New Directions in Diagnosis and Treatment of Canine Acute Pancreatitis

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Declaration

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

Caroline Sarah Mansfield

Date: 20/11/2011
Abstract

Acute pancreatitis is an important disease in companion animal medicine, and diagnostic methodology available to veterinary practitioners is often limited. Evidence based principles for the management of this common disease are also lacking. This thesis explores the current diagnostics of canine pancreatitis and management of this condition, reviewing the literature across both the veterinary and human medical fields.

Assessment of the specificity of canine pancreatic-specific lipase (cPL) was made in a post-mortem study and calculated to be 82-92%, with a correlating sensitivity of 45-55%. A multi-centre study of dogs presenting with clinical signs consistent with acute pancreatitis to assess a new laboratory test, serum canine pancreatic elastase-1 (cPE-1) was also performed. This test had a sensitivity ranging from 66-79%, with a specificity of 92%. The sensitivity of both laboratory tests was greater in dogs with severe disease.

To assess potential treatment options, a clinical severity score was established, with gut health, respiratory complications, cardiac complications, and blood pressure determining the final score. Retrospectively, plasma administration did not appear to be associated with treatment success, but this conclusion was limited by the retrospective nature of the study and small numbers of dogs. Out of the other factors, fasting for 3 or more days was the one most significantly associated with mortality. To begin assessment of nutritional modalities, pancreatic responses in healthy dogs to varying dietary fat composition (ranging from 4%DW to 16% DW) was assessed, with no statistical difference determined. On the basis of this, a pilot study of 10 dogs with severe pancreatitis was undertaken, with 5 dogs fed enterally and another 5 dogs were given total parenteral nutrition (TPN). No differences in mortality or days of hospitalisation between the two were found, but there were significantly less episodes of vomiting or regurgitation in the dogs given food (p < 0.001). There were also more
severe complications (4/5) in the TPN group compared to the enteral feeding group (2/5).

In all, this thesis supports the new premise of enteral feeding of dogs with acute pancreatitis early in the course of disease, determines the sensitivity and specificity of two diagnostic tests and has established an objective marker of disease severity.
Acknowledgements

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**Introduction**

Pancreatitis is an important disease of dogs. Acute pancreatitis can cause profuse vomiting with resultant dehydration and hypovolaemia (Simpson, 1993; Williams and Steiner, 2005). There is a high mortality rate associated with systemic effects of the disease, and dogs often require intensive treatment and hospitalisation (Ruaux and Atwell, 1998b).

Predicting which animals will develop severe complications is difficult, although there is a significant association between fatality and existence of concurrent diseases such as diabetes mellitus, hyperadrenocorticism, and epilepsy (Cook et al., 1993; Hess et al., 1998; Ruaux and Atwell, 1998b). Traditional biochemical methods of diagnosing pancreatitis such as elevation of serum amylase and lipase concentrations are poor predictors of mortality (Mansfield et al., 2003). Despite an increase in the number of studies in the past decade addressing the diagnostic difficulties faced in canine pancreatitis, it is still unclear how sensitive and specific the currently used diagnostic modalities are. Part of this difficulty is a lack of a true gold standard, as pancreatic histopathology is seldom performed in severely unwell dogs with pancreatitis. It is also uncertain as to how the pancreatic inflammation seen histologically correlates to clinical severity, or indeed if it is the primary reason for presentation of the animal.

Treatment of acute pancreatitis is non-specific and aimed at correcting secondary consequences of the disease (such as hypovolaemia or pain for example) rather than directly treating the pancreatic inflammation. There is a lack of controlled studies in treatment of this condition, and most recommendations are extrapolated from animal experimental models and human gastroenterology. An important aspect of developing any prospective treatment trials is to ensure that animals of equal clinical
severity are compared in studies, in order to ensure that any benefits are due to the treatment intervention rather than the study population.
**Purpose**

This body of work aims to answer the following questions

- In a cohort of dogs who display clinical signs that could be consistent with canine pancreatitis, what is the specificity and sensitivity of serum canine pancreatic-elastase 1, a potentially new diagnostic test?

- In a sufficiently large cohort of dogs with diseases of similar severity to acute pancreatitis, what is the clinical specificity and sensitivity of canine pancreatic lipase?

- Is there an effective and robust way to characterise the clinical severity of canine pancreatitis to aid in the development of future research into optimisation of treatment strategies?

- Does the administration of plasma or minimal enteral nutrition have any potential benefits in the treatment of acute pancreatitis?

- Does differing fat content of diet cause differing responses of the canine pancreas, and necessitate specific nutritional strategies?

- Is early interventional enteral nutrition delivered proximal to the pylorus well tolerated by dogs with acute pancreatitis?

- Is there evidence that early interventional enteral nutrition may be of benefit in treating dogs with severe acute pancreatitis?
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APACHE</td>
<td>Acute Physiology and Chronic Health Evaluation</td>
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<td>APN</td>
<td>Acute pancreatic necrosis</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<td>CIRCI</td>
<td>Critical illness related corticosteroid insufficiency</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
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<tr>
<td>EN</td>
<td>Enteral nutrition</td>
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<tr>
<td>ERCP</td>
<td>Endoscopic Retrograde Cholangiopancreatography</td>
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<tr>
<td>FFP</td>
<td>Fresh frozen plasma</td>
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<tr>
<td>ICAM</td>
<td>Intercellular adhesion molecule</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>MEN</td>
<td>Minimal (micro) enteral nutrition</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
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<tr>
<td>MODS</td>
<td>Multiple organ dysfunction syndrome</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NG</td>
<td>Nasogastric (delivery of EN)</td>
</tr>
<tr>
<td>NJ</td>
<td>Nasojejunal (delivery of EN)</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative Predictive Value</td>
</tr>
<tr>
<td>PE-1</td>
<td>Pancreatic Elastase-1</td>
</tr>
<tr>
<td>PLA</td>
<td>Phospholipase</td>
</tr>
<tr>
<td>PLI</td>
<td>Pancreatic lipase immunoreactivity</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PN</td>
<td>Parenteral nutrition</td>
</tr>
<tr>
<td>PPN</td>
<td>Partial parenteral nutrition</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive Predictive Value</td>
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<tr>
<td>PSTI</td>
<td>Pancreatic secretory trypsin inhibitor</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operator characteristics</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>RT-PCR</td>
<td>Real-time polymerase chain reaction</td>
</tr>
<tr>
<td>SAA</td>
<td>Serum amyloid A</td>
</tr>
<tr>
<td>SIRS</td>
<td>Systemic inflammatory response</td>
</tr>
<tr>
<td>Spec-cPL</td>
<td>Specific canine pancreatic lipase</td>
</tr>
<tr>
<td>TAP</td>
<td>Trypsinogen activation peptide</td>
</tr>
<tr>
<td>TLI</td>
<td>Trypsin-like immunoreactivity</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
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1.1 Normal anatomy and physiology of the canine pancreas

In order to fully understand the potential complications of pancreatitis in dogs and devise treatment options, it is important to be cognisant of the role and location of the pancreas. The anatomical location and control of exocrine pancreatic functions are directly relevant when considering the aetiologies of pancreatitis, as well as the resultant clinical signs and optimal treatments.

1.1.1 Normal Canine Pancreatic Anatomy

The pancreas is composed of a left and right lobe, joined in a small central body (Williams, 1996). Embryologically, the right lobe is formed from the ventral primordium (bud) and contains a majority of polypeptide-producing cells (Evans and Christensen, 1979; Williams, 1996). The left lobe develops from the dorsal bud, and predominantly contains glucagon secreting cells (Evans and Christensen, 1979; Williams, 1996). In the dog there are usually two pancreatic ducts leading to the small intestine. The duct that originates from the ventral bud is the pancreatic, or Wirsung’s, duct and opens adjacent to the bile duct on the major duodenal papilla (Evans and Christensen, 1979; Williams, 1996). The accessory, or Santorini, duct originating from the left lobe opens distally to this at the minor duodenal papilla (Evans and Christensen, 1979; Williams, 1996). In some dogs only one duct (generally the accessory as it is the largest), is present and so all pancreatic juice enters the intestine through the minor duodenal papilla (Evans and Christensen, 1979; Williams, 1996). This differs from people, who normally have one duct only and are predisposed to obstructions of this duct (Al Mofleh, 2008).

The pancreas is situated in the cranial part of the abdomen (Figure 1.1). The body follows along the mesenteric surface of the duodenum, curving at the first
duodenal flexure (Evans and Christensen, 1979; Williams, 1996). The right lobe then travels alongside the duodenum, and may reach the caecum. The left lobe is deeper in the mesentery, and sits adjacent to the pylorus. This results in the pancreas having direct physical contact with the liver, transverse colon, left kidney and spleen, as well as the surrounding omentum (Evans and Christensen, 1979; Williams, 1996). Inflammation surrounding the pancreas therefore has the potential to directly affect multiple organs within the abdomen.

The functional units of the exocrine pancreas are acinar cells. The bulk of the exocrine function is performed by the central acinar cells, with an extensive duct system allowing complex communication between the exocrine and endocrine cells of
the pancreas. The endocrine function, which will not be discussed in detail in this thesis, is performed by the islets of Langerhans, which are scattered throughout the tissue. There is close communication between the exocrine and endocrine cells, ensuring coordination of digestion and the production of endocrine hormones such as insulin (Charles, 2007; Evans and Christensen, 1979; Williams, 1996).

The blood supply to the pancreas originates from the coeliac and cranial mesenteric arteries. They branch off to form the cranial and caudal pancreatico-duodenal arteries respectively, and enter the right limb of the pancreas (Evans and Christensen, 1979; Williams, 1996). The splenic artery, which itself arises from the coeliac artery, supplies the left limb. There are parallel venous systems that drain into the portal vein (Evans and Christensen, 1979; Williams, 1996). The pancreatic lobule is supplied by a single arterial branch, and the acinar cells are supplied by a continuous network of capillaries (Charels, 2007; Evans and Christensen, 1979). The artery often supplies islets first and therefore bathes the acinar cell in relevant hormones, or the so-called ‘insuloacinar’ portal system’ (Charles, 2007; Cuthbertson and Christophi, 2006). The downstream supply to acinar cells makes them uniquely susceptible to poor perfusion and hypotension, an important factor in the development of pancreatitis, to be discussed in section 1.2.2.4.

