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Exocrine Pancreatic Insufficiency

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Abbreviations

EPI: Exocrine pancreatic insufficiency

PAA: Pancreatic acinar atrophy

DM: Diabetes mellitus

cTLI: canine serum trypsin-like immunoreactivity

RIA: Radioimmunoassay

RIT: Response to initial treatment

MST: Median survival time

SIBO: Small Intestinal Bacterial Overgrowth

BT-PABA: n-benzoyl-L-tyrosine-p-aminobenzoic acid

SST: Soybean Stimulation Test

TST: TLI-stimulation test

RED: Radial Enzyme Diffusion

SEPI: Subclinical Exocrine Pancreatic Insufficiency

DM: Diabetes Mellitus

DLA: Dog Leukocyte Antigen

GWA: Genome-wide association

SNP: Single Nucleotide Polymorphism

LDC: Lowest Detectable Concentration

CV: Coefficients of Variation

OST: Overall Survival Time

RER: Rough endoplasmic reticulum

Introduction

Exocrine pancreatic insufficiency (EPI) is a disorder where the pancreas is not able to secrete enough pancreatic enzymes. The most common cause for the maldigestion signs of EPI is pancreatic acinar atrophy (PAA) (Westermarck & Wiberg, 2012).
The first breakthrough regarding the pancreas was made in 1856 by Claude Bernard. He discovered that fatty stools could be a symptom of pancreatic disease as the pancreatic juice is essential to absorb fat. In 1895, Alexander Fles figured out that ingesting raw calf pancreas with each meal could treat EPI on humans (Westermarck & Wiberg, 2012).

Although PAA is believed to be the most common cause of EPI in dogs, end-stage chronic pancreatitis is also an important cause. Isolated enzyme deficiency, in particular lipase, pancreatic tumors and hyperacidity of the duodenum inactivating lipase can also cause EPI although rare (Nelson and Couto, 2019). EPI may also be a consequence of autoimmune or fibrosis destruction and end-stage pancreatic inflammation (Mansfield, 2015).

Previously, EPI in cats has been considered rare, but after the start of using serum feline trypsin-like immunoreactivity (fTLI) concentration as a test for EPI the diagnosis has been more frequent (Xenoulis et al., 2016). It is being increasingly recognized (Mansfield, 2015). Typically middle-aged domestic short-haired cats are most predisposed for EPI, but it can also occur in other breeds and ages (Nelson and Couto, 2019).

There is a breed predisposition in dogs. German Shepherd, Rough Collies and Chow Chows are overly represented with EPI caused by PAA. Usually middle-aged to older, small- or medium-breed dogs like Collies, English Cocker Spaniels and Cavalier King Charles have a higher possibility of getting EPI as a result of end-stage chronic pancreatitis (Nelson and Couto, 2019).

The diagnosis of EPI is typically based on clinical signs and history of the patient and confirmed with pancreatic function tests (Westermarck & Wiberg, 2012). The measurement of serum trypsin-like immunoreactivity is pancreas- and species-specific, this is why in the latest years it has become the most commonly used test of pancreatic function which can be used to diagnose canine EPI (Westermarck & Wiberg, 2012). Lipase is primarily from the pancreas,

that is why weight loss and fat maldigestion with fatty feces (steatorrhea) are the most common clinical signs of EPI (Nelson and Couto, 2019).

Enzyme replacement therapy is suggested when clinical signs of maldigestion caused by EPI appear. Powdered enzymes or raw chopped pancreas as nonenteric-coated supplements gives the highest enzyme activity in the duodenum in dogs. The most common adjunctive medication are antibiotics (Westermarck & Wiberg, 2012).

Goals

Goals for this thesis is a literature review of exocrine pancreatic insufficiency and an evaluation of different study cases on the topic. Comparing clinical signs, diagnostic tools, breed predisposition and treatment methods to establish the optimal diagnostic and treatment methods based on large number cases.

Literature review

Exocrine pancreatic insufficiency

Exocrine pancreatic insufficiency is a disease which appears when the pancreas fails to secrete sufficient amount of digestive enzymes (Clark & Cox, 2012). Inadequate production of digestive enzymes can be caused by chronic diseases diminishing the exocrine pancreatic function (Westermarck & Wiberg, 2012). This causes clinical signs that are characteristic for EPI like weight loss, polyphagia and increased fecal volume with loose, poorly digested feces (Batchelor, Noble, Cripps, et al., 2007).

In dogs the most common underlying cause for EPI results from selective loss of the digestive enzyme-producing acinar cells of the pancreas, although there are several underlying causes (Clark & Cox, 2012). Pancreatic acinar atrophy (PAA) is an autoimmune disorder, most likely. Because lymphocytic infiltration of the pancreatic acinar tissue with active tissue destruction occurs concurrently, it is diagnosed through histologic examination of the pancreas (Clark & Cox, 2012). PAA is theorized to progress through these 2 stages: 1- when there is active destruction of acinar tissue, lymphocytic pancreatitis, and 2- when adipose tissue, ductal structures and atypical parenchyma replace acinar tissue, end-stage EPI (Moeller et al., 2002).

Studies show that because of this PAA in German Shepherd Dogs may be caused by a gene inherited in an autosomal recessive fashion which represent an autoimmune disorder (Moeller et al., 2002). PAA has a complex mode of inheritance and is responsible for the hereditary forms of EPI (Westermarck et al., 2010). German Shepherd Dogs and Rough Coated Collies are known to be predisposed to PAA (Batchelor, Noble, Cripps, et al., 2007). While EPI has been found to affect many breeds of dog (Batchelor et al., 2007b), EPI attributed to PAA has a documented higher prevalence in the German Shepherd Dog (GSD) population (Tsai et al., 2013) as a result GSDs comprise two thirds of cases of EPI due to pancreatic acinar atrophy in dogs (Hall et al., 2003). Recent studies have identified associations between four-locus dog leukocyte antigen (DLA) haplotypes and EPI in German shepherd dogs (Tsai et al., 2013). The canine major histocompatibility complex, which is a superlocus of genes involved in immune function, includes the DLA locus. A risk haplotype has been identified and it contains a DLA-DQB1 allele which is not found in other haplotypes. In at least 15 breeds, including several reported to have EPI, have been reported with this allele. As Pembroke Welsh Corgis have a

young average age of onset, as in German Shepherd dogs, this may imply a similar pathogenesis (Evans et al., 2015).
Several single nucleotide polymorphisms (SNP) associated with EPI were proximal to the DLA, this is why current genetic studies are focused on chromosome. The DLA contain several genes important for immune regulation and recognition, several associations with alleles of these genes have been identified for other autoimmune diseases in canines. The involvement of the DLA is proven further by the overexpression of DLA-88 (Clark & Cox, 2012).
In German Shepherd dogs, DLA class I and II haplotypes and alleles have been identified to influence the development of PAA. Studies indicate that loci on multiple chromosomes are associated with EPI. However, more studies have to be done for the identification of other genetic factors (Tsai et al., 2013). In Pembroke Welsh Corgis, identification of both protective and risk alleles contributing to EPI have been done. These findings combined shows that in multiple dog breeds, DLA-DQB1 duplication is associated with EPI (Evans et al., 2015).

Although rarer, EPI can also be caused by chronic pancreatitis and very rarely by pancreatic neoplasia (Westermarck & Wiberg, 2012). Pancreatic hypoplasia can also cause EPI (Batchelor, Noble, Cripps, et al., 2007). Pancreatic tumors can result in EPI caused by a combination of destruction acinar tissue, associated pancreatitis and compression of pancreatic ducts by the mass, however patients with these tumors usually present for other reasons. This is rarely a cause of EPI, as is isolated enzyme deficiency, particularly lipase and hyperacidity of the duodenum inactivating lipase (Nelson and Couto, 2019). EPI may also be a consequence of autoimmune or fibrosis destruction and end-stage pancreatic inflammation (Mansfield, 2015).

Chronic pancreatitis may be a more common cause of EPI than currently realized, however given the difficulty with diagnosis it may be underdiagnosed. Cavalier King Charles Spaniel and Jack Russel Terrier have a strong breed association with chronic pancreatitis (Batchelor, Noble, Cripps, et al., 2007).
EPI was historically thought to be a simple autosomal recessive disorder; however, a study demonstrated that EPI has a more complex mode of inheritance, in which multiple genes combined with environmental factors may contribute towards the variability in clinical presentation and disease progression (Clark & Cox, 2012). Current studies are focused on alleles of the canine major histocompatibility complex and dog leukocyte antigen (Tsai et al., 2013).

The most common cause of feline EPI is believed to be end-stage pancreatitis, PAA has only been certainly reported once in cats (Nelson and Couto, 2019). However, reports in cats as young as 3 months old propose other acquired or congenital causes for EPI (Nelson and Couto, 2019). In cats in eastern USA, end-stage fibrosis and EPI has been reported as causes of the racoon pancreatic fluke, Eurytrema procyonis (Nelson and Couto, 2019).

EPI is a functional diagnosis based on pancreatic function test which measures decreased pancreatic secretion capacity (Westermarck & Wiberg, 2012). Signs of maldigestion are usually not seen until 90% of the pancreas´ secretory capacity is lost, as the exocrine pancreas has a large reserve secretory capacity (Westermarck, Wiberg and Watson 2012). The occurrence of clinical EPI needs approximately 90% reduction in lipase production , meaning that there will be substantial loss of pancreatic acini (Nelson and Couto, 2019). Because this is usually a result from a chronic ongoing disease, it is extremely unlikely to transpire after a solitary round of pancreatitis (Nelson and Couto, 2019). However, the level of underlying pancreatic damage can be underestimated as the chronic disease may be mostly subclinical or only present as occasional clinical acute-on-chronic episodes (Nelson and Couto, 2019).
There is a variability in the age of onset of EPI although 93% of affected dogs will by the age of 4, show signs of maldigestion. A very small portion of dogs affected by EPI will be asymptomatic throughout their lives (Hall et al 2003, (Clark & Cox, 2012).

Up to 70% of dogs with EPI have concurrent small intestinal bacterial overgrowth (SIBO), due to unabsorbed nutrients caused by EPI this can lead to small intestinal dysbiosis, this was suggested by early studies. When treating an affected dog, this should be considered as this can contribute to clinical signs. Dogs with EPI can show signs of both large and small bowel diarrhea. This is because bile salts are deconjugated by bacteria therefore decreasing fat emulsification and also fat digestion. The undigested fat is also broken down to hydroxy fatty acids by bacteria. These and deconjugated bile salts may cause large intestinal diarrhea by stimulating secretion as it irritates the colonic mucosa (Nelson and Couto, 2019).
In time, EPI can lead to severe malnutrition because affected dogs, while eating normally, may not be able to digest and absorb sufficient nutrients – namely vitamins B12 (cobalamin), folate (another B vitamin), E and K (Williams et al., 1996) – due to the role of the pancreatic duct cells in secretion of bicarbonate and intrinsic factor (IF). Intrinsic factor is mainly produced by the pancreas in dogs, however it is produced solely in the pancreas of cats. Bicarbonate

regulates the pH of the intestinal lumen environment and IF, which is necessary for the absorption of cobalamin in the small intestine, functions at pH 7; therefore, in EPI the decreased or absent secretion of bicarbonate in this location results in decreased vitamin and cobalamin absorption (Simpson, 2005).

A negative prognostic indicator in dogs if untreated seems to be cobalamin deficiency which is common in dogs and also in cats with EPI. Intrinsic factor binds to cobalamin so it is able to be absorbed from the distal ileum. It is therefore important to measure serum folate and cobalamin levels when investigating the dog with possible EPI. This is why most, but not all dogs with EPI have hypocobalaminemia and most cats are expected to be vitamin B12-deficient. In cats cobalamin deficiency causes weight loss, diarrhea, villous atrophy and reduced gastrointestinal function (Nelson and Couto, 2019).

The diagnosis of EPI is based on clinical signs and typical history. Pancreatic function testing confirms it as canine serum trypsin-like immunoreactivity (cTLI) by radioimmunoassay (RIA) is both highly specific and sensitive for EPI (Batchelor, Noble, Cripps, et al., 2007).

Pancreatic acinar atrophy (PAA)

In dogs the most common cause for EPI is selective loss of the digestive enzyme-producing acinar cells of the pancreas (PAA) (Clark & Cox, 2012). Pancreatic acinar atrophy is characterized by a selective destruction of the digestive enzyme which produce acinar cells (Westermarck & Wiberg, 2012). This will lead to inadequate secretion of pancreatic enzymes and therefore it will cause maldigestion signs which are typical for EPI (Wiberg, 2004). Through histologic examination of the pancreas, PAA is diagnosed with certainty (Clark & Cox, 2012).

