy, although tissue destruction appears to be largely mediated by cellular immune mechanisms.[26] Immunohistochemical analysis showed that at the onset of acinar cell destruction, most of the infiltrating lymphocytes were T-cells, with an almost equal number of CD4+ T-helper and CD8+ cytotoxic T-lymphocytes. Cytotoxic T-cells predominated in sections in which the gradual destruction of the acinar parenchyma was present.[26] The role of the humoral immune response was previously studied, in which serum pancreatic-specific antibodies in dogs with clinical signs of EPI were compared with those of healthy controls, but the study found no differences between these 2 groups.[28] A recent study showed that serum auto-antibodies reacting at low intensity with pancreatic acinar cells were found in some dogs with partial and end-stage PAA, but not in healthy control dogs, suggesting that the humoral immune response was also activated.[26]

As lymphocytic pancreatitis with active destruction of acinar structures preceded the end-stage atrophy, the term "autoimmune-mediated atrophic lymphocytic pancreatitis" has been suggested to describe the pathologic findings.[25,26] The rate of progression of the atrophy from the subclinical to the clinical phase is highly unpredictable, and the factors affecting it are not yet identified. Long-term follow-up of dogs with partial PAA shows that they may remain in the subclinical phase for years or sometimes for life. No diagnostic markers predicting which dogs will develop clinical disease have been found.[29] Autoimmune diseases are often multifactorial. Genetic susceptibility, environmental factors, and immunologic abnormalities are all involved in this pathogenesis. Environmental factors, either microbial or nonmicrobial, are usually needed to initiate a clinical autoimmune disease in genetically susceptible individuals.[30] The possible contribution of various environmental factors, such as feeding, housing, training, stress, and viruses, in the pathogenesis of PAA has been proposed, but there are no comprehensive studies available on their roles. A survey failed to show any common triggering environmental factors in the histories of dogs with EPI.[21]

Chronic Pancreatitis

Chronic pancreatitis is infrequently the underlying cause for EPI in dogs. Unlike the situation in autoimmune atrophic pancreatitis, there is usually a progressive destruction of both exocrine and endocrine pancreas in chronic pancreatitis. Clinical history usually shows more nonspecific gastrointestinal signs, or the signs of EPI also can develop after those of diabetes mellitus. The pathologic findings in chronic pancreatitis are clearly different from those of PAA. Macroscopically, the pancreas is usually hard, shrunken, and nodular, and there may be adhesions to adjacent abdominal organs. The characteristic histological findings in chronic pancreatitis involve an increase in interlobular and intralobular fibrosis and disorganized acinar lobuli, with or without inflammatory cells in the interstitium.[3,4,7,31]

Clinical Signs

The typical clinical signs of EPI include increased fecal volume and defecation frequency, yellowish feces, weight loss, and flatulence. Other common signs are polyphagia, poorly digested, loose, and pulpy feces, and coprophagia. Nervousness or aggressiveness may occur and these are suspected to result from abdominal discomfort because of increased intestinal gas. Severe, watery diarrhea is usually only temporary. Although atypical, some patients vomit and may rarely be intermittently anorexic, perhaps also reflecting abdominal discomfort. Skin disorders including seborrhea have also been reported. Although these signs of EPI are characteristic, they are not pathognomonic for the disease, because small intestinal diseases may show similar signs of malabsorption.[8,21]

Diagnosis

The diagnosis of exocrine pancreatic dysfunction is based on typical findings in clinical histories and clinical signs and is confirmed with a pancreatic function test. Complete blood cell count and routine serum biochemistry often show unremarkable changes. Serum amylase and lipase activities are not useful in the diagnosis of EPI. Various pancreatic function tests, which measure pancreatic enzyme concentrations in the blood and feces, have been used to diagnose canine EPI. The diagnostic value of these tests lies in their ability to distinguish whether the maldigestion signs are caused by exocrine pancreatic or small intestinal disease, as well as in their practicality. When needed to verify the underlying pathologic process causing the clinical signs, morphologic examination of the pancreas should be performed.[32]

The measurement of canine serum TLI has become one of the most commonly used pancreatic function tests in the diagnosis of canine EPI.[33] Serum TLI assays are species- and pancreas-specific. Current reference ranges for canine TLI (cTLI) in healthy dogs are 5.7 to 45.2 μg/L (Texas A&M University, Gastrointestinal Lab, College Station, TX). Abnormally low serum TLI concentrations (< 2.5 μg/L), with the typical clinical signs of maldigestion, are considered highly diagnostic for severe EPI and indicate severe loss of the digestive enzyme-producing acinar cells.[33] In general, the lower the TLI value, the more valuable a single measurement is in assessing pancreatic dysfunction.

Fecal proteolytic activity measurement has been used for the diagnosis of EPI. The reliability of the different tests varies, and a common problem with these tests is that sometimes normal dogs also show decreased proteolytic activity.[8,34,35] To avoid this problem, fecal proteolytic activity was measured from repeated fecal samples and after using pancreatic stimulation by including ground raw soybeans in the food during the test period.[35] This soybean stimulation test is valuable for diagnosing severe EPI, but the test is insufficiently sensitive to detect subclinical EPI. A recent study showed that in dogs with protein-losing enteropathy, increased fecal loss of α_1-proteinase inhibitor is associated with a decrease in fecal proteolytic activity and may result in a false diagnosis of EPI.[36]

