Evaluation of the SNAP® CPL and Spec CPL® Canine Pancreatic Lipase Tests for Acute Pancreatitis in Dogs Presenting with Clinical Signs of Acute Abdominal Disease

Thesis
Submitted to the Faculty of the Murdoch University School of Veterinary and Life Sciences in the partial fulfilment of the requirements for the degree of Research Masters with Training

By

Mark D. Haworth

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DECLARATION
I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Mark Haworth
ABSTRACT

Canine acute pancreatitis (AP) is commonly encountered in veterinary clinical practice. Acute pancreatitis can occur on its own as a primary disease or can be secondary to other diseases. Early recognition and appropriate management of dogs with AP can be hampered by difficulty in not only diagnosing the disease, but also differentiating primary versus secondary pancreatitis. Failure to recognise that AP is actually the result of another underlying disease may lead to inappropriate management of the patient. This is especially important in critically ill dogs that present to an emergency service.

Historically, catalytic assays have been used to diagnose AP but the tests have shown poor reliability. These tests have largely been criticised for lack of sensitivity and specificity, as pancreatic enzyme activity may not solely represent pancreatic leakage. Recently, a laboratory-based canine specific pancreatic lipase test (Spec cPL®) has been developed to aid diagnosis of AP, which is specific to the detection of pancreatic lipase leakage. To date, there are few studies evaluating the Spec cPL®, but it was developed in the hope it would provide a more definitive diagnosis of AP. Questions have been raised as to its accuracy as a diagnostic test, and it still does not differentiate between primary and secondary pancreatitis.

An in-house canine pancreatic lipase test (SNAP® cPL) has also become available to clinicians. The manufacturer has reported very good agreement between the Spec cPL® and SNAP® cPL, using recombinant canine pancreatic lipase (cPL), but this is yet to be tested clinically. The first aim of this study therefore was to measure agreement between these two tests in dogs presenting with clinical signs of acute abdominal disease. The second aim was to determine the degree of agreement (if any) between a clinical diagnosis of primary AP and increased cPL concentration, as measured by the above two tests. The third aim was to establish the sensitivity and specificity for these tests in dogs with clinically diagnosed primary AP.
The results of this study demonstrated that the SNAP® cPL and Spec cPL® tests had good agreement with each other for the entire cohort of dogs. However, there was poor agreement between each of these tests and a clinical diagnosis of pancreatitis. While sensitivity did not differ markedly from previous reports on Spec cPL®, specificity results were much lower. Therefore, it appears that these tests can detect the presence of cPL but cannot be used in isolation to diagnose primary pancreatitis.
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DEDICATION

To Catriona and my daughters Aurora and Freya. Thank you.
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CHAPTER 1: INTRODUCTION AND OVERVIEW

Canine pancreatitis, in all its forms, has remained an area of intense research for decades and it is likely to continue to generate much debate and research in this decade and the next. Many diagnostic tests have been suggested as important for the investigation of canine pancreatitis. However, no single test to date has been demonstrated to clearly possess a high sensitivity and many suffer poor specificity. Much of the research focusing on singular tests lacks a comparison to a ‘gold standard’. Obtaining diagnostic material for the gold standard test of pancreatitis is also problematic given the invasiveness necessary for pancreatic histology. In addition, despite the publication of studies comparing the test under investigation to a gold standard, there now appears to be emerging discord among researchers as to the accuracy of the gold standard diagnosis of pancreatitis itself (Mansfield et al., 2012). This could potentially confound interpretation of previous research and prohibit meaningful meta-analysis of studies concerned with the diagnosis of pancreatitis.

A non-invasive, cheap, sensitive and specific cage-side test in the emergency setting is desirable for detection of canine pancreatitis, a disease which can present with non-specific clinical signs (Hess et al., 1998). Therefore, detection of pancreatic lipase has recently been posited as an important diagnostic tool for canine pancreatitis as this enzyme is specific to the pancreas (Steiner et al., 2006; Steiner et al., 2008). The canine pancreas-specific lipase (Spec cPL®) laboratory test designed to do this often requires a 24 hour wait for the result, potentially delaying timely and specific intervention required for non-pancreatic disease. The new SNAP® cPL is a cage-side test designed for immediate assessment, and can later be verified with the Spec cPL®.

Over-reliance on these tests by clinicians, particularly in isolation, can lead to misdiagnoses. A plethora of research on the methods for diagnosis of pancreatitis in dogs has been published over a period of decades utilising various cohorts and methodologies. Discerning both the most useful and most relevant information from this body of research is challenging. Consequently, the interpretation of the clinical utility of these diagnostic tests may be subject to misconceptions. The canine specific pancreatic lipase assays (Spec cPL® and SNAP® cPL)
were developed to directly detect the presence of pancreatic lipase, and not just the activity of lipases in general (Steiner et al., 2002, Steiner et al., 2003). These tests initially appeared to offer an exciting development, garnering much interest amongst veterinary clinicians. However, most of the research conducted on these nascent tests has not applied stringent diagnostic guidelines, as discussed in Chapter 2, therefore raising questions about their accuracy. For example, some cohorts of dogs under study have included individuals that, by some standards, would not be considered suitable candidates for these tests in the clinical setting (see Chapter 2).

The prevalence of primary acute canine pancreatitis, as well as its presence as a co-morbid process in a range of canine diseases, remains unknown. The Spec cPL® and SNAP® cPL have been available worldwide for several years, and as more data is generated from their clinical use, more questions arise. Anecdotal communication between the author and many veterinary clinicians has revealed frustration; some veterinarians report dogs testing positive for increased pancreatic lipase, which then had final diagnoses that required specific intervention for diseases other than pancreatitis. Initially it was thought that measuring pancreatic lipase would be the silver bullet for diagnosis, as this enzyme is unique to the pancreas. However, the clinical significance of canine pancreatic lipase elevations as a sole marker for primary pancreatitis should be approached with caution given pancreatic inflammation can occur secondary to other pathologic processes. Although veterinary practitioners should consider all potential diagnoses for dogs with appropriate clinical signs, over-reliance on a diagnosis of primary pancreatitis, based on these tests, could decrease recognition of a co-morbid disease. Failure to recognise a potentially devastating underlying disease could have a severe, or even fatal, outcome for the patient, and prove financially onerous for the owner.

The volume of published information available; the often conflicting nature of this information; and the ever-changing nature of the investigation of acute canine pancreatitis, demands a comprehensive review of the literature. Therefore, the first objective of this thesis was to provide a robust and comprehensive review of the literature surrounding acute canine pancreatitis, focussing particularly on its diagnosis.
The second objective was to determine the sensitivity, specificity and accuracy of the SNAP® and Spec cPL® tests in dogs presenting with acute abdominal disease to an emergency centre. The study undertaken here differs from previous investigations of these tests. Previous research has used pancreatic histopathology that could only be gained post-mortem, and correlated the changes with certain blood markers, such as canine pancreatic lipase. This limits the cohort of dogs under study to those that have died of their disease, or were euthanased, and often the latter predominates. In many dogs in these reports, the reason for euthanasia was not reported, or included shelter dogs. The determination of sensitivity and specificity of a diagnostic test should include a subpopulation of dogs representative of the greater population that are suspected of having the disease in question. Canine pancreatitis often presents with non-specific signs which include vomiting, anorexia, and abdominal pain. It was the intention of this study to include dogs presenting with signs consistent with acute pancreatitis in order to assess the accuracy of canine pancreatic lipase in a representative clinical cohort.

The third objective of this thesis was to quantify the agreement between the SNAP® and Spec cPL® tests using paired serum samples taken from dogs in the cohort described. The newer point-of-care SNAP® cPL test has been investigated in only one recent publication and its agreement with the laboratory Spec cPL® was not independently verified. The two tests utilise different methodologies applying the same basic principles. It is important to establish that these tests are indeed measuring the same target enzyme, in order to validate the point-of-care results.
CHAPTER 2: REVIEW OF THE LITERATURE – CANINE ACUTE PANCREATITIS

2.1 Introduction

Acute abdominal disease is usually defined as severe abdominal pain of less than 24-hours duration. Dogs that present to a veterinarian with acute abdominal disease require timely intervention. After initial clinical evaluation, it is vital to differentiate between medical and surgical cases early in the management process as this may have a significant effect on outcome. This differentiation can be difficult given that the choice of diagnostic tests is often limited in the emergency setting and the history, clinical examination and routine laboratory abnormalities can be non-specific. In cases of acute pancreatitis, the majority of dogs can be managed successfully medically (Thompson et al., 2009). Inappropriate surgical intervention can be costly and may increase morbidity and mortality. But, as noted in the previous chapter, accurate diagnosis of acute pancreatitis has proven challenging over the years. This thesis will focus on acute pancreatitis and current diagnostic testing and will not attempt to characterise or contrast this acute process with chronic pancreatitis.

There are practical limitations associated with research into the diagnostic process for acute pancreatitis. There remain many areas where a consensus has yet to be reached, including determination of the most appropriate "gold standard" test and also the most accurate point-of-care (POC) diagnostic test for this disease. The patient history, clinical examination findings and routine clinical pathology in canine acute pancreatitis are often non-specific; hence there is a need for a simple cage-side test that provides a rapid and reliable diagnosis. Enzyme-linked immunosorbent assay (ELISA) tests have been developed for this purpose by Idexx Laboratories (Westbrook, Maine, USA). The test originally devised was the canine pancreatic lipase immunoreactivity (cPLI), however, the commercial application demanded a more efficient and cost effective method. The quantitative Spec cPL® and then the semi-quantitative point-of-care (POC) SNAP® cPL were developed, which utilise the same capture and detection antibodies. However, research focused on the sensitivity and specificity of these tests has indicated that they may have limited clinical usefulness as sole diagnostic tests for acute
2.2 Definition of Acute Pancreatitis

In people, acute pancreatitis has been defined as an acute inflammatory or necrotising process of the pancreas that does not permanently disrupt the pancreatic architecture and has variable systemic complications, including death (Bradley 1993). Histological changes in canine acute pancreatitis primarily include the presence of neutrophils in the pancreas or peripancreatic fat without evidence of fibrosis or exocrine pancreatic atrophy (Newman et al., 2006). Acute pancreatitis may vary from mild to severe forms, such as acute necrotising pancreatitis, which is characterised by substantial pancreatic and peri-pancreatic necrosis (Feldman et al., 1981). This severe form can lead to necrosis of pancreatic acinar tissue and systemic complications, including multiple organ failure and, frequently, death of the dog (Mansfield et al., 2003).

Other underlying diseases, such as those causing inflammation regional to the pancreas, can also cause acute pancreatitis (Frossard 2008). For the purpose of this thesis, this sort of acute pancreatitis is defined as secondary pancreatitis, as opposed to primary pancreatitis where an underlying cause is not identified.

2.3 Aetiology and Risk Factors

The precise cause of canine pancreatitis in the majority of cases is usually unknown and therefore most are considered to be idiopathic (Hess et al., 1998; Watson 2004; Steiner 2010). Many risk factors for the development of acute pancreatitis have been investigated yet most of these associations remain tenuous at best.

Signalment of the patient is considered an important risk factor. In a necropsy study examining breed disposition for the development of pancreatitis, a total of 151 pancreata of dogs from first opinion practices were sequentially examined, after autolysed cases were removed from the analysis. Three histological sections from each dog were examined for evidence of pancreatic
pathology. Cavalier King Charles Spaniels, Collies, and Boxers showed evidence of chronic pancreatitis, and Cocker Spaniels had evidence for both acute and chronic pancreatitis (Watson et al., 2007). Miniature Schnauzers and Yorkshire Terriers have been reported to be over-represented for pancreatitis in other studies (Schaer 1979; Cook et al., 1993; Hess et al., 1999; Steiner JM et al., 2010). In one of these studies involving 70 dogs (Hess et al., 1999), the odds ratio for Yorkshire Terriers developing fatal acute pancreatitis was calculated to be 41.8 (95% CI: 1.0-1731). However, there appears to be conflicting evidence for breed susceptibility in the available literature; these same authors (Hess et al., 1999), considered the Miniature Poodle to be at decreased risk for fatal acute pancreatitis yet another study including 101 dogs identified this breed at increased risk for pancreatitis (Cook et al., 1993). Such differences in the veterinary literature may reflect other factors such as the genetic composition and breed frequencies of the populations under study, rather than true variations in susceptibility. However, not all dogs in the study conducted by Cook et al. were confirmed pancreatitis and some dogs were included on the basis of clinical signs and elevations in amylase and lipase activities alone. Conversely, all dogs in the study by Hess et al. were confirmed pancreatitis. However, all the dogs in this study had fatal pancreatitis. It is difficult to compare these two studies on the basis of the disparity between severity of disease in each population and for possible misallocation of dogs into the pancreatitis group in the Cook et al. study.

Despite the evidence that some breeds may be at increased risk of developing acute pancreatitis, hereditary predisposition has not been proven conclusively. Pancreatic secretory trypsin inhibitor (PSTI) is a protective protein encoded by the SPINK1 gene that serves to protect against premature activation of trypsinogen within the pancreas (Bishop et al., 2010). In a study (Bishop et al., 2010) comprising sixty-four Miniature Schnauzers (39 with a history of pancreatitis and 25 without), the SPINK1 gene was sequenced in each dog and three variants were detected in each group. Those with a history of pancreatitis were 9.5 times more likely to have at least a single copy of the three variant alleles compared to the healthy group. Further, the proportion of dogs with homozygous alleles for these variants compared with dogs heterozygous or having wild-type alleles, yielded an odds ratio (OR) for pancreatitis in the homozygous group of 3.4. The authors concluded that the variants in the SPINK1 gene likely played a role in the development of pancreatitis in Miniature Schnauzers but conceded that other genetic and environmental factors may also contribute (Bishop et al., 2010).
A subsequent study investigating heritability in Miniature Schnauzers found no relationship between variations in the SPINK1 gene and pancreatitis (Furrow et al., 2012). This case control study assessed 17 Miniature Schnauzers with pancreatitis and 60 Miniature Schnauzers with no significant gastrointestinal history. Further, the control dogs were older in this study compared to the study performed by Bishop et al. and therefore more representative of the population of dogs that are likely to be diagnosed with pancreatitis.

Another study investigating a genetic relationship with canine pancreatitis focussed on the cystic fibrosis transmembrane conductance regulator (CFTR) gene (Spadafora et al., 2010). Atypical CFTR mutations are associated with pancreatitis in humans. This study screened for canine CFTR in 400 dogs. The study included 203 dogs with pancreatitis and 174 control dogs that were unwell for various reasons. Twenty-eight dogs were identified with one of four CFTR missense mutations. No relationship was identified between dogs with pancreatitis and control dogs.

Age has also been suggested as a risk factor for acute pancreatitis; middle aged to older dogs have been identified at increased risk in some retrospective studies (Cook et al., 1993; Hess et al., 1999). Hess et al., (1999) reported pancreatitis was more likely to develop in older dogs than in dogs less the one year of age, with an odds ratio of 27.5 (95% CI: 3.5-219) for dogs 5 to 9 years old, and 36.9 (95% CI: 4.4-307) for dogs 10 years or older, and Cook et al., (1993) reported pancreatitis was more likely in dogs older than 7 years.

One investigation into sex predilection for the development of pancreatitis reported that neutered males, sexually intact males and neutered females were more at risk of fatal acute pancreatitis than sexually intact females with odds ratios of 7.9 (95% CI: 0.5-128.6), 11.3 (95% CI: 0.9-140.4), and 22.0 (95% CI: 1.8-272.4) respectively, when adjusted for age (Hess et al., 1999). In contrast, the study by Cook et al., (1993) involved a larger sample size and reported neutered males, females, and sexually intact females were at greater risk of pancreatitis than sexually intact males with odds ratios of 2.54 (95% CI: 0.97-6.65), 2.30 (95% CI: 0.75-7.08), and 2.31 (95% CI: 1.09-4.89), respectively.
There have been many reports linking dietary indiscretion to pancreatitis in dogs. In one recent retrospective study, 198 dogs with a clinical diagnosis of pancreatitis (and 187 control dogs with a diagnosis of renal failure) demonstrated that ingestion of unusual food, such as table food or food different from the dog's usual diet, increased the risk for developing pancreatitis (OR: 4.3 95% CI: 1.7-10.7). This risk increased even further to 13.2 (95% CI: > 2.1) if the dog had access to "garbage" (Lem et al., 2008).

The ingestion of a meal with a high fat content may precede canine acute pancreatitis and lipaemia may be present at the time of diagnosis (Cook et al., 1999; Hess et al., 1999). In some studies it has been shown that pancreatitis is more likely to develop in obese dogs after feeding high-fat diets or when they have high serum triglyceride concentrations, and has an increased severity when induced in dogs being fed a high-fat diet (Lindsay et al., 1948; Goodhead 1971; Hess et al., 1999; Xenoulis et al., 2010). Obesity may be more prevalent in older dogs and therefore may be a confounding factor (Mason 1970).