PAA is especially acknowledged in young German Shepherd dogs. An autosomal mode of inheritance has been suggested although a recent study suggests that the inheritance is more complex (Nelson and Couto, 2019). PAA in German shepherd dogs and, in Finland, also rough-coated Collies has been proven to be inherited (Wiberg, 2004). An autosomal recessive inheritance model has been suggested by pedigree analyses. These breeds have been reported to have the highest PAA representation , although it has been reported in many other breeds as well (Wiberg, 2004). Young Chow Chows were overrepresented in Britain shown by a large study of EPI (Nelson and Couto, 2019). The juvenile onset suggest a congenital defect in this breed or PAA, although the pathogenesis was unknown (Nelson and Couto, 2019).

Studies have indicated that PAA is not a sex-linked disease. This is proven by the almost even distribution of PAA between females and males. If PAA were inherited as an Y-linked disease, only males would be affected. Also, we would see many more males affected than females with X-linked inheritance, which is not the case (Moeller et al., 2002).

PAA has been reported in humans, but in association with diseases like Shwachman-Diamond and Sjogren´s syndrome which are multiorgan diseases. Canine PAA is a unique disease compared to other species (Westermarck & Wiberg, 2012).

Although PAA is the most common cause of EPI in dogs, it has been reported in a very small number of cats with EPI, this may be because it is underdiagnosed in young cats (Xenoulis et al., 2016). PAA, Eurytrema procyonis and pancreatic aplasia or hypoplasia may all be a cause of EPI in younger cats, however, in cats there are not a lot of studies done (Xenoulis et al., 2016).

Studies have shown that ischemia, pancreatic duct obstruction, defective secretory stimuli, toxic situations and nutritional imbalances or deficiencies among others can cause PAA as an end-result (Westermarck & Wiberg, 2012). These, however, have not been proven to be a factor in naturally occurring PAA in dogs. The common belief has been that PAA is a degenerative disease build on the morphological findings during the end-stage phase of the disease (Wiberg, 2004). However, it has also been suggested to be a hypoplastic disease because of the young age of the affected dogs (Wiberg, 2004). Some reports describe compound exocrine or congenital exocrine and endocrine pancreatic hypoplasia in young puppies (Wiberg, 2004). Some German Shepherds remain subclinical for a long period of time before the disease is apparent, however most dogs develop the disease in young adulthood (Nelson and Couto, 2019).

Pancreatic hypoplasia and PAA is indistinguishable in the histopathologic appearance of the pancreas. However pancreatic hypoplasia is a rather infrequent cause of EPI in dogs as it portrays an evident failure of acinar development, so that these dogs are seriously affected as puppies and thus are unable to grow normally. A study done by observing the progress of acinar atrophy in a German Shepherd has confirmed that PAA, in contrast, usually occur in fully grown adults between 1 and 5 years or older, suggesting that there is a loss of acinar tissue from a formerly functioning normal pancreas (Batt, 1993).

Westermarck et al. used a German shepherd dog puppy where both parents had PAA to follow morphological changes in the pancreas (Wiberg, 2004). The puppy developed EPI later in life, but was born with a histologically and grossly normal pancreas (Westermarck & Wiberg, 2012). Gross and histological abnormalities were not seen until the age of 2 years when severe acinar atrophy was apparent and the clinical signs of EPI appeared, although ultrastructural findings were detected at the age of 6 weeks (Wiberg, 2004). These results support the hypothesis that PAA in German shepherd dogs are due to a progressive and degenerative disease, and is not hypoplastic (Wiberg, 2004).

PAA in German Shepherd dogs is an autoimmune disease directed against the acini, which is suggested by histologic studies. Dogs with PAA are not usually diabetic, because the islets are spared as the disease is directed against the acini mentioned earlier (Nelson and Couto, 2019). Lymphocytic infiltration of the pancreatic acinar tissue transpire simultaneously with active

tissue destruction, which is why PAA is most likely an autoimmune disorder (Clark & Cox, 2012). Other immune-mediated glandular diseases such as immune-mediated polyarthritis and steroid-responsive meningitis have similar age of onset as PAA (German, 2012). Affected dogs do not respond to immunosuppressive therapy however (Nelson and Couto, 2019).

German Shepherds, Eurasian and Rough-coated Collies have been suggested by pedigree analyses to have PAA inheritated by an autosomal recessive trait (Westermarck & Wiberg, 2012). EPI has been suggestedto be a polygenic disease not a single-gene disease. This has been proven by results of a test mating between two German Shepherds with PAA, where only 2 out of 6 offspring were affected (Westermarck & Wiberg, 2012). PAA in Rough-coated Collies and German Shepherds has some features of autoimmune disease shown by recent etiopathogenetic studies. During progression of disease these features include genetic susceptibility to disease and characteristic immunologic and morphologic findings (Westermarck & Wiberg, 2012).

The development of PAA was divided into a clinical phase with severe end-stage atrophy and a subclinical phase with partial acinar atrophy. Both normal and atrophied acinar parenchyma were found in the subclinical phase (Westermarck & Wiberg, 2012). There was no fibrotic or hemorrhagic tissue observed. The histologic findings during the development of atrophy showed marked lymphocytic inflammation into the partly atrophied acinar parenchyma which are typical for an autoimmune disease (Westermarck & Wiberg, 2012). In association with the inflammatory reaction, gradual destruction of acinar structure was found. Only in the end stages of PAA can we see clinical signs (Westermarck & Wiberg, 2012). Thin and transparent pancreas with no signs of fibrosis, pancreatic ducts which are clearly visible and a normal glandular structure which is barely recognizable are the typical gross pathologic findings. The pancreas of dogs with PAA usually have a well preserved endocrine part (Westermarck & Wiberg, 2012)(Nelson and Couto, 2019).

Although tissue destruction seems to be mostly mediated by cellular immune mechanisms, immunologic studies done on dogs with partial PAA have suggested that both humoral and cellular immune responses play a role in the pathogenesis of acinar atrophy. At the beginning of acinar cell destruction, most of the infiltrating lymphocytes were T-cells, with a nearly identical number of CD8+ cytotoxic T-lymphocytes and CD4+ T-helper showed an immunohistochemical analysis (Westermarck & Wiberg, 2012). In sections where the gradual destruction of the acinar parenchyma was present, cytotoxic T-cells predominated. In some

dogs with partial and end-stage PAA, serum auto-antibodies react at low intensity with pancreatic acinar cells. This was not the case in healthy control dogs shown by a recent study, suggesting that the humoral immune response was also activated (Westermarck & Wiberg, 2012).

The factors affecting the rate of progression of the atrophy from the subclinical to the clinical phase are not yet identified, meaning it is highly unpredictable. They may remain in the subclinical phase for years or sometimes even for life shown by long-term follow-up of dogs with partial PAA (Westermarck & Wiberg, 2012).

Chronic pancreatitis

In dogs, chronic pancreatitis is occasionally the underlying cause for EPI. There is usually a progressive destruction of both endocrine and exocrine pancreas in chronic pancreatitis, unlike the situation in autoimmune atrophic pancreatitis (Westermarck & Wiberg, 2012). These animals are likely to be accompanied by diabetes mellitus (DM) because of the progressive loss of exocrine tissue in addition to endocrine cells (Batt, 1993).

The clinical signs of EPI can develop after the clinical signs of DM, however clinical history usually shows more nonspecific gastrointestinal signs. There are clearly different pathologic findings in chronic pancreatitis and PAA. The pancreas is usually shrunken, hard and nodular macroscopically, and there might be adhesions to nearby lying organs in the abdomen. An increase in intralobular and interlobular fibrosis and disorganized acinar lobuli, with or without inflammatory cells in the interstitium is the characteristic histological findings in chronic pancreatitis (Westermarck & Wiberg, 2012).

As a result of concurrent islet cell destruction, many dogs with end-stage chronic pancreatitis develop DM before or after EPI. In cats with end-stage chronic pancreatitis the situation is similar. Middle-aged to older small- or medium-breed dogs, particularly Collies, English Cocker Spaniels and Cavalier King Charles Spaniels are usually the dogs with EPI as a result of end-stage chronic pancreatitis. Labrador Retrievers, Golden Retrievers, Weimaraners and Rottweilers are underrepresented breeds. Boxers rarely develop EPI even though studies have shown that Boxers have a high prevalence of chronic pancreatitis. Among dogs with DM they are also significantly underrepresented. In this breed, this suggests that their chronic pancreatitis does not advance to end-stage disease. Other possible causes, such as inflammatory bowel disease or chronic infection should be thought of before EPI in these breeds if clinical signs for EPI is shown (Nelson and Couto, 2019).

In cats, there is no found breed relationship. Domestic short-haired cats are however most commonly affected, but a variety of pedigree cats has also been reported with the disease. Cats with EPI range from 3 months to 18.8 years, the median age is 7.7 years reported in the largest published study. They are typically middle aged (Nelson and Couto, 2019).
When EPI is diagnosed in cats it is usually due to chronic pancreatitis although EPI is relatively uncommon in cats (Batt, 1993).

Pathogenesis

EPI can either be acquired, usually following severe pancreatic damage such as chronic pancreatitis or inherited, secondary to lymphoplasmocytic acinar destruction with a genetic cause that remains poorly understood (Soetart et al., 2019). End-stage chronic pancreatitis and PAA is believed to be the most common causes of EPI in dogs. It is highly unlikely that a single round of pancreatitis causes EPI as clinical EPI requires approximately 90% reduction in lipase reduction. A chronic ongoing disease is more likely the cause. Although rare, isolated enzyme deficiency; particularly lipase, hyperacidity of the duodenum inactivating lipase and pancreatic tumors can also cause EPI (Nelson and Couto, 2019).
Usually after more than 90% of exocrine function has been lost is when the diagnosis of EPI is made (Mansfield, 2015).

SIBO, or antibiotic-responsive enteropathy, is present in a majority of dogs with EPI. Unabsorbed nutrients in EPI causes small intestinal dysbiosis. This is because bile salts are deconjugated by bacteria therefore decreasing fat emulsification and also fat digestion. The undigested fat is also broken down to hydroxy fatty acids by bacteria. These and deconjugated bile salts may cause large intestinal diarrhea by stimulating secretion as it irritates the colonic mucosa. This is why dogs with EPI tend to present with small and large bowel diarrhea (Nelson and Couto, 2019).

Intrinsic factor is necessary for cobalamin absorption in the ileum, the loss of exocrine pancreatic function can lead to decreased intrinsic factor production. This in addition to impaired intestinal dysbiosis and digestion, which commonly accompanies EPI, is why dogs with EPI are at high risk of low serum cobalamin concentration (Soetart et al., 2019). Reduced duodenal enzyme activity is common in a high proportion of dogs, those with low body condition in particular. This can be caused by the loss of the trophic influence of pancreatic secretions and the effects of malnutrition on the gut (Nelson and Couto, 2019).

Also, a known risk factor for a negative outcome in dogs with EPI and chronic digestive diseases is low serum cobalamin concentration (Soetart et al., 2019). If untreated in dogs, it seems to be a negative prognostic indicator (Nelson and Couto, 2019). Intrinsic factor is mainly produced by the pancreas in dogs, however it is produced solely in the pancreas of cats. Bicarbonate regulates the pH of the intestinal lumen environment and IF, which is necessary

for the absorption of cobalamin in the small intestine, functions at pH 7; therefore, in EPI the decreased or absent secretion of bicarbonate in this location results in decreased vitamin and cobalamin absorption (Simpson, 2005). Intrinsic factor binds to cobalamin so it is able to be absorbed from the distal ileum. This is why most, but not all dogs with EPI have hypocobalaminemia and most cats are expected to be vitamin B12-deficient (Nelson and Couto, 2019). Cobalamin is essential for cellular renewal, particularly in biological systems with high cellular turnover, such as enterocytes, as it acts as a co-enzyme in nucleic synthesis (Soetart et al., 2019). Suboptimal treatment may be the case if there is failure to recognize the potential for hypocobalaminemia. This is especially the case in cats (Mansfield, 2015).
Folate is specifically absorbed in the proximal intestine. It is a B vitamin of bacterial and vegetable origin. Because of intestinal dysbiosis, in EPI, serum folate concentration could be increased leading to elevated bacterial content in the intestine. Extensive gastrointestinal lesions could impair folate absorption (Soetart et al., 2019). A study has identified a significantly lower serum concentration of retinol and 25-hydrocholecalciferol in dogs with EPI compared to healthy dogs (Barko & Williams, 2018).

Genetic test would be desirable for a disease such as EPI. Through the early identification of carriers of recessive mutations and diseased dogs breeders can make educated choices to reduce undesirable traits and diseases. EPI is strongly suggestive of a heritable disease as it has an increased incidence within breeding lines and among certain breeds. A test-mating excludes the possibility of an autosomal recessive mode of inheritance as had been compatible with data from numerous independent studies, instead it suggests a polygenic mode. A major locus was not identified by a genome-wide association (GWA) analysis in a large population of unrelated German Shepherd dogs, however numerous associated chromosomal regions were revealed. Whether there are multiple genetic variants with additive effects, different genetic causes in different populations or both environmental and genetic factors remains unclear. These data do however clearly point to the involvement of environmental and/or multiple genetic factors in EPI. Several single nucleotide polymorphisms (SNP) associated with EPI were proximal to the DLA, this is why current genetic studies are focused on chromosome. The DLA contain several genes important for immune regulation and recognition, several associations with alleles of these genes have been identified for other autoimmune diseases in canines. The involvement of the DLA is proven further by the overexpression of DLA-88 (Clark & Cox, 2012).