The nervous supply to the pancreas is not discretely localised, but rather forms a large web of nerve fibres, with many nerve trunks throughout the parenchyma of the pancreas (Evans and Christensen, 1979; Williams, 1996). This nervous supply originates from the vagus and splanchnic nerves, which travel alongside the coeliac and caudal mesenteric arteries (Evans and Christensen, 1979; Williams, 1996). The neurogenic supply is also intricately involved in the exocrine function of the pancreas.
1.1.2 Normal pancreatic physiology

The pancreas has a number of exocrine functions. The pancreas is responsible for secreting enzymes into the small intestine to assist in degradation of proteins, fats and carbohydrates. To protect itself from the digestive nature of these enzymes the pancreatic enzymes are stored as zymogens within the pancreatic acinar cells. Zymogens are catalytically inactive precursors of digestive enzymes that are secreted into the lumen of the small intestine in response to food. Enterokinase, a peptide produced by small intestinal mucosal cells, activates trypsin. Trypsin subsequently activates an enzyme cascade, cleaving the activation peptides from other digestive zymogens, as shown in Figure 1.2 (Rinderknecht, 1986).

![Diagram](image)

**Figure 1.2: Diagrammatic representation of trypsin activating the pancreatic cascade within the duodenum**

The pancreas is able to protect itself from auto-digestion in a number of ways. As already mentioned, firstly the digestive enzymes are stored in the acinar cells as inert zymogens, and theoretically are only activated within the lumen of the duodenum. Secondly, within the acinar cell the zymogen granules are kept separately from the lysosomal granules enclosed in membrane bound organelles (Steer and Meldolesi, 1988). Lysosomal enzymes are produced in the ribosomes attached to the rough endoplasmic reticulum, in the same manner that zymogens are, but are additionally glycosolated and phosphorylated as they pass through the Golgi complex (Frossard and
Pastor, 2002; Steer and Meldolesi, 1988). Studies have shown that these two enzyme groups are kept physically apart throughout all stages of their production (Scheele, 1980). Thirdly, location of pancreatic secretory trypsin inhibitor (PSTI) within the acinar cells allows for immediate inhibition of trypsin should it be prematurely activated within the acinar cells. PSTI is produced and stored in the same location as the digestive enzymes (Laskowski and Kato, 1980; Rinderknecht, 1986). Finally, should any activated trypsin reach the circulation, larger anti-proteases in the blood have the capacity to deactivate some circulating trypsin (Rinderknecht, 1986; Williams, 1996).

1.1.2.1 Control of exocrine function

The control of exocrine secretion is an important aspect in considering nutritional management of pancreatitis. There are four phases of pancreatic secretion: basal, cephalic, gastric and intestinal (Abou-Assi and O’Keefe, 2001). The basal rate is slow and present during fasting; the cephalic is vagally mediated and stimulated by sight, smell and taste; the gastric is stimulated by the stomach distending with food causing gastrin release; and the intestinal phase occurs when chyme enters the duodenum, via secretin and cholecystokinin (CCK) production. It would appear that secretin stimulates production of the aqueous component of pancreatic secretion in the ducts, whilst CCK stimulates production of digestive enzymes in the acinar cells (Frossard and Pastor, 2002). There is also a pathway not controlled by CCK that stimulates pancreatic secretion when food is in the duodenal lumen. The amount of digestive enzymes contained in pancreatic secretions increases relative to the amount of bicarbonate 1-2 hours following a meal, with a second peak 8-11 hours later comprising a larger volume and containing a greater amount of bicarbonate (Williams, 1996). There are other substances that can also stimulate secretion, such as acetylcholine,
gastrin, substance P, and vasoactive peptide (VIP); but their complete role is not yet fully understood (Abou-Assi and O’Keefe, 2001).

The enteropancreatic pathway has been studied a lot in rodent models, and to some extent in people, but very little in dogs. One early experimental study (utilizing a fistula model in 4 dogs) measured pancreatic secretion and quantified proteins and bicarbonates in those secretions (Konturek et al., 1979). They found that there was an increase in pancreatic protein secretion when amino acids and fat were delivered both intravenously and directly into the duodenum. This response seemed to be particularly triggered by L-tryptophan and L-phenylalanine. This suggested that end-products of protein digestion may be more potent stimulants than protein as a whole. In dogs therefore, there could also be an enterohepatic reflex that stimulates pancreatic secretory function after absorption of L-isomers of amino acids from the gut.

One canine experimental study also assessed the interaction of various substances on pancreatic secretion (Beglinger et al., 1984). They determined that in dogs, CCK alone did not increase pancreatic protease or bicarbonate secretion, but when given with secretin there was an increase. This didn’t change when atropine was administered, except in the presence of phenylalanine, suggesting the cholinergic control of pancreatic secretion is probably not present in dogs. This has some clinical relevance, as it is thought that the cephalic (or anticipation) phase of pancreatic secretion is vagally mediated. If vagal (cholinergic) mediation doesn’t occur in dogs, then exposure to smells and sight of food may have little effect on pancreatic secretion.

In a landmark study that helped to shape the nutritional management of acute pancreatitis for decades, the effects of diet and anatomical location of food delivery were assessed in healthy dogs (Ragins et al., 1973). It was concluded that maximal pancreatic stimulation occurred in dogs when an acidic fluid containing amino acids
was delivered to the duodenum, but not when delivered to the jejunum. This led to the avoidance of feeding methods that resulted in food being present in the duodenum.

Further, the concept of an ‘ileal brake’ has been proposed. This is when nutrients reach the distal ileum or colon there is negative feedback on a wide range of intestinal functions. In a canine experimental study this was demonstrated when a complete human liquid convalescent diet was infused into the colon and ileum (Wen et al., 1995). This delayed phase 3 of the migrating motor complex (MMC) in the duodenum and adjacent ileum and increased the release of inhibitory substances on pancreatic secretion. These substances in the dog also inhibit gastric secretions, gastric emptying and the normal fasting motility of the stomach. The ileal brake concept was proven in another canine experimental model, and appeared to be most strongly stimulated by tryptophan (Niebergall-Roth et al., 2000). This suggests that although food within the intestine may initially cause increased pancreatic secretions, once it reaches the distal ileum or colon, there is negative feedback on the pancreas, perhaps ameliorating any adverse effects.

1.2 Pancreatitis: The Disease
1.2.1 Animal experimental models

In order to fully evaluate the advances made in understanding the pathophysiology of pancreatitis it is important to have an understanding of the experimental models used, and how they may relate to canine pancreatitis as it naturally occurs. These methods were well summarised by Su et al in 2006, and are reviewed briefly here, with both invasive and non-invasive methods for inducing pancreatitis (Su et al., 2006). The most commonly applied non-invasive method is administration of a CCK analogue (cerulein) intravenously, subcutaneously or into the peritoneum. It has been applied to a number of species, including dogs (Simpson et al., 1995). This results in a mild, self-limiting form of pancreatitis that is useful for studying lung-associated
pathology, and so is likely reflective of only a small proportion of dogs seen in veterinary practice. A dietary model feeding a choline-deficient diet with ethionine (CDE diet) is simple and highly reproducible, and causes a severe, necrotizing form of pancreatitis (Lombardi et al., 1975). This correlates well with the severe acute pancreatitis seen in dogs, as it results in hypovolaemia, hypoxia, pancreatic necrosis and acidosis. This model is very useful for assessing interventions that might reduce mortality, but to date is considered species-specific and sex-specific, as it only affects young female mice (Niederau et al., 1992). Other non-invasive methods such as ethanol administration, gene knockout or immune modulation are difficult to reproduce and costly to perform, so are seldom undertaken unless studying a specific genetic abnormality or pharmacological intervention, and are typically limited to rodents. A newer developed protocol in mice is the administration of high-dose arginine, which results in dose-dependent pancreatic necrosis (Hegyi et al., 2004). Long-term administration results in chronic pancreatitis, which may also be of benefit in canine medicine as it could allow for evaluation of the insulin-acinar axis (Weaver et al., 1994).

Invasive methods of inducing pancreatitis are probably more applicable for relating to gallstone induced disease in people, but there may be some relevancy to dogs. Invasive methods have been performed in a range of species, including dogs and cats. These invasive methods include a closed duodenal loop model that closes off the pancreatic duct and diverts bile to the jejunum (Pfeffer et al., 1957). This model induces necrosis rather than inflammation and carries a high mortality rate. If the loop is temporarily closed and trypsin along with sodium taurocholate is injected into the duodenum, the pancreatitis is milder and interventions are easier to study (Orda et al., 1980). It is unknown how much of a role duodenal reflux has in causing pancreatitis in dogs, and so this may not be a valid method for comparative studies. Perhaps the most
promising methodology for canine disease is combined hypersecretion (injection of CCK) and low-dose infusion of glycodeoxycholic acid into the pancreatic ducts (GDOC) (Schmidt et al., 1992). This method is perhaps the most appropriate for comparative purposes as it causes widespread necrosis and inflammation of the organ; however it poses some technical challenges. Other invasive methodologies are technically complex, such as antegrade pancreatic duct perfusion, duct ligation, ischaemia-perfusion models or biliopancreatic duct injections of taurocholate, and all of which produce severe pancreatitis.

1.2.2 Pathophysiology of pancreatitis

There has been a considerable amount of work elucidating the cellular events that initiate pancreatitis and the resultant inflammatory cascades over the past three decades. These investigations have been on experimental rodent models (unless otherwise stated), with a resultant extrapolation to naturally occurring disease that may not always be appropriate (Su et al., 2006).

1.2.2.1 Initiating cellular events

Pancreatitis develops when there is excessive activation of trypsin and other pancreatic proteases within the pancreas, which overwhelm the safeguards of both PSTI within the acinar cell and anti-proteases in the circulation (Lasson and Ohlsson, 1984). The local and distant inflammatory effects of pancreatitis are mediated by a range of inflammatory pathways, and severe disease is perpetuated by splanchnic circulatory failure, as discussed further in this section.