A new fecal test for diagnosing exocrine pancreatic dysfunction is the enzyme-linked immunosorbent assay determination of fecal pancreatic elastase. Canine fecal pancreatic elastase is a species- and pancreas-specific test with high sensitivity but relatively low specificity. A single fecal elastase concentration > 20 μg/g can be used to exclude EPI in dogs with chronic diarrhea. Values < 20 μg/g in association with typical clinical signs of EPI are suggestive of severe pancreatic dysfunction.[37-39] This test is not sufficiently sensitive to diagnose subclinical EPI and partial PAA.[40]

Accurate diagnosis may be made by assay of stimulated output of pancreatic enzymes in the lumen of the proximal small intestine, and this approach is considered the gold standard for the diagnosis of EPI in humans.[14] However, this relatively cumbersome approach is not necessary to demonstrate the severe loss of pancreatic enzymes typically seen in dogs with EPI. To avoid duodenal intubation, in vivo assay has been achieved in the dog by oral administration of the chymotrypsin substrate bentiromide (*N*-benzoyl-L-tyrosyl-p-aminobenzoic acid, BT-PABA) and subsequent assay of PABA in blood or urine: low levels of PABA indicate EPI.[41] This test was introduced in the 1970s and was considered an important advance in diagnosis of EPI for the first few years after its introduction, although its use remained largely restricted to university referral centers because it is relatively impractical for widespread application. However, some problems in interpretation emerged, because it was found that there could be overlap between results from dogs with EPI and those with small intestinal disease. This test is now really only of historical interest.

Diagnosis and Treatment of Subclinical EPI and Partial PAA

Wiberg et al[11] showed that it is possible to diagnose exocrine pancreatic dysfunction in the early phase, before severe destruction of acinar structure and typical clinical signs occur. In this study, which included dogs from different breeds, the prevalence of a subnormal but not diagnostic serum TLI concentration (2.5-5.0 μg/L) was 6% of all samples in which serum TLI was measured. To study whether a subnormal TLI value was a predictor of impending decline of pancreatic function, repeated determinations of serum TLI were made. This study demonstrated that a single TLI concentration in the range of the subnormal TLI has questionable value in evaluation of the long-term status of the exocrine pancreas, and normal concentrations were found on retesting in about 50% of the dogs with previous TLI concentrations in the range of 2.5 to 5.0 μg/L. The explanation for these transient subnormal TLI concentrations remains unknown. When first presented, most of these dogs had no typical clinical signs of EPI, but many had either sporadic or chronic gastrointestinal signs. On retesting, most of these dogs were clinically normal or only occasionally had gastrointestinal signs. The remaining 50% of dogs in this study displayed a repeatedly low serum TLI ($<$ 5.0 μg/L but usually above 2.5 μg/L), but still showed no clinical signs indicative of EPI. Pancreatic pathology was verified by laparotomy and pancreatic biopsy, revealing partial PAA in all dogs. The dogs were all either German Shepherd Dogs or Rough Collies. Despite the low fasting serum TLI values and the reduced pancreatic mass, the residual secretory capacity of the pancreas was apparently sufficient to prevent the appearance of clinical signs, thus a diagnosis of subclinical EPI (SEPI) was made. Some of these dogs with subclinical disease sometimes showed TLI values as low as those found in dogs already showing clinical signs (less than 2.5 μg/L). Presumably, this variation in the expression of clinical signs reflects variation between dogs in the extrapancreatic digestive capacity due to gastric and intestinal enzymes. Wiberg et al[11] concluded that repeatedly low serum TLI values ($<$ 5.0 μg/L) in clinically healthy dogs are a valuable marker of the subclinical EPI and highly suggestive of partial PAA. Because changes in serum TLI concentration in these dogs are so unpredictable, repeated determinations are needed to increase the accuracy of diagnosis, especially when subnormal serum cTLI values (2.5-5.0 μg/L) are found.

At present, cTLI measurement is the most valuable pancreatic function test for diagnosing subnormal pancreatic function in dogs. The diagnostic value of other tests, i.e., fecal proteolytic activity, canine fecal pancreatic elastase measurements, and BT-PABA, were found to be insufficiently sensitive to detect subclinical EPI, although the tests are good indicators of clinical EPI. With regard to fecal elastase measurement, subclinical dogs showed marked day-to-day fluctuations with overlap between subclinical and control dogs.[40]

To further evaluate the secretory capacity of exocrine pancreas, the TLI-stimulation test (TST) has been used.[11] The TST is performed as follows. After fasting overnight, serum samples for TLI assay are collected. Directly after, the pancreas is stimulated by intravenous administration of both secretin and cholecystokinin infused over 1 to 2 minutes, and a serum sample for the second TLI assay is collected 20 minutes after stimulation. Secretin stimulates secretion of pancreatic bicarbonate-rich fluid, which increases pressure in pancreatic ducts, and results in leakage of enzymes into the bloodstream. Cholecystokinin acts as a direct stimulant of enzyme secretion. In dogs with clinical EPI, the pancreas has no reserve capacity to respond to stimuli, but control dogs and dogs with SEPI show a significant response. However, the TST is not more valuable in diagnosing SEPI than repeated TLI measurements. In dogs with SEPI, the true clinical value of TST may be as a predictive test. By following TST results in an individual dog, it may be possible to predict more precisely gradual impairment of exocrine pancreatic function.