Severe hypertriglyceridaemia is an established risk factor for pancreatitis in people (Cameron et al., 1974; Toskes 1990; Linares et al., 2008). Serum triglycerides may be hydrolysed by pancreatic lipase, producing excessive amounts of free fatty acids, which are toxic to the pancreas (Havel 1969; Saharia et al., 1977; Xenoulis et al., 2010). An alternate theory is hyperviscosity in the capillaries of the pancreas secondary to elevated concentrations of chylomicrons (Tsuang et al., 2009). These theories have been called into question because although there is a recognised threshold blood concentration of triglycerides which will predispose to pancreatitis in humans, there is no correlation above that threshold with severity (Talukdar et al., 2009).

In an experimental model using isolated perfused canine pancreata, increased concentration of triglycerides induced pancreatic injury (Saharia et al., 1977). Despite this, hypertriglyceridaemia is not conclusively a cause of pancreatitis in dogs and may be simply a secondary effect of the pancreatitis itself. Recently (Xenoulis et al., 2011), triglyceride concentrations in 17 Miniature Schnauzers with a history of pancreatitis and 34 age-matched controls (also Miniature Schnauzers) without a history of pancreatitis were compared. Hypertriglyceridaemia was documented, even during quiescent periods, for the dogs with a history of pancreatitis, with an
odds ratio of 5.02 compared to the controls. The study concluded that further investigation was required to clarify the role of hypertriglyceridaemia as a predisposing factor of acute canine pancreatitis (Xenoulis et al., 2011).

Concurrent disease is a common finding in cases of canine acute pancreatitis but it is difficult to determine if this is a significant and specific risk factor, since many diseases such as endocrinopathies, renal disease and neoplasia are more common in older dogs. Diabetes mellitus, hyperadrenocorticism, and hypothyroidism have been reported as risk factors for pancreatitis in two studies with a combined total of 171 dogs (Cook et al., 1993, Hess et al., 1999). It is plausible that disorders of fat metabolism that predispose to hypertriglyceridaemia may also result in the development of pancreatitis. However, these associations have been described as weak at best (Steiner 2010).

Certain drugs and medications have been implicated in canine pancreatitis, but a direct causal link is usually lacking. Reports of some drugs inducing pancreatitis in people appear to have been adopted into the veterinary literature, not always with sufficiently robust evidence. Drugs commonly cited in both people and dogs as potentially causing pancreatitis include organophosphates, clomipramine, L-asparaginase, azathioprine, thiazides, frusemide, oestrogens, sulpha drugs, tetracycline, procainamide, and propofol (Frick et al., 1987; Teske et al., 1990; Lankisch et al., 1995; Trepanier 2004, Watson 2004; Steiner et al., 2008; Kook et al., 2009; Steiner 2010, Schleis et al., 2011). In the veterinary literature, specific investigation of this association has been limited to azathioprine, potassium bromide, phenobarbital, and corticosteroids (Broe et al., 1983; Moriello et al., 1987; Housten et al., 1991; Gaskill et al., 2000; Steiner et al., 2008). Prednisone and dexamethasone have been investigated as a cause of canine pancreatitis but no evidence has supported this hypothesis (Parent 1982; Fittschen et al., 1984; Steiner et al., 2009). Elevated canine pancreatic lipase immunoreactivity concentrations were recorded in dogs receiving either potassium bromide, phenobarbitone, or a combination of the two drugs; however the authors were unable to establish a direct link with pancreatitis (Steiner et al., 2008). Azathioprine has also been implicated in case reports of dogs undergoing immunosuppression, but many of these dogs were concurrently receiving prednisolone, thus confounding any robust conclusions (Houston et al., 1991; Moriello et al., 1987). Furthermore, a definitive confirmation of pancreatitis was lacking in both of these reports.
To investigate this further an isolated ex-vivo canine pancreas model was used to study the effect of azathioprine on the secretory functions of the pancreas (Broe et al., 1983). These authors demonstrated a significant increase in the secretory volume and bicarbonate output, and a profound decrease in trypsin output in azathioprine-perfused pancreata but there was no change in the appearance or weight compared to controls. They concluded that azathioprine had a marked effect on pancreatic function but did not elaborate further.

Pancreatitis has also been linked to hypercalcaemia, after a dog with idiopathic hypocalcaemia that was supplemented with calcium developed the disease (Neuman, 1975). Zinc-induced pancreatic lesions appear to be well documented in many species, including the dog (Charles 2007), and pancreatitis has been associated with zinc toxicosis in a Beagle, but this dog was also receiving potassium bromide for seizures, thus complicating the determination of causality (Mikszewski et al., 2003).

Pancreatitis secondary to pancreatic duct obstruction may be due to neoplasia, chronic inflammation and fibrosis, as well as luminal occlusion of the pancreatic or common bile duct by parasites, inflammatory exudate, scar tissue, and pancreatoliths (Charles 2007). The majority of non-alcoholism-associated cases of human pancreatitis are attributed to gallstone obstruction of the bile or pancreatic ducts leading to increased intra-ductal pressure and subsequent premature activation of pancreatic enzymes (Cappell 2008). One study evaluating risk factors for pancreatitis concluded that obstruction of the pancreatic duct was the most likely inciting cause in two dogs with pancreatic neoplasia (Cook et al., 1993). However, conclusive cases of acute pancreatitis caused by naturally-occurring partial or complete obstruction of the pancreatic duct have not been reported. A study of surgical ligation of the bile duct in eight dogs that were subsequently necropsied at 8 weeks (4 dogs) and at 20 weeks (4 dogs) revealed fibrosis and atrophy of the pancreas, but no evidence of pancreatitis (Simpson et al., 1989).

Duodenal reflux into the pancreatic duct occurs in normal dogs within 1 to 2 hours of feeding (Hendricks et al., 1980) and it has been suggested that in some dogs this backflow of activated pancreatic enzymes and bile salts may cause ductal necrosis, predisposing to the development of acute pancreatitis (Williams 1996; Watson 2004).
Finally, ischaemia and reperfusion have been demonstrated to cause pancreatitis in experimental models (Broe et al., 1982; Sanfey et al., 1985), and a meta-analysis of the human medical literature from 1966-2005 (Cuthbertson et al., 2006) revealed evidence that disturbances of both systemic and pancreatic microvasculature contribute to the development of pancreatitis. Similar studies have not been undertaken in dogs. Postoperative pancreatitis has also been reported in people with direct trauma, embolisation, and hypotension (White et al., 1970). Trauma associated with biopsy of the pancreas has also been a concern for triggering pancreatitis in dogs. However, in a small study including four healthy Beagles, biopsy of the pancreas in three individuals combined with careful tissue handling was shown to be safe, with no changes in clinical health in the dogs during or after the procedure (Harmoinen et al., 2002).

2.4 Pathogenesis

Although the cause(s) of acute canine pancreatitis remain uncertain (see 2.3 above) it is generally accepted that pancreatitis develops as a result of autodigestion consequent to the premature activation of enzymes within the pancreatic acinar cells, irrespective of inciting cause (Simpson 1993).

Pancreatic enzymes are secreted via exocytosis from the apices of acinar cells, as inactivated zymogen granules, into the pancreatic ducts (Ganong 2005, Mix et al., 2006). The zymogen trypsinogen is activated by enzymatic cleavage of trypsin activation peptide (TAP) from the amino terminal of the polypeptide chain. Enteropeptidase, an enzyme produced by enterocytes, is responsible for this cleavage, therefore, the process usually occurs once trypsinogen reaches the small intestinal lumen (Ganong 2005; Mix et al., 2006). Once cleaved, trypsin is able to convert other pro-enzymes, including trypsinogen, starting an autocatalytic chain reaction (Ganong 2005; Mix et al., 2006). However, other enzymes from a variety of sources, such as lysosomal proteases, are also able to activate zymogens. Abnormal fusion of lysosomes and zymogen granules in the acinar cells form giant cytoplasmic vacuoles (Saluja et al., 1987; Saluja et al., 1989; Steer 1992) and subsequent exposure of zymogen granules to an acidic environment in these giant vacuoles causes lysosomal hydrolysis and conversion to active enzymes, initiating pancreatic injury. This culminates in acute pancreatitis as evidenced by
inflammatory infiltrates, pancreatic oedema and haemorrhage, pancreatic necrosis and peripancreatic necrosis (Steer 1992; Simpson 1993).

Protective mechanisms exist to prevent premature activation of trypsinogen to trypsin. These include: the synthesis of trypsin as the inactive pro-enzyme trypsinogen; rapid autolysis of activated trypsin; compartmentalization of activating enzymes in intracellular lysosomes; the production of counter-regulatory proteins, such as pancreatic secretory trypsin inhibitor (PSTI), by acinar cells (Simpson 1993); and a low intracellular ionised calcium concentration in acinar cells (Frossard et al., 2008; Petersen et al., 2011). If premature activation of trypsinogen occurs, PSTI, which is secreted simultaneously with trypsinogen, blocks the cascade of enzyme activation (Simpson 1993). Further, normal pancreatic secretions move along the pancreatic duct with unidirectional flow, minimizing reflux events. Pancreatic injury occurs once these protective mechanisms are exhausted and the digestive enzymes are prematurely activated.

It is recognised that pancreatitis can progress to multiple organ dysfunction (Ruaux 2000). The release of cytokines initiates the systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS) and death (Ruaux 2000). In monitoring the progression of these syndromes in critically ill human patients with pancreatitis, the sequential organ failure assessment (SOFA) score and other clinicopathological indicators of MODS are useful prognostic indicators (Marshall et al., 1995; Vincent et al., 1998; Frossard et al., 2008).

Abnormalities of a patient’s coagulation status are not specific for pancreatitis, but may be indicative of its severity. For example, Hess et al (1998) demonstrated that the activated partial thromboplastin time (aPTT) was prolonged in 61% (17/28) and prothrombin time (PT) was prolonged in 43% (12/28) of dogs with fatal acute pancreatitis. Of the 20 dogs with thrombocytopenia in this study, 55% (11/20) had prolonged aPTT or PT, but no relationship was found between platelet counts and aPTT or PT (Hess et al., 1998). In the same study, 16% (4/25) of dogs had an increased concentration of fibrin-split products. All four of these dogs had thrombocytopenia, but only 3 had either a prolonged aPTT or PT. In a more recent study of 61 dogs with pancreatitis, haematopoietic complications such as thromboembolic events were not correlated with survival (Mansfield et al., 2008). Only 7 dogs in this study showed evidence of coagulation abnormalities, with 5 recovering to discharge. Although the measurement of these
parameters was recommended by Hess et al., (1998) these latter authors remained circumspect about their usefulness.

2.5 The Diagnosis of Canine Pancreatitis

2.5.1 Introduction

The diagnosis of canine pancreatitis can be challenging and is influenced by many patient and non-patient factors. As stated previously (and described below, in 2.5.3), the clinical signs are variable and non-specific (Cook et al., 1993; Hess et al., 1999) and are shared with other diseases such as septic peritonitis or intestinal obstruction. The ability of the veterinarian to make a correct diagnosis of pancreatitis is paramount so that timely interventional treatment can be implemented. The implications of misdiagnosis in cases of acute abdomen may contribute to mortality. Thus, point-of-care testing may expedite the diagnostic process leading to more favourable outcomes.

The use of history and physical examination, the application of diagnostic imaging modalities (radiology, ultrasound and computerised tomography), measurement of analytes (e.g. serum amylase and lipase activities), and, most recently, the evaluation of enzyme immunoassays that measure canine pancreatic lipase are the cornerstones of investigation for canine pancreatitis. These diagnostic tools will be reviewed in the following pages.

There have been some diagnostic tests that, despite initially showing promise, have not gained acceptance for the diagnosis of pancreatitis. These include tests for trypsin-like immunoreactivity (TLI), trypsin activation peptide (TAP), trypsin alpha-1 proteinase inhibitor and alpha-2 macroglobulins (Ruaux et al., 1999; Mansfield et al., 2000; Suchodolski et al., 2001; Mansfield et al., 2003; Steiner et al., 2008). These tests are no longer available to veterinarians and will not be discussed further here.

Any discussion regarding the accuracy of diagnostic testing procedures requires reference to a procedure that is often referred to as the ‘gold standard’ against which the sensitivity and
specificity is evaluated. For pancreatitis this alone is not without controversy and this section starts with a review of the value of pancreatic histopathology.

2.5.2 The ‘Gold Standard’ of Diagnosis: Pancreatic Histology

The term ‘gold standard’ is defined as “any standardised clinical assessment, method, procedure, intervention or measurement of known validity and reliability which is generally taken to be the best available, against which new tests or results and protocols are compared” (Segen 1992). In canine pancreatitis, pancreatic histology has been generally accepted as the gold standard for many years; however concerns regarding this parameter have been raised recently. It has been suggested that this diagnostic test has its limitations regarding sensitivity since the parameters defining an acceptable level of canine pancreatic inflammation, as determined by histology, have been questioned (Mansfield et al., 2012).

The accepted histological definition of acute pancreatitis in people is “an acute inflammatory process of the pancreas with variable involvement of other regional tissues or remote organ systems that does not lead to permanent changes” (Bradley 1993). In the dog, histologic characteristics that define acute pancreatitis are marked by neutrophilic inflammation with an absence of fibrosis or exocrine atrophy (Neuman et al., 2006). This neutrophilic inflammation is usually present within the parenchyma of the pancreas, peripancreatic fat, or both (Neuman et al., 2006).

The inflammatory process can be restricted to small focal areas of inflammation and necrosis within the pancreas, or can extend over larger areas (Neuman et al., 2006), and herein lies the concern about the potential limitation of biopsy-derived histopathology as a tool for ante-mortem diagnosis. In a seminal post-mortem study by Newman et al (2004), pancreata were collected from 74 dogs consecutively presented for post mortem but without reported diagnosis. The pancreata were sectioned every 2cm from the tip of the right limb. Of the 47 dogs in the study with microscopic evidence of pancreatitis, very few had diffuse disease. In fact, less than one quarter of all sections from individual dogs displayed histological evidence of pancreatic inflammation and no single area of the pancreas could be recommended as a biopsy site, given that the location of inflammation varied (Newman et al., 2004). In addition, the gross
Macroscopic appearance of the pancreas cannot be used reliably as a means of identifying an affected area to biopsy, given that only 5.5% of these dogs had gross changes yet 64% had microscopic evidence of disease. However, of those with macroscopic evidence of pancreatitis, this area was a productive biopsy site in all dogs tested (n= 4). Therefore, the use of a single biopsy of the pancreas as a means of diagnosing acute pancreatitis, either via exploratory laparotomy or ultrasound-guidance, appears to be insensitive. Only dogs with gross lesions visible macroscopically, or by ultrasonographic imaging, could be expected to produce a diagnostic biopsy, and without this a large number of false negatives would be expected (Newman et al., 2006). In order to reliably diagnose pancreatitis histologically, a post-mortem would be required, with the pancreas transversely sectioned every 2 cm (Newman et al., 2006). This is obviously not applicable in the clinical practice setting.

In a follow-up study to the one described above, histological assessment and grading of the pancreas was the focus of a 2006 paper by the same researchers (Newman et al, 2006), with the stated aim to advance the classification of pancreatic disease. A standardised grading system was proposed for canine pancreatic inflammatory disease after examination of 101 pancreata collected within 6 hours of death from dogs presenting for necropsy to a referral hospital. The grades included not only the presence of neutrophilic inflammation, lymphocytic inflammation and necrosis mentioned above, but also pancreatic fat necrosis, oedema, fibrosis, atrophy and hyperplastic nodules. In this study, tissue sections were obtained every 2cm from the tip of the right limb of the pancreas. Historical information was compatible with pancreatitis in some of these dogs. Grades were allocated as 0 for the absence of lesions or 1, 2, or 3 based on increasing severity for each section. Scores of 1, 2, or 3 were defined as; less than 10% of the section affected, 10 – 40% affected, or greater than 40% affected, respectively. A mean cumulative score (MCS) was then determined by summing the score of single sections and dividing this by the number of sections evaluated for that pancreas. An MCS > 0.0 but ≤ 1.0 was considered mild, >1.0 but ≤ 2.0 considered moderate, and > 2.0 was considered severe. Pancreatic lesions were identified in 92% of all dogs in this study using this system of grading. Pancreatic nodules followed by lymphocytic inflammation, fibrosis and atrophy were the most common lesions, followed then by neutrophilic inflammation, pancreatic fat necrosis, pancreatic necrosis and, finally, oedema. This grading system has been adopted by three of four recent studies evaluating the sensitivity and specificity of canine pancreatic lipase (Steiner et al., 2008;
Neilsen-Carley et al., 2011; Trivedi et al., 2011), although Neilsen-Carley et al (2011) were the only authors to use pancreatic nodules as an inclusion criteria.

Recently, Mansfield et al. (2012) suggested that this approach may present limitations, as a lesion in any pancreatic section automatically constituted mild pancreatic disease, yet this may simply represent normal variation. The histological grading system used for Mansfield et al.’s study was adapted from two previous studies (Newman et al., 2006; Watson et al., 2007). This approach allows for a score of 0 for absence of inflammation or if less than two small foci of mononuclear cells were present with no disruption to the architecture in any of three sections from the left, right, and body of the pancreas (Mansfield et al., 2012). Scores of 0 to 4 are added from the three sections to provide a total score out of 12. A score of 1 indicated less than 5% neutrophilic or lymphoplasmacytic inflammation; 2 indicated 5 to 50% neutrophilic or lymphoplasmacytic inflammation; 3 indicated > 50% neutrophilic or lymphoplasmacytic inflammation; and 4 indicated necrosis of pancreatic tissue or peripancreatic necrosis / steatitis.