Predisposed breeds, age and sex

Some breeds appear to be more predisposed to EPI than other dogs even though it has been reported in many different breeds. German Shepherd Dogs are the breed that EPI is most commonly found (Batchelor, Noble, Cripps, et al., 2007)(Westermarck & Wiberg, 2012). It generally becomes apparent between 1 and 5 years of age in this breed (Clark & Cox, 2012). Rough-Coated Collies, Cavalier King Charles Spaniels and Chow Chows are also breeds that EPI is commonly found in, although rarer than in German Shepherd Dogs. Approximately 50-70% of all dogs diagnosed with EPI were German Shepherds. Rough-Coated Collies were 20% of the cases with EPI in Finland. Both breeds usually have PAA as the underlying cause for EPI. Approximately 1% within these 2 breeds are the estimated prevalence of the disease. Overly represented with EPI are female dogs (Westermarck & Wiberg, 2012).

Clinical signs

The clinical signs depend on the severity, nature and duration of EPI (Batt, 1993).
Up to 70% of dogs with EPI have concurrent small intestinal bacterial overgrowth (SIBO), due to unabsorbed nutrients caused by EPI this can lead to small intestinal dysbiosis, this was suggested by early studies. When treating an affected dog, this should be considered as this can contribute to clinical signs. Dogs with EPI can show signs of both large and small bowel diarrhea. This is because bile salts are deconjugated by bacteria therefore decreasing fat emulsification and also fat digestion. The undigested fat are also broken down to hydroxy fatty acids by bacteria. These and deconjugated bile salts may cause large intestinal diarrhea by stimulating secretion as it irritates the colonic mucosa (Nelson and Couto, 2019).

Increased defecation frequency and fecal volume are typical clinical signs of EPI. As is flatulence, weight loss and yellowish, poorly digested, pulpy and loose feces (Westermarck & Wiberg, 2012). As a direct consequence of passage of malabsorbed dietary constituents along the intestinal tract, severe and watery diarrhea may occur. Secondary alterations in the transmucosal flux of fluid may also cause this (Batt, 1993).
Polyphagia and coprophagia are also common signs. Seborrhea have been reported (Westermarck & Wiberg, 2012). Other skin disorders, eczemas and poor coat have also been reported (Wiberg, 2004).

Figure 2 - The feces of EPI patients are usually dicolored, highly pungent, large volume and loose consistency. (Anon 2008)

Figure 1 - A German Shepherd dogs with EPI showing weight loss. (Nelson and Cuoto 2019)

Abdominal discomfort because of increases intestinal gas may cause aggressiveness and nervousness in some dogs (Westermarck & Wiberg, 2012). Increased bowel movements and gas formation can result in abdominal discomfort (Wiberg, 2004). Perhaps also reflecting abdominal discomfort, some patients vomit and may rarely intermittently anorexic, although atypical. These signs are not pathognomonic for EPI, although these signs are characteristics, because small intestinal diseases may show similar signs of malabsorption (Westermarck & Wiberg, 2012).

In some cases where diabetes mellitus is associated with chronic pancreatitis, animals can be clinically affected. However, typically there are no signs of systemic disease and the animals are not lethargic or depressed. Similar clinical signs can occur in association with other conditions which can be problematic when trying to find a diagnosis (Batt, 1993). Clinical signs for EPI are not pathognomonic for exocrine pancreatic dysfunction, although they are considered typical (Wiberg, 2004). In particular chronic small intestinal diseases can cause the most confusion when setting a diagnosis (Batt, 1993). The typical clinical signs of EPI may sometimes be preceded by non-specific and prolonged gastrointestinal signs (Wiberg, 2004).

In cats, weight loss is the most common clinical sign. In some cats, weight loss is the only clinical sign. Unformed feces, increased appetite, anorexia, vomiting, poor hair coat and lethargy are other clinical signs (Xenoulis et al., 2016). Greasy soiling of the hair coat in the perianal region can be seen in some cats (Steiner, 2012). Studies show that approximately half of the cats presented with a combination of unformed feces and weight loss. However, only approximately 30% of the cats presented with the combination of unformed feces, increased appetite and weight loss (Xenoulis et al., 2016).

Diagnostic tools

Laboratory testing

Clinical signs and clinical histories are used for the diagnosis of EPI and confirmed with a pancreatic function test (Westermarck & Wiberg, 2012). Clinical signs of maldigestion are usually present when canine EPI is diagnosed, which is usually at the end-stage phase of the disease (Wiberg, Nurmi, et al., 1999).

Measurement of serum canine trypsin-like immunoreactivity (cTLI) is used to diagnose EPI (Clark & Cox, 2012). This test usually delivers accurate results and it was developed for the diagnosis of EPI in dogs in particular (Spillmann et al., 2001). It is a highly specific and sensitive test (Wiberg, Nurmi, et al., 1999). In addition it is also pancreas- and species-specific (Westermarck & Wiberg, 2012). It is one of the best tests for detecting EPI. Trypsin and trypsinogen that have entered the bloodstream directly from the pancreas is what is being measured by the TLI assay (Wiberg, Nurmi, et al., 1999). Normal values are between 5.0 and 35.0 µg/L in dogs. Diagnostic for EPI are very low levels less than 2.5 µg/L. Levels between 2.5 and 5.0 µg/L might predict the presence of PAA, however it is possible to have one or more unusually low serum cTLI values, even though the dogs are pathologically and clinically normal, and later have normal test results (Clark & Cox, 2012). Studies have shown that clinically healthy dogs with repeatedly low serum TLI values less than 5.0 µg/L is highly suggestive of partial PAA and a valuable marker of the subclinical EPI. Repeated testing need to be done to increase the accuracy of diagnosis as changes in serum TLI concentration in these dogs are unpredictable (Westermarck & Wiberg, 2012). In cats, a serum TLI concentration with a result of 8 µg/L is considered diagnostic (Mansfield, 2015).

Intestinal diseases do not affect cTLI measurement as trypsinogen does not need to be absorbed from the intestinal lumen. Because the absorption of the orally given enzymes used in enzyme replacement treatment into the bloodstream is limited and the species-specific antibodies used in the assay do not cross-react immunologically with enzymes in supplementations, enzyme replacement treatment does not affect serum cTLI measurement.
Even a transient and slight postprandial increase of serum trypsinogen level may occur, this is why serum samples for TLI measurement is recommended taken after fasting for 8 to 12 hours (Wiberg, 2004). As exogenous enzymes should not be absorbed from the gut into the circulation, it is not necessary to stop exogenous pancreatic enzyme supplementation (Nelson

and Couto, 2019). Because trypsinogen is eliminated by glomerular filtration, a rise in serum TLI can be caused by a renal dysfunction associated with pancreatic disease.
As a result of pancreatic duct obstruction or acute inflammatory attacks in the remaining exocrine tissue, serum TLI can increase. This is why, despite severe pancreatic dysfunction, higher TLI values may be detected in chronic pancreatitis (Wiberg, 2004).

Typical clinical signs of maldigestion and abnormally low serum TLI concentrations are considered highly diagnostic for severe EPI. Severe loss of the digestive enzyme-producing acinar cells are indicated (Westermarck & Wiberg, 2012). By measuring TLI and being able to use it to diagnose PAA before manifestation of clinical maldigestion signs and total acinar atrophy develops allows the progression of atrophy to be monitored (Westermarck & Wiberg, 2012).

Other tests like N-benzoyl-L-tyrosyl-P-aminobenzoic acid absorption, fecal proteolytic activity and fecal soybean stimulation test have been used for the diagnosis of EPI. However all of these tests are either unreliable, difficult to perform or both and have been replaced by serum cTLI by radioimmunoassay. An assay for fecal elastase measurement has recently been introduced. However, it is proven inferior to the measurement of serum cTLI concentration as well because of some false-positive results (Moeller et al., 2002). The ability to distinguish whether the maldigestion signs are caused by small intestinal disease or exocrine pancreatic disease is the diagnostic value of these tests. In addition these tests are practical. They are good indicators of clinical EPI, however they are insufficiently sensitive to detect subclinical EPI (Westermarck & Wiberg, 2012). Determination of serum lipase activity is neither specific nor sensitive for diagnosis of pancreatitis, however, it has been used for this purpose in both dogs and humans for decades (Steiner et al., 2006).

Because of a deficiency of pancreatic IF, serum cobalamin concentration is often decreased in dogs with EPI. It can therefore be advisable to measure this as well. It should either be supplemented enterally or parenterally if the concentration is low. In about one-third of dogs with EPI, serum folate concentrations are high which may indicate SIBO or dysbiosis. However, sometimes serum folate concentration may be low as well. This can suggest concurrent inflammatory or infiltrative disease in the jejunum, dietary deficiency or potentially metabolism by bacteria (Nelson and Couto, 2019).

Dogs with EPI are more susceptible to GI pathogens as they have reduced gut immunity. In particular if a dog have a history of being fed raw pancreas or raw diet, a fecal culture would be indicated if the dog is failing to respond to standard therapy (Nelson and Couto, 2019).

Gross pathologic findings can be found when severe atrophy of the exocrine pancreas is the cause of EPI. The pancreas has no normal glandular structure visible and it is thin and transparent (Wiberg, Nurmi, et al., 1999). It is normal in length. Usually the pancreas is shrunken, hard and nodular. There is no hemorrhagic or fibrotic tissue. There might be adhesions. The pancreatic ducts are clearly visible and the normal glandular structure is hardly recognizable (Wiberg, 2004).

Cytology, histopathology

Normal tissue, if present in the end-stage of EPI, is found in small isolated lobuli histologically. However, usually no normal acinar tissue is left. Normally, atypical tissue will replace the normal acinar parenchyma. It will be replaced by disorganized small round cells with light acidophilic, often slightly granular cytoplasm and a central nucleus. In addition it will have dilated and prominent ductal structures. In some cases adipose tissue replace normal tissue. Generally, fibrous tissue is not increased.
In small infiltrations or scattered among atrophied tissue, lymphocytes, inflammatory cells and plasma cells can be found.
In dogs with PAA, some evidence for degenerative changes in the endocrine cells has been reported, however usually the endocrine part of the pancreas is well preserved (Wiberg, 2004).

In the clinical phase of PAA, the histological findings can be divided into three stages. Among the atrophied parenchyma some sparse normal acinar tissue was found in the first stage. The gradual transition of acinar cells in the border zone between the atrophied and normal tissue was associated with lymphocytic infiltration.
Some normal acini were found, however atypical atrophic tissue dominated in the second stage. In comparison to the earlier stage, the inflammatory reaction was less intense.
No inflammation or normal tissue was present in the end-stage atrophy. However, the ductular structures were prominent (Wiberg, 2004).

For the ultrastructural changes in dogs with end-stage PAA, degenerative changes can be found in the remaining acinar cells. Loss of zymogen granules, dilatation of the rough endoplasmic reticulum (RER) and whorl formation characterizes the findings (Wiberg, 2004).
EPI is less commonly seen with an ultrasonography than diseases like pancreatitis and neoplastic lesions. Unfortunately, various pancreatic disorders overlap in ultrasonographic findings. Age-related changes and incidental findings may mimic pancreatic disease. This is why ultrasonographic findings have to be judged based on history, laboratory data and signalment as well. Ultrasonography can however be useful when monitoring response to treatment (Hecht & Henry, 2007).

The progressive degenerative changes of acinar cells can be divided into four stages. Slight dilatation of RER is showed in the first stage. RER becomes more disorganized and dilated in the second stage. In the cytoplasm, round condensing vacuoles were found. In addition, zymogen granules and mitochondria were larger than normal. The chromatin condensed and the nucleus was crenated. Dilatation of RER was increased in the third stage. There were extensive fusion of zymogen granules and the nucleus was pyknotic. Acinar cells were necrotic in the fourth stage (Wiberg, 2004).

In chronic pancreatitis, both the endocrine and exocrine pancreas may become affected, unlike in PAA. Pathological findings in chronic pancreatitis are clearly different from those in end-stage PAA. Disorganized acinar lobuli and an increase in the intralobular and interlobular fibrosis, with or without inflammatory cells in the interstitium are characteristic histological finding in chronic pancreatitis. It may be classified as pancreatic fibrosis, chronic fibrotic pancreatitis, chronic interstitial pancreatitis and pancreatic atrophic cirrhosis (Wiberg, 2004). Exocrine pancreas is affected by infiltrative lymphocytic inflammation before total atrophy occurs which gradually leads to selective atrophy and destruction of the acinar cells. This is seen in the subclinical phase of EPI (Wiberg et al., 2000). Studies suggest that lymphocytic pancreatitis leads to atrophy of the pancreas as it was clearly shown that the inflammatory reaction precede the pancreatic acinar atrophy (Wiberg, Saari, et al., 1999).