Verification of Underlying Pathological Process

Pancreatic morphological examination is performed when it is necessary to identify the underlying pathological process causing pancreatic dysfunction. In dogs with either partial or end-stage PAA, the clinical histories, clinical signs, and results of function tests are usually already highly suggestive of the underlying pathological process, and at least with breeds predisposed to PAA, morphological examination is rarely needed. Morphological examination can, however, be useful in some atypical cases. The disadvantage of laparotomy and laparoscopy is that they are invasive. Further, the morphological changes in the pancreas are usually unevenly distributed, thus the severity of findings is greatly dependent on the site of biopsy. Pancreatic biopsies can be taken without further complications by laparotomy to dissect a small pancreatic sample or by laparoscopy using crushing forceps. No complications have been observed even when repeated biopsies are taken from the same dog during follow-up of disease progression.[24,42]

Treatment and Prognosis of Subclinical EPI with Partial PAA

The ability to diagnose PAA in the subclinical phase raised the question of whether stopping the autoimmune-mediated destruction of acinar tissue is possible, thus preventing the appearance of clinical signs. A long-term follow-up study (from 1 to 6 years) was performed comparing 2 groups of dogs with SEPI due to partial PAA.[29] One group of 7 dogs received long-term immunosuppressive therapy with azathioprine and the other group of 13 dogs received no treatment. While receiving azathioprine, dogs had no clinical signs of EPI; however, 2 dogs showed signs some months after immunosuppressive treatment was withdrawn. The other dogs in the group remained clinically healthy the whole follow-up time. In the untreated group, 5 dogs developed clinical disease during follow-up, whereas others remained in the subclinical phase. It was concluded that dogs may stay in the subclinical phase of EPI for years or sometimes for life, with or without immunosuppressive treatment, and that the rate of this natural progression to the clinical phase can vary markedly. No markers that predict which dogs will go on to develop clinical disease have been identified. The value of immunosuppressive treatment in slowing acinar atrophy is therefore questionable.[29] Further studies are needed to identify what factors are involved in initiating clinical disease.

When dogs with partial PAA show no gastrointestinal signs (SEPI), no treatment is needed. However, some dogs with partial PAA do show chronic or intermittent gastrointestinal signs not typical of EPI. A diagnostic dilemma is whether the signs are due to subnormal pancreatic function and presumed autoimmune pancreatitis, or due to concurrent small intestinal disease, or a combination of both. For these dogs, a diagnostic workup should be done first, followed by treatment for concurrent small intestinal disease. If there is no response, a trial treatment with enzymes should be initiated.

Treatment of Clinical EPI and End-stage PAA

Replacement therapy with enzyme supplements is needed to compensate for the lack of enzyme production in dogs with EPI.[43]

Around the turn of the 20th century, missing enzymes were for the first time shown to be successfully replaced by oral administration, thus resolving the signs resulting from their absence, and preventing the ultimate starvation of affected patients. Some pancreatic digestive enzymes, most notably pancreatic lipase, are very sensitive to acid pH, and so passage through the stomach in such a way that they do not lose significant activity is a challenge. Both amylase and lipase are destroyed very rapidly at a pH below 3.5. Trypsin is very tolerant of acid pH, and thus remains unaffected by normal conditions of the stomach. In humans only 17% of lipase ingested with a meal can be

recovered intact from the duodenum.[44] To improve passage of functional pancreatic enzymes through the stomach, many companies have developed different kinds of enteric-coated pancreatic enzyme preparations. Given the fragility of pancreatic enzymes, the manufacturing process must be gentle, and many factors must be considered to ensure that the enzymes are not destroyed. Although much is already known about pancreatic enzyme supplementation, modern replacement therapy must be improved. Despite adequate enzyme supplementation, digestion capacity does not return to normal in either human beings or dogs with EPI. Only a small portion of the orally administered enzymes are delivered functionally intact to the small intestine.[43] For maximum digestive efficiency, pancreatic enzyme preparations should be formulated 1) to protect acid-labile enzymes from gastric inactivation, 2) to provide concomitant gastric emptying of the enzymes with the meal, and 3) to deliver maximal enzyme activity to the proximal duodenum. To fulfill these criteria, formulation of a sustained-release preparation of pancreatic extracts that releases enzymes over a prolonged period to a site favorable for their function is underway. Unfortunately, this preparation is not yet commercially available, and preparations formulated for human beings may not work similarly in dogs.[45]

Several studies have been published concerning the gastric emptying rate of nondisintegrating dosage forms of enzymes. Earlier studies have stated that particles reduced to a size of 2 mm or less are emptied from the full stomach of dogs as rapidly and randomly as liquids.[46] More recently, however, in normal dogs and a dog with EPI, most multiple-unit preparations (diameter 1-1.7 mm) have been shown to remain in the stomach of dogs for up to 6 to 8 hours before being swept into the duodenum by the intestinal "housekeeper wave."[47] Most pancreatic enzymes prepared in granule form obviously would never be emptied from the stomach simultaneously with food. Even a reduction of granule size to 0.3 mm had no clear effect on the gastric-emptying time of these preparations. It can thus be concluded that even smaller granules than the present ones are needed to optimize the gastric emptying of multiple-unit preparations in pancreatic therapy. However, such developments would aggravate the existing problems in manufacture associated with the granulation and coating of particles.[45] In humans with chronic pancreatitis or cystic fibrosis, encapsulated enteric-coated microspheres and minimicrospheres (diameter < 1.7 mm) are considered the enzyme treatment of choice. However, full reversal of EPI with enzyme replacement therapy cannot be obtained in all patients.[48]