The earliest event in pancreatitis occurs when there is activation of trypsinogen to trypsin within the acinar cell, as confirmed by electron microscopy and ultrastructural studies (Lerch et al., 1992; Rinderknecht, 1986; Saluja et al., 1997; Steer and Meldolesi, 1988). A secretory (or apical) block develops, where zymogen granules
are not secreted into the intestinal lumen as normally for degradation by enterokinase (Saluja and Steer, 1999). This block is likely due to microtubular dysfunction, and may result from oxidative stress in some cases (Windsor and Hammodat, 2000).

As a result of this block, co-localisation of zymogen granules and lysosomal proteases develops, where trypsinogen is activated to trypsin by lysosomal proteases (Rinderknecht, 1986; Steer and Meldolesi, 1988). This is depicted in Figure 1.3, and has become widely accepted as the main initiating step in pancreatitis. As discussed in section 1.1.2, lysosomes are normally kept physically separated from zymogen granules within the acinar cell. Trypsin has been shown to be activated by cathepsin B, a lysosomal protease (Rinderknecht, 1986). Although concurrent administration of a cathepsin B inhibitor significantly reduced the development of pancreatitis in one study, it didn’t completely prevent it (Saluja et al., 1997). It is likely the inhibitor used in this experiment was only a partial antagonist, as confirmed later in an in vitro model (Saluja and Steer, 1999). There may also be other mechanisms involved, as cathepsin-B deficient mice can also develop pancreatitis, albeit to a lesser severity than mice without that deficiency (Halangk et al., 2000). Additionally, hypotension has been shown to cause trypsin activation prior to the appearance of proteases, suggesting there are other cellular mechanisms that have not been fully elucidated that lead to this premature trypsin activation (Mithofer et al., 1995; Rinderknecht, 1986). Co-localisation may also occur due to a dysfunction of the normally coded separation of the two components (Steer et al., 1984).

The role of inappropriate trypsin activation in initiating pancreatitis is exemplified by a mutation in the cationic trypsinogen gene that is linked to recurrent pancreatitis in people (Whitcomb et al., 1996). It is suspected that this mutation results in failure of the protective mechanism against activation, and so prematurely activated trypsin accumulates in the acinar cell (Bhatia et al., 2000).
Experimental models have shown that the pH of the acinar cell is very important in determining the likelihood of the lysosomal and zymogen granules co-localising (Bhoomagoud et al., 2009; Noble et al., 2008; Sherwood et al., 2007). The most recent of these studies showed that low acinar pH alone did not lead to the development of pancreatitis, but it did heighten zymogen activation following cerulein stimulation. Trypsinogen activation also appears to require the presence of intracytosolic calcium in some in vitro experiments (Kruger et al., 2000; Mooren et al., 2003). It is proposed that Ca\(^{2+}\) located in the zymogen granules has a protective function against activation of trypsin, but within the cytosol acts in conjunction with CCK as an intra-cellular messenger for the release of lysosomal proteases (Kruger et al., 2000). In fact, blockade of the calcium signalling system within the cells stops production of trypsinogen activation peptide (TAP) (Mooren et al., 2003; Raraty et al., 2000; Sherwood et al., 2007).

**Figure 1.3**: Demonstration of the co-localisation theory. In the normal cell on the left, the zymogen granules and lysosomes are manufactured within the Golgi apparatus, but processed and transported to the apex separately. In the abnormal cell on the right, there is an apical block, which allows zymogen granules to fuse with lysosomes. Cathepsin B, a lysosomal protease is then able to activate trypsinogen to trypsin in the acinar cells. Pancreatitis develops when the local safeguard pancreatic secretory trypsin inhibitor (PSTI) is overwhelmed by trypsin, and pancreatic enzymes are then activated within the acinar cell.
Trypsin and other activated pancreatic enzymes are likely to be directly damaging to the acinar cell when production overwhelms the PSTI present in the acinar cells. The potential direct actions of these enzymes are detailed in Table 1.1.

Table 1.1

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>PATHOPHYSIOLOGICAL ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>Activation of other proteases</td>
</tr>
<tr>
<td></td>
<td>Coagulation and fibrinolysis (disseminated intravascular coagulation)</td>
</tr>
<tr>
<td>Phospholipase A_2</td>
<td>Hydrolysis of cell membrane phospholipids</td>
</tr>
<tr>
<td></td>
<td>Pulmonary surfactant degradation (cell necrosis and liberation of toxic substances such as myocardial depressant factor, respiratory distress, neurological signs)</td>
</tr>
<tr>
<td>Elastase</td>
<td>Degradation of elastin in blood vessel walls (haemorrhage, oedema, respiratory distress)</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Activation of xanthine oxidase and subsequent generation of oxygen-derived free radicals (membrane damage)</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>Kinin generation from kininogens</td>
</tr>
<tr>
<td>Kinins</td>
<td>Vasodilation, pancreatic oedema (hypotension, shock)</td>
</tr>
<tr>
<td>Complement</td>
<td>Cell membrane damage, aggregation of leucocytes (local inflammation)</td>
</tr>
<tr>
<td>Lipase</td>
<td>Fat hydrolysis (local fat necrosis, hypocalcaemia)</td>
</tr>
</tbody>
</table>

Role of pancreatic enzymes in the systemic pathophysiology of pancreatitis. Adapted from Williams DA (1996)

Early experimental models favour the notion that the activated enzymes spilling over into the interstitium is the triggering factor for progression from mild to severe pancreatitis (Halangk et al., 2000).

1.2.2.2 The recruitment of neutrophils and production of reactive oxygen species

Following the direct damage to the pancreas, inflammation ensues. Inflammation is defined as vasodilation of local blood vessels, increased permeability of local capillaries allowing leakage to the interstitial spaces, clotting of fluid in the interstitial spaces, migration of granulocytes to affected tissue and swelling of cells (Guyton and Hall, 2006). Neutrophils contain agents such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ions (·OH) that are collectively termed
reactive oxygen species (ROS). ROS are directly toxic to cells and further increase cellular and vascular permeability, as well as enhancing the expression of inflammatory mediators.

Pancreatitis has been proven to be less severe in mice with neutrophil depletion (Bhatia et al., 1998a). However, other studies have shown that blockage of ROS doesn’t ameliorate pancreatitis, and so other factors must also contribute to development of severe disease (Wang et al., 1996; Wisner et al., 1988). One possible mechanism is via nitric oxide (NO) as discussed in the next section.

Chemokines are a group of small molecular weight cytokines that have chemotactic properties, and therefore play a large role in recruiting leucocytes to areas of injury. Generally they are subdivided into the CXC and CC subfamilies based on their amino acid sequence (Bhatia et al., 2000). The CXC chemokines that possess a conserved amino acid sequence (ELR) at their N terminus are potent neutrophil attractors, and the best example of this group is interleukin-8 (IL-8). Neutrophil migration to the pancreas is thought to be mainly initiated by IL-8 early in the course of inflammation (Frossard and Pastor, 2002; Gross et al., 1992).

The adhesion of leucocytes to the endothelial wall is via expression of intercellular molecule adhesion-1 (ICAM-1) and other selectins mediated by IL-8 and others (Bhatia et al., 2000; Makhija and Kingsnorth, 2002). ICAM-1 is an inducible molecule normally only expressed at low levels on endothelial surfaces, but has been shown to be increased in experimental pancreatitis (Frossard et al., 1999). ICAM-1 knockout mice are protected to some extent against the development of acute pancreatitis and associated organ damage, emphasising the role of adhesion molecules in this disease (Frossard et al., 1999).

One study recently analysed the role elastase and trypsin play in neutrophil migration in acute pancreatitis (Keck et al., 2005). They developed an in vitro
assessment of neutrophils and cultured endothelial cells, then exposed them to increasing doses of trypsin and elastase, as well as in a correlating in vivo model of severe pancreatitis in rats. Using confocal microscopy it was found that both trypsin and elastase up-regulate expression of adhesion molecules on white blood cells and endothelial cells. What is most striking is that this was done in the absence of pro-inflammatory cytokines or free radicals and so was a direct action. As this effect only occurred in the presence of serum, a co-factor such as complement may be necessary.

Activated neutrophils then follow a chemokine gradient into the pancreatic tissue (Bhatia et al., 2000). The activated neutrophils and other leucocytes such as macrophages, may contribute to the development of inflammation in distant organs as they are produced by the bone marrow and are present in the circulation (Gloor et al., 1998a). Leucocyte accumulation occurs initially in the perivascular areas of the pancreas, and then following oedema and the resultant change in permeability, the leucocytes egress to the pancreatic body (Pezzilli, 2009).

Neutrophils have also been implicated in the shift from apoptosis to necrosis in pancreatic cells. Necrotic cells release cytosolic contents into the extra-cellular space and elicit a profound inflammatory response (Frossard and Pastor, 2002). Apoptotic cells, on the other hand, are rapidly phagocytosed by macrophages and don’t elicit an inflammatory response (Anderson and Wang, 1998; Majno and Joris, 1995). Milder forms of experimental pancreatitis have more apoptosis than necrosis (Bhatia, 2004; Kaiser et al., 1995). It may be that recruitment of neutrophils into the acinar cells promotes a shift from acinar cell apoptosis to necrosis (Windsor and Hammodat, 2000). This is probably intricately linked to pancreatic microcirculation (discussed in this 1.2.2.4) and proportional to the degree of oxidative stress (Frossard and Pastor, 2002). There are also vasoactive mediators that have been implicated in the development of necrosis, such as endothelin-1 and PLA-2 (Al Mofleh, 2008).
1.2.2.3 Nitric oxide (NO) and further oxidative stress

Nitric oxide (NO) is a small inorganic molecular compound that has been shown to regulate pancreatic exocrine secretion, promote capillary integrity, inhibit leucocyte adhesion and modulate pancreatic microvascular blood flow (Ang et al., 2009; DiMagno, 2007; Dobosz et al., 2005). The biological functions of NO are diverse, as it can be regulatory or cytotoxic and the function is determined by the NO synthase (NOS) isoform involved in synthesis of NO, the location of NO production and the amount produced (Mashimo and Goyal, 1999). NOS isoforms are named after their initial location in the vascular endothelium (eNOS), neurons (nNOS) or macrophages (iNOS). Genetic mouse models with each isoform deleted have been developed to assess the role of each of these in a variety of inflammatory conditions. In the pancreas, eNOS and nNOS are constitutively expressed and are calcium-dependent enzymes that stimulate low levels of NO production. On the other hand, iNOS is calcium insensitive and produces high amounts of NO that circulates throughout the body (DiMagno, 2007).