In summary, this latest grading system signals a movement away from the stringent nature of the previous system to allow for some lesions to be present without having to classify the pancreas as mildly affected, yet retains pancreatic histopathology as the gold standard against which all other testing procedures can be evaluated. Furthermore, the focus in this grading system is on inflammation rather than oedema, nodules, atrophy and fibrosis.

### 2.5.3 Patient Anamnesis

Pancreatitis in dogs can present with a nebulous history. In a report examining 70 cases of fatal acute pancreatitis, 91% of dogs had a history of anorexia, 90% had a history of vomiting, 79% weakness, 50% polyuria and polydipsia, 33% diarrhoea, 20% neurologic abnormalities, 16% melaena, 11% seizures, 10% haematemesis, and 4% haematochezia (Hess et al., 1998). This study is skewed towards the more severe cases, as these are more likely to be fatal, and therefore likely to have more severe signs in their history. Another more recent report supports these findings (Papa et al., 2011). It is generally accepted that while certain clinical signs, as reported in the history, are consistent with a diagnosis of pancreatitis, none if these are specific and, therefore, patient anamnesis alone is non-diagnostic.
2.5.4 Physical Examination

The physical examination findings in dogs with pancreatitis are non-specific and similar to that found in animals with other causes of abdominal disease. Physical examination abnormalities at the time of presentation include abdominal pain, moderate to severe dehydration, obesity, pyrexia, and icterus (Hess et al., 1998; Pápa et al., 2011).

2.5.5 Diagnostic Imaging

2.5.5.1 Radiography

Abdominal radiography plays an important role in determining the underlying causes of the canine acute abdomen. However, its usefulness in discerning pancreatitis from other causes is somewhat limited. Abdominal radiographs were assessed in 209 dogs determined (by the standards of the day) to have pancreatitis in a retrospective study gathering cases from 1967 to 1976 (Kleine et al., 1978). Of these dogs, 17 had biopsy-proven pancreatitis, 72 were proven by necropsy, and 120 displayed abdominal pain, fever, a serum amylase level of at least twice the reference range, with the authors reporting the exclusion of other causes of elevated amylase. Of these, 182 dogs had technically satisfactory radiographs and were included in further analyses.

The most common abnormality identified was increased density, diminished contrast, and granularity in the right cranial abdomen in 58% of dogs (106/182). The stomach was displaced toward the left side or the pyloric antral border was truncated in 55% of dogs (100/182). A mass-effect medial to the proximal descending duodenum or duodenal displacement toward the right side was observed in 42% (77/182) dogs. A gas pattern within, or thickened walls of, the descending duodenum were noted in 23% (42/182) of dogs and finally, a gas pattern in, or caudal displacement of, the transverse colon was noted in 9% (17/182) of dogs (Kleine et al., 1978). The authors concluded that radiography is an important adjunct in the diagnosis of pancreatitis as it may arouse suspicion of acute pancreatitis while allowing for investigation of other disorders that present as acute abdominal disease. Many of these disorders require emergency surgical intervention and may be detected by radiography. Also, radiographic
evidence may guide the clinician away from the potentially harmful effects of surgery in dogs with acute pancreatitis (Kleine et al., 1978).

In a retrospective study of 70 fatal cases of acute pancreatitis, 41 dogs had abdominal radiographs available for review (Hess et al., 1998). A single board certified veterinary radiologist determined that only 24% (10/41) had abnormalities suggestive of acute pancreatitis, concluding that conventional radiography is an insensitive test for the diagnosis of pancreatitis.

### 2.5.5.2 Abdominal Ultrasonography

Ultrasonographic techniques and equipment have improved considerably over the last 30 years. The development of ultrasonographic technique to establish the presence or absence of pancreatic disease has relied on establishment of normal landmarks in conjunction with experimentally induced and naturally occurring lesions. Early studies focussed on the ability to detect the pancreas in the first place, together with descriptions of sonographic changes that were deemed to occur during pancreatitis, and later studies have evaluated the accuracy of such testing.

Ultrasonographic features of experimentally-induced acute pancreatitis in three dogs were described in 1983 (Nyland et al., 1983). Pancreatitis was induced by oleic acid infused directly into the pancreas and twice daily ultrasound examinations were performed for 5 days until euthanasia. In-homogenous masses were noted in the right pancreatic lobe, with increased density of the left pancreatic lobe. At this early point in the development of veterinary ultrasonography, the authors stressed that the normal canine pancreas could not be accurately delineated by this modality. They were cautious about drawing conclusions between the image characteristics of experimentally induced pancreatitis and naturally occurring pancreatitis (Nyland et al., 1983). A similar experimental study a few years later (Murtaugh et al., 1985) also noted difficulty in obtaining a clear image of the normal pancreas, which was likely due to the equipment limitations of the time. However, identification of ill-defined masses with in-homogeneous echogenicity accompanying an overall decrease in echogenicity was detected in the right and left lobes once pancreatitis had developed. The pancreata were imaged by
ultrasonography ex vivo during post mortem to confirm the echotexture observed during the experiment.

In a study by Lamb et al. (1995), four dogs were administered IV cholecystokinin to induce pancreatitis and compared with four control dogs given IV saline. Ultrasonography of the pancreas was performed at 0, 2, 4, and 6 hours after the infusion. Immediately after the final ultrasound the dogs were euthanized and the pancreas was removed for histological examination. Rapid onset of pancreatitis was noted in all 4 dogs given cholecystokinin and confirmed by histology. Heterogeneous hypoechoic lesions and oedema were detected by ultrasound at either 2 or 4 hours after the start of infusion, and the authors describe ‘tiger stripe’ interlobular oedema to be characteristic of the cholecystokinin-induced pancreatitis. There were no significant changes in the control dogs. More recent ultrasonographic abnormalities of acute pancreatitis were described as a hypoechoic pancreas and hyperechoic peripancreatic mesentery (Saunders 1991).

The landmark study of fatal acute pancreatitis which included abdominal ultrasound evaluations was published in 1998 (Hess et al., 1998). These authors gathered retrospective data from dogs with pancreatitis between 1986 and 1995. Of the 70 cases of fatal acute pancreatitis, as confirmed by necropsy, 34 dogs had abdominal ultrasound examinations. Of these, 68% (23/34) had sonographic evidence suggestive of acute pancreatitis, as defined by Saunders (1991). Later, in another retrospective study (Hess et al., 2000) evaluating concurrent disorders in canine diabetes, four dogs with histopathology of the pancreas consistent with pancreatitis also had abdominal ultrasound abnormalities consistent with pancreatitis. The ultrasound images were interpreted by the same radiologist interpreting the images from the previously described study.

These results have been further supported by the two most recent studies (Steiner et al., 2008; Trivedi et al., 2011), which confirm the usefulness of ultrasonography as a diagnostic modality for pancreatitis. In 22 dogs with confirmed pancreatitis by histopathology, nine underwent abdominal ultrasonography prior to euthanasia. These 22 dogs were selected for examination due to macroscopic evidence of pancreatitis from a group of 208 dogs presented for necropsy. Of the 22 dogs, 20 had at least one of the following clinical signs reported prior to euthanasia;
vomiting, anorexia, abdominal pain, or diarrhoea. The reason for presentation was never reported for these dogs (Steiner et al., 2008). Of these, 66% (6/9) displayed sonographic changes consistent with pancreatitis. The severity of their pancreatitis was assessed histologically by grading the percentage of sections affected. Virtually all of the dogs in this study had very mild pancreatitis as assessed by histopathology, yet histological severity of pancreatitis was increased in the dogs with ultrasonographic evidence of pancreatitis. The most recent study by Trivedi et al., (2011) reported that 100% (8/8) of dogs with histologically confirmed pancreatitis had ultrasonographic changes consistent with pancreatitis. Interestingly, the Spec cPL® result was lower than 400μg/L in 50% (4/8) of these dogs, suggesting an increased sensitivity of ultrasound examination compared with this enzyme immunoassay (see section 2.5.7.4).

2.5.5.3 Computed Tomography

A case report of two dogs (Jaeger et al., 2003) introduced the use of computed tomography (CT) for assistance in the diagnosis of acute necrotizing pancreatitis. In one dog, the left pancreatic lobe formed an irregular mass that was hypoattenuating relative to the spleen. Images obtained after iodinated contrast administration showed the central portion of the pancreas greatly enhanced, surrounded by non-enhancing, hypo-attenuated peripheral tissue, which was interpreted as necrosis of the pancreas. The pancreas was also enlarged. A limitation of this report is the absence of confirmed diagnosis by histopathology or post mortem. Cytological evaluation of the pancreas in one case was consistent with pancreatic necrosis although the sensitivity of this method to establish a diagnosis of pancreatitis is unknown. In the second dog, the pancreas was enlarged and heterogeneous, and there was also thickened mesentery and free abdominal fluid. The right lobe and body of the pancreas were markedly enhanced after iodinated contrast, with the left limb non-enhancing, consistent with necrosis of the left limb of the pancreas. Similar to the first case, cytology of the pancreatic tissue was consistent with pancreatic necrosis. The advantages of CT also include the identification of vascular thrombosis, although colour doppler ultrasound has proven more sensitive in humans (Dorffel et al., 2000).
The disadvantages associated with using CT include general anaesthesia and the use of contrast material. Subsequent hypotension or disturbances in the microcirculation that may occur due to these drugs makes this procedure relatively contraindicated in dogs with pancreatitis. The use of helical CT by Jaeger et al., (2003) allowed for rapid acquisition of images and only required the use of short acting sedation. A combination of fentanyl, diazepam and ketamine for sedation successfully avoided hypotension that may be seen with inhalational anaesthetics. Regardless, the use of CT is also limited by its cost, access, and interpretation of images by qualified personnel.

2.5.6 Clinical Pathology

2.5.6.1 Routine Clinical Pathology

Routine complete blood count (CBC) collected from dogs with acute pancreatitis most often shows a leukocytosis. In the report by Hess et al., (1998), 62% of dogs (37/60) had a leukocytosis, with 84% (31/37) of these exhibiting a left shift. Only 3.3% (2/60) of dogs had a leukopaenia. Anaemia was reported in 8 of 63 dogs and five of these had a perceived inadequate reticulocyte response. Thrombocytopenia was observed in 59% (20/34) of dogs in the same study. In a study describing experimentally-induced pancreatitis, leukocytosis was demonstrated in each of six dogs, with neutrophil numbers peaking 2-3 days after pancreatic insult (Brobst et al., 1970).

Serum biochemistry in the study by Hess et al., (1998) revealed elevated urea and creatinine in 53% (33/62) and 59% (36/61) of dogs, respectively, that died due to fatal acute pancreatitis. Serum phosphate was also elevated in 68% (41/60) of their cases. There were also elevations in alanine aminotransferase (ALT) 61% (35/57) and alkaline phosphatase (ALP) 79% (46/58) activities, total bilirubin 53% (31/58), and cholesterol 48% (29/60). Serum albumin was low in 50% (30/60) of dogs.

Interestingly, despite 97% of dogs in the same study being assessed as either moderately or severely dehydrated, 90% (35/39) had either isothenuric or hyposthenuric urine. It is unknown if these dogs had received intravenous fluids prior to urinalysis and the reasons for this finding were not reported by the study.
Taken together, these results derived from a total of 70 dogs with fatal acute pancreatitis demonstrate that no single parameter within the framework of routine clinical pathology (haematology, serum biochemistry or urinalysis) is specific for the diagnosis of pancreatitis (serum amylase and lipase will be discussed in the next sections). They are, however, useful for assessing the current health status of the dog and may be indicative of existing co-morbidities, or guide further diagnostics after ruling out other possible differential diagnoses.

### 2.5.6.2 Serum Amylase Activity

Amylase is an enzyme that catalyses the hydrolysis of starch into sugars. It is found in fluid from the exocrine pancreas, where it is synthesised and stored in acinar cells with pancreatic lipase. Both amylase and lipase form an important component of the digestive process. Four isoforms of amylase have been identified in the dog (Murtaugh et al., 1985) and both have been studied extensively for its usefulness in the diagnosis of pancreatitis due the presumption that its serum activity would be elevated during pancreatic insult (Brobst et al., 1970; Mia et al., 1978; Strombeck et al., 1981; Simpson et al., 1989; Simpson et al., 1991; Hess et al., 1998; Mansfield et al., 2000; Steiner et al., 2008; Trivedi et al., 2011). An increased plasma level of amylase activity was therefore thought to be indicative of pancreatic inflammation, however this hypothesis has been investigated and challenged by many authors.

Early studies revealed that pancreatectomy in dogs did not significantly reduce serum amylase activity, indicating that there are sources of amylase other than the pancreas (Simpson et al., 1991). It was also recognised early that hyperamylasaemia may be present in dogs without pancreatitis, in animals with decreased renal function and with gastrointestinal and hepatobiliary diseases (Polzin et al., 1983; Strombeck et al., 1981).

Brobst et al. (1970) used an experimental model to investigate amylase activity in pancreatitis. Pancreatitis was induced in dogs by either injecting carbon tetrachloride into the pancreatic parenchyma or by infusing chyme, trypsin, or a combination of these, in a retrograde direction from the duodenum into the pancreatic duct. Serum amylase activity was maximal 1-2 days after pancreatic insult in 100% (6/6) of dogs and values increased by a mean of 8 times
baseline values. Later, Mia et al. (1978) established a normal reference interval for serum amylase activity of 250-1500 Caraway units/dl, using 44 healthy dogs and the iodometric method. Pancreatitis was experimentally induced in these dogs and peak serum amylase activities were measured at between 4,500 to 14,000 Caraway units/dl. These studies illustrated that serum amylase elevations could be produced by pancreatic injury.

The pancreatic isoform of serum amylase was found to be elevated in canine pancreatitis compared to control dogs (Murtaugh et al., 1985). Measurement of the pancreatic isoform requires agarose-gel electrophoresis. Its measurement was thought to be of value in cases of suspected pancreatitis, to identify pancreatic acinar leakage when normoamylasaemia was present and potentially distinguish cases of extra-pancreatic hyperamylasaemia (Murtaugh et al., 1985). Measurement of this isoform of amylase however has not become routine practice, due in part to its complexity and cost.

Reporting serum amylase activity for the diagnosis of pancreatitis has continued to this day, but largely in the context of comparing its usefulness (or otherwise) to the spectrum of diagnostic tests that are available today (Trivedi et al., 2011). In a large retrospective study, Strombeck et al. (1981) investigated the relationship between canine serum lipase and amylase activities in 713 routine clinic cases where these values were measured together. A range of 0 to 100 U/L of lipase activity was used to classify dogs as not having pancreatitis (none of these had pancreatic lesions at necropsy). The authors reported normal mean serum amylase activity of 2,980 U/L ± 1,490 (standard deviation, SD) with a range of serum amylase activity determined as 0 to 4,029 U/L. Serum lipase values of less than 800 U/L were measured in 567 dogs and only 0.82% of total variation in serum amylase could be explained by the corresponding lipase values (r = 0.09). The serum amylase activity was then correlated to paired serum lipase activities for values over 800 U/L (146 dogs) and 30.6% of the amylase activity variation could be explained by lipase in this group (r = 0.55). This was then compared to dogs with serum lipase concentrations of between 700-799 U/L as these were grouped as “probably having” pancreatitis. For this group, the mean serum amylase activity ± 2 SD ranged from 857 to 4,869 U/L. The mean was not reported by the authors. The authors concluded that the range of amylase activities were essentially the same for dogs from both groups, and did not endorse its use for diagnosing pancreatitis.
Surgical ligation of the pancreatic duct in eight dogs was used to induce pancreatitis and 100% (8/8) of dogs showed increase amylase activity (Simpson et al., 1989). Baseline values for lipase activity ranged from 294 to 1,232 IU/L with a mean of 638 IU/L. Post-ligation lipase activities increased by 13.7 fold, with a range of 3,400 to 11,400 U/L. Amylase elevations persisted above the reference range for more than 14 days after surgery in only 2 of 8 dogs. As there have been no confirmed reports of naturally occurring pancreatic duct obstruction-induced pancreatitis in the dog (refer to earlier review), the use of this model to determine the clinical utility of serum amylase for the diagnosis of canine pancreatitis appears dubious, in retrospect.

Serum amylase activity was measured in 39 dogs with naturally occurring fatal acute pancreatitis and 69% (27/39) of dogs had elevated concentrations (Hess et al., 1998). As with lipase, the authors concluded amylase to have limited diagnostic usefulness. Steiner et al. (2008) showed that in dogs with naturally occurring macroscopic evidence of pancreatitis, serum amylase concentrations were above the reference interval in 40.9% (9/22). When a value three times the reference limit was applied, the figure was reduced to 18.2% (4/22) of dogs.