To study the effect of different enzyme preparations in the treatment of dogs with EPI, 2 dogs had the cranial part of their jejunum cannulated.[49] Their food was supplemented with commercial enzyme preparations in turn as follows: powder, granules, capsules, enteric-coated tablets, and finely chopped raw pig pancreas. Jejunal samples were withdrawn through the cannula at 0.5-hour intervals for 6 hours after feeding. The activities of proteases, amylase, and lipase were determined in jejunal samples and in feces. The control subjects comprised 14 healthy dogs and 1 subclinical EPI dog. This dog had about 90% of the pancreas atrophied, but showed no symptoms of poor digestion. The jejunal enzymatic activity of this dog represented the minimal activity with which a dog would normally manage. The highest lipase activities in the jejunal samples were achieved with raw pig pancreas. Powder achieved the second highest activities, but the other commercial porcine enzyme preparations yielded activities that were only one tenth of those attained with the raw pancreas. With the raw pancreas, the jejunal lipase activities were not as high as those in control dogs but exceeded those in the subclinical EPI dog. Raw pancreas and commercial enzyme preparations increased the activities of proteases and amylase well beyond those found in the jejunum of the subclinical EPI dog. With the commercial powder preparation and with the raw pancreas, jejunal enzyme activity was detected immediately after feeding. Capsules and granules delayed the appearance of jejunal enzymatic activity by 1 to 2 hours and enteric-coated tablets by 5 hours. Measuring enzymatic activity in feces was not reliable for evaluating the potency of pancreatic enzyme preparations added to food. Lipase activity was seldom found, and amylase and protease activities showed substantial variation.

The effect of the 2 uncoated enzyme supplements, raw chopped pancreas, and porcine pancreatic powder preparation were compared in a long-term clinical study.[50] The study included 76 dogs with EPI, and powdered enzymes were given to 40 dogs and raw chopped pancreas to 36 dogs. When comparing the prevalences of 11 gastrointestinal tract signs classified as typical of EPI, no significant difference was observed between the 2 groups of dogs. The study showed that in practice, selection between these supplements is usually based on economics and practicality. The raw chopped pancreas was only one fourth as expensive, but practical difficulties, mainly in availability, handling, and storing, were considerable, compared with powdered enzyme supplements. The most common source of raw chopped pancreas was from pigs (72%), followed by cattle (19%) and sheep or reindeer (9%). The mean amount of raw chopped pancreas was 87 g/meal. Raw pancreas can be stored frozen for several months before feeding. All dogs given powdered enzyme were given the same product (Viokase V; Fort Dodge Laboratories, Fort Doge, IA), and the mean amount was 3.0 g/meal. No significant correlation was found between body weight and amount of enzyme fed.

Because raw chopped pancreas is not available in many countries, powder enzyme supplementation is the most common treatment regime for dogs with EPI.

Widely accepted recommendations in the literature are that treatment in dogs with EPI be initiated at a dose of 2 teaspoons of powdered pancreatic extract per 20 kg body weight at each meal.[51] Enzymes should be mixed with food immediately before the meal is fed. Owners are able to decrease the dose of pancreatic enzymes based on their pet's response. Most dogs require at least 1 teaspoon of enzymes per meal. Little additional improvement was observed after doubling or quadrupling this dose in EPI dogs with ligated pancreatic ducts.[52] Side effects of porcine pancreatic extracts are rare. It has, however, been reported that high doses of pancreatic enzyme supplements can cause oral bleeding in dogs with EPI, but that oral bleeding can be successfully managed by dose reduction in most dogs.[53]

Numerous attempts have been made to increase the effectiveness of enzyme supplementation in dogs. Antacids or H2-receptor blockers have been recommended in the therapeutic regimen to reduce gastric acid–induced destruction of orally administered enzymes. This practice is, however, costly and does not increase efficacy of pancreatic enzyme supplementation.[51,54] In humans, a double-blind, placebo-controlled crossover study was conducted to measure the effect of acid suppressant therapy (ranitidine or omeprazole) on fat absorption in patients with cystic fibrosis. No overall significant improvement in fat absorption could be demonstrated with adjuvant therapy.[55] Concurrent oral administration of bile salts and pre-incubation of the meal with pancreatic enzymes for 20 to 30 minutes before feeding did not improve the response.[56]

Aside from porcine pancreatic extracts, bacterial lipase has been reported to be effective in correcting steatorrhea in dogs with experimental EPI.[57] Bacterial lipase is secreted by *Burkholderia plantarii* during fermentation. It is resistant to acid denaturation and protease digestion and does not require colipase for lipolytic activity. Bacterial lipase maintains activity also in the presence of bile acids. The effects of bacterial lipase were compared with those of a powered porcine pancreatic enzyme preparation (Viokase powder; A. H. Robins Company, Richmond, VA) in alleviating steatorrhea in dogs with EPI. The results showed that correcting steatorrhea required 75 times more porcine lipase than bacterial lipase by weight (18 vs. 240 mg). Improved fat absorption with use of bacterial lipase does not, however, improve absorption of the other nutrients, and thus proteases and

amylase are necessary to correct protein and carbohydrate malabsorption, if present. The study[57] further showed that in using bacterial or porcine lipase to treat dogs with experimental EPI a high-fat and high-protein diet improved fat absorption more efficiently than when the dogs were fed a low-fat, low-protein diet. Enzyme preparations from plants and fungi are also available and it is sometimes stated that they are as effective as ordinary pancreatic extracts; these preparations contain lipases extracted from molds and the protease papain from the tropical papaya tree. Nevertheless, preparations obtained from an animal (porcine) pancreas are demonstrated to be the ideal replacement enzymes and can never be replaced by vegetable ferments.[58] An experimental study in dogs has, however, shown that fungal lipase may prove to be useful in treating dogs with EPI.[59]