Most acini contain cells whose stainability varies greatly and are reduced in volume. Isolated in loose connective tissue and fat, markedly changed lobules can be found. This is observed in chronic pancreatitis when using light microscopy. Zymogen granules are completely absent or reduced in number (Pfister et al., 1980).

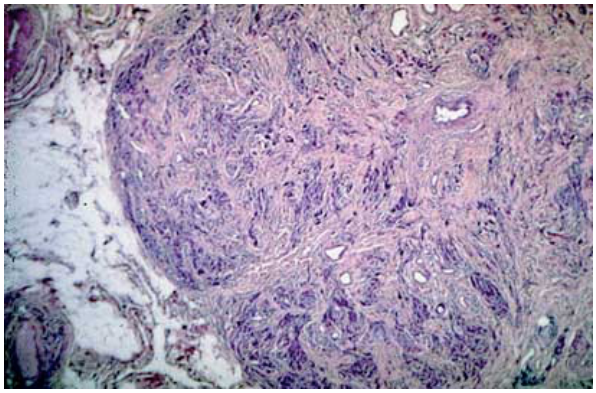


Figure 3 - In this section from the pancreas of a dog with EPI secondary to pancreatitis fibrosis and atrophy are evident. (Anon 2008)

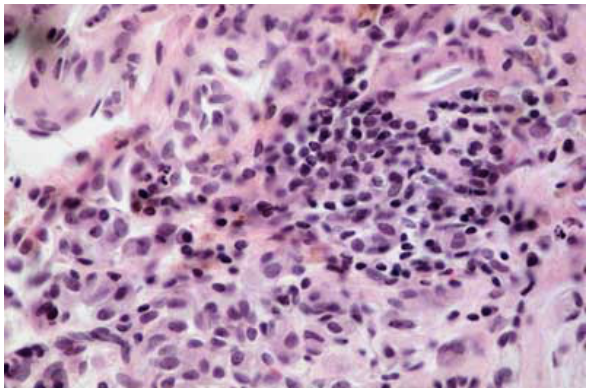


Figure 4 - The development of EPI in dogs with familial acinar atrophy is preceded with lymphocytic inflammation. (Anon 2008)

Treatment

The treatment of EPI appears to be inconstant and the optimal treatment regimen is not known although therapeutic options are well described. Pancreatic enzyme replacement is usually the initial therapy. Not all dogs respond well enough to this. Why there is a variable response to some dogs with this treatment and why it fails in other dogs are not completely understood (Batchelor et al., 2007).

The primary treatment to EPI is to supplement each meal with pancreatic enzyme preparations as the clinical signs of EPI are due to the inadequate production of digestive enzymes (Wiberg, 2004). Enzyme supplementation for the rest of their lives is required for all dogs and cats with clinical EPI (Nelson and Couto, 2019). Digestion capacity does not return to normal despite accurate enzyme supplementation. Of the orally-given enzymes, only a small portion are delivered functionally intact into the small intestine. Nonenteric-coated supplements has achieved the best results in dogs. Supplementation with powdered enzymes or raw chopped pancreas has achieved the highest enzyme activity in the duodenum (Wiberg, 2004). Although there is the potential for acquiring gastrointestinal infections like Campylobacter and Salmonella when using raw pancreas (Nelson and Couto, 2019). Because of the delayed gastric emptying of the preparations, the value of enteric-coated supplements has been limited in dogs (Wiberg, 2004). As a consequence of the acidic pH in the stomach, a large portion of enzyme activity is lost when entering the stomach. An H2 blocker is administered to increase the gastric pH or the dosage of enzymes given is increased to overcome this (Nelson and Couto, 2019).

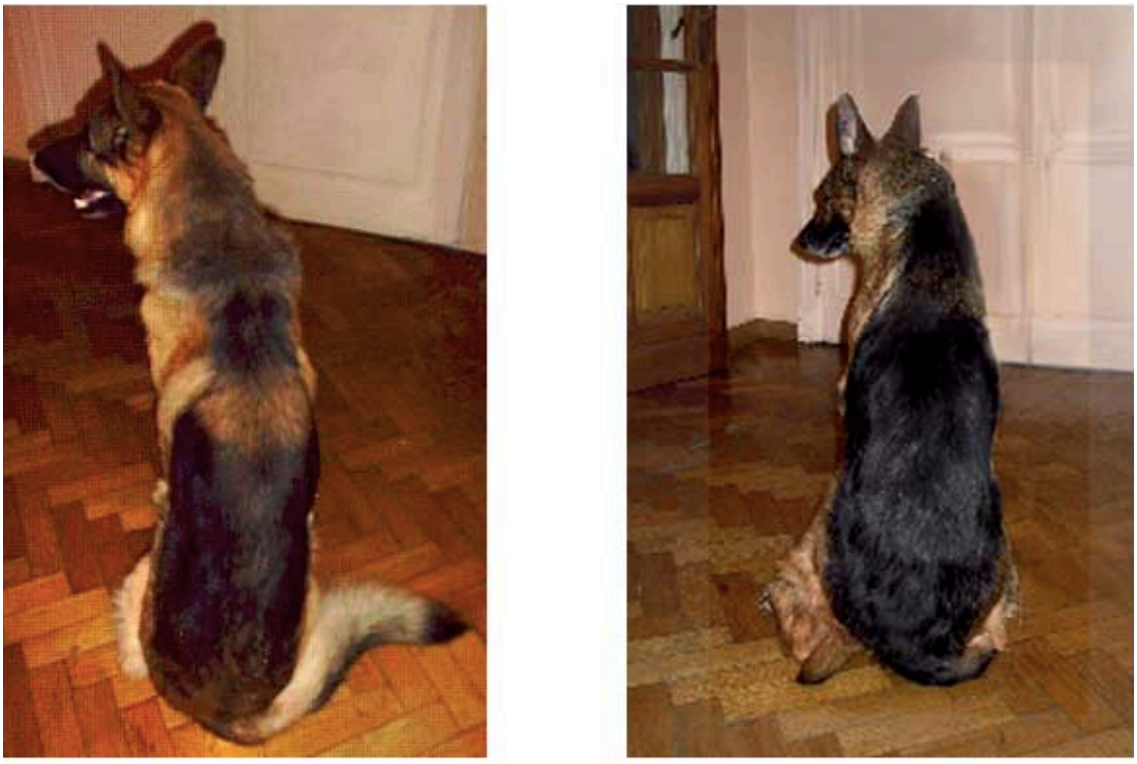
Clinical feeding studies have shown controversial results of using special diets and the actual benefits. The commonly recommended feeding for EPI is a highly digestible, low fat and low fiber diet however. A low fat diet has been considered necessary because enzyme supplements alone are unable to restore normal fat absorption. The bacterial deconjugation of bile salts in a small intestinal disease may affect the fat absorption, which can result in diarrhea as it produces metabolites (Wiberg, 2004). However, weight gain may be difficult to achieve in large-breed dogs with cachexia and EPI on a low-fat diet. Feeding a low-fat diet, clinical signs may resolute faster than a normal-fat diet. Otherwise there is no proof that low-fat diet improves the outcome in dogs with PAA more than normal-fat diet. High-fat diets should be avoided however (Nelson and Couto, 2019).

Undigested carbohydrates act as substrates for intestinal bacteria and may produce osmotic diarrhea, this is why highly digestible diets are recommended (Wiberg, 2004). Fiber impairs the activity of pancreatic enzymes and therefore the diet should be low in fiber. Soluble fiber may also absorb pancreatic enzymes. The activity of brush border enzymes and the small intestinal absorption may be reduced by fiber (Nelson and Couto, 2019).
Clinical signs such as increased fecal volume, borborygmi, flatulence and defecation frequency have been proven to be alleviated by feeding highly digestible, low fiber diets.
Hypoallergenic diets may benefit some dogs as a consequence of EPI may be dietary sensitivities (Wiberg, 2004). Each meal should be with enzymes added and it is best to feed two or more meals a day (Nelson and Couto, 2019).

Dietary advice is slightly different in dogs with EPI as a result of chronic pancreatitis. Long-term feeding of a low-fat diet which seems to reduce acute flare-ups of disease and postprandial pain seems to benefit these dogs (Nelson and Couto, 2019).

A valuable supportive treatment for EPI is antibiotics, in particular metronidazole, tylosin and oxytetracycline. In cases where clinical signs have recurred during enzyme treatment and poor treatment response to enzymes alone, antibiotics have been used. Especially during initial treatment (Wiberg, 2004). If the dogs fail to respond to cobalamin and enzyme treatment and only if necessary, is it advisable to administer antibiotics (Nelson and Couto, 2019).
Vitamin B_{12} supplementation is required for dogs and cats with hypocobalaminemia, which can be given orally or by parenteral injections (Nelson and Couto, 2019). A critical aspect of managing patients with EPI is correcting micronutrient deficiencies like cobalamin (Barko & Williams, 2018).

Enzyme supplementation with either raw pancreas or a powdered pancreatic extract is the used treatment for EPI in cats. In addition, most cats require lifelong parenteral cobalamin supplementation as they have severely decreased serum cobalamin concentrations (Steiner, 2012).



(A) Pre-treatment

(B) Post-treatment

Figure 5 - German Shepherd suffering from EPI before and after treatment (Anon 2008)

Prognosis

The loss of functional pancreatic tissue is almost total when dog with PAA show clinical signs of EPI. Lifelong enzyme replacement treatment is usually required, however the changes are considered to be irreversible (Wiberg, 2004). As the disease can be successfully treated in most dogs, the prognosis for EPI is good (Nelson and Couto, 2019). Studies focused on the response to initial treatment with enzyme supplements has been reported to be poor in 17% and good in 64% of the cases. The effect of treatment was assessed according to its ability to increase bodyweight and decrease polyphagia and diarrhea. During the first weeks of treatment a resolution of these signs could be found in many dogs (Wiberg, 2004).
For most dogs with PAA, lifelong treatment is indicated. However a study has shown that the discontinuation of enzyme supplements did not cause a recurrence of clinical signs if after a good initial treatment response. In these dogs, however, continuous enzyme replacement treatment was unnecessary as some of the pancreatic tissue with reserve secretory capacity had remained (Wiberg, 2004).

A study shows that approximately 30% of the dogs were euthanized during the first two years after the diagnosis of EPI. The principal reasons for euthanasia were high cost of treatment, refusal by owners to continue treatment and a failure to respond to treatment.
Another study reported mesenteric torsion as a severe, but rare complication of EPI. Excessive intestinal gas and disorders in intestinal motility is probably the cause for this (Wiberg, 2004). Another study showed that for the dogs that responded very well to the treatment the median survival time was more than 5 years. This can prove that scheduling regular follow-up appointments is very important. To address all concurrent problems during treatment, evaluate the progress and change management as necessary (Nelson and Couto, 2019).

A recent study has compared the general well-being of Rough-coated Collies and German Shepherd dogs treated with long-term enzyme replacement treatment for EPI and healthy control dogs of the same breed. In half of the dogs nonenteric-coated enzyme supplements almost completely kept the gastrointestinal tract signs considered typical for dogs with EPI in control. In 20% of the dogs, poor response to treatment was observed.
During long-term treatment, short relapses of clinical signs may develop. Antibiotics were administered to half of the dogs during treatment to control these signs. During long-term treatment, permanent deterioration of the clinical condition in dogs with EPI is uncommon.

During the first months of treatment, response to treatment usually remained fairly stable during long-term treatment. The prognosis of nonenteric-coated enzyme supplements as long-term treatment of EPI was considered to be good (Wiberg, 2004).

EPI as a result of end-stage chronic pancreatitis, the prognosis for dogs and cats is good in most cases. Survival of several years in most cases, even if it is complicated by concurrent DM (Nelson and Couto, 2019). Normal quality of life and life expectancy can be expected in cats as most cats respond well to therapy (Steiner, 2012).

Materials and methods

Case report from Batchelor (Batchelor, Noble, Cripps, et al. 2007)

This case study has a record of 13 069 dogs tested with cTLI (canine serum trypsin-like immunoreactivity) by RIA (radioimmunoassay) which is highly specific and sensitive for EPI. The testing was done from January 1990 and September 2002 at the Comparative Gastroenterology laboratory at the University of Liverpool.

The goal of the study was to get an answer to the hypothesis that for EPI, important breed associations exist and that EPI is common in certain breeds and rare in other dogs with clinical signs of gastrointestinal disease undergoing cTLI assay. The main aim of this study was to compare the proportion of positive tested dogs for EPI between breeds in a population of considerable size of clinically affected dogs undergoing cTLI assay. Another aim was to compare the respective proportion of each breed in this affected population with that in a large control population.