Amylase activity was measured in healthy dogs (n = 36), in dogs with renal disease (n = 7), in dogs with histologically confirmed pancreatitis (n=15), and in dogs with various other diseases (n= 26) in a retrospective study (Mansfield et al., 2000). The sensitivity and specificity of serum amylase was found to be 62.1% and 55%, respectively. The authors also demonstrated a concomitant elevation in lipase in 66.6% of dogs with pancreatitis. Despite this, it was concluded there was significant overlap between dogs with pancreatitis and those with other diseases, making the diagnostic utility alone or together with lipase unreliable (Mansfield et al., 2000).

In the most recent report evaluating the value of enzyme activity for the diagnosis of pancreatitis, Trivedi et al., (2011) used 70 dogs with and without histological evidence of pancreatitis. Serum amylase was found to have a sensitivity and specificity of 14% and 100%, respectively, for moderate to severe pancreatitis using a cut-off value of 1,240 U/L. However, there were only seven dogs in this report without pancreatitis, making the strength of the specificity result questionable (Trivedi et al., 2011). Further, many of the dogs necropsied in this
study may have pancreatic inflammation secondary to other disease processes, as the primary diagnoses were not reported.

2.5.6.3 Serum Lipase

Lipases are water-soluble enzymes that hydrolyse lipids into polar lipolysis products (Svendsen 1994) and are produced by many tissues, including the pancreas (Steiner et al., 2002). Serum lipase activity, as an indicator of pancreatic lipase activity, has been evaluated for decades in the diagnosis of canine pancreatitis (Brobst et al., 1970; Mia et al., 1978; Strombeck et al., 1981; Simpson et al., 1989; Simpson et al., 1991; Hess et al., 1998; Mansfield et al., 2000; Steiner et al., 2008; Trivedi et al., 2011) but its use as a diagnostic tool has remained controversial.

In people, there have been many sources and types of this enzyme identified, such as gastric lipase, hepatic lipase, lipoprotein lipase, hormone sensitive lipase, in addition to pancreatic lipase (Svendsen 1994). Although lipases have limited amino acid homology they share a highly conserved sequence of 3 amino acids that serve as the catalytic triad, or hydrolysis mechanism, formed by serine, aspartic acid and histidine (Svendsen 1994). This makes catalytic assays vulnerable to measurement of lipases derived from sources other than the pancreas. In support of this notion, Simpson et al. (1991) found that serum lipase activities were not decreased in dogs that underwent pancreatectomy, indicating that, like in humans, there are other sources of lipase in the dog (Simpson et al., 1991). In addition, there were no differences in serum lipase concentrations between 75 healthy dogs and 25 dogs diagnosed with exocrine pancreatic insufficiency (Steiner et al., 2006).

In the study of 6 dogs performed in 1970 by Brobst et al., referred to previously, lipase activity peaked 1-2 days after pancreatic insult in 83% (5/6) of dogs and values increased by a mean of 57 times the baseline values. The remaining dog’s peak lipase value was found on day 8. The study further demonstrated that serum lipase activity closely paralleled serum amylase activity in 5 of 6 dogs in experimentally-induced pancreatitis.
In addition to measurement of serum amylase activity, the study by Mia et al. (1978) also evaluated normal serum lipase values in 44 healthy dogs and, also, elevations during experimentally induced pancreatitis in 8 dogs (Mia et al., 1978). Serum lipase activity in normal dogs was determined to be 0 to 50 IU/L and ranged from 325 to 810 IU/L post pancreatic injury. This study also showed parallel increases in amylase and lipase in 6 of 8 dogs, with serum amylase activities returning to normal earlier than that of serum lipase.

At the time of the retrospective report published by Strombeck et al. 1981, which investigated the relationship between serum lipase and amylase values, canine serum lipase activity reference intervals were only tentatively established. Therefore the authors’ laboratory determined the mean activity of lipase in healthy dogs to be 150 U/L ± 125 U/L SD. In 713 cases that were assessed from hospital records, serum lipase activity had a tenuous relationship with serum amylase activity when increased above 800 U/L. Above this level, the estimated correlation coefficient (r) between lipase and amylase was = 0.55 and approximately 30% of the variation in the amylase values could be explained by changes in the lipase values (Strombeck et al., 1981). Necropsy findings were reported in 92 dogs. Serum lipase was greater than 800 U/L in 25 of these dogs, with pancreatic lesions in 19 and only 10 of these with evidence of pancreatitis. For lipase activities between 500 and 799 U/L in the necropsy group, 8 of 14 had pancreatitis or pancreatic carcinoma. Of the 53 remaining dogs with lipase activities below 500 U/L only 1 had pancreatitis. The authors concluded that low serum lipase activity values almost always ruled out pancreatitis but that elevated levels of lipase were not pathognomonic for pancreatitis. In fact, necropsy results illustrated that elevations in lipase were also associated with renal and hepatic disease in the absence of pancreatic involvement, analogous to serum amylase activity, which was most likely due to decreased clearance.

Lipase activity was measured in a study of 8 dogs after pancreatic duct ligation (Simpson et al., 1989). Baseline values ranged from 68 to 560 U/L with a mean of 265 U/L. Post ligation lipase values increased by a mean of 25.6 fold with a range of 4,500 to 13,600 U/L. Lipase elevations persisted for more than 14 days after surgery in 6 of 8 dogs. The authors of this study concluded that lipase activity was a more reliable indicator of pancreatitis than amylase activity due to higher peak increases in all dogs. The limitations of this study with reference to ductal ligation as a cause of pancreatitis in dogs have been discussed previously.
In a retrospective study of 70 histologically confirmed cases of fatal acute pancreatitis, only 39% (16/41) of dogs had elevated serum lipase activity. The authors also concluded that the sensitivity appeared to be poor (Hess et al., 1998). There were no values of lipase activity below the normal range in this study.

Lipase activities were also evaluated in the retrospective study by Mansfield et al. (2000). The sensitivity of serum lipase was found to be 73% and specificity 55%. Many dogs without pancreatitis had elevations greater than fivefold. The authors concluded that there was significant overlap between dogs with pancreatitis and those with other diseases, but there was no guarantee that a dog with pancreatitis would have elevated lipase activity (Mansfield et al., 2000). At about the same time, in a study reported only in conference proceedings (Steiner et al., 2001), lipase activity in 11 dogs with histologically-confirmed, naturally occurring pancreatitis was reported to be significantly higher than in a group of 74 healthy dogs, with mean ± SD lipase activity found to be 319 ± 146 U/L and 4512 ± 5375, respectively. However, only 7/11 dogs had serum lipase activity above the reference interval and a cut-off using 3 times the reference range reduced this number to 6/11, producing a sensitivity of only 54.5%.

A more recent study evaluating canine pancreata displaying macroscopic evidence of pancreatitis revealed that only 41% (9/22) of dogs had elevated ante-mortem lipase activities (Steiner et al., 2008). Macroscopic pancreatitis inclusion criteria included peripancreatic fat necrosis, pancreatic haemorrhage, presence of pus when the organ was cut, or a dull granular capsular surface in this study. The authors suggested a cut-off value of 3 times the reference for serum lipase activity in order to exclude false positives that had been demonstrated in other studies. This reduced the sensitivity to 13.6% (3/22) however. One of the limitations of this study involved the nature of acquisition of the pancreata, which were obtained solely due to their gross evidence of pancreatitis from a larger necropsy group. The reasons for necropsy were not reported, and concurrent disease could not be ruled out.

Trivedi et al., 2011 improved on the previous study by using dogs with histological evidence of pancreatitis to evaluate the diagnostic accuracy of serum lipase activity. Unfortunately, similar to the report by Steiner et al. (2008), this study also included necropsies of dogs where the reasons for presentation were not reported. Therefore, many cases were unlikely to have had
primary pancreatitis. The authors grouped dogs by severity of histological pancreatic lesions into mild and moderate-to-severe. The sensitivity and specificity of lipase activity was found to be 54% and 43% for the mild group and 71% and 43% for the moderate-to-severe group, respectively.

Investigations examining lipase activity in various diseases other than pancreatitis have also been undertaken. Studies of serum lipase activity in dogs with chronic renal failure have reported contrasting results. One study by Polzin et al. (1983) found that an increase in lipase activity was associated with renal failure, whereas Steiner et al. (2010) found there was no significant difference in lipase activity in dogs with experimentally-induced chronic renal failure compared with a control population. In the more recent study of 16 dogs with chronic renal failure, experimentally induced by nephrectomy, serum lipase activity was within the reference range for all dogs, with a median value of 266.5 U/L versus the healthy control group of 74 dogs (294.5 U/L). Unfortunately, no pre-nephrectomy lipase activities were measured in this study as the serum was collected from dogs in an unrelated project 20 years earlier.

The differences between the lipase assay results reported in these 2 studies could be explained by the differences in substrates used, as these studies are separated by greater than 20 years. The authors (Steiner at al., 2010) acknowledged that a particular lipase in the study performed in 1983 may have reacted with the test substrate of the time, yielding the elevations not seen with the current methodology. Only mild azotaemia was detected in many dogs in the more recent study, possibly indicating that lack of severity in renal dysfunction may have been responsible for the discord in results. However, there was no direct correlation detected in this study between creatinine concentration and serum lipase activity, which the authors used to refute this notion. Further, the stability of lipase stored at minus 80 degrees for 20 years is not known, which may also have affected the results.

Elevations in serum lipase have also been demonstrated in dogs with gastritis, gastroenteritis, or duodenal foreign body. In a group of 48 young dogs, 16 with canine parvovirus enteritis and 32 with enteritis or gastroenteritis having no definable underlying cause, 27.1% (13/48) had serum lipase activities above the laboratory reference range (Rallis et al., 1996). The authors were unable to rule out pancreatic involvement but concluded that hyperlipasaemia may also be attributed to “gastrointestinal disturbance”. Further, in a case report of two dogs with duodenal
foreign bodies, both had serum lipase activities greater than twice the reference range (Willard et al., 1993). One limitation of these studies is the possible decrease in glomerular filtration rate in affected dogs, leading to decreased lipase excretion. These studies do not adequately report renal function in these dogs and impaired function could have contributed to lipase elevations. Further, dehydration leading to decreased pancreatic perfusion and secondary pancreatic inflammation could contribute to increased serum lipase activity.

Pancreatic and hepatic neoplasia was also proposed to have been responsible for increases in serum lipase activity, with minimal concurrent increase in serum amylase activity, in a small study of six dogs. Serum lipase activity ranged from 5410 U/L to 42,900 U/L, 11 to 93 times the upper reference limit. Histochemical and immunohistochemical staining patterns suggested tumour lipase production in 5 of the 6 dogs. The authors concluded that marked, unexplained hyperlipasaemia may be a non-invasive indicator for neoplasia of the pancreas and liver in dogs (Quigley et al., 2001).

Anecdotally, corticosteroids have been mooted to cause pancreatitis. A prospective study evaluating the effect of dexamethasone on serum lipase demonstrated a significant increase in lipase activity in dogs treated with a high dose (2mg/kg) subcutaneously 3 times on day 8 (n=6), and with a low dose (0.2mg/kg) given subcutaneously 3 times a day (n=6) for 22 days, compared with control dogs (2 groups of n=6) that received the dexamethasone vehicle only (Parent 1982). Measurements were taken on days 3, 5, and 8 for all dogs and continued for days 12, 15, 19, and 22 in the low dose group. All dogs were necropsied after the conclusion of the injections and no evidence of pancreatitis was found. In another similar study, 30 dogs were divided into 5 groups of 6, with group 1 receiving no intervention, group 2 receiving 0.6mg/kg prednisone twice daily by mouth, group 3 receiving 2mg/kg prednisone twice daily by mouth, group 4 receiving the prednisone injectable vehicle to the same volume as group 5, which received 4mg/kg prednisone intramuscularly once a day (Fittschen et al., 1984). Serum lipase was measured on days 0, 6, 11 and 15. Only on day 6 in the intramuscular prednisone group (group 5) were serum lipase concentrations significantly increased above baseline.
All dogs were necropsied and no ultrastructural changes were observed in the pancreata of dogs receiving prednisone. One dog in group 5 had evidence of mild pancreatitis, as defined by a few foci of fat necrosis surrounded by sparse neutrophilic infiltrate (Fittschen et al., 1984).

In the previously mentioned studies examining the effects of dexamethasone on serum lipase activity, serum amylase was also evaluated (Parent 1982, Fittschen et al., 1984). Both studies showed a significant decrease in serum amylase in treated dogs, compared to controls, on all days measured.

Elevated serum lipase activity has also been linked with potassium bromide and phenobarbital therapy. Two separate retrospective studies in dogs showed increases in serum lipase activity with potassium bromide and phenobarbital therapy, separately and in combination (Gaskill et al., 2000, Steiner et al., 2008). The presence or absence of pancreatic inflammation could not be demonstrated, as pancreatic histology was performed in very few dogs.

2.5.7 Enzyme-linked Immunosorbent Assay (ELISA) for the Diagnosis of Pancreatitis

2.5.7.1 Principles

The ELISA is a form of enzyme immunoassay that utilises antibodies as reagents to identify antigens (e.g. canine pancreatic lipase) in order to allow quantification (Crowther 2001). The methodology of interest in this thesis is a direct ‘sandwich’ ELISA that detects canine pancreatic lipase (cPL) (Figure 1). A sandwich ELISA derives its name from the sandwiching effect of the antibodies on the antigen (in this case cPL). Here, the antibody used for capture of the antigen is attached to the solid surface of a plate. Once the antigen is bound to the capture antibody, a second (conjugated) antibody is used to facilitate detection and quantification. The conjugate antibody is linked to horseradish peroxidase (HPO) which exerts its enzymatic effects on an added substrate that then changes colour in its presence. The result may be read by eye or a spectrophotometer. A spectrophotometer can measure adsorption of light at a specific wavelength, yielded by substrate catalysis, which is then compared to a known calibration curve to determine the concentration of the antigen. If read by the eye, the test yields either a strong colour (strong positive result, indicting higher levels of cPL), a partial colour indicating a
weak positive result, or no colour, interpreted as a negative reading, thereby providing a semi-quantitative result. Visual interpretation by this method can vary between operators and results may be considered subjective in comparison to the spectrophotometric method.

Use of monoclonal antibodies as a single species of immunoglobulin, ensures that the interaction between the paratope of the immunoglobulin with the epitope of the antigen is consistent. This property enables standardisation between tests. Different monoclonal antibodies may be used for the capture antibody (on the solid phase) and the detecting antibody, facilitating an orientation of the antigen that increases the probability that the detecting antibody will bind.
Figure 2.1: An illustration of the direct sandwich ELISA technique used for detection of canine pancreatic lipase

Monoclonal anti-cPL antibodies attached to ELISA plate (the solid phase).

Serum sample mixed with monoclonal anti-cPL antibodies for capture of desired antigen (cPL) with HPO attached (conjugate).

Mixed conjugate and serum sample added to ELISA plate for detection.

ELISA plate washed leaving only sandwiched cPL and removing unwanted HPO.

Chromogen added. Enzymatic process with horseradish peroxidase causes colour change.

Optical density of colour change quantified visually or by spectrophotometry for concentration determination of cPL.
2.5.7.2 Measurement of cPL by radioimmunoassay (RIA)

The canine pancreatic lipase gene was first sequenced by Mickel et al. (1989). The protein itself was purified by Steiner and colleagues in 2002. In their study, antisera against canine pancreatic lipase (cPL) and canine gastric lipase (cGL) was obtained by inoculating rabbits with purified cPL and cGL. Sacrificed dogs from an unrelated study allowed for application of these antibodies to a wide variety of canine tissues including striated muscles, skin, eyes, cerebral cortex, cerebellum, medulla, hypophysis, spinal cord, thyroid gland, parathyroid glands, adrenal glands, lymph nodes, spleen, cardiac muscle, aorta, vena cava, epiglottis, trachea, bronchi, lungs, kidneys, urinary bladder, salivary glands, tongue, oesophagus, stomach, all sections of the small intestine, colon, liver, gall bladder, pancreas, prostate, and testes. Only pancreatic acinar cells had positive immunofluorescence for cPL. The conclusion by the authors was that there is exclusive expression of cPL in the pancreas only, and the high specificity of their antibodies meant that there was no cross-immunoreactivity with other lipases.