Supportive Treatments

Aside from dietary enzyme supplements, dietary changes are often made to improve clinical response (cross reference to Alex German's chapter), but sometimes weight gain and stool quality remain suboptimal.[60-62] Other medications for treatment of gastrointestinal tract signs are often used in such dogs with EPI. Antibiotics are the most common adjunctive medication. Of the antibiotics administered, tylosin is used in Finland almost exclusively.[63-67]

Clinical Pharmacology

Tylosin is a macrolide, bacteriostatic antibiotic that has activity against most gram-positive and gram-negative cocci, gram-positive rods, and *Mycoplasma*. However, the gram-negative bacteria *Escherichia coli* and *Salmonella* spp. are intrinsically tylosin resistant. Tylosin is used only in veterinary medicine, and the most common indications are for treating pigs with diarrhea or poultry with chronic respiratory diseases. Tylosin has also been used as a feed additive in food animal production, and it has been shown to increase weight gain and feed efficiency, especially in pigs. There is debate into the mechanisms underlying tylosin-mediated growth enhancement.

Tylosin is used in a powder form for pigs and poultry. For small animals tylosin powder is usually reconstituted in capsules or mixed with the food for administration. In some European countries, tylosin is also available in tablet form, which facilitates its use in dogs.

Therapeutic Use

Our experience with tylosin was first derived from studies in dogs having EPI and continued diarrhea after appropriate enzyme therapy.[63] These studies found that tylosin had a favorable effect as an adjunctive therapy for dogs with EPI and diarrhea. Although most of the studies below involved dogs without EPI, the clinical responses are similar in dogs with EPI and we believe that the underlying mechanisms for tylosin-responsive diarrhea (TRD) are probably similar.[64-67]

Recently in Finland, tylosin has become the most frequent antibiotic used in the treatment of idiopathic intermittent or chronic diarrhea in dogs. Anecdotal reports from veterinarians and dog owners reveal that many dogs with diarrhea respond quickly to tylosin treatment, generally within a few days of initiation of therapy. When tylosin is discontinued, the diarrhea often reappears within a matter of weeks or months. Some of these dogs require ongoing treatment for very long periods. It was also observed that tylosin's effect in controlling diarrhea signs does not appear to diminish with time and the need for an increased dosage is not required. No apparent tylosin-associated adverse effects have been reported.

Our clinical experience indicates that TRD affects dogs of all breeds and ages, but is most often observed in middle-aged, large-breed dogs. Signs often begin as intermittent diarrhea that becomes progressively more frequent and often persistent. Abnormal, loose fecal consistency is the predominant sign. Most of the owners describe their dogs' feces as having a watery or mucoid consistency, suggesting that TRD involves both small- and large-bowel mechanisms. Increased frequency of borborygmus and flatulence typically are also described, and vomiting is reported occasionally during the diarrheal outbreaks.

Diagnostic evaluation of TRD cases has failed to identify an underlying etiology. Routine blood parameters, fecal examinations, and diagnostic imaging studies are usually normal. When intestinal biopsies are obtained, the histologic findings are considered either to be normal or to have only mild inflammatory changes.

Clinical Studies Using Tylosin for Chronic Diarrhea

Only a few studies treating diarrhea in dogs with tylosin have been published. In 1976, Van Kruiningen reported that tylosin was effective in the treatment of unspecific canine diarrhea.[68] Recently, our study group performed 3 prospective clinical trials to obtain more information on TRD.[64-66] The first study[64] consisted of 14 adult client–owned dogs of 12 different breeds. Each dog's diet remained unchanged throughout the study. All dogs had chronic or intermittent diarrheal signs for a period of longer than 1 year. All dogs previously had been treated successfully with tylosin for at least 6 months. Tylosin had been discontinued at least twice, but the diarrhea had always recurred. These dogs were then considered to have TRD. When the study commenced, all dogs had been on tylosin for at least 1 month and were healthy and free of diarrhea. Tylosin was then discontinued, and the dogs were monitored for a period of up to 1 month to determine whether signs of diarrhea would reappear. Diarrhea reappeared in 12 of 14 dogs (85.7%) within 30 days. Tylosin, prednisone, or a probiotic treatment trial was initiated when diarrhea was present. Tylosin resulted in resolution of diarrhea in all dogs within 3 days of initiation of therapy, with most resolving within 24 hours. In contrast, prednisone did not completely resolve diarrheal signs, and the probiotic *Lactobacillus rhamnosus* GG did not prevent the relapse of diarrhea in any of the dogs.

In a second study,[65] 7 beagles with chronic diarrhea for a duration of at least 1 month were identified from an experimental dog colony. Treatment trials of antibiotics, prednisone, and diet were used. Tylosin was administered for 10 days, and during that time the feces became significantly firmer, although they remained unacceptably loose. When the treatment was discontinued, diarrhea reappeared within 3 weeks. Next, treatment trials with other antibiotics (metronidazole, trimethoprim-sulfadiazine, or doxycycline) and prednisone had almost no effect on fecal consistency in these dogs. The diet was then changed for a 10-day period from a highly digestible, moist pet food to a dry food developed for normal adult dogs. The feces again became significantly firmer, although the fecal consistency remained loose in some dogs. The dry food feeding period was then extended to 3 months, and the fecal consistency continued to fluctuate from ideal to diarrhea. Because the consistency was not satisfactory on diet alone, tylosin was added to the therapy for 10 days. The fecal consistency became normal and remained so throughout the 3-month follow-up time of this study. The study demonstrated that, in this group of experimental dogs having chronic diarrhea, the fecal consistency became significantly firmer with tylosin in conjunction with dietary modification. Neither treatment alone was sufficient to obtain ideal fecal consistency, but when the dogs were treated simultaneously with both regimens, ideal fecal consistency was achieved and maintained. The study suggests that tylosin and feeding regimens had a synergic effect. In our latest placebo-controlled, randomized, double-blinded, prospective clinical trial[67] on dogs with suspected TRD, we aimed to assess the effect of tylosin on fecal consistency compared with a placebo treatment and also to establish whether tylosin in dogs with recurrent diarrhea is as effective as empiric studies and anec-