Studies in experimental acute pancreatitis have had differing conclusions about the role of NO. Some have shown NO to confer a benefit (Molero et al., 1995; Werner et al., 1997), whilst others showed a minimal or a negative effect (Dabrowski and Gabryelewicz, 1994; Lomis et al., 1995). These differences may be explained by the different biological effects of the different NOS isoforms. Decreased eNOS function causes a shift from low-level production of NO to high-level ROS production and oxidative stress, a phenomenon that has been demonstrated in vascular diseases in people (DiMagno, 2007). One study using mice showed that early up-regulation of eNOS was associated with amelioration of pancreatitis severity, which was proposed to be due to maintenance of pancreatic microvascular blood flow as eNOS is localised to the vascular endothelium in the pancreas (DiMagno et al., 2004). Studies have also shown that eNOS is down-regulated in the latter stages of pancreatitis, whilst iNOS
was greatly increased in the acinar cells and lungs, in proportion to the disease severity (Al-Mufti et al., 1998; Ang et al., 2009; Cheng et al., 2010; Ueno et al., 2005).

When NO concentration increases within cells due to increased iNOS expression, mitochondrial respiratory function is impaired and NO becomes peroxynitrite. Peroxynitrite is an oxidant that can cause lipid peroxidation, protein nitration, DNA strand damage and cell necrosis (Szabo, 2003). As well, NO can directly cause production of ROS, thereby perpetuating oxidative stress, and also stimulate prostaglandin production and activity.

It has been shown that tumour necrosis factor-α (TNF-α) reduces expression of eNOS in bovine endothelial aortic cells and both Nuclear Factor kappa-B (NF-κB) and IL-8 increase expression of iNOS in the lung and pancreas during experimental rodent pancreatitis (Anderson et al., 2004; Leindler et al., 2004; Long et al., 2005; Ueno et al., 2005). NO derived from nNOS is poorly studied in pancreatitis, but could potentially play a role in amplifying the neurogenic inflammation mediated by substance P, as discussed in section 1.2.2.7 (Bhatia et al., 1998b).

It is still unclear the exact role NO plays in naturally occurring human pancreatitis, and there may be different roles associated with the aetiology and severity (Ang et al., 2009). One recent study showed a significant positive correlation between plasma NO and severity in a group of people with acute pancreatitis (Que et al., 2010). Oxidative stress was greatest in the group that developed systemic complications. Therapeutic manipulation of the NO system has had unproven benefit to date in human medicine (DiMagno, 2007). Further studies elucidating the role of NO and the various NOS isoforms are needed in canine pancreatitis.

1.2.2.4 Pancreatic microcirculation

Disturbed pancreatic microcirculation plays a very important role in pancreatic inflammation and permeability (Bassi et al., 1994; Borodin et al., 2006; Gardner et al.,
In animal models, vasoconstriction within the pancreas appears to be an early event in acute pancreatitis (Kusterer et al., 1993; Schröder et al., 1985). Additionally, administration of phenylepinephrine (a potent vasoconstrictor) converts mild, oedematous pancreatitis to a severe, necrotising form (Klar et al., 1991). In people, early onset spasm of large pancreatic vessels has been shown to correlate with poorly perfused areas of the pancreas, and subsequent high mortality rates (Takeda et al., 2005). One experimental study in rats also identified that lower pancreatic perfusion was associated with more severe pancreatitis (Foitzik et al., 1995).

The vascular disturbance in pancreatitis is likely to be multi-factorial in origin and may occur as a result of increased vascular permeability resulting from inflammatory cytokines (to be discussed), the direct effects of pancreatic proteases, and/or microthrombi formation resulting from hypercoaguability (Gardner et al., 2008; Keck et al., 2005). In addition to these mechanisms, the pancreas is intrinsically susceptible to ischaemia, as demonstrated by subclinical pancreatitis being identified in people with hypovolaemia (Cuthbertson and Christophi, 2006). Following ischaemia or reperfusion there is increased adherence of leucocytes to endothelium and formation of ROS (Zhou and Chen, 2002). It is likely that a similar process occurs in dogs as well. Impaired lymphatic drainage could also contribute to poor circulation, as red blood cells can enter the lymph and enhance leucocyte adhesion (Foitzik et al., 1995). These factors are depicted in Figure 1.4.

An additional cause of the disturbances in the circulation of the pancreas is the intricate exocrine-endocrine balance (Windsor and Hammodat, 2000). Due to the complicated and collateral blood supply, acinar cells are exposed to high concentrations of hormones produced in the islet cells. The capillary network in the pancreas is very permeable, facilitating this communication. It may well be that amylin, an inflammatory product originating from islet cells, could favour acinar necrosis by
causing a selective reduction in pancreatic blood flow (Svensson et al., 1994).

Metalloproteinase-9 (MMP) may also play a role in altering the permeability of the blood vessels and perpetuating the neutrophilic response (Al Mofleh, 2008).

**Figure 1.4** Demonstration of the changes in pancreatic microcirculation during pancreatitis. $O_2$ = oxygen; $CO_2$ = carbon dioxide; PMN = neutrophils; ICAM = Intercellular adhesion molecule
1.2.2.5 Kallin-kallikrein system

An early and direct action of pancreatic proteases is to cause the kallikrein-kinin system to become activated, which is depicted in Figure 1.5. The pancreas has a high tissue concentration of kallikrein, generally stored as prekallikrein (Griesbacher, 2000). Pancreatic tissue kallikrein releases kallidin, whilst plasma kallikrein releases bradykinin. Kinins precipitate oedema via vasodilation of arteries and increased capillary permeability. They are also potent activators of nociceptive neurones, attract cytotoxic factors into tissue and enhance production of prostaglandins (Griesbacher, 2000). There are two major kinin receptor types, with Bɔ constitutively expressed in tissue that becomes the major receptor during acute inflammation. The nociceptive and vascular effects of the kinin system are predominantly mediated by Bɔ receptors. Production of the kallikreins in acute pancreatitis has been confirmed in experimental models (Griesbacher, 2000; Shimizu et al., 1993). Kinins have a short biological half-life, and are degraded by kininase II which is structurally identical to angiotensin-converting enzyme (Thal et al., 1963). Along similar lines, Bɔ receptor antagonists appear experimentally to have beneficial effects on pancreatic blood flow, necrosis and survival in necrotising models even when given after induction of disease (Bloechle et al., 1998; Hoffmann et al., 1996; Satake et al., 1996).
Figure 1.5 Representation of the kallikrein-kinin system, the actions of enzymes are solid arrows, whilst the metabolic pathways are broken arrows. Adapted from Griesbacher; 2000. There is the potential for blockage of the $B_1$ and $B_2$ receptors to have some beneficial effect in pancreatitis. LMW= low molecular weight; HMW = high molecular weight

1.2.2.6 The complement system

Whilst it is unknown to what extent or in what fashion activation of the complement system occurs in canine acute pancreatitis, it has been shown to be activated early in people with acute pancreatitis, and to a greater extent in those with severe disease (Gloor et al., 2003). It is most likely that the alternative complement pathway is initiated in pancreatitis, as there is an absence of antibodies or microbes as the primary trigger. C5a is one of the most potent mediators of the complement system, and substantially enhances vascular permeability. Surprisingly, in a study using a mouse model where C5a receptors were genetically ‘removed’ it was actually found that the resultant pancreatitis was worse (Bhatia et al., 2001). It is not entirely elucidated as to why C5a appeared to be anti-inflammatory, but it may well be that it limits the recruitment of pro-inflammatory mediators (Bhatia et al., 2000; Saluja and
In contrast to this, one group of researchers found a strong association between expression of C3a and necrotising versus oedematous pancreatitis (Hartwig et al., 2006). Additionally, blockade of the complement receptor 1 led to amelioration of the local pancreatic inflammation and down-regulation of the leucocyte-endothelial interaction. It is possible the conflicting conclusions of these studies are due to the different experimental model used. Alternatively it may be that the complement system is a complex one, with opposing and synergistic actions of various components within it.

1.2.2.7 Substance P and the Renin Angiotensin System

Substance P is a peptide produced by nerve endings that acts by binding to Neurokinin-1 (NK-1) receptors. As well as mediating pain, it is thought to increase vascular permeability, therefore potentially playing a role in progression of pancreatitis. A mouse study has shown up-regulation of NK-1 receptors and the amount of Substance P in acute pancreatitis (Bhatia et al., 1998b). It appears to be particularly related to lung injury, and genetic deletion of NK-1 receptors in mice is protective against the systemic effects of pancreatitis. It is also proposed that NO and Substance P interact and synergistically amplify pain and inflammation (Ang et al., 2009). No studies to date have assessed the role of Substance P in canine pancreatitis.

One area that has been poorly investigated in both experimental and naturally occurring disease is the role the renin-angiotensin system (RAS) may play in the initiation and perpetuation of pancreatic inflammation (Pezzilli, 2009). Angiotensin-II receptors have been identified in the pancreas (Leung et al., 1999). Expression of genes encoding these receptors and metabolites of the RAS has been shown to be up-regulated in acute pancreatitis (Leung et al., 2000). As RAS metabolites may trigger ROS production, this is an area that warrants further evaluation.
1.2.2.8 Cytokine ‘storm’

Prior to, during, and after inflammatory cells have appeared within the pancreas there occurs activation of multiple cytokines and chemokines. This is a complexly mediated phenomenon, and has been referred to as a ‘cytokine storm’, with a multitude of cytokines all contributing to the clinical consequences (Makhija and Kingsnorth, 2002; Mayer et al., 2000; Norman, 1998). Cytokines are small proteins produced in response to stimuli, and act to up or down regulate various aspects of the inflammatory process. There are an increasingly discovered number of cytokines that are produced by T cells to either drive or facilitate a Th1 or Th2 immune response. Th1 responses mediate cellular immunity and activate neutrophils, macrophages and natural killer cells. Th2 responses mediate humoral immunity via B cell activation or the recruitment of eosinophils. Each cytokine has the capacity to act synergistically with others, and there is a large overlap in their functions, as represented in Figure 1.6.