In the following year, Steiner et al. (2003) reported the development of a radioimmunoassay (RIA) for the measurement of cPL in an attempt to circumvent the need for catalytic assays that could not discriminate between different lipases from different tissues. Antibodies against cPL obtained from rabbits were iodinated with I\(^{125}\). A reference range was obtained from 47 healthy dogs using the central 95th percentile as 4.4 to 276.1μg/L. Difficulties encountered with this assay included limited accuracy in the high end of the working range, but sample concentrations were consistently overestimated by the RIA in this range which would not negatively impact on its clinical application. Limited precision and reproducibility in the low end of the working range was also noted, but was tested at very low concentrations with a working range of 1 to 863 μg/L. Again, the clinical relevance of this issue was questionable. Although the RIA for cPL was validated, the practicality of working with radioactive materials was a major disadvantage. Radioactive materials used in this assay are a cause for concern in regard to safety of laboratory personnel, public perception, and regulatory restrictions on laboratories using this technology. An alternative was sought for commercial viability that brought about the emergence of the ELISA cPLI test.
The canine pancreatic lipase immunoreactivity (cPLI) test, based on ELISA methodology, was the forerunner of a now well-established commercial variant, the canine specific pancreatic lipase assay (Spec cPL® ELISA, Idexx Laboratories, see Section 2.5.7.4). For the development of the cPLI, purified cPL was obtained from sacrificed dogs and antiserum directed against cPL was developed in rabbits, as described above. Purified rabbit polyclonal antibodies were bound to microtitre plates and used to capture cPL. Purified polyclonal antibodies were also biotinylated, where biotin (vitamin B7) is attached covalently to the antibody and used to detect cPL already captured by the antibody bound to the microtitre plates in a sandwich ELISA. This process of detection was enabled by streptavidin, a protein derived from the bacterium *Streptomyces avidinii*, which has an extremely high affinity for a non-covalent bond with biotin. Streptavidin labelled with HPO and a suitable substrate/chromogen could then be used for visual detection of the sandwiched cPL, and the optical density of the reaction determined. A standard curve of optical densities for various concentrations of cPL was derived using known concentrations of cPL. Sensitivity, working range, accuracy, precision, and reproducibility were all validated for the assay. Seventy-four healthy dogs were used to establish the normal reference interval, which was taken from the central 95th percentile of the group, and found to be 2.2 to 102.1μg/L (Steiner et al., 2003).

The reference intervals between the RIA and ELISA (cPLI) were compared and have been reported to differ (Steiner et al., 2003). It was noted that the reference populations were different, but when healthy dogs from a single population were compared, the RIA values were consistently higher (Steiner et al., 2003). Although the authors acknowledged that different immunologic assays for the same substance can produce different results, correlation between the assays was high with a Spearman coefficient \( r = 0.9708 \) (Steiner et al., 2003). The explanation offered by the authors alluded to steric hindrance when pancreatic lipase is fixed to the bottom of the wells of the ELISA plates. The cPLI has shown good reproducibility, ease of performance, cost effectiveness, long-term stability, has no requirement for radioactive materials, and is better suited to discriminating dogs with exocrine pancreatic disorders. Therefore, the cPLI ELISA superseded the RIA.
The cPLI has not been subjected to extensive, rigorous and independent evaluation. These researchers produced an abstract evaluating the cPLI in dogs with naturally occurring pancreatitis confirmed by histopathology (Steiner et al., 2001). When compared to the control population of 74 dogs, 11 dogs with histologically confirmed pancreatitis had a median cPLI value of 676.8μg/L, which was significantly higher than the control group of 16.3μg/L. The cPLI was also above the upper limit of the reference range of 102 μg/L in all dogs (n=11). For the diagnosis of pancreatitis, the authors decided an empirical cut-off value of 250μg/L, yielding a sensitivity of 81.8% in 9 of 11 dogs with pancreatitis (Steiner et al., 2001).

Studies investigating the utility of the cPLI in dogs that have clinical signs consistent with pancreatitis are scarce. Of 22 dogs with macroscopic evidence of pancreatitis presented for necropsy, where the diagnoses were unknown, 20 had clinical signs that could have been attributable to pancreatitis such as vomiting, abdominal pain, anorexia and diarrhoea (Steiner et al., 2008). Sensitivity of the cPLI in this group of dogs was determined to be 63.6%. Steiner et al., (2008) argued that while the number of dogs in the study was low, nearly 82% of dogs had mild histopathologic evidence of pancreatitis and none had severe pancreatitis using the scoring system that was developed by Newman et al., (2006).

The specificity of the cPLI has been investigated in a single study. Gastritis was confirmed in 25 dogs by biopsy and subsequent serum testing of cPLI was performed on each dog. Of 25 dogs with biopsy confirmed gastritis only 1 had cPLI above the cut-off for pancreatitis (>200μg/L) (Steiner 2000). None of these dogs had pancreatic histology performed to rule in or out pancreatitis (refer to the earlier section on ‘gold standard’ diagnosis of pancreatitis).

Another retrospective study that has investigated the use of cPLI in dogs with confirmed diseases other than pancreatitis evaluated dogs with histologically confirmed inflammatory bowel disease (IBD) (Kathrani et al., 2009). A cPLI concentration above the reference interval was identified in 32% (15/47) of dogs with IBD, with a mean value of 427μg/L, which is above the manufacturer’s cut-off concentration for pancreatitis. The remainder of samples had concentrations within the normal reference range. Only 20% (3/15) of dogs with cPLI concentration above the reference range had ultrasonographic evidence of pancreatitis and no dogs with a low cPLI concentration showed ultrasonographic evidence of pancreatitis. There
was no significant difference between these two groups with respect to ultrasound findings. In addition, there was no significant difference in histology severity for IBD between the groups. The authors themselves point out that the clinical signs of pancreatitis and IBD are non-specific and easily confused with other conditions. Although the study concluded that a subset of dogs with IBD may have subclinical pancreatitis, diagnostics pursued in reverse order may actually result in misdiagnosis of IBD for pancreatitis.

The 2010 study by Steiner et al. that investigated the effect of chronic renal failure on serum lipase activity (Steiner et al., 2010), also evaluated cPLI. Only 12.5% (2/16) of dogs had cPLI concentrations above the reference interval and none were above a suggested cut-off for pancreatitis of approximately twice the reference range. However, cPLI was significantly higher in dogs with experimentally induced renal failure than the control group. The authors concluded that these elevations in canine pancreatic lipase concentration were not clinically relevant. The stability of cPL over 20 years stored at minus 80 degrees Celsius is unknown.

The issue of canine pancreatic lipase stability after storage of samples, or testing of samples by laboratories after interstate transport, needed to be addressed. Thus the stability of canine pancreatic lipase was investigated in serum samples stored at a variety of temperatures over a time period of 21 days (Steiner et al., 2009). On days 0, 3, 7, 14, and 21, at temperatures of 24°C, 4°C, -20°C, and -80°C, samples from 8 randomly selected dogs were tested for cPLI. No significant variation was found over 21 days for individual dog’s serum samples. However, a larger variation between results was found at low and high mean concentrations of canine pancreatic lipase. The highest mean concentration of 407.8 μg/L was well above the reference interval of 2.2-102.1 μg/L and the variation between results at this concentration was determined to be a mean of only 20.8 μg/L. This is unlikely to have a significant clinical impact on decision making, although the cPLI test never went on to be used commercially. The authors also compared 30 paired serum and plasma samples from randomly selected dogs and reported no difference in the median values of cPL and a strong correlation of r = 0.977 between serum and plasma cPL concentrations.

Steiner et al. (2009) continued their investigations into the purported benefits of the measurement of canine pancreatic lipase compared with non-specific catalytic assays for
general lipase. Since lipase activities derived by catalytic assays increase with glucocorticoids, the effect of prednisone on serum cPL, as measured by cPLI, was also assessed. Pre-treatment samples were obtained from 6 dogs on days 0 and 14 and subsequent samples were obtained on days 28 and 42 after administration of 2.2 mg/kg prednisone per day from day 15 to 42. Additional samples were obtained on days 56 and 70. No samples were recorded with cPLI over the reference interval and the mean cPLI concentrations for all dogs over all days did not differ significantly, thus, establishing another benefit of cPL over general lipase measurement.

As the cPLI assay uses small scale manufacturing processes not suitable for commercialisation, another ELISA was developed to circumvent this. It was then called the canine specific pancreatic lipase test (Spec cPL®), which incorporates dual monoclonal antibodies derived from mice (Huth et al., 2010). Further, a rapid in-clinic semi-quantitative assay (SNAP® cPL) has also been developed using the same dual monoclonal antibodies for capture and detection of pancreatic lipase as the Spec cPL®.

2.5.7.4 Canine specific pancreatic lipase (Spec cPL®)

The Spec cPL® was developed by Idexx Laboratories for its suitability in commercial application (Huth et al., 2010). During development, a recombinant antigen was synthesised for use instead of cPL (rcPL) (Beall et al., 2010). Mice were immunised with purified native canine pancreatic lipase (cPL) and 2 monoclonal antibodies were obtained for the capture and conjugated phases of the ELISA (Huth et al., 2010). A sandwich ELISA for Spec cPL® was developed; optical densities were derived from an HPO detection system and a standard curve was constructed from known concentrations of cPL. The original ELISA, the cPLI, was used as the gold standard assay. Analysis of the performance of the Spec cPL® by Idexx Laboratories showed reliability for a dynamic range of cPL concentrations 36 to 954μg/L. Intra- and inter-assay variability was analysed over this range, with production of acceptable coefficient of variations for ELISA technology. It was also determined that bilirubin, haemolysis and excess lipid, at concentrations consistent with canine pancreatitis, did not affect the results (Huth et al., 2010). In addition, any variability was thought comparable to the cPLI assay, though, a bland-Altman plot comparing the assays showed the Spec cPL® to read consistently higher at concentrations above 200μg/L, which is the upper limit of the reference range. The authors concluded that this bias was
clinically irrelevant as it occurred higher that the reference interval. However, Idexx Laboratories (McCord et al., 2009, Beall et al., 2011) have adopted the following guidelines for interpretation of Spec cPL results:

- Normal animals - reference interval 0 to 200μg/L,
- Equivocal - if results fall between 200 to 400μg/L, and
- Consistent with pancreatitis - results of cPL at concentrations > 400μg/L

The specificity of the Spec cPL has been investigated in two recent studies. The first by Neilson-Carley et al. (2011), involved dogs of varying health status, the majority of which were obtained from an animal shelter (27/42), with the remaining 15 dogs used from a referral hospital with disease states including dystocia, neoplasia, anaemia, and trauma. Histological assessment was based on 1-2cm interval slices of all pancreata. Ninety-five percent (40/42) of these dogs revealed no lesions compatible with pancreatitis. Spec cPL concentrations in 95% (38/40) of these dogs were below the upper reference limit of 200 μg/L, yielding a specificity of 95%. Only one of the 2 remaining dogs had a Spec cPL above 400 μg/L, which is consistent with pancreatitis. This dog was young and the only abnormality detected was haemoconcentration. It had no evidence of pancreatic inflammation and was from the animal shelter. The authors conceded in their discussion that this population of dogs would be unlikely candidates for the Spec cPL test, as they exhibited no clinical signs of pancreatitis. Therefore the authors could only conclude that the cut-off values used may be useful for diagnosing pancreatitis in dogs with a lack of histopathologic lesions consistent with pancreatitis, and for which pancreatitis is not considered a major differential diagnosis. They also recommended further studies to evaluate specificity of this test in a population of dogs where pancreatitis is considered a differential diagnosis.

The second study evaluating Spec cPL for its specificity performed histological examination on 3 sections of canine pancreas to confirm pancreatitis (Mansfield et al., 2012). A new scoring system for histological pancreatitis was proposed in this study (and was described previously – section 2.5.2). Of 32 dogs included in the study, 20 were classified as having subclinical pancreatitis of no clinical relevance. Using cPL cut-off concentrations of 200 and 400 μg/L, there were 4 and 2 false positive results, yielding a specificity of 80% and 90% respectively. The
remaining 12 dogs had a histopathologic diagnosis of pancreatitis. Using the same cut-off values (200 and 400 µg/L) for cPL, there were 7 and 4 true positives, respectively, yielding a sensitivity of 58% and 33%, respectively. Of all the dogs designated true positives (7 dogs), only 3 had pancreatitis as the primary cause of their clinical signs.

Trivedi et al. (2011) evaluated both the sensitivity and specificity of the Spec cPL® in 70 dogs with and without histopathologic evidence of pancreatitis. This study also enrolled dogs that were euthanized for a variety of reasons, and required collection of a serum sample within 24 hours before euthanasia and the removal of the entire pancreas for histopathologic evaluation within 4 hours of euthanasia. Necropsy was performed on 69 of 70 dogs enrolled. Histopathologic evidence of mild pancreatitis was observed in 56 of 70 dogs, moderate pancreatitis in 6 of 70 dogs, and severe pancreatitis in 1 of 70 dogs. Of the 7 dogs with no histopathologic evidence of pancreatitis, only 1 had a Spec cPL® above 200 µg/L, which was also below the cut-off for being consistent with pancreatitis (400 µg/L). The study also showed that the correlation between histopathologic indicators of acute pancreatitis, such as suppurative inflammation, peripancreatic fat necrosis and acinar cell necrosis, and the Spec cPL® concentration were stronger than those for chronic pancreatitis, such as pancreatic fibrosis and atrophy. However, nearly all dogs in this study with acute pancreatitis had evidence of chronic pancreatitis and could not be considered separately. When the authors used the manufacturer’s recommended cut-off of 400 µg/L, the sensitivity for mild pancreatitis was only 21% (12/56), and when using 200 µg/L was 43% (24/56) (Trivedi et al., 2011). When applied to the moderate-to-severe group for cPL concentrations, 71% (5/7) was obtained for both cut-offs. The authors noted that the strict nature of the histological scoring system used might have contributed to a poor sensitivity for dogs with mild pancreatitis. This system required any dog with 1 or more lesions in any section of the pancreas to be classified as mild pancreatitis, despite the authors’ suspicions that this may represent normal variation. Specificity for a cut-off concentration of greater than 400 µg/L was 100% (7/7) for both mild and moderate-to-severe pancreatitis, and 86% (6/7) for both these groups using a cut-off of greater than 200 µg/L. Interestingly, this study also reported that only 65% (11/17) of dogs with Spec cPL® concentrations greater than 400 µg/L exhibited clinical signs consistent with pancreatitis, such as vomiting and anorexia. Further, only 35% (6/17) displayed abdominal pain on physical exam. The diagnoses assigned to the dogs included in this study were unknown and the authors
acknowledged that they were necropsied for a variety of reasons. The majority of cases were mild, which may have skewed the sensitivity data. Importantly, 21% (12/56) of dogs with mild histologic evidence of pancreatitis had a Spec cPL® > 400 μg/L (consistent with pancreatitis), and all of them had histological evidence of hepatopathy. Three of these dogs had histologic evidence of intestinal lesions. Only one dog in this subgroup had macroscopic evidence of pancreatitis. The authors recommended further assessment of the Spec cPL® in dogs with extra-pancreatic disease, especially those with hepatopathy.

In the study by Steiner et al., (2006) including dogs with macroscopic pancreatitis, the sensitivity of the Spec cPL® was 63.6%. As specified earlier, the authors believed this low sensitivity may be a reflection of the mild scoring of pancreatitis. They also pointed out that the cPLI and Spec cPL® had the highest sensitivity for detecting pancreatic inflammation than the other tests evaluated; serum amylase, lipase, cTLI and trypsin alpha 1 proteinase inhibitor.

McCord et al., (2012) introduced the assessment of the Spec cPL® for sensitivity and specificity in diagnosing acute pancreatitis in dogs that presented with an initial differential diagnosis of acute pancreatitis. The history, physical exam, laboratory findings, abdominal ultrasound and clinical course were assessed by 4 internists blinded to the Spec cPL® results. Still ultrasound images were reviewed by a board certified radiologist. These dogs were then assigned to 1 of 5 groups of either not pancreatitis (group 0), not primary pancreatitis (group 1), possibly pancreatitis (group 2), probably pancreatitis (group 3), or pancreatitis (group 4). A control group of dogs deemed not to have pancreatitis was also included for categorization into these 5 groups. There was a high level of agreement in the categorization between internists for grouping, with a kappa value of 0.87. Sensitivity and specificity were reported for the Spec cPL® as 78 and 88% respectively using 400 μg/L cPL as the cut-off. The authors concluded that the test was superior to amylase and lipase for the diagnosis of pancreatitis, and further, that dogs with Spec cPL® values under 200 μg/L were unlikely to have pancreatitis. The study lacked histopathologic evaluation of pancreata for all dogs bar 3. This study draws attention to the notion of non-primary pancreatitis, where it may run concurrent to a more clinically relevant disease process. That is, dogs with extra-pancreatic disease, or disease that does not originate under the umbrella term of pancreatitis, may develop inflammatory infiltrates of the pancreas.
secondary to the nature of the inciting disease. This has not been thoroughly investigated as far as the Spec cPL® is concerned.

2.5.7.5 SNAP® cPL

Idexx Laboratories developed a semi-quantitative immunochromatographic version of the Spec cPL®, the SNAP® cPL, in order to offer an in-house test that provides an immediate result for use in emergency settings. The SNAP® cPL test is registered to screen dogs for pancreatitis when they present to a veterinary care facility, with subsequent follow up by Spec cPL® quantification.

A brief communication by researchers at Idexx Laboratories (Beall et al., 2011) has been published on the performance of the in-clinic SNAP® cPL. The SNAP® cPL is described as a semi-quantitative assay which generates a coloured test spot on a bench top device that is compared visually to the reference spot. If the test spot is the same colour intensity or darker than the reference spot, the test is determined to have a concentration of cPL greater than 200 μg/L and is therefore deemed to be positive, or abnormal. The cut-off value of 200 μg/L was selected so that fewer dogs with elevated cPL would go undetected. The manufacturer advises that a positive SNAP® cPL results is then followed by the Spec cPL® to derive an absolute value. The perceived benefits of the SNAP® cPL include its rapid test time in the clinic and ease of use.