dotal reports suggest. The proportion of dogs with normal fecal consistency at the end of the 7-days-long treatment period was 85% in the tylosin group and 29% in the placebo group. Our findings indicate that tylosin is significantly more effective than placebo in treating recurrent diarrhea in dogs, and it remains our antibiotic of choice for EPI patients with persistent or recurrent diarrhea after enzyme supplementation. In the placebo-controlled study we used the tylosin dosage 25 mg/kg every 24 hours, but the results of a recent study (unpublished) indicate that a suitable dose could be as low as 5 mg/kg once daily.

Other Supportive Treatments

Cobalamin (vitamin B12) deficiency in dogs with EPI is probably largely a result of increased uptake of cobalamin by the intestinal bacteria and perhaps partly to reduce secretion of pancreatic intrinsic factor, which is required for normal absorption of cobalamin. Enzyme treatment alone does not lead to increasing serum cobalamin levels.[69-71] Because cobalamin deficiency is common in canine EPI, serum cobalamin should be measured in dogs that are clinically suspected of having EPI or that do not respond satisfactorily to enzyme treatment, and probably every 1 to 2 years after diagnosis. Once subnormal serum concentrations are observed, cobalamin should be administered by subcutaneous injection and the dose currently recommended is 250 to 1000 μg depending on the size of the dog, weekly initially and then monthly.[72]

Although malabsorption of fat-soluble vitamins may be expected with EPI, the clinical importance of vitamin A, D, E, and K deficiency in this syndrome has not been reported. When the treatment response to enzymes and supportive therapies is still unsatisfactory, concomitant small intestinal disease should be suspected, and further diagnostic studies and treatment should be performed.[8]