The initiating step of this process in pancreatitis, as in many other inflammatory diseases, appears to be the activation of NF-κB (Gukovsky et al., 1998; Steinle et al., 1999). NF-κB is a transcription factor that modulates the expression of most cytokines. High NF-κB expression in peripheral blood mononuclear cells from people with acute pancreatitis has been demonstrated, with a correlation to the development of systemic complications (Satoh et al., 2003). It also stimulates production of adhesion molecules as well as iNOS (Long et al., 2005).

In one rodent model, cytokine activity within the pancreas was determined by tissue mRNA and real-time polymerase chain reaction (RT-PCR) (Norman et al., 1997b). Early in the onset of pancreatic inflammation TNF-α and IL-1β were detected. IL-6 was located in the pancreas after some time, whilst distant expression of all these cytokines was evident in the lung, liver and spleen after a delayed period; but never in
the kidney, heart or skeletal muscle. The amplitude of the cytokine expression correlated with the severity of pancreatic inflammation.

Pancreatic acinar cells have been shown to be capable of producing TNF-α (Gukovskaya et al., 1997; Norman et al., 1995). TNF-α is synergistic with many other inflammatory cytokines, and also promotes cell adhesion (via ICAM) and programmed cell death (Makhija and Kingsnorth, 2002). IL-1 is a pro-inflammatory cytokine that is synergistic with TNF. The production of IL-1 requires IL-1β and two IL-1 receptors that have similarity to toll-like receptors. This production is mediated by a group of enzymes called caspases, which also cause the production of other inflammatory cytokines such as IL-8. The caspase family (previously known as interleukin-1 converting enzyme) appears to favour necrosis over apoptosis compared to TNF-mediated inflammation in acinar cells, and worsens severity (Norman et al., 1997a; Rau et al., 2001a; Rau et al., 2001b).
Figure 1.6 (previous page) Demonstration of the activation of the cytokine storm in acute pancreatitis. Systemic Inflammatory Response Syndrome (SIRS) results from the cytotoxic effects when the pro-inflammatory mediators (red) overwhelm the anti-inflammatory mediators (blue) and is manifested as vasodilatation and increased capillary permeability. TNF; tumour necrosis factor; IL; interleukin; IL-ra, IL-1 receptor antagonist; NO, nitric oxide; ROS, reactive oxygen species or superoxide, AA, arachidonic acid; MCP-1, monocyte chemoattractant protein-1; RANTES, regulated on activation, normal T cell expressed and secreted. SIRS (Systemic inflammatory response syndrome) is a syndrome characterised by presence of capillary permeability, hypotension and subsequent organ changes.

IL-1β is a potent facilitator of neutrophil migration and strongly associated with the development of systemic inflammatory response syndrome (SIRS) (Dinarello, 1996). Additionally, it activates the transcription of ICAM-1 and NF-κB, further perpetuating the inflammatory cascade. The early activation of this cytokine has been demonstrated in experimental models of acute pancreatitis (Fink and Norman, 1997; Norman et al., 1997b; Tanaka et al., 1995). Its action is counteracted by an IL-1 receptor antagonist (IL-1ra). In people with acute pancreatitis, an imbalance in IL-1β:IL-1ra was highly predictive of pulmonary failure (Mayer et al., 2000). Further to that, IL-1 knockout mice did not develop pancreatitis, emphasising the role this cytokine has in the early initiation and progression of acute pancreatitis (Norman et al., 1996).

IL-6 is produced by a variety of mononuclear cells, endothelial cells and fibroblasts. It is produced in response to stimulation by TNF and IL-1β (Makhija and Kingsnorth, 2002). It is increased early in the course of experimental pancreatitis (Norman et al., 1997b). As it is more reliably measured than either TNF or IL-1β, it has been negatively correlated with prognosis in naturally occurring human acute pancreatitis (Chen et al., 1999a; De Beaux et al., 1996; Leser et al., 1991; Mayer et al., 2000; Pezzilli et al., 1995).

IL-8 is a pro-inflammatory chemokine intimately involved in neutrophil actions, both as an inciter and product of the Th1 profile. Similar to IL-6, concentration of IL-8
has been correlated with the severity of naturally occurring pancreatitis in people (De Beaux et al., 1996; Pezzilli et al., 1995; Rau et al., 1997). Platelet activating factor (PAF) is a cytokine produced predominantly by neutrophils that has been extensively studied in experimental models. It appears to be particularly associated with lung injury (Formela et al., 1994). Blockade of this cytokine has led to reduction of lung-associated complications in naturally occurring disease in some studies, although there is not uniform agreement with this (Heinrich et al., 2006; Johnson et al., 2001; McKay and Imrie, 2004).

In experimental studies, IL-10 has been assessed due to its anti-inflammatory effects, which favours a shift to a Th2 cytokine profile and down-regulates NF-κB transcription. There were some promising results with IL-10 ameliorating the severity of pancreatitis in experimental models (Kusske et al., 1996; Van Laethem et al., 1998). Additionally, IL-10 knockout mice were shown to get a more severe form of pancreatitis (Gloor et al., 1998b). Pre-treatment of rats with IL-10 also reduced iNOS levels, and increased TGF-β1, which correlated to an increase in the rate of apoptosis (Zhang et al., 2007b). This along with a demonstration that serum IL-10 was increased in people with severe pancreatitis suggested a potential treatment avenue (Chen et al., 1999b). Unfortunately, IL-10 therapy has had limited success in treatment of human acute pancreatitis (Pezzilli, 2009).

As the knowledge in this area continues to expand there are a multitude of other cytokines that are also being evaluated in pancreatitis; such as IL-2, -4, -11, -18 (Mayer et al., 2000; Rau et al., 2001a). Regulated on activation, normal T cell expressed and secreted (RANTES) is now known as CC chemokine ligand 5 (CCL5), and is produced by circulating T cells under the stimulation of TNF-α and IL-1. It is responsible for protection against viruses, and is a potent chemoattractant for recruiting
leucocytes (Makhija and Kingsnorth, 2002). Similarly, monocyte chemoattractant protein-1 (MCP-1) is a potent leucocyte recruiter as well.

Whether any of these inflammatory mediators become clinically important in canine pancreatitis, either from a treatment or aetiological point of view, remains to be seen. Certainly, it is highly likely that they contribute to systemic effects of disease to some extent. Frossard summarized very well the various genetic knockout studies that have been performed to elucidate the inflammatory process that occurs in acute pancreatitis (Frossard and Pastor, 2002). This has been reproduced with permission in Figure 1.7. This is a clear demonstration of the complexity and interaction of the inflammatory pathways, which emphasises that targeting just one cytokine would be unlikely to result in a substantial clinical benefit.

**Figure 1.7** reprinted with kind permission from Frossard and Pastor 2002. Dotted lines represent a positive or beneficial effect, whilst plain lines indicate a deleterious effect. In summary, the genetic knockout models have shown that genetic deletion of cathepsin B reduces intra-pancreatic production of trypsin; Deletion of the NK1R gene reduces lung associated injury; Deletion of TNF-α protects mice with pancreatitis via down-regulation of ICAM-1; ICAM-1 deletion also protects against pancreatitis and distant organ disease; Over expression of metallothionein-1 (MT-1), which scavenges ROS is associated with less severe pancreatitis; Deletion of IL-1 protects against lung injury; Over-expression of IL-10 is protective to some extent.
1.2.2.9 Perpetuation of disease

Pancreatic and peritoneal inflammation may cause vomiting due to stimulation of peripheral chemoreceptors in the mesentery and circulating emetic agents may also reach the chemoreceptor trigger zone (CTZ) adjacent to the vomiting centre in the medulla in pancreatitis. Vomiting results in significant third space losses, and splanchnic circulation subsequently decreases as part of circulatory shock. The body can adjust for this by increasing the oxygen extraction from systemic circulation up to 90% (Takala, 1996). It is thought that prolonged oxygen extraction > 70% can lead to regional ischaemia (Rowell et al., 1984). Ischaemia of the intestine increases permeability and mucosal acidosis, which as it progresses increases the rate of apoptosis of enterocytes and decreases nutrient transport of intestinal epithelial cells (Fink, 1991). Additionally, the increased bowel permeability that ensues leads to cytokine release, and skeletal muscle lysis (Wilmore et al., 1988).

There are currently two proposed models as to how the intestine may contribute to the inflammatory state, although in reality it is likely to be a combination (Flint and Windsor, 2003).

1. The Gut Starter model: Neutrophils are primed during reperfusion of ischaemic intestine, and then are provoked by exposure to endotoxin in systemic circulation (Moore et al., 1994). This is mediated via PLA-2 in the mesenteric blood system (Gonzalez et al., 2001). The primed neutrophils are then potent mediators of distant organ dysfunction.

2. The Gut Motor model: Disruption of the intestinal barrier leads to translocation of bacteria and inflammatory cells to the portal venous and lymphatic systems, which activates downstream immune cells in the absence of infective foci (Meakins and Marshall, 1986).
One porcine experimental pancreatitis study showed decreased intestinal blood flow caused mucosal acidosis (Juvonen et al., 1999). In people with severe acute pancreatitis, low mucosal pH has been shown to be closely related to development of multiple organ dysfunction syndrome (MODS) and death (Bonham et al., 1997). A calculated intramucosal pH ≤ 7.25 predicted death with an overall accuracy of 82% (sensitivity 100%, specificity 77%). This is a higher association than many of the clinical scoring systems or measurement of serum inflammatory markers described in later sections (Al Mofleh, 2008). It is also proposed that xanthine oxidase is produced on reperfusion of ischaemic intestine, leading to production of free radicals which perpetuate the inflammatory process (Fink, 1991).