The SNAP® cPL test was developed from the same ELISA technology used for the Spec cPL®, using the same two monoclonal antibodies to capture and detect cPL for the test ‘spot’. A predetermined volume of canine serum is added to a solution containing chromagen-conjugated antibodies against cPL, and the mixture is then added to the testing device. The mixture flows along the solid phase support matrix, which allows the cPL-antibody complex (if present) to bind to the solid phase capture anti-cPL antibody. When the mixture reaches a certain distance along the solid phase, the device is depressed to allow release of a wash solution followed by a substrate solution (3,3’,5,5’-tetramethylbenzidine), back through the matrix. The reference spot on the solid phase of the test comprises chicken immunoglobulin G (IgG) that is recognised by a goat-derived anti-chicken IgG antibody in the liquid phase reagents, and serves as an internal
control, generating a fixed colour intensity regardless of the canine serum sample. This colour intensity is designed to match a sample containing approximately 200 μg/L cPL. The liquid phase antibodies are conjugated to horse radish peroxidase for the colorimetric interpretation, using the substrate 3,3',5,5'-tetramethylbenzidine, producing a blue colour.

Recombinant cPL (rcPL) was used to calibrate the test. The reference standards contain rcPL at 0, 100, 200, 500, 1000 μg/L. The colour intensity produced by the standards is measured by a reflective densitometer to generate readings for known rcPL concentrations. Densitometer colour intensity ratios of known rcPL concentrations, to reference spot concentrations, for each of the known rcPL samples were used to produce a calibration curve. This enables estimation of an unknown canine serum cPL concentration using the optical density ratio derived from the patient's test spot compared to the reference spots. In essence, a progressively darker test spot is a reflection of a higher concentration of cPL in the sample. The use of a reflective densitometer can then derive a specific concentration for the sample based on the difference between the test spot and the reference spot.

Several testing conditions were used to evaluate the clinical utility of the SNAP® cPL; it was determined that commonly encountered serum components in dogs with pancreatitis, such as increased bilirubin, lipid and haemoglobin, did not interfere with the test results (Beall et al., 2011). The visual results of the SNAP® cPL were also shown to be precise with 5 canine samples of known cPL concentrations, tested 10 times each on 3 separate days, for a total of 150 tests. The test was consistent for 149 of the samples when using a densitometer and agreed with the Spec cPL® on all 149 occasions.

Further, the visual results of the SNAP® cPL were assessed for its agreement with the Spec cPL® using three separately prepared batches of the SNAP® cPL on 49 canine samples (Beall et al., 2011). A broad distribution of cPL concentrations was determined by the Spec cPL® reference method and run in duplicate, with the mean concentration used to calculate the agreement. The authors reported 96-100% and 88-92% agreement for the normal and abnormal samples, respectively (Beall et al., 2011). The kappa coefficient showed a very good agreement (κ=0.878) between the SNAP® and Spec cPL® tests, and was further confirmed by a correlation coefficient of $r = 0.92$ to 0.948. It was noted that samples determined by the Spec
cPL® as >200 μg/L but <300 μg/L, could be reported as ‘normal’ on the SNAP® cPL, possibly due to a visual discordance, as the test and reference spots were nearly identical. These 49 samples were run once on each of 3 batches of SNAP® cPL and duplicate on one batch. Results showed good correlation between batches and between duplicates within the batch.

Interestingly, the visual results for a 214 μg/L serum pool and the 200 μg/L rcPL pool were split between abnormal and normal, with approximately 30% reading as normal throughout testing. It was concluded that samples with concentrations approximating the cut-off for abnormal may be difficult to interpret due to subjective evaluation.

The stability of the SNAP® cPL assay itself when stored at 2-7°C was also tested over 15 months at months 1, 2, 4, 6, 8, 10, 12, 15, using rcPL and serum cPL, and there was no difference compared to baseline, making the test stable for storage over this time period (Steiner et al., 2009).

A single study (McCord et al., 2012) has evaluated the sensitivity and specificity of the SNAP® cPL in dogs that presented to multiple veterinary centres with, and without, a differential diagnosis of pancreatitis. All dogs were allocated to groups based on the likelihood of the presence or absence of pancreatitis by 4 Board-certified internists. Ultrasound examinations were performed in each case, but not always by a veterinary radiologist. However, still images were evaluated by the radiologist in each case. Of 84 dogs evaluated, 57 dogs had suspected pancreatitis. However, pancreatitis in 37 of these 57 dogs was still considered unlikely; these individuals were determined to have a convincing primary alternative disease present to explain the clinical findings. The remaining 20 dogs fell into 3 groups of “possible pancreatitis” (n= 9), “probable pancreatitis” (n=8) and “definite pancreatitis” (n=3) and were used to determine the sensitivity of the tests. The group with “possible pancreatitis” had inconsistent clinicopathological evidence supporting the diagnosis and no ultrasonographic evidence of pancreatitis, but no alternative disease could be determined. The group “probable pancreatitis” had supportive clinicopathological and ultrasonographic abnormalities, but did not have cytological or histopathologic evidence to support a diagnosis. Only the remaining three (of 20) dogs demonstrated cytological or histopathologic evidence of pancreatitis. The sensitivity and specificity of the SNAP® cPL was determined to be 94 and 77.5% respectively. The authors noted the important influence of the relationship between the prevalence of pancreatitis and the
predictive values of the tests. To emphasise this point they stated that using laboratory tests to aid the diagnosis of acute pancreatitis where the index of suspicion for the disease is low, could result in false positive results. The authors also concluded the reverse to be true in dogs. The predictive value is likely to be low when the index of suspicion for acute pancreatitis is high in conjunction with a negative test result (McCord et al., 2012).

Despite the SNAP® and Spec cPL® tests showing promise in their ability to identify the presence of cPL, their capacity to identify acute pancreatitis appears far from conclusive. Non-specific lipase activity may increase with acute pancreatitis, but it has been shown to be too inaccurate for clinical application. This may equally be true of cPL. It is hypothesised that cPL serum concentration may fluctuate with primary pancreatic disease as well as local or systemic disease that may impact directly on the pancreas. The relationship of cPL concentration with acute abdominal disease in dogs requires further investigation.
CHAPTER 3: APPLICATION OF STATISTICAL METHODS TO THE DIAGNOSIS OF CANINE ACUTE PANCREATITIS

3.1 Introduction

Accurate diagnosis relies on the clinician’s ability to assimilate a spectrum of data, generated from a subject’s problem list, and choose the most likely explanation for the subject’s problems. The process of arriving at the diagnosis relies on applications of probabilities, or likelihoods, of the changes recognised in the data to fit with the diagnosis. These probabilities are based on previous studies that characterise the pathophysiology, and the pattern of clinical signs, progression and outcome. The purpose of reaching a diagnosis is to create an opportunity to intervene and change the outcome, or establish a prognosis.

Selection of an appropriate test for deriving a diagnosis is based on assessment of the diagnostic accuracy of a test, its sensitivity and its specificity. Certain conditions can make it difficult to establish the sensitivity and specificity of a diagnostic test, one of which is the absence of a gold standard. The application of diagnostic tests for the diagnosis of canine pancreatitis is impeded by certain conditions, many of which have been discussed in Chapter 2. This chapter will summarise these difficulties as they apply to the statistical methods used for assessing a diagnostic test.

3.2 Sensitivity and specificity

Sensitivity is the proportion of subjects with the target disorder that have a positive test result (Haynes et al., 2006). Specificity is the proportion of subjects without the target disorder that have a negative test result (Haynes et al., 2006). A test with a high sensitivity will detect most subjects with the target disorder and is preferred for a diagnostic test. A test with high specificity will detect most subjects without the target disorder and is preferred for a screening test (Haynes et al., 2006). Ideally, a test would have both high sensitivity and specificity, making the test highly accurate.
The determination of sensitivity and specificity for any given study is reliant on the population in which the test is being conducted, in particular the prevalence of the target disorder. In addition, the evaluation of the test in question should be compared against the “gold standard”, that is, the testing method that confirms the true presence or absence of the target disorder in the population. Thus, the two key factors required for unbiased estimations of sensitivity and specificity are (1) selection of an appropriate sample population, and (2) comparison with a ‘gold’ standard.

3.3 Selection of the appropriate ‘intended use’ population

The challenge of evaluating a test in the clinical setting is that there may be a variety of diseases that present with similar signs. The important question is whether the test can discriminate between subjects that are suspected to have the disease, which do not, and those that do have the disease. Ideally, the population of individuals used to determine a test’s operating characteristics should be similar to the population in which the test will be used, and should include individuals that display a broad spectrum of clinical features of the disease in question, from mild to severe (Haynes et al., 2006).

To illustrate this point, design-related bias occurs if selection of a normal population without the disease is compared with subjects that have severe forms of the disease; this may overestimate the power of the test. One example of a study with design-related bias from the literature pertaining to the diagnosis of pancreatitis is by Neilson-Carley et al. (2011). These authors compared a cohort of dogs (27/42) obtained from an animal shelter as their normal population (i.e. a group expected to have a low prevalence of pancreatitis) with the remaining 15 dogs that were sourced from a referral hospital, with varying disease states. These circumstances may lead to an over-estimation of the specificity of the test. In another study, Trivedi et al. (2011) also included a cohort of dogs that was not the intended-use population. The entire cohort appeared to consist of sick dogs with a broad spectrum of disease and only 65% of these dogs had clinical signs consistent with pancreatitis. A further limitation of this study was that most of the dogs had only mild pancreatitis on histopathology, which may have also skewed the sensitivity and specificity data.
Similar challenges in study design make it difficult to assess the diagnostic accuracy of the Spec cPL® and SNAP® cPL. An ideal population to evaluate the test would be one that comprised dogs with signs of acute abdominal disease, and included individuals with pancreatitis that showed a broad spectrum of severity. One further, common hindrance to any study involving dogs with pancreatitis is the small sample size, which is usually due to the difficulty of obtaining a definitive diagnosis, that is, a gold standard result.

3.4 The role of the gold standard in test evaluation

A ‘gold’, or reference, standard must provide definitive diagnosis of the disease in question, and is generally more time-consuming, expensive, invasive or otherwise difficult to perform than the test being compared to it; hence the motivation for development of the test (Haynes et al., 2006). Sometimes the gold standard cannot be obtained in client-owned subjects, for example, when necropsy is required. It is common in clinical studies to use a composite reference standard, which may be a combination of other tests and expert opinion (Reitsma et al., 2009). As discussed previously (Section 2.5.2), the traditionally accepted gold standard for acute pancreatitis is presence of inflammation within the pancreas viewed on light microscopy examination of tissue sections. It can be difficult to apply the stringent histopathological scoring systems that have been developed for acute pancreatitis, as these can only be achieved from tissue samples harvested at necropsy. Therefore, using a population of subjects that underwent necropsy not only limits sample size, but also may introduce bias towards a population of more severely affected subjects.

Given the difficulties of applying the gold standard to studies evaluating acute pancreatitis, it may be better to apply a composite reference standard that includes data such as history, physical examination, ultrasonography and other clinico-pathologic test results. This would rely on systematic expert review being employed to confirm the clinical diagnosis. Unfortunately, there is no consensus currently on such a reference standard, and therefore the disease is variably defined within the literature (as previously discussed).
This problem of lack of a gold standard for an appropriate sample population brings into question the validity of using sensitivity and specificity as appropriate measures of a diagnostic test’s merit for pancreatitis. It may be that measures of agreement, such as Cohen’s kappa statistic, with clinical diagnosis may be more useful and provide a more relevant interpretation. Cohen (1960) stated that for any problem in nominal scale agreement between two judges, there are only two relevant quantities: $p_o$ = the proportion of units in which the judges agreed, and $p_e$ = the proportion of units for which agreement is expected by chance. Therefore Cohen’s kappa is used to determine the degree of agreement between two observers or tests, taking into account that some agreement may occur by chance. This also allows for a comparison to the presence of a clinical syndrome rather than a gold standard. In the case of acute pancreatitis, it may be present but may not be the primary reason for the subject being ill. Therefore, characterising that the subject is showing the clinical syndrome of acute pancreatitis, rather than another serious illness that is causing some degree of pancreatitis, is vital in order to determine a further diagnostic or treatment plan.

Given the inherent difficulties of interpreting the results of evaluation of diagnostic test accuracy of Spec cPL® and SNAP® cPL in the literature, our goal therefore was to demonstrate the limitations of these tests applied to an appropriate sample population and compare results of these tests to a composite reference standard that represented a clinical diagnosis, rather than the historical gold standard.
CHAPTER 4: DIAGNOSTIC ACCURACY OF THE SNAP AND SPEC CANINE PANCREATIC LIPASE (CPL) TESTS FOR PANCREATITIS IN DOGS PRESENTING WITH CLINICAL SIGNS OF ACUTE ABDOMINAL DISEASE.

(This chapter describes the clinical research project undertaken for the RMT degree, and forms the text of the following publication: Haworth MD, et al. (2014). Diagnostic Accuracy of the SNAP and Spec Canine Pancreatic Lipase (cPL) Tests for Pancreatitis in Dogs Presenting with Clinical Signs of Acute Abdominal Disease J Vet Emerg Crit Care. In Press.)

4.1 Introduction

Acute pancreatitis (AP) is an important disease of dogs, with variable and non-specific clinical signs such as abdominal pain, vomiting and diarrhea (Cook et al., 1993; Hess et al., 1998). These clinical signs are also present in conditions such as septic peritonitis or intestinal obstruction, which require specific and timely interventional treatment. Traditional diagnostic methodologies, such as total serum lipase and amylase, have poor sensitivities and specificities for the diagnosis of AP in dogs (Mansfield et al., 2000; Steiner et al., 2008; Trivedi et al., 2011).

The canine pancreatic lipase test measures lipase that originates in the pancreas, and theoretically should only be increased during times of pancreatic inflammation (Steiner et al., 2003). The canine pancreatic lipase immunoreactivity (cPLI) assay (first a radioimmunoassay, and subsequently an enzyme immunoassay) has been validated in dogs (Steiner et al., 2003). The cPLI assay was then developed into a commercially available specific canine pancreatic lipase (Spec cPL) assay, using a recombinant peptide as the antigen and dual monoclonal antibodies for capture and detection (Huth et al., 2010). Spec cPL shows good correlation to and high reproducibility with cPLI (Huth et al., 2010). Spec cPL results < 200 µg/L are considered to be consistent with an absence of pancreatic inflammation (Huth et al., 2010; McCord et al., 2012), while results ≥ 400 µg/L are considered consistent with a diagnosis of pancreatitis, and a result in the range 200-399 µg/L is considered equivocal (Steiner et al., 2001; McCord et al., 2012). A rapid in-clinic semi-quantitative assay (SNAP cPL) has also been developed using the same dual monoclonal antibodies for capture and detection of pancreatic lipase as Spec cPL (Steiner et al., 2001). SNAP cPL is reported to show good correlation and reproducibility compared to the laboratory-based Spec cPL (Steiner et al., 2001). A negative
SNAP cPL result corresponds to a Spec cPL concentration < 200 µg/L, and a positive result with a concentration ≥ 200 µg/L (Steiner et al., 2001).

The reported sensitivity for cPLI/Spec cPL ranges from 21-82% (Steiner et al., 2001; Steiner et al., 2008; Trivedi et al., 2011; McCord et al., 2012; Mansfield et al, 2012), whilst specificity for Spec cPL is reported to range from 86-100% (Trivedi et al., 2011; McCord et al., 2012; Mansfield et al., 2012). All but one of these studies based a diagnosis of AP on histological demonstration of pancreatic inflammation. As a result, the sensitivities and specificities may not be accurate as pancreatic inflammation was often very low or mild. The sensitivity of Spec cPL (or cPLI) has been shown to be higher in dogs with increasing histological severity (Newman et al., 2004; Steiner et al., 2008; Trivedi et al., 2011). A recent study reported the sensitivity and specificity of the SNAP cPL to be 94% and 77% respectively in dogs that presented both with suspicion and without suspicion of pancreatitis (McCord et al., 2012).

The true diagnostic accuracy of non-invasive methodologies for AP is unknown due to the difficulty in obtaining a gold standard. Abdominal ultrasound is used extensively in veterinary practice. The main finding in acute pancreatitis is peri-pancreatic hyperechogenicity associated with peri-pancreatic fat necrosis in the acute necrotizing form (Hecht et al., 2007). Pancreatic inflammation may also develop due to duodenal reflux, ischemia or generalized peritonitis in association with other diseases such as septic peritonitis, abdominal hemorrhage or intestinal foreign bodies. Therefore, despite the presence of histological and ultrasonographic severe pancreatic inflammation, pancreatitis may only be secondary and not be the cause of the clinical presentation in dogs. Sensitivity of ultrasound may be influenced by animal factors (obesity, ingesta interfering with image quality), operator factors (level of experience) and technical factors (ultrasound equipment). Reported sensitivities for ultrasound are 66-68% (Hess et al., 1998; Steiner et al., 2008). Higher median histological grading of pancreatic inflammation has been reported to correlate with ultrasonographic evidence of pancreatitis (Steiner et al., 2008). In another recent study, a small subgroup of dogs with histologically confirmed pancreatitis of varying severity all had ultrasonographic evidence of pancreatitis (Trivedi et al., 2011). Therefore history, clinical signs, laboratory testing, and abdominal imaging are often used together to make a clinical diagnosis of AP. Conversely, it is also possible that reliance on histological evaluation of the pancreas may bias studies towards dogs with more severe disease
and a fatal outcome). Additionally, pancreatic biopsies are seldom obtained in critically ill dogs unless at post-mortem, and inflammation may be unevenly distributed throughout the pancreas, or just be located in the peri-pancreatic fat (Newman et al., 2004; Hecht et al., 2007).