References

1. Bernard C: Memoir on the Pancreas, Monographs of the Physiological Society No. 42. London, UK, Academic Press, 1985
2. Kuhlmann H: The magic potions in the belly gland, in Rudmann MA (ed). The magic potions in the belly gland. Hannover, Germany, Solvay Pharmaceutical, GmbH, 2000
3. Thordal-Christensen AA, Coffin DL: Pancreatic disease in the dog. *Nord Vet Med* **8:**89–114, 1956
4. Holroyd JB: Canine exocrine pancreatic disease. *J Small Anim Pract* **9:**269–281, 1968
5. Freudiger U: Diseases of exocrine pancreas in dogs. *Kleintierpraxis* **16:**201–228, 1971
6. Dimagno EP, Go VL, Summerskill WH: Relations between pancreatic enzyme outputs and malabsorption in severe pancreatic insufficiency. *N Engl J Med* **288:**813–815, 1973
7. Rimaila-Pärnänen E, Westermarck E: Pancreatic degenerative atrophy and chronic pancreatitis in dogs. A comparative study of 60 cases. *Acta Vet Scand* **23:**400–406, 1982
8. Williams DA: Exocrine pancreatic disease, in Ettinger SJ, Feldman EC (eds): Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, 5th ed. Philadelphia, PA, W. B. Saunders, pp 1345–1367, 2000
9. Westermarck E: The hereditary nature of canine pancreatic degenerative atrophy in the German shepherd dog. *Acta Vet Scand* **21:**389–394, 1980
10. Westermarck E, Pamilo P, Wiberg M: Pancreatic degenerative atrophy in the Collie breed: a hereditary disease. *Zentralbl Veterinarmed A* **36:**549–554, 1989
11. Wiberg ME, Nurmi AK, Westermarck E: Serum trypsinlike immunoreactivity measurement for the diagnosis of subclinical exocrine pancreatic insufficiency. *J Vet Intern Med* **13:**426–432, 1999
12. Batchelor DJ, Noble PJ, Cripps PJ, et al: Breed associations for canine exocrine pancreatic insufficiency. *J Vet Intern Med* **21:**207–214, 2007
13. Proschowsky HF, Fredholm M: Exocrine pancreatic insufficiency in the Eurasian dog breed—inheritance and exclusion of two candidate genes. *Anim Genet* **38:**171–173, 2007
14. Säteri H: Investigations on the exocrine pancreatic function in dogs suffering from chronic exocrine pancreatic insufficiency. *Acta Vet Scand Suppl* **53:**1–86, 1975
15. Durie PR: Inherited causes of exocrine pancreatic dysfunction. *Can J Gastroenterol* **11:**145–152, 1997
16. Washabau RJ, Callan MB, Williams DA: Cholecystokinin secretion is preserved in canine pancreatic insufficiency [abstract]. *J Vet Intern Med* **9:**193, 1995
17. Boari A, Williams DA, Famigli-Bergamini P: Observations on exocrine pancreatic insufficiency in a family of English Setter dogs. *J Small Anim Pract* **35:**247–250, 1994
18. Boari A, Williams DA, Bergamini PF: Diagnosis and management of concurrent exocrine pancreatic insufficiency (EPI) and diabetes mellitus (DM) in a young Rottweiler dog. *Eur J Comp Gastroenterol* **1:**29–31, 1997
19. Neiger R, Jaunin VB, Boujon CE: Exocrine pancreatic insufficiency combined with insulin-dependent diabetes mellitus in a juvenile German Shepherd Dog. *J Small Anim Pract* **37:**344–349, 1996
20. Westermarck E, Batt RM, Vaillant C, Wiberg M: Sequential study of pancreatic structure and function during development of pancreatic acinar atrophy in a German Shepherd Dog. *Am J Vet Res* **54:**1088–1094, 1993
21. Räihä M, Westermarck E: The signs of pancreatic degenerative atrophy in dogs and the role of external factors in the etiology of the disease. *Acta Vet Scand* **30:**447–452, 1989
22. Weber W, Freudiger U: Erbanalytische Untersuchungen uber die chronische exocrine Pancreasinsuffizienz beim Deutschen Schäferhund. *Schweiz Arch Tierheilk* **119:**257–263, 1977
23. Moeller EM, Steiner JM, Clark LA, et al: Inheritance of pancreatic acinar atrophy in German shepherd dogs. *Am J Vet Res* **63:**1429–1434, 2002
24. Westermarck E, Saari SA, Wiberg ME: Heritability of exocrine pancreatic insufficiency in German shepherd dogs. *J Vet Intern Med* **24:**450–452, 2010
25. Wiberg ME, Saari SA, Westermarck E: Exocrine pancreatic atrophy in German Shepherd Dogs and Rough-coated Collies: an end-result of lymphocytic pancreatitis. *Vet Pathol* **36:**530–541, 1999
26. Wiberg ME, Saari SA, Westermarck E, Meri S: Cellular and humoral immune responses in atrophic lymphocytic pancreatitis in German shepherd dogs and rough-coated collies. *Vet Immunol Immunopathol* **76:**103–115, 2000
27. Hill FW, Osborne AD, Kidder DE: Pancreatic degenerative atrophy in dogs. *J Comp Pathol* **81:**321–330, 1971
28. Simpson KW, Cobb MA: Investigation of the relationship of circulating anti-pancreatic antibodies to exocrine pancreatic insufficiency in dogs. *Eur J Comp Gastroenterol* **3:**37–42, 1998
29. Wiberg ME, Westermarck E: Subclinical exocrine pancreatic insufficiency in dogs. *J Am Vet Med Assoc* **220:**1183–1187, 2002
30. Janeway CA, Travers P, Walport M, et al: Janeway CA, et al (eds): Immunobiology, 4th ed. London, Elsevier Science, pp 262-303, 489-509, 1990
31. Watson PJ: Exocrine pancreatic insufficiency as an end stage of pancreatitis in four dogs. *J Small Anim Pract* **44:**306–312, 2003
32. Newman SJ, Steiner JM, Woosley K, Williams DA, Barton L: Histologic assessment and grading of the exocrine pancreas in the dog. *J Vet Diagn Invest* **18:**115–118, 2006
33. Williams DA, Batt RM: Sensitivity and specificity of radioimmunoassay of serum trypsin-like immunoreactivity for the diagnosis of canine exocrine pancreatic insufficiency. *J Am Vet Med Assoc* **192:**195–200, 1988
34. Hill FW, Kidder DE: The estimation of daily faecal trypsin levels in dogs as an indicator of gross pancreatic exocrine insufficiency. *J Small Anim Pract* **11:**191–195, 1970
35. Westermarck E, Sandholm M: Faecal hydrolase activity as determined by radial enzyme diffusion: A new method for detecting pancreatic dysfunction in the dog. *Res Vet Sci* **28:**341–346, 1980
36. Ruaux CG, Steiner JM, Williams DA: Protein-losing enteropathy in dogs is associated with decreased fecal proteolytic activity. *Vet Clin Pathol* **33:**20–22, 2004
37. Spillmann T, Wiberg ME, Teigelkamp S, et al: Canine faecal pancreatic elastase (CE 1) in dogs with clinical exocrine pancreatic insufficiency, normal dogs and dogs with chronic enteropathies. *Eur J Comp Gastroenterol* **2:**5–10, 2001
38. Spillmann T, Wittker A, Teigelkamp S, et al: An immunoassay for canine pancreatic elastase 1 as an indicator for exocrine pancreatic insufficiency in dogs. *J Vet Diagn Invest* **13:**468–474, 2001
39. Battersby IA, Peters IR, Day MJ, German AJ, Hall EJ: Effect of intestinal inflammation on fecal elastase concentration in dogs. *Vet Clin Pathol* **34:**49–51, 2005
40. Wiberg ME, Westermarck E, Spillmann T, et al: Canine faecal pancreatic elastase (CE 1) for the diagnosis of subclinical exocrine pancreatic insufficiency in dogs. *Eur J Comp Gastroenterol* **2:**21–25, 2001
41. Freudiger U, Bigler B: The diagnosis of chronic exocrine pancreatic insufficiency by the PABA test. *Klintierprax* **22:**73–79, 1977
42. Harmoinen J, Saari S, Rinkinen M, Westermarck E: Evaluation of pancreatic forceps biopsy by laparoscopy in healthy beagles. *Vet Ther* **3:**31–36, 2002
43. Wiberg ME: Pancreatic acinar atrophy in German shepherd dogs and rough-coated collies. Etiopathogenesis, diagnosis and treatment. A review. *Vet Q* **26:**61–75, 2004
44. Dimagno EP, Layer P, Clain JE: Chronic pancreatitis, in Go VLW, DiMagno EP, Gardner JD (eds): The Pancreas: Biology, Pathobiology and Disease. New York, NY, Raven, pp 665–706, 1993
45. Heinämäki J: Formulation and radiological imaging of oral solid drug products intended for the treatment of exocrine pancreatic insufficiency in dogs. Thesis. Helsinki, Finland, Yliopistopainos, 1991
46. Hinder RA, Kelly KA: Canine gastric emptying of solids and liquids. *Am J Physiol* **233:**335–340, 1977
47. Heinämäki J, Marvola M, Happonen I, et al: The fate of multiple-unit enteric-coated formulations in the stomach of the dog. *Int J Pharm* **42:**105–115, 1988
48. Trifan A, Balan G, Stanciu C: Pancreatic enzymes replacement therapy in chronic pancreatitis: an update. *Rev Med Chir Soc Med Nat Iasi* **105:**646–650, 2001
49. Westermarck E: Treatment of pancreatic degenerative atrophy with raw pancreas homogenate and various enzyme preparations. *Zentralbl Veterinarmed A* **34:**728–733, 1987
50. Wiberg ME, Lautala HM, Westermarck E: Response to long-term enzyme replacement treatment in dogs with exocrine pancreatic insufficiency. *J Am Vet Med Assoc* **213:**86–90, 1998