The intestinal barrier is a functional rather than strictly anatomical unit that is maintained by intestinal microflora, appropriate gut motility, digestive enzymes, mucus layer, epithelial layer, endothelial layer, mucosal-associated lymphatic tissue and the gut-liver axis. It is thought the epithelial layer is the most important part of the intestinal barrier, as it is a single cell layer that is very prone to disturbances in microcirculation (Flint and Windsor, 2003). In people and cats it has been shown the oxygen counter-current exchange system and the anatomical position of the villi circulation predispose to hypoperfusion during times of decreased splanchnic circulation (Fink, 1991; Kampp et al., 1968). This is because in intestinal villi the artery and vein run parallel, but in opposite directions, with a dense capillary network close to the top of the villus. This results in a decreasing tissue PO\textsubscript{2} gradient from the base up to the tip of the villi (Takala, 1996). The change in PO\textsubscript{2} may also be due to the higher metabolic activity of cells located in the tip. Regardless of the mechanism, this low PO\textsubscript{2} tension makes the villi tips susceptible to tissue hypoxia if vasoconstriction occurs. In dogs, a similar vascular anatomy in the villus is present, with a single arteriole unbranched to the tip of the villus (Strombeck, 1996). It is possible that dogs may be
exquisitely sensitive to villus hypoxia as the drainage from the capillary network to the veins occurs closer to the tip of the villus than in other species (Strombeck, 1996).

The epithelial layer forms tight junctions that prevent passage of molecules > 11.5Å in diameter, which includes lipopolysaccharides and other inflammatory mediators (Ravin and Fine, 1962). It is hypothesised that bacteria may not be able to pass through the gut in healthy rodent models, due to the size of these junctions (Wolochow et al., 1966), but other properties such as polarity of the microbes and surveillance by the gut-associated lymphoid tissue, are probably as important.

1.2.3 Classification of pancreatitis

There is a lot of confusion about the nomenclature surrounding pancreatitis and its various definitions in the veterinary literature. This is probably because pathological classifications are dependent on histological descriptions. As the different types of pancreatitis overlap in their clinical presentation and biopsy specimens are rarely obtained ante-mortem in acute pancreatitis, a clinical bias in terminology currently exists in the veterinary literature.

The current edition of a major reference veterinary pathology textbook discusses acute pancreatic necrosis (APN) in a different sub-section from pancreatitis (Charles, 2007). They make the definition of APN based on necrosis, not inflammation, being the predominant histological feature. They also state that the aetiology and pathogenesis is different for APN and acute pancreatitis, although this has not been proven. What is most likely is that the response is to the same stimuli, but the progression and final outcome is different between the two.

The initial histological events for APN are described as perilobular necrosis and reactive inflammation at the periphery of affected lobes, with a variable degree of involvement of adjacent adipose and other tissue. The duct system and centrilocublar parenchyma appear to be spared in the early stages (Charles, 2007).
A specific histological form, acute haemorrhagic pancreatic necrosis is also described (Charles, 2007). This term is rarely used anymore, and only in specific instances such as secondary to a scorpion bite or an L-asparaginase reaction. The necrosis in this form is centred on the ducts.

Acute interstitial pancreatitis is defined as a swollen organ due to diffuse interlobular oedema, and occasionally haemorrhage or necrosis, and is most commonly seen in *Toxoplasma*-associated pancreatitis in cats (Mergener and Baillie, 1998). Chronic interstitial pancreatitis arises by extension of an inflammatory process that commences in the ducts, and is usually of minor clinical consequence.

The biggest and most confusing contradiction is that in pathology-based descriptions, APN is stated to smoulder and persist, and then cause EPI or diabetes mellitus as a result (Charles, 2007). This is in contrast to the definition in many human and veterinary medical textbooks where acute pancreatitis is considered to be a completely reversible condition (Mergener and Baillie, 1998; Williams, 1996). Additionally, in recently published veterinary medical literature acute pancreatitis is defined as having no underlying fibrosis (Kalli et al., 2009; Newman et al., 2006; Watson et al., 2007; Williams, 1996). Chronic pancreatitis, which is diagnosed when there is fibrosis, is instead cited as being the major cause of diabetes mellitus or exocrine pancreatic insufficiency (Simpson and Lamb, 1995; Watson, 2004; Watson, 2003; Watson et al., 2010; Williams, 1996; Xenoulis et al., 2008). Chronic pancreatitis is more likely to be recurrent as the fibrosis renders the pancreas less distensible, and so some duct obstruction develops, predisposing to ductular based inflammation (Watson, 2004). This then becomes recurrent acute pancreatitis, termed ‘acute-on-chronic’ by some authors (Kalli et al., 2009; Watson et al., 2007).

Clinically, this histological differentiation is seldom determined as pancreatic biopsy is not undertaken ante-mortem. Therefore, the medical nomenclature usually
relates to the severity and longevity of clinical signs. Where this becomes important is
that not all acute cases have severe disease, and not all chronic cases have mild disease.

Mild acute pancreatitis causes no multi-system failure and has an uncomplicated
recovery, whilst severe acute pancreatitis causes multi-system failure or development
of complications. It is recommended that the term necrotising is used as a sub-category
of acute pancreatitis only when it is histological proven, acknowledging that this is
most likely to be present in severe disease (Watson, 2004).

Again this definition is similar to that in people, where acute pancreatitis is an
inflammatory process centred on the pancreas, with possible multi-organ involvement
(Al Mofleh, 2008). The process is considered reversible, with no fibrosis present
(Bradley, 1993). Acute pancreatitis is sometimes sub-classified into necrotising or non-
necrotising, with the consensus being that there is a worse outcome with the former (Al
Mofleh, 2008; Blum et al., 2001). Necrosis is generally diagnosed by computed
tomographic (CT) evaluation in people. Severe acute pancreatitis in people is defined
as being associated with organ failure, local complications (such as local fluid
collections, infected necrosis, pseudocysts) or both (Windsor and Hammoudat, 2000).
The presence of infected necrosis and the extent of the necrosis are the two most
important determinants of outcome in people. Such a determinant has not been made in
dogs, partly due to the difficulty in assessing the amount of necrosis.

1.2.4 Aetiology and risk factors in dogs

The list of potential aetiologies that can cause pancreatitis in dogs is long and
includes nutritional excesses, hyperlipoproteinaemia, drugs, toxins, hypercalcaemia,
duct obstruction, duodenal/biliary reflux, pancreatic trauma, ischaemia/reperfusion and
miscellaneous (Charles, 2007; Williams, 1996). Specific diseases such as babesiosis
and leishmaniasis are also reported to cause pancreatitis in dogs, although for the latter
it is unclear whether it is the disease or the treatment that is responsible (Aste et al., 2005; Carrasco et al., 1997; Mohr et al., 2000).

Drugs that have been reported in the veterinary literature to cause pancreatitis include azathioprine, chlorthiazine, hydrochlorthiazide, zinc, potassium bromide, vinblastine, sulphonamides, cisplatin, organophosphates, L-asparaginase and 5-aminosalicylate amongst others (Charles, 2007; Dalefield et al., 1999; Hammond et al., 2004; Kook et al., 2009; Mikszewski et al., 2003; Segev et al., 2008; Stewart, 1994; Trepanier et al., 2003; Williams, 1994; Williams, 1996).

The potential of glucocorticoids to cause pancreatitis in cats and dogs is controversial, with some authors believing there is a connection between glucocorticoid administration and pancreatitis (Schaer 1979). Pancreatitis has been reported in a small percentage (3.1%) of dogs with intervertebral disc disease treated with intravenous corticosteroids, primarily dexamethasone (Moore and Withrow 1982). Only 2 of these dogs had pancreatitis confirmed at post-mortem, but there may have been other precipitating factors for pancreatitis, such as hypotension associated with severe spinal cord disease, that caused this inflammation, rather than the corticosteroids per se. Corticosteroid treatment in healthy dogs or those with neurological signs failed to induce clinical pancreatitis but did result in an increase in serum pancreatic enzyme concentrations in at least three studies (Fittschon and Bellamy, 1984; Lucena et al., 1999; Parent, 1982).

There is often evidence cited that low-protein, high-fat diets induce pancreatitis and high-fat diets in dogs cause a more severe pancreatitis (Haig, 1970; Yago et al., 1997). These studies did not assess whether there was pancreatic necrosis or inflammation, rather the volume of pancreatic secretion and the pancreatic enzymes within those secretions. Thus the clinical validity of this extrapolation is questionable.
All of the test diets (the high protein, high fat, and high starch diet) increased the volume of pancreatic juice produced, but the high-fat diet didn’t change the protein composition.

Overweight dogs are at greater risk of pancreatitis, and this may be associated with abnormal dietary intake or indicate a general predisposition to inflammation (Lem et al., 2008). A chronic inflammatory state is associated with adipose tissue, and adipokines in people, and likely also occurs in dogs (Federico et al., 2010; Radin et al., 2009). This may be an important factor in canine pancreatitis especially in consideration of the peri-pancreatic fat involvement, but this has yet to be evaluated.

In one retrospective survey, dogs with recent ingestion of unusual food items and garbage ingestion all showed an increased risk of developing pancreatitis, rather than dogs that appeared to have a higher intake of treats and snacks (Lem et al., 2008). This study suggested, but could not prove, that inappropriate food rather than the fat/protein content of food per se may be most important in the development of pancreatitis. To date, the connection between high fat diets and pancreatitis in dogs remains anecdotal (Shukla, 2010). However, it is possible that increased CCK production could result from increased fat content in the diet. Additionally, it has been observed that feeding a low-protein, high fat diet to dogs for prevention of struvite urolithiasis (Hill’s SD®) causes and increased incidence of pancreatitis (D Williams, personal communication).

Acute pancreatitis may also directly result from hypoxia, and ductal hypertension, via an effect on pancreatic microcirculation as previously discussed (Charles, 2007; Cuthbertson and Christophi, 2006; Mithofer et al., 1995). This is reflected in the high incidence of pancreatitis reported after abdominal surgery, especially adrenalectomy (Lem et al., 2008; Schwartz et al., 2008). Despite the
experimental association between premature trypsin activation and hypercalcaemia, common conditions such as lymphoma that cause hypercalcaemia are not reported to cause pancreatitis in dogs. This may be that the increase in calcium is more gradual than seen in people, where hypercalcaemia-associated pancreatitis is most often seen in cardiopulmonary bypass (Dervenis et al., 1999). It may also be that the disease is subclinical and escapes diagnosis in those dogs.