Analysis within the sampled population and comparison with the pet dog population as a whole were the two methods used to identify individual breeds underrepresented or overrepresented of dogs with EPI. When a breed was proven to be either underrepresented or overrepresented by both methods, evidence was considered as a genuine association with EPI. However, if there was no correspondence between the results of the two methods, the breed was considered potentially underrepresented or overrepresented.

13,069 cTLI assays records were reviewed. In total 1,127/13,069 dogs were diagnosed with EPI. Dogs of 132 breeds were tested. EPI was detected in 59 different breeds. Of all the samples 3,537 was from German Shepherd dogs. Of the dogs that the breed was known, 1,064 out of 12,259 tested positive for EPI.

The analysis by breed within the sampled population method observed prevalence of EPI in German Shepherd dogs, Cavalier King Charles Spaniel, Cocker Spaniels, Rough Coated Collies, West Highland White terriers, Chows and mixed breed dogs in the sampled population was significantly higher than in the within-data control population. Although with fewer affected individuals, Cairn Terrier and Corgi breeds were also identified as overrepresented. In Labrador Retrievers, Golden Retrievers, Weimaraners, Rottweilers, Great Danes and Boxers,

the observed prevalence was significantly lower in the sampled population than in the within-data control population, most notably in Boxers where none had EPI out of the 524 individuals tested.

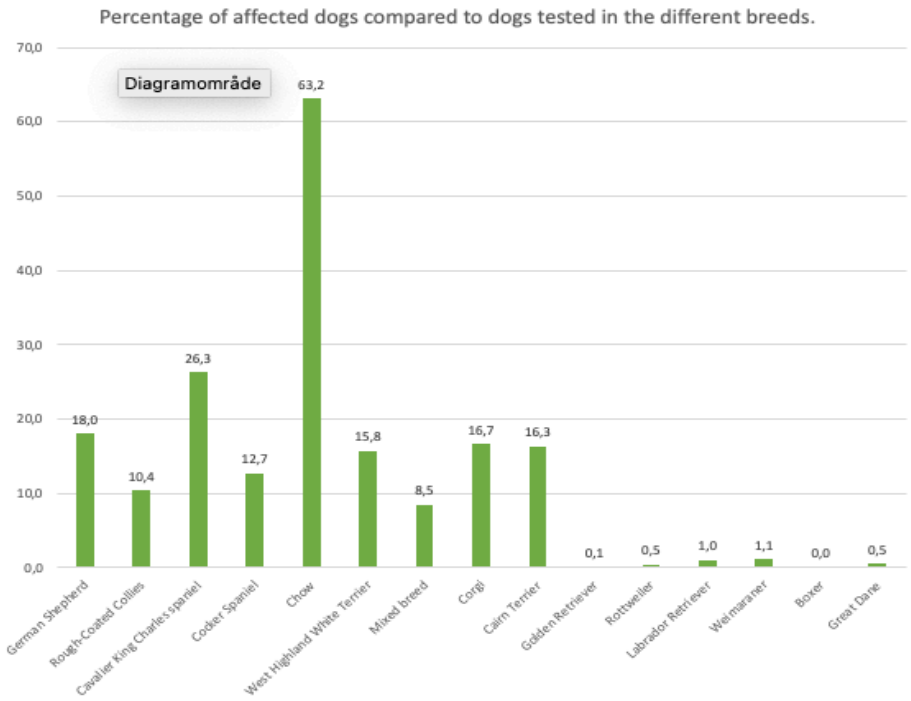


Figure 6 - The breeds in the study (Batchelor et al. 2007)

German Shepherd dogs, Rough Coated Collies, Corgis, Chows and Cavalier King Charles Spaniels were overrepresented in the affected population when compared with the pet insurance database. Here, Labrador Retrievers, Golden Retriever, Rottweilers, Weimaraners, Boxers and mixed breeds were underrepresented in the affected population. Great Danes, Cairn Terriers, West Highland White terriers and Cocker Spaniels were not significantly different.

Comparing both methods in the affected population, German Shepherd dogs, Rough Coated Collies, Cavalier King Charles spaniel and Chows were overrepresented, and Labrador Retrievers, Golden Retrievers, Rottweilers, Weimaraners and Boxers were underrepresented.
Out of all the affected dogs, the age was known for 1,023 of them. The overall median age was 42 months at the time of diagnosis, although the range was from 3-204 months. Cavalier King Charles spaniels were older than German Shepherd dogs, Chows and Rough Coated Collies at the time of diagnosis proven by posthoc analysis with the Mann-Whitney test. Between German Shepherd dogs, Cows and Rough Coated Collies, the age at diagnosis was not significantly different.

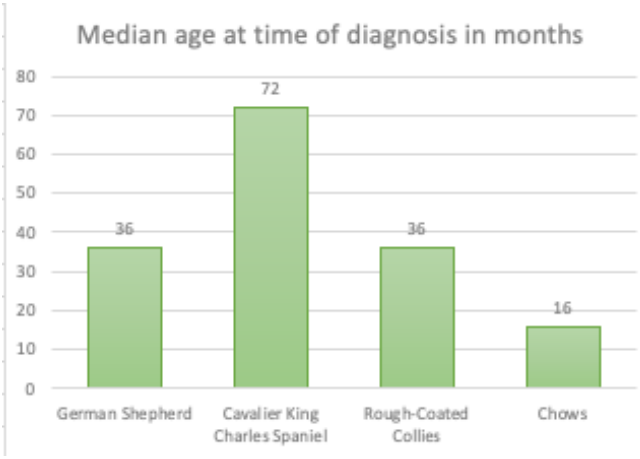


Figure 7 - Time of diagnosis for the different breeds (Batchelor et al. 2007)

In the sampled population there was an overrepresentation of male dogs with 58,8%. Female dogs were overrepresented among the affected dogs however, with 56,6% positive tested of a total of 1 063. In German Shepherd dogs, Chows and Cavalier King Charles spaniels overrepresentation of females was seen. However, in Rough Coated Collies there was no sex association observed.

Case report from Batchelor (Batchelor, Noble, Taylor, et al. 2007)

In this study 743 dogs diagnosed with EPI was recorded, however only 178 out of these dogs had enough information to be useful for the study. The Comparative Gastroenterology Laboratory of the University of Liverpool created a database of canine trypsin-like immunoreactivity assays used to identify dogs with EPI, which was when serum TLI was lower than 2.5 µg/L. A questionnaire was sent out to the submitting veterinarian for each dog where information like the age of diagnosis, sex, breed, medical therapy used, clinical signs at the time of diagnosis and at various time points after treatment and any concurrent medical problems was written. Serum folate and cobalamin assays performed at the same time as the cTLI assay was recorded when available. Response to initial treatment (RIT) was graded by the veterinarian as poor, partial or good for each dog. The time between the diagnosis of EPI and death was also recorded.

Out of the 178 dogs, 18 breeds were represented where over half of the cases were German Shepherd dogs. 58% in the study were females, meaning they were overrepresented. In German Shepherd dogs the median at the time of diagnosis was 3 years, whereas in non-German Shepherd dogs the median was 4 years. On an average German Shepherd dogs were diagnosed at a younger age.

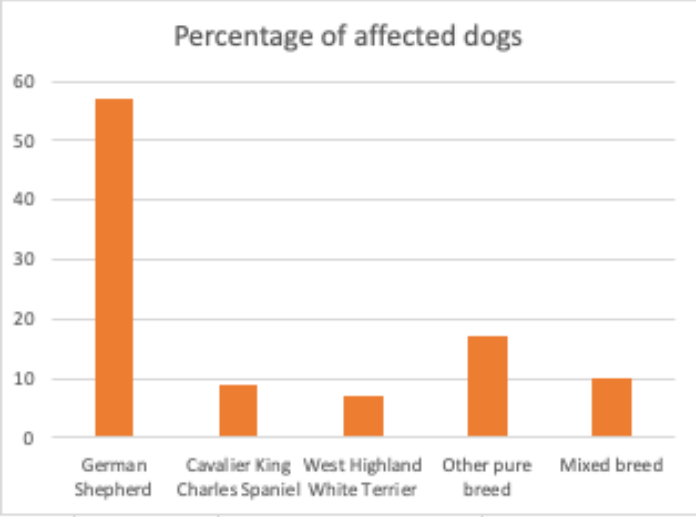


Figure 8 - The breeds in the study (Batchelor et al. 2007)

146 out of the 178 dogs had information available on the clinical signs. At the time of diagnosis diarrhea was very common. Vomiting was present in some cases. Polyphagia was common, some dogs were inappetent but many dogs had normal appetite. Most of the dogs were underweight while a very small portion was overweight. A few dogs were nervous and aggressive.

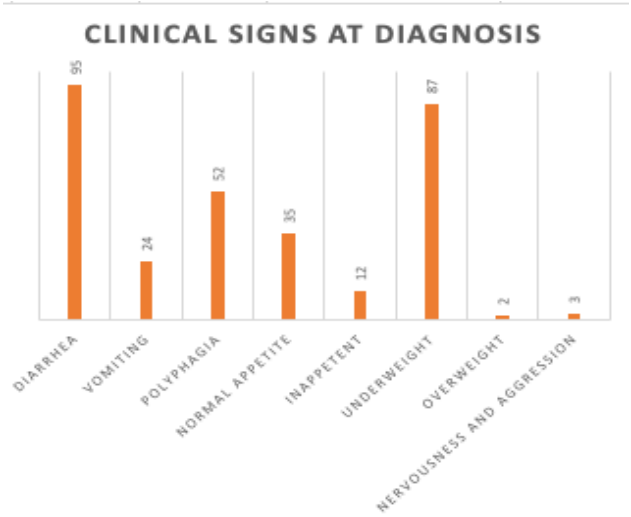


Figure 9 - Clinical signs seen at the time of diagnosis (Batchelor et al. 2007)

At the time of therapy clinical signs frequently persisted. Out of the surviving dogs diarrhea persisted in some of the dogs whereas body condition and appetite normalized in the majority of dogs. A portion remained underweight out of the dogs alive 1 year after diagnosis.

At the same time as cTLI was measured in 163 dogs, cobalamin and serum folate concentrations were measured. Serum folate concentration was high in the majority of dogs and the majority was low on serum cobalamin. The combination of high folate and low cobalamin concentrations was seen in almost half of the cases. At the time of diagnosis a portion of the dogs also had hypocobalaminemia.

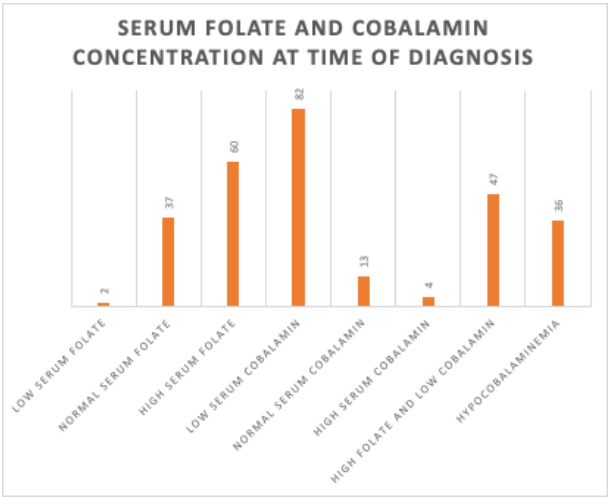


Figure 10 - Concentration of serum folate and cobalamin at time of diagnosis (Batchelor et al. 2007)

As for therapy, accurate information was available for 172 dogs. Several different products were used as enzyme replacement therapy. Some dogs were given enteric-coated preparations, most dogs were given uncoated, enzyme powder and a few dogs were fed fresh pancreas or incubated enzymes with the food. 11 dogs were not given pancreatic enzymes, - out of these 7 were euthanized and 1 was not properly followed-up. The 3 dogs left lived for approximately 4.5, 8.5 and 9.5 years respectively.

The diet was changed in approximately half of the 178 dogs where information was available. A majority of those were fed fat-restricted diet, while the rest were fed selected protein or high-fiber foods, low-fat foods or home-cooked food.

Almost half of the 171 dogs that had accurate information available for treatment with antibacterials. Some were treated with amoxicillin/clavulanate, tylosin, trimethoprim/sulfonamide and enrofloxacin. However the most common was metronidazole and oxytetracycline.
A small portion of the dogs were given H2-receptors, most commonly cimetidine, out of the 174 dogs where information was available.
A small portion of the dogs were given cobalamin by injection out of the 168 dogs where information was available. Out of the dogs with hypocobalaminemia only a few were given cobalamin.

Additional treatment, like glucocorticoids, probiotics, antidiarrheals and sulfasalazine were given to some of the 173 dogs where information was available. Rarely used treatments were stool softeners, acupuncture, folate supplements, anthelminthics and anabolic steroids.
The majority of the 118 dogs that received enzyme replacement therapy and had accurate information available showed good RIT. Some showed partial RIT and a some showed poor RIT. Dogs that had high serum folate concentration or received antibiotics at the time of diagnosis had a worse RIT shown by analysis.