51. Williams DA: Exocrine pancreatic disease, in Ettinger SJ, Feldman EC (eds): Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, 5th ed. Philadelphia, PA, W. B. Saunders, pp 1345–1367, 2000
52. Pidgeon G, Strombeck DR: Evaluation of treatment for pancreatic exocrine insufficiency in dogs with ligated pancreatic ducts. *Am J Vet Res* **43:**461–464, 1982
53. Rutz GM, Steiner JM, Williams DA: Oral bleeding associated with pancreatic enzyme supplementation in three dogs with exocrine pancreatic insufficiency. *J Am Vet Med Assoc* **221:**1716–1718, 2002
54. Pidgeon G: Malassimilation syndrome: maldigestion/malabsorption, in Kirk RW (ed): Current Veterinary Therapy; II. Philadelphia, PA, W. B. Saunders, pp 930–935, 1980
55. Francisco MP, Wagner MH, Sherman JM, Theriaque D, Bowser E, Novak DA: Ranitidine and omeprazole as adjuvant therapy to pancrelipase to improve fat absorption in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* **35:**79–83, 2002
56. Williams DA: The pancreas, in Guilford WG, Center SA, Strombeck DR, Williams Meyer DJ (eds): Strombeck's Small Animal Gastroenterology, Philadelphia, PA, W. B. Saunders, pp 400–401, 1997
57. Suzuki A, Mizumoto A, Rerknimitr R, Sarr MG, DiMango EP: Effect of bacterial or porcine lipase with low- or high-fat diets on nutrient absorption in pancreatic-insufficient dogs. *Gastroenterology* **116:**431–437, 1999
58. Layer P, Keller J: Lipase supplementation therapy: standards, alternatives, and perspectives. *Pancreas* **26:**1–7, 2003
59. Griffin SM, Alderson D, Farndon JR: Acid resistant lipase as replacement therapy in chronic pancreatic insufficiency: a study in dogs. *Gut* **30:**1012–1015, 1989
60. Westermarck E, Wiberg M, Junttila J: Role of feeding in the treatment of dogs with pancreatic degenerative atrophy. *Acta Vet Scand* **31:**325–331, 1990
61. Westermarck E, Junttila JT, Wiberg ME: Role of low dietary fat in the treatment of dogs with exocrine pancreatic insufficiency. *Am J Vet Res* **56:**600–605, 1995
62. Westermarck E, Wiberg M: Effects of diet on clinical signs of exocrine pancreatic insufficiency in dogs. *J Am Vet Med Assoc* **228:**225–229, 2006
63. Westermarck E, Myllys V, Aho M: Effect of treatment on the jejunal and colonic bacterial flora of dogs with exocrine pancreatic insufficiency. *Pancreas* **8:**559–562, 1993
64. Westermarck E, Skrzypczak T, Harmoinen J, et al: Tylosin-responsive chronic diarrhea in dogs. *J Vet Intern Med* **19:**177–186, 2005
65. Westermarck E, Frias R, Skrzypczak T: Effect of diet and tylosin on chronic diarrhea in beagles. *Intern Med* **19:**822–827, 2005
66. Kilpinen S, Spillmann T, Syrja P: Effect of tylosin on dogs with suspected tylosin-responsive diarrhea: a placebo-controlled, randomized, double-blinded, prospective clinical trial. *Acta Vet Scand* **53:**26, 2011
67. Westermarck E: Tylosin-responsive diarrhea, in Bonagura JD, Twedt DC, Kirk RW (eds): Kirk's Current Veterinary Therapy, 14th ed. St. Louis, MO, Saunders Elsevier; 2009, p 1388
68. Van Kruiningen HJ: Clinical efficacy of tylosin in canine inflammatory bowel disease. *J Am Anim Hosp Assoc* **12:**498–501, 1976
69. Batt RM, Morgan JO: Role of serum folate and vitamin B_{12} concentrations in the differentiation of small intestinal abnormalities in the dog. *Res Vet Sci* **32:**17–22, 1982
70. Batt RM, Horadagoda NU, McLean L, Morton DB, Simpson KW: Identification and characterization of a pancreatic intrinsic factor in the dog. *Am J Physiol* **256:**517–523, 1989
71. Simpson KW, Morton DB, Batt RM: Effect of exocrine pancreatic insufficiency on cobalamin absorption in dogs. *Am J Vet Res* **50:**1233–1236, 1989
72. Ruaux CG: Cobalamin and gastrointestinal disease. Proceedings of the 20th ACVIM Congress, Dallas, TX, 2002