One review of canine pancreatitis found that Yorkshire terriers were at increased risk, whilst Labrador retrievers and Miniature poodles had a decreased risk of developing pancreatitis (Hess et al., 1999). Another review found that Miniature schnauzers, Miniature poodles and terriers were more often affected (Cook et al., 1993), and other studies have also identified terriers to have a higher incidence (Lem et al., 2008; Ruaux and Atwell, 1998a). Whether this reflects a true predisposition, or breed preferences in those localities cannot be determined from the available data. Hereditary pancreatitis has been proven in people (Blackstone, 1998; Chen and Ferec, 2000; Whitcomb et al., 1996), and is suspected in some breeds especially Miniature Schnauzers, but a genetic abnormality has yet to be established in dogs (Bishop et al., 2004; Bishop et al., 2010).

Concurrent disease, particularly diabetes mellitus, hypothyroidism and hyperadrenocorticism are commonly reported in canine pancreatitis (Cook et al., 1993; Hess et al., 1998; Lem et al., 2008). Some possible connections between diabetes mellitus and pancreatitis include sub-clinical pancreatitis causing progressive islet cell destruction or autoantibodies directed against insulin secreting cells promoting a generalised pancreatic inflammation. All three of these endocrinopathies are associated with changes in serum lipid concentrations, and the association between hyperlipidaemia and pancreatitis cannot be discounted (Xenoulis and Steiner, 2010).
Uraemia has been associated with pancreatitis but the severity of the pancreatitis is generally mild and often an incidental finding (Williams 1995).

### 1.2.5 Prevalence and mortality rates in dogs

Prevalence of pancreatitis is very difficult to determine. One older reference quoted 3.2%, but this is likely to be grossly inaccurate as both under diagnosis of chronic pancreatitis and over diagnosis of acute pancreatitis likely occurs (Strombeck, 1990). Prevalence of chronic pancreatic inflammation in one post-mortem study was 34%, but there was no determination of how clinically important these changes were (Watson et al., 2007). Other studies identified between 75 and 92% of dogs in a referral institution to have pancreatic inflammation on post-mortem examination (Newman et al., 2005; Newman et al., 2006). This is likely to be an over-estimation, as most of the reported inflammation is likely to have been incidental, and also reflects a referral bias.

The reported mortality rate in dogs ranges from 27% to 58% (Charles, 2007; Cook et al., 1993; Ruaux and Atwell, 1998a; Schaer, 1979). This reported rate is also questionable as the reports were from referral centres and therefore predisposing to more severe cases, or there was a lack of definitive gold standard diagnosis. Euthanasia for non-medical reasons may also influence the true mortality of this condition. Even taking those factors into account, it is a higher mortality rate than the 5-15% reported in human studies (Al Mofleh, 2008).

### 1.2.6 Clinical signs

Dogs with acute pancreatitis generally present with a sudden onset of anorexia, depression and vomiting (Hess et al., 1998; Watson, 2004; Williams and Steiner, 2005). Fresh blood may be in the vomitus when vomiting is prolonged as for any other severe gastrointestinal disease, and occasionally diarrhoea will be present. Veterinary textbooks often quote that ingestion of a fatty meal precedes clinical signs (Williams
and Steiner, 2005). However, a recent retrospective analysis found that recent exposure to unusual food items rather than fat content of the food is more likely to be the inciting cause (Lem et al., 2008). Most dogs will have abdominal pain (Hess et al., 1998).

The findings on clinical examination vary considerably with the severity and stage of the disease, and the associated degree of dehydration and shock. Severely affected dogs will have signs of dehydration and shock such as tachycardia, tachypnoea, prolonged capillary refill time, hypothermia and dry mucous membranes (Williams and Steiner, 2005). Mildly affected dogs may have less dramatic signs. Complications such as icterus due to extra-hepatic bile duct obstruction develop 2-3 days after the onset of vomiting (Watson, 2004).

In a retrospective review by Hess et al (1999) of 70 dogs with fatal acute pancreatitis, the history and physical examination findings were reported. These have been tabulated (Table 1.2).

Table 1.2

<table>
<thead>
<tr>
<th>HISTORICAL FINDING</th>
<th>NUMBER OF CASES</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anorexia</td>
<td>64</td>
<td>91</td>
</tr>
<tr>
<td>Vomiting</td>
<td>63</td>
<td>90</td>
</tr>
<tr>
<td>Weakness</td>
<td>55</td>
<td>79</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>23</td>
<td>33</td>
</tr>
<tr>
<td>Polyuria/polydipsia</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Neurological abnormalities</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Melena</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>Weight loss</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Haematemesis</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Haematochezia</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Summary of clinical findings of 60 dogs with fatal acute pancreatitis. Adapted from Hess et al (1999)
1.2.7 Complications

1.2.7.1 Systemic

Systemic inflammatory response syndrome (SIRS) is a generalised systemic reaction, with resultant organ dysfunction that is characterised by peripheral vasodilation and increased capillary permeability (Barton, 2005). The development of systemic complications in acute pancreatitis is due to an imbalance between the development of SIRS and the compensatory anti-inflammatory response syndrome (CARS) (Windsor and Hammodat, 2000). This is likely to be mediated by the cytokine storm, NO and other inflammatory mediators as previously discussed. The clinical criteria for SIRS in dogs is tachycardia (heart rate > 120 beats per minute), tachypnoea (respiratory rate > 40 breaths per minute), pyrexia (>39˚C) or leucocyte abnormalities (> 18,000 or < 5000 WBC/µL) (Barton, 2005). Multiple organ dysfunction (MODS) is an extension of SIRS, and is defined as altered organ function such that homeostasis cannot be maintained without intervention. It is unknown how many dogs with pancreatitis have SIRS, but approximately 60% of people with acute pancreatitis have been reported to have SIRS, and it is considered a sign of severity (Singh et al., 2009).

Acute renal failure may develop secondary to the hypovolaemia and ischaemia resulting from vomiting in acute pancreatitis as well as potential development of intravascular coagulopathy and direct inflammation (Zhang et al., 2008a). It has been postulated that NF-κB activation may also cause aggregation of activated neutrophils in the glomeruli (Satoh et al., 2003). Endotoxin released during intestinal ischaemia may promote renal vasoconstriction (and thereby contributing to renal failure) due to high affinity for binding on the renal artery (Windsor et al., 1993). Acute respiratory injury is most closely linked to platelet activating factor, although PLA-2, TNF-α and IL-1β may also play a role (Lopez et al., 1995). The histological changes in lung from rodents with experimentally induced acute pancreatitis include substantial neutrophil
infiltration, damage of endothelial cells, interstitial oedema and intra-alveolar haemorrhage (Gomez Cambronero et al., 2002). Additionally, there may be change in pulmonary surfactant or apoptosis of type II pneumocytes (Gomez Cambronero et al., 2002). Other systemic complications include disseminated intravascular coagulation and cardiac arrhythmias, all mediated by the many systemic inflammatory cascades initiated by acute pancreatitis (Ruaux, 2000; Williams and Steiner, 2005).

Diabetes ketoacidosis (DKA) is a commonly reported complication in canine pancreatitis. It is not possible to determine if DKA occurs before, or in conjunction with the pancreatic inflammation. It is possible that the acidosis present in DKA may cause trypsin activation and then acinar cell necrosis, rather than the exocrine inflammation destroying the acinar cells (Bhoomagoud et al., 2009).

Late onset complications include chronic relapsing pancreatitis and the subsequent development of exocrine pancreatic insufficiency or diabetes mellitus (Hylands, 2006; Watson, 2003). Recently in people, sub-clinical exocrine insufficiency has been demonstrated following a bout of acute pancreatitis (Boreham and Ammori, 2003; Pezzilli et al., 2009). This may not be adequately appreciated in dogs, as sub-clinical exocrine insufficiency is difficult to diagnose (Wiberg and Westermarck, 2002). The reported systemic complications of pancreatitis in dogs are detailed in Table 1.3, as extracted from (Williams, 1994).
Table 1.3

<table>
<thead>
<tr>
<th>Organ system</th>
<th>Complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Hypovolaemic shock</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Cardiac arrhythmias</td>
</tr>
<tr>
<td></td>
<td>Myocardial depression</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Haematemesis</td>
</tr>
<tr>
<td></td>
<td>Haemorrhagic diarrhoea</td>
</tr>
<tr>
<td></td>
<td>Ileus</td>
</tr>
<tr>
<td>Renal</td>
<td>Pre-renal azotaemia with oliguria</td>
</tr>
<tr>
<td></td>
<td>Associated renal parenchymal necrosis</td>
</tr>
<tr>
<td></td>
<td>Thrombi</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Diabetes ketoacidosis</td>
</tr>
<tr>
<td></td>
<td>Hypocalcaemia</td>
</tr>
<tr>
<td></td>
<td>Hyperlipidaemia</td>
</tr>
<tr>
<td>Haematological</td>
<td>Disseminated Intravascular Coagulation (DIC)</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>Cerebral vascular accident (secondary to DIC)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>Pulmonary oedema</td>
</tr>
</tbody>
</table>

Systemic complications in canine acute pancreatitis

1.2.7.2 Local complications

Acute fluid collections are defined in the human medical literature as fluid accumulations within the pancreatic parenchyma that develops in the first 6 weeks following a bout of acute pancreatitis (Wu and Conwell, 2010b). A pseudocyst on the other hand, develops at least 6 weeks after an episode, does not contain an epithelial lining and its contents are composed of amylase-rich pancreatic secretion, generally occurring in milder cases of acute pancreatitis (Wu and Conwell, 2010b). Another late development local complication is a walled off area containing necrotic tissue. In people, this may become a nidus for infection, but in dogs this late onset change is rarely diagnosed.