The primary objective of this study was to determine the accuracy, sensitivity and specificity of SNAP cPL and Spec cPL in dogs presenting with acute abdominal disease to an emergency centre. A secondary objective of this study was to quantify the agreement between the Spec cPL and the SNAP cPL for paired serum samples taken from the same cohort of dogs.

4.2 Materials and Methods

Client-owned dogs presenting to a first-opinion and referral emergency center at a university teaching hospital between March 2009 and April 2010 were recruited. Dogs were initially included if they had two or more of the following clinical signs: acute (< 2 days) onset of abdominal pain, vomiting, abdominal distension or diarrhea. Dogs were excluded from the analysis if they did not have a definitive diagnosis made during hospitalization.

Blood was collected via jugular, cephalic or saphenous venepuncture from all dogs within 24 hours of admission as part of diagnostic investigation. If additional samples of blood were collected specifically for the purposes of the study, signed owner consent was obtained. The study was approved by the institutional Animal Ethics Committee, fulfilling NHMRC regulations. Two mLs of blood was collected into plain serum tubes initially, centrifuged at 5800 rpm (3120g) for 10 minutes, and allowed to equilibrate to room temperature prior to serum collection.

The storage of the SNAP cPL (SNAP cPL Test Kit, Idexx Laboratories Inc., Westbrook, ME) kits, sample handling and testing procedure was according to manufacturer’s instructions (Packet Insert, Idexx Laboratories Inc., Westbrook, ME). Testing of SNAP cPL was either performed at the time of collection and the remaining serum frozen at minus 20°C, or the serum sample was kept refrigerated for less than one week and then allowed to equilibrate to room temperature before being tested and then frozen. One of two qualified veterinary nurses and one of the authors (MH) performed all in-house SNAP cPL testing and were unaware of the final diagnoses at the time. Results of the SNAP and Spec cPL tests were not paired with individual
dogs at the time a diagnosis was assigned, and the test result was not revealed to veterinarians in charge of the clinical case. If the person performing the in-house test was unsure of the result due to test and reference spot intensity similarities, the test was repeated. If similar results were obtained a second time, the result was recorded as an abnormal. A later batch analysis of Spec cPL (Spec cPL ELISA, Idexx Laboratories, Brisbane, Queensland, Australia) concentration was performed on the frozen serum that had been stored up to 18 months. This was shipped overnight, refrigerated, to a regional laboratory (Idexx Laboratories, Brisbane, Queensland) for analysis.

The SNAP cPL was recorded as either visually normal or abnormal, where abnormal is equivalent to a canine pancreatic lipase ≥ 200 µg/L. The agreement between a clinical diagnosis of pancreatitis and a visually abnormal SNAP cPL was quantified by the kappa (κ) coefficient. Further, the agreement between a clinical diagnosis of pancreatitis and a Spec cPL ≥ 400 µg/L was also quantified by the kappa (κ) coefficient. A Spec cPL ≥ 400 is considered consistent with pancreatitis (Steiner et al., 2001, Trivedi et al., 2011, McCord et al., 2012).

A cut-off concentration of cPL ≥ 200 µg/L, as measured by Spec cPL, was used for the agreement between the SNAP cPL and Spec cPL for all dogs. Spec cPL above or below this concentration, with visually abnormal or normal SNAP cPL respectively, was considered necessary for agreement between the two tests.

All agreements were made using McNemar’s test, and quantified by the kappa (κ) coefficient (PROC FREQ, SAS v9.1, SAS Institute, Cary, NC). Results for the Spec cPL were between the values of 30 µg/L and 1000 µg/L, which represents the limits of the range reported by the laboratory performing the assays. Reported results of ≤ 30 µg/L or ≥ 1000 µg/L were calculated as 30 µg/L or 1000 µg/L respectively for all analyses.

Minimal testing to diagnose dogs with AP included history, physical exam, complete blood count, biochemical analysis, and abdominal ultrasound by a veterinary radiologist. Diagnostics for dogs without AP were performed as indicated for each individual dog to enable a diagnosis. These tests included history, physical exam, complete blood count, biochemical analysis,
abdominal ultrasound (by either a veterinary radiologist or emergency clinician), blood gases and electrolytes, thoracic and abdominal radiography and computed tomography, echocardiography, coagulation function, body fluid analysis and culture and sensitivity, cytology, biopsy, histopathology, immunohistochemistry, and surgery.

The results of these tests were reviewed by 3 of the authors (2 board-certified ECC and 1 board-certified internal medicine) upon completion of the study to determine the definitive diagnosis. The authors were blinded to the results of the SNAP and Spec cPL tests at that time. Dogs were diagnosed with AP if they had ultrasonographic and/or histological support for pancreatic inflammation or necrosis with no other identifiable disease. Supportive ultrasonographic evidence of AP was defined and reported by the veterinary radiologist to include the presence of an enlarged, hypoechoic pancreatic tissue surrounded by hyperechoic peripancreatic mesentery, with or without peritoneal effusion, biliary duct dilatation and corrugation or thickening of the duodenal wall (Hess et al., 1998; Hecht et al., 2007). Additionally, in order to be given a final diagnosis of clinical AP, a minimum of 6 months follow-up was required to ensure exocrine pancreatic neoplasia was unlikely.

For analysis, dogs were allocated to one of two groups, based on the above criteria:

- **Group 1:** Dogs with AP as their primary disease.
- **Group 2:** Dogs with confirmed disease other than AP. These dogs may have had pancreatic inflammation but was considered inconsequential and not the primary cause of their clinical presentation.

### 4.3 Results

Samples were collected from 64 client-owned dogs, with 26 dogs excluded as no definitive diagnosis could be made, leaving 38 dogs for analysis (Figure 4.1). Vomiting was present in 28 (74%), diarrhea in 8 (21%), abdominal pain in 33 (87%) and abdominal distension in 7 dogs (18%) (Table 4.1). No sample on testing had an indeterminate SNAP cPL result. Serum was available for Spec cPL measurement in 36 of 38 dogs. Twenty-nine (76%) of these samples were either haemolysed \( n = 25/38 \) (66%), icteric \( n = 3/38 \) (8%) or lipaemic \( n = 6/38 \) (16%), or had a combination of these characteristics \( n = 6/38 \) (16%).
Eleven dogs were diagnosed with AP (Group 1). Breeds in this group included Australian Cattle Dog (n=2), Fox terrier (n=1), Border Collie (n=1), Jack Russell terrier (n=1), Labrador (n=1), Maltese cross (n=1), Akita (n=1), Siberian Husky (n=1), Miniature Schnauzer (n=1) and Cocker Spaniel Cross (n=1). Ages ranged from 1.5 to 13 years (median = 9 years, mean = 8 years). There were 7 females (6 neutered), and 4 males (3 neutered). Abdominal ultrasonography was consistent with AP in all 11 dogs, and no dog underwent surgery. Three dogs were euthanatized (post mortem confirmed diagnosis in 1; no post-mortem examination was permitted in 2), with no clinical recurrence in the surviving 8 dogs at 6 months follow-up. No dog
Table 4.1: Presenting clinical and clinicopathologic characteristics of 38 dogs presenting with acute abdominal disease grouped by a clinical diagnosis of acute pancreatitis (Group 1) or disease other than acute pancreatitis (Group 2) and their corresponding SNAP canine pancreatic lipase (cPL) results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (Pancreatitis)</th>
<th>Group 2 (Not pancreatitis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SNAP cPL abnormal (n = 9)</td>
<td>SNAP cPL normal (n = 2)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gross hemolysis</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Gross lipemia</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gross icterus</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Azotaemia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Elevated urea</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal Effusions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Septic or Inflammatory</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Transudate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not classified</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Euthanatized</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

with AP had azotemia. Abdominal effusions were noted in 5 (45%) of these dogs, but no abdominal fluid was collected for analysis.

Primary disease other than AP was diagnosed in 27 dogs (Group 2). Breeds included Labrador (n=3), Siberian Husky (n=2), Border Collie (n=2), Rottweiler (n=2), German shepherd (n=2), and the remaining 16 dogs were represented by single or mixed breeds. Ages ranged from 17 weeks to 15 years (median = 10 years; mean = 9 years). There were 9 neutered females, 3 entire females, 12 neutered males and 3 entire males. Fifteen of these dogs were euthanatized, and 1 dog died.

Five of the dogs from group 2 had full post mortem performed, which confirmed the absence of pancreatic or peri-pancreatic inflammation or necrosis. Diagnoses included anaplastic large T-cell lymphoma of the liver (n=1); small intestinal infarction with bilateral adrenomegaly (n=1); pancreatic islet cell carcinoma with erosive enterocolitis (n=1); pancreatic islet cell carcinoma with hepatic metastasis (n=1); pancreatic carcinoma with hepatic, duodenal, lymph node and
lung metastasis (n=1). Of the 3 dogs with pancreatic islet cell carcinoma, histology did not identify inflammation or necrosis associated with pancreatic tissue, although in 1 there was virtually no recognisable pancreatic tissue present. None of the dogs with pancreatic carcinoma tested positive with either SNAP cPL or Spec cPL. Both remaining dogs had abnormal SNAP cPL tests but only the dog with small intestinal infarction had an elevated Spec cPL consistent with pancreatitis.

The 22 dogs that did not have post-mortem examination in group 2 were diagnosed with small intestinal foreign bodies (n=6), hemoperitoneum due to splenic and/or concurrent hepatic masses (n= 3), pyometra (n=2), hepatic abscessation (n=2), emphysematous cholecystitis (n=2), abdominal mass and concurrent septic peritonitis (n=1), large solitary hepatic mass invading the caudal vena cava (n=1), septic peritonitis due to a ruptured jejunal mass (n=1), septic peritonitis due to intestinal foreign body (n=1), prostatic abscessation (n=1), pericardial effusion (n=1), and hepatic lymphoma (n=1).

Abdominal ultrasound by a veterinary radiologist was performed in a total 14 dogs of group 2. Abdominal surgery was performed in 14 dogs in group 2, of which 8 dogs did not undergo abdominal ultrasonography prior. One dog had abdominal and thoracic computed tomographic examination. No surgery or specialist abdominal imaging was performed in 4 dogs of group 2. A final diagnosis of hemoperitoneum was confirmed in 3 of the 4 by abdominocentesis. All 3 had large abdominal masses as ultrasound by the emergency resident, and were reported as splenic (n=2) or hepatic (n=1). The dog identified as having a hepatic mass also displayed a septic component as evidenced by intracellular bacteria by cytology. The remaining dog had a post-mortem only. The final diagnosis was pancreatic carcinoma with hepatic metastasis.

In the 11 dogs of group 1, 9 (82%) tested positive with SNAP cPL and 2 (18%) tested negative (Table 4.2). Pancreatic lipase was measured in 8 of 9 positive SNAP dogs, and 100% (8/8) had Spec cPL concentrations ≥ 200 µg/L (range 320 to 1000 µg/L; median 800 µg/L; mean 748 µg/L). There was insufficient serum for Spec cPL testing in the remaining dog. The two dogs with negative SNAP cPL results both had Spec cPL concentrations of 30 µg/L.
Table 4.2: Specific canine pancreatic lipase (Spec cPL) serum concentrations in 11 dogs presenting for acute abdomen with a diagnosis of primary pancreatitis grouped by SNAP cPL result (positive or negative) and listed in ascending order by Spec cPL concentration.

<table>
<thead>
<tr>
<th>Spec-cPL result (µg/L)</th>
<th>n= 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog SNAPP cPL positive</td>
<td>Dog SNAP cPL negative</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Insufficient sample</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
</tr>
</tbody>
</table>

In the 27 dogs of group 2, 11 (41%) tested positive with SNAP cPL and 16 (59%) tested negative (Table 4.3). Pancreatic lipase was measured in 10 of 11 positive SNAP dogs, and 6 had Spec cPL concentrations ≥ 400 µg/L, and 4 had Spec cPL < 200 µg/L. There was insufficient serum for Spec cPL testing in one SNAP positive dog in group 2. The remaining 16 dogs in group 2 with a negative SNAP cPL all had Spec cPL concentration < 200 µg/L (median 30 µg/L, mean 51 µg/L, range 30 to 121 µg/L). Eight of the 11 dogs (73%) with a positive SNAP cPL in group 2 had abdominal effusions (septic/inflammatory in 6). In the 16 dogs with a negative SNAP cPL in group 2, 8 (50%) had abdominal effusions (with 3 being septic/inflammatory). Therefore 9/27 (33%) of dogs in group 2 had septic or inflammatory abdominal effusions, with 6 of these 9 having a positive SNAP cPL. Of the 4 dogs with positive SNAP cPL and normal Spec cPL results, 2 (50%) had septic or inflammatory abdominal effusions.
Table 4.3: Diagnosis and specific canine pancreatic lipase (Spec cPL) serum concentration (µg/L) in 27 dogs presenting for acute abdomen where pancreatitis was not the primary cause for presentation, grouped by SNAP cPL result (positive or negative) and listed by organ system involved. * Pancreatic histology and post mortem were performed and no pancreatic inflammation was present.

<table>
<thead>
<tr>
<th>SNAP cPL positive</th>
<th>SNAP cPL negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>Diagnosis</td>
</tr>
<tr>
<td>1</td>
<td>Small intestinal foreign body</td>
</tr>
<tr>
<td>2</td>
<td>Small intestinal foreign body</td>
</tr>
<tr>
<td>3</td>
<td>Small intestinal foreign body and septic peritonitis</td>
</tr>
<tr>
<td>4</td>
<td>Small intestinal infarction with bilateral adrenomegaly*</td>
</tr>
<tr>
<td>5</td>
<td>Hepatic T-cell lymphoma*</td>
</tr>
<tr>
<td>6</td>
<td>Hepatic mass</td>
</tr>
<tr>
<td>7</td>
<td>Hepatic masses / septic peritonitis</td>
</tr>
<tr>
<td>8</td>
<td>Hepatic/splenic masses with hemoperitoneum</td>
</tr>
<tr>
<td>9</td>
<td>Hepatic abscess</td>
</tr>
<tr>
<td>10</td>
<td>Hemoperitoneum / septic peritonitis</td>
</tr>
<tr>
<td>11</td>
<td>Pyometra and septic peritonitis</td>
</tr>
<tr>
<td>23</td>
<td>Pericardial effusion</td>
</tr>
<tr>
<td>24</td>
<td>Prostatic abscess</td>
</tr>
<tr>
<td>25</td>
<td>Pancreatic carcinoma*</td>
</tr>
<tr>
<td>26</td>
<td>Pancreatic carcinoma with ulcerative enterocolitis*</td>
</tr>
<tr>
<td>27</td>
<td>Pancreatic carcinoma with hemoperitoneum*</td>
</tr>
</tbody>
</table>

Azotaemia was present in 3 dogs in group 2, with creatinine ranging from 220 to 323 µmol/L (ref: 44-159 µmol/L) and urea ranging from 10 to 28.2 mmol/L (ref: 2.5-9.6 mmol/L). These 3 dogs all had positive SNAP cPL results, with 2/3 also having Spec cPL concentration ≥ 200 µg/L. Urea alone was increased in 4 dogs in this group ranging from 10.1 to 26.9 mmol/L (ref: 2.5-9.6 mmol/L). Only 2 of these dogs (50%) had a positive SNAP cPL test, and none had Spec cPL ≥ 200 µg/L.

The clinical sensitivity and specificity for SNAP cPL was 82% (9/11 dogs of group 1) and 59% (16/27 dogs of group 2), respectively. The clinical sensitivity and specificity for Spec cPL was
70% (7/10 dogs of group 1) and 77% (20/26 dogs of group 2), respectively. Accuracy of the SNAP and Spec cPL with a clinical diagnosis of AP was 66% and 75% respectively.

The agreement of SNAP cPL with a clinical diagnosis of primary AP in all dogs (Table 4.4) resulted in a κ of 0.33 (95% CI: 0.06-0.61).

Table 4.4: Cross-tabulation of the agreement (κ) between a clinical diagnosis of pancreatitis (Group 1) and SNAP cPL for 38 dogs presented with signs of acute abdominal disease (Groups 1 and 2).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAP positive</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>SNAP negative</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

κ = 0.33 (95% CI: 0.06-0.61)

Agreement was also calculated to assume the 2 dogs in group 1 that tested normal on SNAP cPL were falsely diagnosed with pancreatitis. If these dogs were moved to group 2 for analysis, agreement of SNAP cPL and a clinical diagnosis of AP resulted in a κ of 0.44 for all dogs (95% CI: 0.20-0.67). The agreement of Spec cPL with a clinical diagnosis of primary AP (Table 4.5) in all dogs resulted in a κ of 0.43 (95% CI: 0.12-0.74).