The survival rate at the end of the study were that the majority of the dogs were dead, some were still alive and some were lost to follow-up. 140 dogs had enough follow-up information to be adequate. 63 months were the overall median survival time (MST) for dogs treated with follow-up available. Within a year, 30 dogs had died, out of these 27 were euthanized, 10 were euthanized because of persistent clinical signs and 5 were euthanized because of cost. As a

worst case scenario, where all the dogs lost to follow-up were dead, 1-year survival rate for EPI in treated dogs was 64%. The 2-year survival rate was 57% and the 3-year survival rate was 46%.

For the dogs with serum cobalamin concentration ≥100ng/L the MST were 2709 days, which were longer than the dogs with serum cobalamin concentration <100ng/L that did not receive supplementation which were 1346 days.

Age, breed, sex at diagnosis, high serum folate concentration and the combination of a low serum cobalamin and high serum folate concentration did not have a significant effect on survival. Neither did the use of fat-restricted diet, any dietary modification, treatment with H2-receptor antagonists, antibiotics or with an enteric-coated enzyme formulation.

For the dogs which had partial or poor RIT the median survival were 458 days which was way less than the dogs that had a good RIT which was 3471 days. At the time of diagnosis, the presence of diarrhea, polyphagia, vomiting, being underweight or anorexia had no significant effect on survival.

Case report from Wiberg, Nurmi and Westermarck (Wiberg et al. 1999)

In this study 1.236 serum samples were analyzed at the Veterinary Laboratory Vetlab in Finland by radioimmunoassay of TLI from 1995 to 1997. The main reason for this study was to research whether or not subnormal TLI concentrations reflect abnormal exocrine pancreatic function in dogs. Diagnosis of EPI in a subclinical phase may offer a better opportunity study the etiology of the disease which might also find a way to stop progression of the disease as some functional capacity and exocrine tissue still remain.

The concentrations of TLI were divided into 3 categories: concentrations < 2.5 µg/L, 2.5-5.0 µg/L and > 5.0 µg/L. Out of these categories, the two first were analyzed further in this study which resulted in 158 dogs. They were divided into 2 groups based on serum TLI concentrations and clinical history: those with clinical EPI and those with suspected EPI respectively.

Clinical signs of maldigestion associated with concentration of serum TLI < 2.5 µg/L were the criterias for the diagnosis of clinical EPI. These dogs were not included in the further study as the reason for this study was to research subclinical EPI.

This remains 44 dogs where the concentration of TLI was 2.5-5.0 µg/L or the concentration of TLI was < 2.5 µg/L but with clinical signs the were atypical for EPI. Out of these 35 dogs had a serum TLI concentration of 2.5-5.0 µg/L and out of these the majority had acute or occasional diarrhea, some had chronic diarrhea and a few had no gastrointestinal signs. A majority of the dogs were also thin or had weight loss. The remaining 9 dogs had a serum TLI concentration of < 2.5 µg/L and out of these the majority had no gastrointestinal signs, some had occasional diarrhea and 1 dog had chronic large bowel diarrhea. In total 9 different breeds were represented with the majority being German Shepherd dogs. The median age was 3.3 years however it ranged from 1 to 8 years. The majority was male dogs. To evaluate the status of the exocrine pancreas, additional studies were performed on all the 44 dogs with suspected EPI.

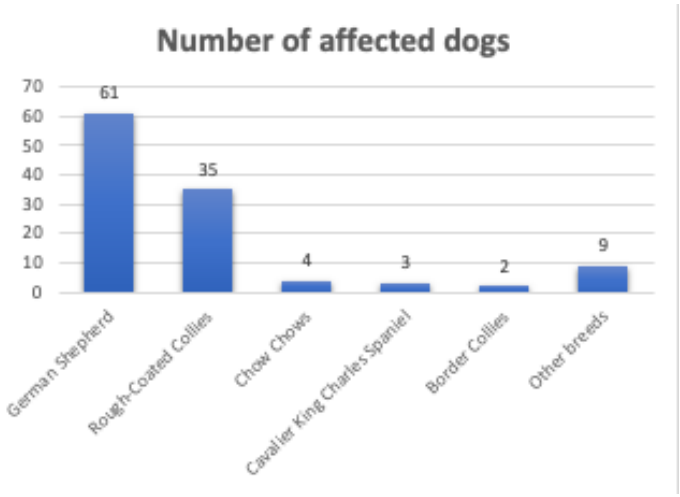


Figure 11 - the breeds in the study (Wiberg et al. 1999)

After they were fasted overnight, the 44 dogs with suspected EPI were retested of serum TLI concentration. The TLI was measured by radioimmunoassay and the clinical histories were reassessed. 20 dogs with suspected subclinical EPI, that had TLI consistently less than 5.0µg/L, remained in the study as the others either had normal serum TLI concentration or they had progressive clinical signs of EPI associated with TLI concentration that were below 2.5µg/L on retesting.

12 dogs out of the 20 that were suspected with subclinical EPI and TLI concentration was persistently lower than 5.0 µg/L were subjected to gross examination of the pancreas and pancreatic biopsy, soybean stimulation test (SST), serum n-benzoyl-L-tyrosine-p-aminobenzoic acid (BT-PABA) test and measurement of serum cobalamin and folate concentration.

Mostly a cranial midline laparotomy or rarely a post mortem examination was performed for the gross examination of the pancreas. On the caudal part of the pancreas a pancreatic biopsy, 5 mm in diameter was taken for the ultrastructural, histologic and immunohistologic studies. The dogs all recovered uneventfully.

On 4 dogs the serum BT-PABA test was performed. The dogs were given 16.5 mg/kg BT-PABA in 10ml water/kg PO after overnight fasting. Post administration serum samples were taken after 1- and 2- hours and analyzed for PABA.

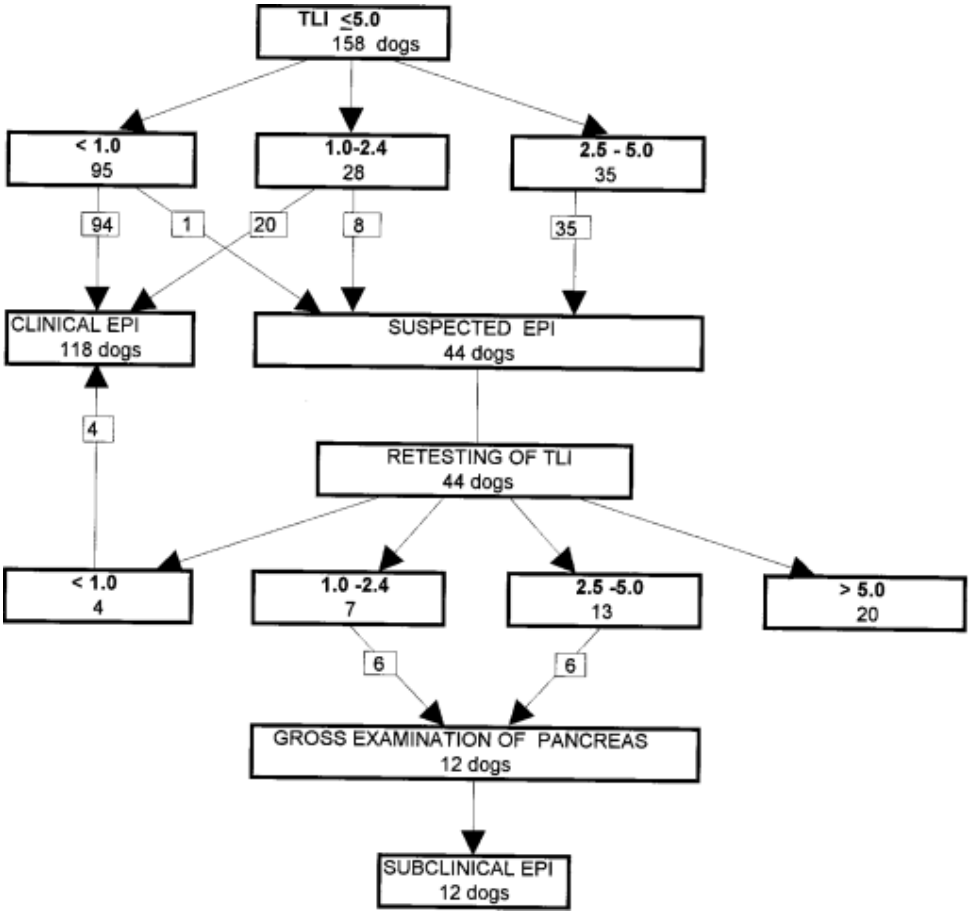


Figure 12 - Number of dogs tested (bottom) and their serum TLI concentrations (top) (Wiberg et al. 1999)

On 10 dogs, fecal SST was performed. Fecal samples were collected on days 2, 3 and 4, after the dogs were fed raw soybeans mixed in food for 4 days. Both X-ray film test and radial enzyme diffusion (RED) in calcium caseinate agar were used to measure fecal proteolytic activity. In 9 dogs, serum cobalamin and folate were measured. After fasting overnight the serum samples were collected.

The TLI-stimulation test (TST) was performed after fasting overnight, the serum samples for TLI assay were collected. It was performed on dogs with clinical EPI, dogs with suspected SEPI (subclinical exocrine pancreatic insufficiency) and on clinically normal dogs. The pancreas was stimulated by both cholecystokinin and secretin given as IV administration immediately after the serum samples were taken. 20 minutes after stimulation a serum sample for the 2nd TLI assay was collected.

After the study, 5 out of the 10 normal control dogs were euthanized and subjected to a postmortem examination. The pancreas was normal in structure and size in all dogs.
During the test, 3 dogs out of the 6 dogs with clinical EPI were supplemented with enzymes. Out of these 1 dog was examined postmortem and the 2 others had laparotomy performed. In all 3, endstage pancreatic atrophy was confirmed.
After the TST the 12 dogs with suspected SEPI were also subjected to postmortem examination or laparotomy.

In 20 dogs, normal TLI concentration were found. In these dogs the 1st measured TLI concentration ranged from 2.5 to 5.0 µg/L and the retested concentrations ranged from 5.2 to 28.4 µg/L. The time ranged from 1 to 27 months between the TLI measurements. Clinically, 1 dog had chronic diarrhea, some dogs had occasional gastrointestinal signs and most dogs had no gastrointestinal signs at the time of retesting.

In 4 dogs, clinical EPI was confirmed. The TLI concentrations in these dogs at the first testing ranged from 0.6 to 2.9 µg/L. The TLI concentrations at the retesting ranged from 0.6 to 0.9 µg/L. The time ranged from 2 to 12 months between TLI measurements. Clinically, these dogs showed typical signs of EPI.

In 20 dogs, persistent TLI concentrations less than 5.0 µg/L were detected. The TLI concentrations in these dogs at the first testing ranged from 1.0 to 4.9 µg/L. In 7 dogs the TLI concentrations at retesting ranged from 1.0 to 2.4 µg/L and in 13 dogs it ranged from 2.5 to 4.9 µg/L. The time ranged from 1 to 24 months between TLI measurements. Clinically, some dogs had signs not resembling typical EPI like food intolerance and occasional diarrhea, while most dogs had no gastrointestinal signs at the time of retesting. The TLI concentrations were additionally measured for the 3rd and 4th time in some of the dogs and it was consistently lower than 5.0 µg/L.

For all the 12 dogs with TLI concentrations persistently lower than 5.0 µg/L they showed diminished pancreatic mass by gross examination. The pancreas was thinner in width, but normal in length. Scattered areas that had lost their glandular appearance were scattered within the normal tissue of the pancreas. A diagnosis of SEPI was made in all 12 dogs based on gross

pancreatic pathology and clinical findings. Pancreatic enzyme supplementation were not required in these dogs.

Before the laparotomy the TLI concentrations ranged from 1.0 to 4.1 μg/L. Between the 1st TLI measurement and laparotomy the time period ranged from 1 to 12 months. In 3 out of 4 dogs the results of BT-PABA were subnormal. In all 10 dogs tested with RED, the SST showed normal fecal proteolytic activity. In all except 1 out of the 10 dogs the result using the X-ray films was analogous with the RED activity. Only 1 out of the 10 dogs had high folate concentration with slightly low cobalamin concentration. However 5 out of the 10 dogs had mildly high serum folate concentration alone.

Case report from Moeller, Steiner and Clark (Moeller et al 2002)

In this case study the goal was to research the heritability of PAA in German Shepherd Dogs. The purpose of this study was to determine the inheritance of PAA in German Shepherd Dogs in the United States as previous studies have been carried out in other countries. In total, 135 German Shepherd Dogs were used.
In mainly German Shepherd Dogs and Rough-Coated Collies, PAA leads to EPI. It is a degenerative disease of the exocrine pancreas.

By 5 years of age, affected dogs usually have clinical signs of EPI, however some dogs show signs as early as around 1 year of age. Steatorrhea, voluminous feces, polyphagia and weight loss are some of the most common clinical signs. Feces are usually loose in texture, light in color and malodorous.

For dogs with PAA, scattering, disorganization and atrophy of pancreatic acinar cells can be findings on histologic evaluation of pancreatic biopsy specimens. Degenerative changes of acinar cells has been revealed as early as 6 weeks of age by electron microscopy of pancreatic tissue. The tissue loss becomes even more extensive and bring about a hasty loss of exocrine pancreatic function while the disease progresses.

Serum cTLI measurement by radioimmunoassay is used for this study as it is 100% sensitive and specific for the diagnosis of EPI. 5.0 to 35.0 µg/L is the reference range for this assay. A value less than 2.5 µg/L is diagnostic of EPI. EPI is an ideal candidate for evaluation as a hereditary disease because of the high specificity and sensitivity of serum cTLI concentration for diagnosing. The disease status of any member of a family can be assessed easily by use of this assay.

PAA might be an autoimmune-mediated disease. This is indicated by recent work. PAA is theorized to progress through these 2 stages: 1- when there is active destruction of acinar tissue, lymphocytic pancreatitis, and 2- when adipose tissue, ductal structures and atypical parenchyma replace acinar tissue, end-stage EPI. This shows that PAA in German Shepherd Dogs may be caused by a gene inherited in an autosomal recessive fashion which represent an autoimmune disorder.

For participation in this study, breeders of German Shepherd Dogs were contacted for family information. Family members with EPI were identified in several German Shepherd dog families. Dogs belonging to several generations were available and 2 pedigrees were selected. As many related dogs as possible were tested for EPI whether they had clinical signs or not. When possible, previously positive tested dogs for EPI were retested.

For the measurement of serum cTLI concentration by radioimmunoassay a single serum sample was collected from each dog. To diagnose EPI for each dog, serum cTLI concentration was used. Dogs considered to have EPI had a serum cTLI concentration less than 2.0 µg/L. PAA was assumed to be the cause of EPI. For future extraction of DNA, additional blood samples were collected.

In this study 135 dogs were evaluated. In 102 of the 135 dogs, serum cTLI concentration was measured. Of the 135 dogs, 19 had EPI. 59 German Shepherd dogs consisted the first family of. Serum cTLI concentrations were measured in 48 of them. Serum cTLI concentration were lower or equal to 2.0 µg/L in 9 of the 59. Two dogs were asymptomatic for EPI but had serum cTLI concentration less or equal to 2.0 µg/L.

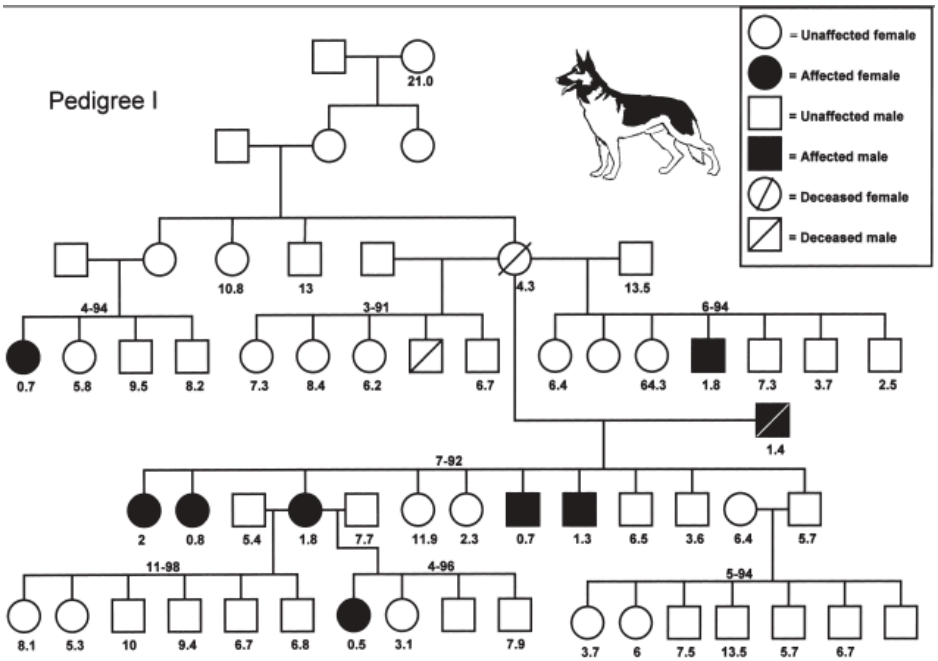


Figure 13 - Pedigree I consisting of 59 German Shepherd dogs (Moeller et al. 2002)

76 German Shepherd Dogs consisted the second family of. Serum cTLI concentrations were measured in 54 of them. Serum concentrations were less or equal to 2.0 µg/L in 10 out of the 76 dogs.

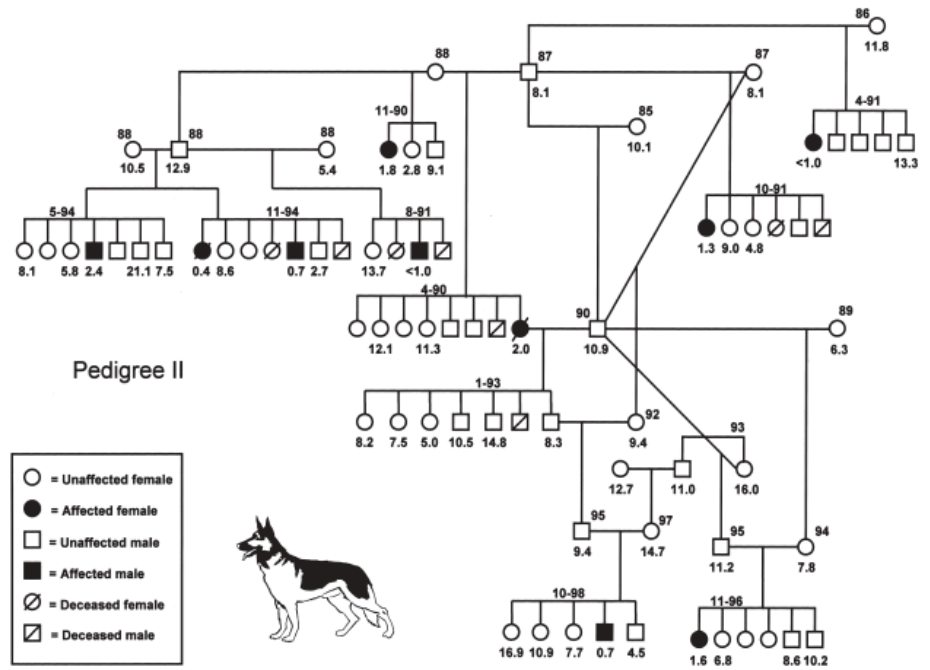


Figure 14 - Pedigree II consisting of 76 German Shepherd dogs (Moeller et al. 2002)

Several litters with unaffected parents had affected individuals. In contrast, in several litters unaffected individuals came from parents, of which at least 1 parent was affected.
Data from 7 complete litters represent pedigree I, while date from 10 complete litters represent pedigree II.

To distinguish affected dogs from unaffected dogs a cutoff point was established, which was a serum cTLI concentration of less or equal to 2.0 µg/L. All dogs with a higher level were considered unaffected while all dogs with an equal or lower level was considered affected.
PAA in German Shepherd Dogs is a hereditary disease from a clinical standpoint. By measuring a single serum cTLI concentration identification of disease status is accomplished and it can easily be diagnosed. In dogs, for diagnosing EPI, cTLI assay has been proven to be 100% specific and sensitive.
In the study of these 2 pedigrees PAA is indicated that it is not a sex-linked disease. This is because of the distribution of PAA between females and males is almost even. Only affected males would be the cause if PAA was Y-linked inheritance, which is not the case. Also many more males than females would be affected if PAA were inherited as an X-linked disease, which is not the case either.

Evidence that the putative trait for PAA is recessive is proven by dogs with EPI are produced from parents lacking clinical signs for EPI. For a simple autosomal recessive inheritance, however, the rate of affected dogs is slightly lower than expected. This can be explained by some of the dogs had not reached 4 years yet by the time of analysis and in affected dogs there was a higher rate of stillbirths. Later in life the dogs that might have been too young for the analysis may develop a low serum cTLI concentration and clinical signs later in life.

In addition, to ensure that all dogs with positive results were affected, a cutoff value was set at 2.0 µg/L for serum cTLI concentration. This may have contributed to decreasing the apparent prevalence of the disease as it may have led to a small increase in false-negative results as the cutoff value for EPI for serum cTLI concentration is less than or equal to 2.5 µg/L.

Case report from Soetart, Rochel, Drut and Jaillardon (Soetart et al. 2019)

In this case study the goal was to find out if the survival times in dogs diagnosed with EPI would be significantly influenced by the alterations in the serum concentrations of folate and/or cobalamin. Intrinsic factor is necessary for cobalamin absorption in the ileum, decreased intrinsic factor production can be caused by the loss of exocrine pancreatic function. Because of this there is a high risk of low serum cobalamin concentration for dogs with EPI. Impaired digestion and intestinal dysbiosis commonly accompanies EPI, this will also contribute in worsening the cobalamin absorption. For dogs with EPI and chronic digestive diseases, low serum cobalamin concentration is a known risk factor for a negative outcome.
Folate is specifically absorbed in the proximal intestine. It is a B-vitamin of bacterial and vegetable origin. Because of intestinal dysbiosis in EPI, serum folate concentration could be increased. In the intestine this can lead to increased bacterial folate content. Contrary, if there are extensive gastrointestinal lesions, folate absorption could be impaired.

The study was done from January 2006 to December 2012 at Oniris – Nantes Atlantic National Veterinary College in France. Clinical signs highly suggestive of EPI – like being underweight despite increased or maintained appetite and/or diarrhea – and serum cTLI level <2.5µg/L are the basis for the study. Another criteria was that the serum cobalamin and folate results had to be available from the time of diagnosis. To collect information, a survey was sent out to the veterinarian who usually cared for each dog as a follow-up.
The initial treatment after diagnosis were recorded, including type of diet, pancreatic enzyme replacement, cobalamin supplementation and antibiotic therapy. A survey using a 4-point scale was sent out to the veterinarians and dog owners to subjectively evaluate the global efficacy of initial treatment: 1 – which was the absence of improvement, to 4 – which was the resolution of all clinical signs. A score was given where 1 or 2 was set as “poor” and 3 or 4 was set as “good” for the response to initial treatment (RIT).

A canine specific chemoluminescence enzyme immunoassay (cTLI canine, Siemens Immulite) was used to asses serum cTLI. The lowest detectable concentration (LDC) was 0.3µg/L and the inter- and intra-assay coefficients of variation (CVs) were 6.2% and 5.0%, respectively.

Using a radioimmunoassay (SimulTRAC-SNB, MP Biomedical), cobalamin and folate assays were performed simultaneously. For cobalamin and folate, LDC was 75ng/L and 0.6µg/L, respectively. CVs for intra- and inter-assay were 5.7% and 8.5% for cobalamin and 3.5% and 13.5% for folate. The upper limits for linearity ranges were 3000ng/L for cobalamin and 20.0µg/L for folate. For cobalaminemia and serum folate concentration, 350-850ng/L and 3-12µg/L, respectively, were the reference ranges for dogs established by the laboratory.

During the study period 882 out of the 11 682 TLI assays that were run, met the inclusion criteria and that is the amount of surveys that were sent out to the veterinarians. 299 out of the 371 surveys that were returned contained enough information to be included in the study.

German Shepherd dogs, Rough Coated Collies and Cavalier King Charles spaniels were the three main breeds reported. Compared to the other breeds the proportion of male German Shepherd dogs was significantly higher. However, in the population as a whole, there was no sex bias. 3.9 years was the median age at diagnosis, however, compared to the other breeds, Cavalier King Charles spaniels were significantly older. This further confirms the fact that in this breed, EPI is most likely due to end-stage chronic pancreatitis. The main clinical signs were polyphagia, underweight and diarrhea. The median was 3 months of duration of clinical signs before diagnosis.