Table 4.5: Cross-tabulation of the agreement (κ) between a clinical diagnosis of pancreatitis (Group 1) and specific canine pancreatic lipase (Spec cPL) for 36 dogs presented with signs of acute abdominal disease (Groups 1 and 2).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spec cPL ≥ 400 µg/L</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Spec cPL &lt; 400 µg/L</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>

κ = 0.43 (95% CI: 0.12-0.74)

The agreement between SNAP cPL and Spec cPL for all dogs (Table 4.6) resulted in a κ of 0.78 (95% CI: 0.59-0.98).
Table 4.6: Cross-tabulation of the agreement (κ) between SNAP cPL and specific canine pancreatic lipase (Spec cPL) for 36 dogs presented with signs of acute abdominal disease.

<table>
<thead>
<tr>
<th></th>
<th>Spec cPL ≥ 200 µg/L</th>
<th>Spec cPL &lt; 200 µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAP positive</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>SNAP negative</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

κ = 0.78 (95% CI: 0.59-0.98)

The agreement between SNAP cPL and Spec cPL concentration for dogs with disease of non-pancreatic origin resulted in κ 0.65 (95% CI: 0.35-0.94). The agreement between SNAP cPL with Spec cPL concentrations in dogs with primary AP resulted in κ =1.0.

4.4 Discussion

This study has demonstrated that SNAP and Spec cPL tests have poor agreement with a clinical diagnosis of primary acute pancreatitis in dogs presenting with compatible history and clinical signs. This was predominantly due to the tests returning a large number of clinically relevant false positives (SNAP cPL: 11/27 dogs or 41%, Spec cPL: 6/26 dogs or 23%).

Sixty percent of SNAP positive dogs without AP had Spec cPL concentrations greater than 400 µg/L, with no dogs having a value between 200-400 µg/L. In this study, having a second reference spot in the SNAP cPL test to indicate concentrations above 400 µg/L would not have improved specificity. The specificity of Spec cPL was greater than SNAP cPL. This was because 4 dogs without AP had visually abnormal SNAP cPLs, but Spec cPL concentrations well below 200 µg/L. The specificity of SNAP cPL and Spec cPL in this study is lower than previously reported (Newman et al., 2004; Trivedi et al., 2011; McCord et al., 2012; Mansfield et al., 2012). The authors feel this is probably due to a population of exclusively sick dogs with similar clinical presentations being tested, without reliance on histologic diagnosis and a known final diagnosis.

The sensitivity of the Spec cPL is consistent with that reported previously (Newman et al., 2004; Steiner et al., 2008; Trivedi et al., 2011; Neilson-Carley et al., 2011,) although that of the SNAP cPL was lower (McCord et al., 2012). There were only a small number of false negatives (2/11
dogs or 18%) with the SNAP cPL. The sensitivity of Spec cPL was lower than SNAP cPL due to 1 dog with a cPL concentration of 320 µg/L being below the diagnostic cut-off of 400 µg/L.

There was good agreement between SNAP cPL and Spec cPL results. This was greater in dogs with a clinical diagnosis of primary pancreatitis than in those without. Further, all dogs testing normal on SNAP cPL had Spec cPL concentrations below 200 µg/L (18/18 dogs or 100%).

There are several possible explanations for the 11/27 dogs without AP that tested positive with SNAP cPL. One possible explanation is that pancreatic inflammation may develop due to diffuse abdominal inflammation, as found in dogs with septic peritonitis. Additionally, any condition that causes hypoperfusion of the pancreas, or ischemia and reperfusion of the splanchnic circulation may cause pancreatic inflammation, as the pancreas is exquisitely sensitive to disturbances of microcirculation (Cuthbertson et al., 2006). Increased total serum lipase activity has been reported in dogs with duodenal foreign bodies (Willard et al., 1993) and acute gastroenteritis (Rallis et al., 1996). This may potentially be due to production of lipase by organs other than the pancreas, or due to duodenal reflux causing sub-clinical pancreatitis. In studies that have assessed specificity of cPL, diagnosis was based on post-mortem analysis from referral centers (Trivedi et al., 2011; Mansfield et al., 2012) and dogs with intestinal foreign bodies were not included in the sample populations. Therefore it is remains unclear whether cPL concentrations are increased in dogs with duodenal foreign bodies. However, none of the dogs with intestinal foreign bodies in this study had elevated Spec cPL, and only 2 were reported in the duodenum. It is possible that other isoforms of lipase were being measured. This is considered unlikely as pancreatic lipase has been localized to the pancreas in immunohistochemical studies (Steiner et al., 2002), and is too low to be quantified in dogs with exocrine pancreatic insufficiency (Steiner et al., 2006).

Dogs with decreased renal function have been shown to have increased serum total lipase activities (Strombeck et al., 1981; Mansfield et al., 2000). One study has shown that Spec cPL is not increased in dogs with experimentally induced chronic renal failure (Steiner et al., 2010), but this has not been verified in dogs where there may be a naturally occurring acute decline in glomerular filtration. In the study cohort reported here there were 3 dogs with azotemia, although none had anuric renal failure. These 3 dogs were not diagnosed with AP, but all had
positive SNAP cPL results, and 2 also had Spec cPL concentrations ≥ 200 µg/L. The clinical diagnoses in these 3 azotemic dogs were splenic and hepatic masses with hemoperitoneum, septic peritonitis secondary to a perforating intestinal foreign body, and small intestinal thrombosis. The dog with intestinal thrombosis underwent post-mortem examination and no histological evidence of pancreatitis was present. The remaining 2 dogs underwent abdominal surgery with no gross evidence of pancreatitis recorded in the surgical reports. However, histological examination of the pancreas was not performed and therefore concurrent microscopic pancreatitis could not be ruled out. Additional studies are required to further elucidate the role of azotemia on cPL concentrations, particularly in acute disease.

All 3 dogs with pancreatic carcinoma had negative SNAP cPL and Spec cPL results. This may be due to very little associated inflammation, as documented in two dogs, or a lack of functional pancreatic tissue, as observed in the remaining dog.

Four dogs in this study had positive SNAP cPL results but Spec cPL concentrations < 200 µg/L. Operator error in interpreting the SNAP cPL results is a possible explanation for this discrepancy, but is considered unlikely. All 3 individuals interpreting the test were trained personnel, and made the observations after performing the test strictly according to the manufacturer guidelines. The manufacturer reports 96-100% agreement between SNAP cPL and Spec cPL for normal samples (cPL < 200 µg/L) and 88-92% agreement for abnormal samples (cPL ≥200 µg/L) (Steiner et al., 2001). It is also reported that visual discrepancy occurs mostly at Spec cPL concentrations around 200 µg/L. For the 4 discrepant results, SNAP cPL was abnormal, but all had Spec cPL concentrations < 105 µg/L. This makes visual discrepancy unlikely. Prolonged storage of serum samples prior to measurement of Spec cPL may also be a contributing factor, as all 4 of these samples were frozen for greater than 6 months. The stability of Spec cPL has been reported to be unchanged after 21 days at room temperature, refrigerated, at -20°C, and at -80°C (Steiner et al., 2009). Additionally, a study evaluating lipase activity and Spec cPL in dogs with experimentally induced chronic renal failure utilized stored samples that were more than 20 years old, and demonstrated significantly elevated pancreatic lipase concentrations in one dog (Steiner et al., 2010). Instability of canine pancreatic lipase in serum frozen at -20°C is therefore thought to be an unlikely cause of the discrepancies in this study.
Hemolysis, icterus and lipemia were frequently present in the samples in this study, but these factors have been shown not to interfere with the visual interpretation of SNAP cPL (Steiner et al., 2001) tests or with measurement of Spec cPL concentrations (Huth et al., 2010). It is theoretically possible that an unknown protein was present in the serum of these 4 dogs that caused interference. However, given that both SNAP cPL and Spec cPL utilize the same dual monoclonal antibodies, abnormal results would be expected for both tests if cross-reactivity was present. However, the stability of a potential cross-reacting inflammatory protein may not be as long lived during storage as cPL, and not be detectable at the time of Spec cPL measurement.

One limitation of this study is the use of ultrasound alone to diagnose AP. The diagnosis of AP by ultrasound has been reported to have a sensitivity ranging from 66 to 68% (Hess et al., 1998; Steiner et al., 2008). In these studies, there may have been primary disease other than AP as no final diagnosis was discussed, and mild or chronic forms of pancreatitis could also have been present, reducing the sensitivity of ultrasound (Steiner et al., 2008). The other study evaluating ultrasound detection of pancreatitis was performed between 1986 and 1995, commencing well over 20 years ago (Hess et al., 1998). The authors believe the diagnostic sensitivity of ultrasound is likely to be much higher now than in the earlier studies due to improved equipment and operator expertise. Additionally, the specificity of ultrasound for the diagnosis of AP due to the presence of hyperechogenicity associated with peri-pancreatic fat necrosis is well accepted (Mansfield et al., 2011). Therefore, the authors feel that the number of false positives in dogs diagnosed with primary AP were negligible. A further limitation was the absence of ultrasonographic evaluation of the pancreas in nearly half of the dogs of group 2. This may have enabled comparison between the positive tests of group 2 and ultrasonographic findings. All dogs in the AP group were treated appropriately for AP, and had no recurrence of clinical signs within 6 months of discharge for all survivors, making concurrent pancreatic neoplasia or other abdominal disease such as septic peritonitis unlikely. Of the 3 dogs in this group that were euthanatized, consent for post-mortem examination was only given for 1 dog. This confirmed the presence of severe pancreatic inflammation and necrosis. To determine if a false positive diagnosis of AP may have influenced this agreement, dogs in group 1 that had a negative SNAP cPL / Spec cPL test were moved to group 2 for analysis. Analysis of agreement for a clinical diagnosis of pancreatitis then gave a χ of 0.44, which still represents poor agreement.
A further limitation of this study was the small number of dogs analyzed. Many dogs were excluded due to an absence of a definitive diagnosis. Therefore, bias towards severe disease may have arisen as dogs with mild pancreatitis may not have had a diagnosis made after full diagnostic work-up. It is unknown what effect inclusion of those dogs would have on the analysis of diagnostic accuracy, sensitivity or specificity.

The manufacturer currently recommends performing a quantitative Spec cPL assay following SNAP cPL at initial presentation. Although there was a good concordance between SNAP cPL and Spec cPL concentration overall in this study, there was some discordance in dogs without primary AP. However, the use of additional testing such as abdominal imaging, along with stringent assessment of clinical and historical findings to make a diagnosis of acute pancreatitis may preclude the necessity of follow-up testing. Currently there is no published data that shows changes in serum concentrations of pancreatic enzymes corresponds to clinical improvement, or should be used to select treatment regimes.

4.5 Conclusion

This study indicated a poorer specificity of cPL for diagnosing AP than previously reported, although sensitivity was similar. There was reasonable agreement between SNAP cPL and Spec cPL results. Measurement of Spec cPL had a better agreement than SNAP cPL for a clinical diagnosis of AP, but overall both produced poor agreement. A positive SNAP cPL or Spec cPL may be indicative of pancreatic inflammation, however this cannot readily determine the primary reason for clinical presentation. Conversely, a negative SNAP cPL or Spec cPL < 200 µg/L appears to be moderately specific, with a small number of dogs (2/11; 18%) diagnosed with AP having false negative results.
Chapter 5: GENERAL DISCUSSION

The complicated challenges of determining test accuracy for the diagnosis of acute pancreatitis were highlighted and discussed in Chapter 3 of this thesis. Historically, these challenges related to the difficulty of creating good study design in an appropriate clinical setting for acute pancreatitis. The prospective study that was conducted (Chapter 4) was designed in an attempt to address some of these issues, and resulted in highlighting others. The aim of this final chapter is to reflect on, and critically evaluate, the study design described in Chapter 4, and to provide further discussion on the problems encountered therein.

One of the common problems encountered by previous studies has been the failure to select an appropriate sample population; firstly lack of an adequate sample size, and secondly the selection of a biased population. This study has also suffered from both of these challenges. The inclusion criteria for this study, reported in Chapter 4, limited the population to subjects where a full diagnostic work-up was performed; this may have not only constrained the sample number, but may have also introduced bias by skewing the selected population towards subjects with more severe disease. The setting for the study was a 24-hour referral hospital, which may have further skewed the population to the more severe end of the spectrum of disease. If the population includes mostly severely affected subjects, then the sensitivity may be falsely increased and specificity falsely decreased. In this study, the sensitivity of the SNAP® cPL test was similar to other studies, but its specificity was much lower, which may reflect this bias. Despite these limitations in sample population, it is suggested that the selection of the population used in this study was more appropriate than previous studies, as it included those with the disease and those with similar clinical signs but without the disease. This falls more closely towards the ‘intended use’ population, although, as mentioned above, it is still likely skewed with regard to severity of disease.

The accepted gold standard of histopathological diagnosis cannot be achieved for a cohort of dogs that are mostly expected to survive. As mentioned previously, even if necropsy is applied to the cohort, there is still the concern about whether the current grading system is too stringent for defining pancreatitis. But further controversy also exists as to whether histopathology should be used at all as the gold standard of diagnosing the syndrome of acute pancreatitis. This study highlighted this controversy by abandoning a gold standard altogether, and using a composite
reference standard instead. This approach was chosen not only due to the lack of availability of histopathology within the cohort, but because it was deemed to be a more appropriate method for determining Spec cPL® accuracy. As such, this is the first study to separate acute pancreatitis into two different categories; primary and secondary. The historical gold standard would not have been useful in separating these two diagnoses; only a composite of diagnostic tests and expert opinion can be used in this clinical setting. Therefore, the choice of a composite reference standard in this study was purposeful and, in the author's opinion, more useful. It was not a goal of the study to disprove that increased cPL indicated pancreatic inflammation, rather that increased cPL did not rule out another, more important, underlying disease. In these cases of secondary pancreatitis, the cPL result was considered to be irrelevant, or not necessarily helpful, as there was other significant underlying disease. By way of analogy, an increased alanine transferase activity does not simply indicate that hepatitis is the diagnosis, but that there may be other reasons for leakage of this enzyme. This study has tried to shift the emphasis towards the ‘clinical interpretation’ of the result. This may be another reason why specificity was much lower in this study compared to previous reports, as the dogs categorised as being ‘disease-negative’ may still have had pancreatic inflammation.

During this study, considerable reliance was placed on ultrasound examination as an inclusion into the composite reference standard. It is the author’s opinion that the exclusion of ultrasound examination in the current gold standard of diagnosis, and reliance on histopathology instead, may have been premature. Since the landmark report for sonographic sensitivity of canine pancreatitis was undertaken nearly 30 years ago (Hess et al., 1998), the ultrasound equipment, operator skills and operator knowledge have most likely vastly improved the accepted sensitivity now. In fact, clinicians currently use ultrasonography as the test of choice for the clinical diagnosis of acute pancreatitis. Focus should be given to providing evidence that modern ultrasonography is a useful tool for diagnosing pancreatitis.

As there is currently no consensus on what elements should be included in a composite reference standard, it brings into question the validity of using certain statistical tests in order to make any comment about accuracy of the Spec cPL®. This study included sensitivity, specificity and kappa agreement to describe the relationship between primary acute pancreatitis and cPL. Strictly, sensitivity and specificity should not be applied where an accepted gold standard has not been used as the comparison. These operational characteristics were included
in this study (Chapter 4) to provide a comparison to the previous reports in literature and to satisfy the needs of the veterinary audience in this regard. However, in reality the study used a composite reference standard and defined the disease as a clinical syndrome. Without the absolute knowledge of the presence or absence of pancreatitis, it may have been inappropriate to report sensitivity and specificity.

This concept of purposefully avoiding sensitivity and specificity was discussed briefly in Chapter 3, and using agreement as the alternative test may have been a better option for this study. Certainly it was appropriate for describing the relationship between Spec cPL® and SNAP® cPL. The use of this test for describing the accuracy of the Spec cPL® and SNAP® cPL for diagnosis of primary pancreatitis is likely a controversial decision. However, it was felt by the authors that sensitivity and specificity might be misinterpreted by the audience as reflective of the test’s accuracy for detecting pancreatic inflammation, not simply agreement with a clinical diagnosis. As it is more important in critically ill patients to distinguish between primary and secondary pancreatitis, choice of a composite reference standard and use of agreement as expression of the accuracy was felt to be the best method to display the Spec cPL®’s and SNAP® cPL’s limitations in the clinical setting.

**Conclusion**

The evidence from the study undertaken here highlights the difficulties in utilising a serum marker for the diagnosis of canine acute pancreatitis. Indeed, the use of any single test in the diagnosis of acute pancreatitis is difficult to recommend. While further investigations of cPL and other serum markers are likely to be undertaken, the value of including other diagnostic modalities such as abdominal ultrasound should be revisited. However, the lack of a universal gold standard is expected to remain unchanged in ante-mortem diagnosis in the near future, which is likely to continue to generate much controversy surrounding the diagnosis of this important canine disease.
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