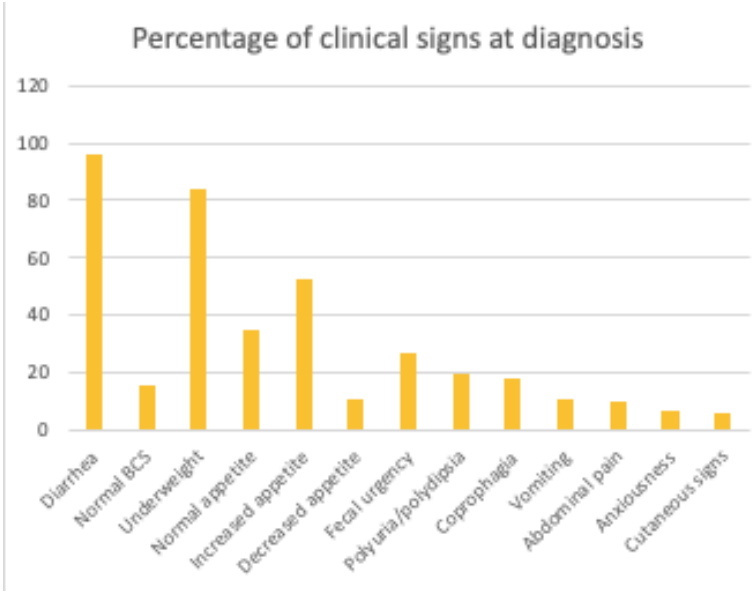


Figure 15 - Clinical signs seen at the time of diagnosis (Soetart et al. 2019)

Prior to the diagnosis, none of the dogs had received cobalamin. In approximately half of the dogs, hypocobalaminemia (cobalamin <350ng/L) occurred. A few of the dogs had hypercobalaminemia (cobalamin >850ng/L). Dogs with hypocobalaminemia, compared to the dogs with normal serum cobalamin concentrations, were significantly older with a median at 4.6 and 2.5 years, respectively.
Some of the dogs had serum folate concentrations within the reference interval while the majority had elevated serum folate concentration. There was no age difference between these two groups.
In approximately a third of the dogs, concurrent decreased serum cobalamin concentrations and elevated serum folate concentration were recorded.
For dogs with hypocobalaminemia and the dogs with elevated serum folate concentrations the duration of clinical signs was longer than the dogs with normal serum cobalamin concentrations.

RIT was considered good for the majority of the dogs out of the 224 dogs where information was available. The majority received a cobalamin supplementation out of the dogs with hypocobalaminemia at diagnosis. Due to lack of information the doses were impossible to calculate.

Approximately half of the dogs were given antimicrobials, mainly the dogs showing hyperfolatemia at diagnosis. A majority of the dogs were given oral enzyme replacement which was the only factor notably correlated with a poor RIT.

Between the diagnosis and interviews the median time was 4.4 years. Approximately the same percentage of dogs were reported dead and alive at the end of the study. However, a portion were lost to follow-up. For dogs with EPI, 4.8 years was the median OST.
The majority of the deceased dogs were euthanized, some because of treatment costs, some because of persistent clinical signs despite treatment and approximately half of them for other causes not associated with EPI directly.

Older age, male sex, decreased appetite, long duration of clinical signs before diagnosis, no enzyme replacement therapy administered, low serum cobalamin concentration and poor RIT was factors associated with an earlier death. Associated with a better prognosis was normal

body condition. Although it did not achieve statistical significance, more dogs with high serum folate concentration at diagnosis had a good prognosis.

Hypocobalaminemia and decreased appetite at diagnosis were significantly associated with a shorter survival time. As was male sex and the absence of enzyme replacement therapy. A good prognostic factor was hyperfolatemia at diagnosis. Dysorexia at diagnosis was clearly associated with premature death, although it was an uncommon clinical sign in this study, otherwise none of the clinical signs of EPI affected the survival time.
The strongest and most significant favorable prognostic factor was the use of an enzyme supplementation therapy.

Discussion

In the Batchelor et al. 2007 report about breed associations for canine exocrine pancreatic insufficiency it was found that German Shepherd dogs was highly overrepresented (Batchelor, Noble, Cripps, et al., 2007). This is also found in the report of the same author about prognostic factors in canine pancreatic exocrine insufficiency, and also in other papers (Soetart et al., 2019; Wiberg, Nurmi, et al., 1999). The study of Moeller is done solely on German Shepherds which indicates that the breed is overrepresented with EPI (Moeller et al., 2002).
Rough Coated Collies and Cavalier King Charles spaniels are also highly overrepresented (Batchelor, Noble, Cripps, et al., 2007; Soetart et al., 2019). However Labrador Retrievers, Golden Retrievers, Rottweilers, Weimaraners and Boxers were underrepresented with EPI (Batchelor, Noble, Cripps, et al., 2007). The other reports did not have data on this.

According to Soetart the proportion between male and female dogs diagnosed with EPI is even (Soetart et al., 2019). However compared to other breeds the proportion of male German Shepherd dogs was significantly higher. In the study of Wiberg the majority of EPI cases are male dogs (Wiberg, Nurmi, et al., 1999). Contrary, Batchelor et al. 2007 report about prognostic factors in canine pancreatic exocrine insufficiency has an overrepresentation of female dogs (Batchelor, Noble, Taylor, et al., 2007). Batchelor et al. 2007 report about breed associations for canine exocrine pancreatic insufficiency there is also seen an overrepresentation of female dogs (Batchelor, Noble, Cripps, et al., 2007). However, this is only seen in German Shepherd dogs, Chows and Cavalier King Charles spaniels. In Rough-Coated Collies there was no sex association observed. Moeller et al. has an almost even distribution of females and males, indicating that it is not a sex-linked disease (Moeller et al., 2002).

In the Batchelor et al. 2007 report about breed associations for canine exocrine pancreatic insufficiency the overall median age was 42 months (3,5 years) at the time of diagnosis, however the range was from 3-204 months. In addition, Cavalier King Charles spaniels were older than German Shepherd dogs, Chows and Rough-Coated Collies (Batchelor, Noble, Cripps, et al., 2007). Soetart et al. also noted that Cavalier King Charles spaniels were significantly older than other breeds at the time of diagnosis. The median age in this study in general was 3.9 years (Soetart et al., 2019). Batchelor et al. 2007 about prognostic factors in canine pancreatic exocrine insufficiency has the median age as 3 years at the time of diagnosis in German Shepherd dogs. However in non-German Shepherd dogs, the median age was 4 years

old (Batchelor, Noble, Taylor, et al., 2007). Wiberg et al. has the age median as 3.3 years however it ranged from 1 to 8 years (Wiberg, Nurmi, et al., 1999).
Comparing the studies, two says that Cavalier King Charles spaniel is diagnosed at an older age compared to other breeds (Batchelor, Noble, Cripps, et al., 2007; Soetart et al., 2019) and the other says that German Shepherd dogs is diagnosed at a younger age compared to other breeds (Batchelor, Noble, Taylor, et al., 2007). All these studies have a median age between 3 and 4 years which amplifies the probability that this is the case.
Moeller et al. concludes that by the age of 5 years affected dogs usually have clinical signs of EPI, which also confirms that the median age is around 3-4 years (Moeller et al., 2002). Some dogs may show signs as early as around 1 year of age also (Moeller et al., 2002).

Wiberg et al. 1999 collected data of 1,236 dogs and the most common clinical signs were diarrhea and weight loss (Wiberg, Nurmi, et al., 1999). The most frequent clinical signs according to Moeller et al. using 135 dogs were steatorrhea, voluminous feces, polyphagia and weight loss. In addition the feces were usually loose in texture, light in color and malodorous (Moeller et al., 2002). Soetart et al. found by summarizing the data of 11,682 patients that polyphagia, underweight and diarrhea are the main clinical signs as well (Soetart et al., 2019). Batchelor et al. 2007 report about prognostic factors in canine pancreatic exocrine insufficiency had diarrhea, polyphagia and underweight as the most common clinical signs in 743 dogs (Batchelor, Noble, Taylor, et al., 2007). In addition some dogs had vomiting, nervousness and aggressiveness as clinical signs. Most dogs were underweight with polyphagia, however some dogs were overweight and some were inappetent or had a normal appetite (Batchelor, Noble, Taylor, et al., 2007). It can be concluded summarizing all together the data of 13,796 dogs, that the most common clinical signs according to all the papers are the diarrhea and weight loss, and the third is the polyphagia.

The Batchelor et al. 2007 study was done from 1990 to 2002 (Batchelor, Noble, Cripps, et al., 2007), the Wiberg et al. 1999 study was done from 1995 to 1997 (Wiberg, Nurmi, et al., 1999) and the Soetart et al. study was done from 2006 to 2012 (Soetart et al., 2019). The Batchelor et al. and Soetart et al. study was done for a longer period of time than the Wiberg et al. 1999 study (Batchelor, Noble, Cripps, et al., 2007; Soetart et al., 2019; Wiberg, Nurmi, et al., 1999). In addition the Batchelor et al. study had a record of over 13 000 dogs tested, the Wiberg et al. study had over 1200 dogs tested and the Soetart et al. study had a record of over 11 000 dogs tested (Batchelor, Noble, Cripps, et al., 2007; Soetart et al., 2019; Wiberg, Nurmi, et al., 1999).

Given the longer time period and a larger number of recorded dogs, this might make the Batchelor et al. and Soetart et al. study more reliable (Batchelor, Noble, Cripps, et al., 2007; Soetart et al., 2019).
Also the Batchelor et al. 2007 report about prognostic factors in canine pancreatic exocrine insufficiency had 743 recorded dogs (Batchelor, Noble, Taylor, et al., 2007), the Moeller et al. study had 135 recorded dogs (Moeller et al., 2002). Which is a lot less than the other studies as well. According to those two papers the most relevant prognostic factors are enzyme replacement therapy, proper follow-up and age collected about a 1000 patients (Batchelor, Noble, Taylor, et al., 2007; Moeller et al., 2002).

In all the reports, the dogs are tested with cTLI by RIA. This is highly specific and sensitive for EPI.

Comparing these study cases to other literary data the breed disposition is most commonly found in German Shepherd dogs, which is also the case in these studies. Rough-Coated Collies, Cavalier King Charles spaniels and Chows are also overrepresented breeds. However, in some of these studies several other breeds are pointed out as being overrepresented as well. There is no other data supporting this.
The general age at the time of diagnosis is 1 to 5 years. In these studies there is an overall median at around 3 to 4 years of age, which is within the same age gap.
In these studies some show an overrepresentation of females (Batchelor, Noble, Cripps, et al., 2007; Batchelor, Noble, Taylor, et al., 2007) while others show an overrepresentation of male dogs (Wiberg, Nurmi, et al., 1999). Two studies show an even distribution of females and males (Moeller et al., 2002; Soetart et al., 2019). Female dogs are overly represented with EPI shown by literary data. The studies with an overrepresentation of males might be explained by an overrepresentation of males in the study or too few dogs in the study to get an accurate percentage.

Increased defecation frequency and fecal volume, loose feces, weight loss, polyphagia, aggressiveness and nervousness are the most common clinical signs in both literary data and the case studies. In addition, literary data has skin disorders which is not found in the study cases.

In conclusion the study cases are fairly accurate to literary data. There are some deviations especially for sex predisposition. The breed predisposition in some of the studies are not proven. In general EPI is diagnosed in young to medium aged dogs. German Shepherd dogs is highly predisposed. Rough-Coated Collies, Cavalier King Charles Spaniels and Chows are also predisposed. Female dogs has a higher representation of EPI compared to males. The most common clinical signs are diarrhea, increased defecation frequency and volume, poorly digested and loose feces, weight loss and polyphagia.

Summary

Exocrine pancreatic insufficiency is a disease where the pancreas is not able to secrete sufficient amount of pancreatic enzymes. The most common causes of EPI are PAA and chronic pancreatitis. German Shepherd dogs in particular is a highly predisposed breed to get EPI caused by PAA. Rough-Coated Collies and Chows are also predisposed breeds. Usually middle-aged to older, small- or medium-breed dogs like Cavalier King Charles spaniels, Collies and English Cocker Spaniels are predisposed breeds to get EPI caused by end-stage chronic pancreatitis. EPI is generally diagnosed between 1 and 5 years of age. Female dogs are overrepresented compared to male dogs.

The most characteristic clinical signs for EPI are diarrhea, increased fecal volume with loose, poorly digested feces, polyphagia and weight loss. The feces is yellowish, poorly digested and pulpy feces. Several skin disorders and vomiting may also be seen. Nervous and aggressive dogs because of abdominal discomfort can also be seen. It can be difficult setting a diagnosis based on clinical signs as small intestinal diseases can cause many of the same clinical signs.

EPI is diagnosed based on clinical signs and history and confirmed with cTLI. It is highly specific and sensitive. It is also pancreas and species-specific. It is considered the best test for detecting EPI. Normal cTLI levels are between 5.0 and 35.0 µg/L. Levels under 2.5 µg/L are diagnostic for EPI. Clinical signs are usually not seen until the end-stage phase of the disease as 90% of the pancreas´ secretory capacity is lost before clinical signs are seen. This is because the pancreas has a large reserve secretory capacity.

The optimal treatment regimen for EPI is not known. Enzyme replacement therapy is suggested as initial treatment for EPI. Powdered enzymes or raw chopped pancreas as nonenteric-coated supplements gives the highest enzyme activity in the duodenum in dogs. However, not all dogs respond well enough to this. Antibiotics is sometimes given as adjunctive medication.

As the disease can be successfully treated in most dogs the prognosis for EPI is good. However, studies show that many dogs are euthanized because of high cost of treatment, refusal by owners to continue treatment and failure to respond to treatment. Scheduling regular follow-up appointments is very important to address concurrent problems during treatment and change management as necessary.

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