Despite the term pancreatic abscess commonly being used in the veterinary literature, on review of all papers discussing pancreatic abscess or pseudocyst, development of fluid in the canine pancreas is invariably acute in onset and sterile in nature (Bellenger et al., 1989; Edwards et al., 1990; Johnson and Mann, 2006;
Marchevsky et al., 2000; Salisbury et al., 1988; Smith and Biller, 1998; VanEnkevort et al., 1999). This suggests that instead they should have been termed acute fluid collections. Additionally, the presumption that these are infected is not borne out in the veterinary literature. Out of a total of 29 with culture results, only one dog had a positive mixed culture, and it is likely this was due to secondary pancreatic infection resulting from a septic peritonitis (Bellenger et al., 1989; Edwards et al., 1990; Johnson and Mann, 2006; Marchevsky et al., 2000; Salisbury et al., 1988; Smith and Biller, 1998; VanEnkevort et al., 1999).

In pathological descriptions, a phlegmon is a solid mass of indurated pancreas and adjacent tissue that results from inflammation, oedema and necrosis. It typically develops within a few days of acute pancreatitis, and resolves spontaneously within 2-3 weeks (Charles, 2007). This term is seldom used clinically.

Another local complication that occurs in acute pancreatitis is the development of extra-hepatic bile duct obstruction (Williams and Steiner, 2005). This may occur due to physical obstruction of the bile duct, or be functional secondary to localised peritonitis. This condition typically manifests as jaundice 3-7 days after the onset of acute pancreatitis. Dogs may be systemically well despite the jaundice, or at times this is associated with a deterioration in clinical status. In most dogs, the jaundice and bile duct obstruction resolves with time (Herman et al., 2005). This may be due to reduction in the size of the pancreas. However, as the pancreas does take weeks to return to normal size, it is more likely to be due to resumption of oral intake, and subsequent gall bladder contraction.
1.3 Pancreatitis: The Diagnosis

1.3.1 Differential diagnosis

The clinical signs of acute pancreatitis in dogs are not pathognomonic. The differential diagnoses for acute pancreatitis that need to be eliminated as a priority are the life threatening conditions such as intestinal obstruction, closed pyometron or septic peritonitis, which all require surgical intervention and have very similar presentations. Other differentials include non-specific gastroenteritis, dietary indiscretion, diabetic ketoacidosis, liver disease, uraemia and other metabolic causes of vomiting.

1.3.2 Routine clinical pathology

Most laboratory abnormalities present in dogs with pancreatitis result from hypovolaemia or inflammation and are therefore not specific for pancreatitis (Whitney, 1993; Williams, 1994). Many of the differential diagnoses for pancreatitis, such as uraemia or gastrointestinal inflammation, will also result in similar laboratory changes (Simpson and Lamb, 1995; Williams, 1994). In brief, these changes include leucocytosis, azotaemia and increased liver enzymes. Decreased calcium has also been documented in dogs with acute pancreatitis, and has been suggested to be associated with a poorer prognosis (Holowaychuk et al., 2009; Jacobs et al., 1985; Schaer, 1979).

1.3.3 Specific biochemical assessment

1.3.3.1 Lipase and Amylase

Serum lipase and amylase concentrations have been shown to increase in experimental and naturally occurring canine pancreatitis (Akuzawa et al., 1994; Jacobs et al., 1985; Whitney, 1993). However, neither enzyme is specific to the pancreas as they also originate from gastrointestinal mucosa and are excreted by the kidneys (Williams, 1996). Serum lipase activity has been shown to be markedly increased in dogs with
acute enteritis, gastroenteritis, liver disease and in renal failure (Mansfield and Jones, 2000; Rallis et al., 1996; Walter et al., 1992). Lipase concentration can also be elevated up to five-fold by the administration of dexamethasone in dogs with no pancreatic inflammation (Williams, 1996). Conversely, dogs with exocrine pancreatic insufficiency have been shown to have measurable serum lipase concentration (Simpson et al., 1991; Steiner et al., 2006). Serum lipase and amylase concentrations can also be normal in dogs that do have pancreatitis. In one retrospective review by Hess et al (1998) less than 50% of dogs with acute fatal pancreatitis had increased lipase concentrations, whilst only 30.8% had increased amylase concentrations (Hess et al., 1998). Other estimates place the value more conservatively at 15-20% of dogs with acute pancreatitis having normal serum lipase and amylase concentrations (Mansfield and Jones, 2000; Stewart, 1994). This value is probably much greater in dogs with chronic pancreatitis (Watson et al., 2007). This dilemma obviously places the clinician in a difficult position. Not only is there a huge overlap of clinical and laboratory findings seen in dogs with pancreatitis and with other diseases, but also there is no guarantee that a dog with pancreatitis will have increased lipase and amylase.

1.3.3.2 Trypsinogen Activation Peptide

Trypsinogen activation peptide (TAP) is the cleavage peptide produced when trypsinogen is cleaved to trypsin (Figure 1.2). Theoretically, in pancreatitis TAP will be released into the abdominal cavity and then the circulation in high concentrations, and subsequently be cleared through the kidneys (Hurley et al., 1988). Several studies in people showed a high correlation between the severity of pancreatitis and urinary TAP concentration, and a high degree of specificity and sensitivity for diagnosis (Gudgeon et al., 1990; Neoptolemos et al., 2000). A reference interval for measurement of TAP in healthy dogs was established and its utility as a diagnostic test for canine pancreatitis was assessed (Mansfield and Jones, 2000). Unfortunately it was not highly sensitive for
diagnosis, although it was specific (Table 1.4). There may be some benefit in establishing clinical severity however, as discussed in a later section (Mansfield et al., 2003).

1.3.3.3 Trypsin-like Immunoreactivity

Serum trypsin-like immunoreactivity (TLI) is an accurate and specific indicator of pancreatic mass and is thought to be entirely pancreatic in origin (Batt, 1993). It has been shown in experimental models of pancreatitis that there is an early increase in the serum TLI concentration, followed by a rapid decrease (Simpson et al., 1989). However, in clinical cases the TLI concentration is often decreased by the time of sampling and can be affected by other diseases such as renal disease so is seldom useful (Mansfield and Jones, 2000; Ruaux and Atwell, 1999). These reasons, along with an often lengthy delay in receiving results, make its usefulness for the diagnosis of pancreatitis questionable.

Table 1.4

<table>
<thead>
<tr>
<th></th>
<th>Plasma TAP</th>
<th>Urinary TAP</th>
<th>UTCR</th>
<th>TLI</th>
<th>Lipase</th>
<th>Amylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.666</td>
<td>0.615</td>
<td>0.640</td>
<td>0.645</td>
<td>0.525</td>
<td>0.556</td>
</tr>
<tr>
<td>Diagnostic Cut-off</td>
<td>4.53 nM</td>
<td>162.6 nM</td>
<td>24.5</td>
<td>100 µg/L</td>
<td>770 U/L</td>
<td>5400 U/L</td>
</tr>
<tr>
<td>Sensitivity %</td>
<td>40.5</td>
<td>29.4</td>
<td>30.8</td>
<td>37.5</td>
<td>63.6</td>
<td>22.7</td>
</tr>
<tr>
<td>Specificity %</td>
<td>76.5</td>
<td>96.6</td>
<td>94.7</td>
<td>89.3</td>
<td>54.8</td>
<td>78.1</td>
</tr>
</tbody>
</table>

Summary of receiver-operating characteristics (ROC) analysis of a selection of laboratory methods for diagnosing pancreatitis. Data were analysed from 22 dogs with histologically confirmed pancreatitis and 34 dogs with other disease (Mansfield and Jones 2000). The diagnostic cut-off for lipase used was three times the upper reference interval. UTCR = Urinary TAP to creatinine ratio; TLI = Trypsin like immunoreactivity; TAP = Trypsinogen activation peptide

1.3.4 Canine Pancreatic Lipase

Canine pancreatic lipase is one of the most recently established laboratory tests in veterinary medicine, and its use is now widespread. The premise of this test is that it measures lipase that originates solely in the pancreas, and so it will only be increased in pancreatic inflammation (Steiner and Williams, 2002). Immunolocalization studies
showed pancreatic lipase was present only within pancreatic tissue of dogs, and serum concentrations in dogs with absent exocrine pancreatic function were decreased (Steiner et al., 2002; Steiner et al., 2006). The assay itself (first a radioimmunoassay, and then a enzyme immunoassay) has been well validated (Steiner et al., 2003; Steiner and Williams, 2003). The canine pancreatic-lipase immunoreactivity (cPLI) assay was then further developed into a commercially available specific canine pancreatic lipase (spec-CPL) sandwich ELISA, using a recombinant peptide as the antigen and monoclonal antibody for measurement. This new commercially available assay shows a good correlation to the original assay, as well as high reproducibility (Huth et al., 2010). The newer assay has caused a change in the reference intervals for the diagnosis of pancreatitis, with results < 200 µg/L expected in healthy dogs, and results > 400 µg/L considered consistent with a diagnosis of pancreatitis (Steiner, 2003; Williams and Steiner, 2005). A new in-clinic rapid semiquantitative assay has also been developed, and shows good alignment and reproducibility with the laboratory-based fully quantitative assay (Beall et al., 2011).

The original abstract published about this test quoted a sensitivity of 88%, much higher than for total lipase in the same group of dogs (Steiner et al., 2001). It is important to put that into context however, as this only assessed 11 dogs, all with relatively severe pancreatitis. In a later study to evaluate the sensitivity of pancreatic lipase, 22 dogs were assessed (Steiner et al., 2008a). These dogs had a post-mortem performed within 6 hours of death, and all demonstrated gross evidence of pancreatic disease. The pancreas was fixed in its entirety and a histological activity index was recorded based on a previously published paper (Newman et al., 2006). According to this classification, out of the 22 dogs there were 4 with moderate disease and the rest had mild disease. It is not possible to determine how clinically severe each case was, and the final cause of death or potential other primary diseases were not discussed.
However, using the Atlanta criteria (discussed in section 1.3.8), it would appear that 8 had severe pancreatitis. Both cPLI and spec-CPL had an overall sensitivity of 63.6% in diagnosing pancreatitis, compared to 40.9% and 31.8% for amylase and total lipase respectively. If only the 8 severely affected dogs were assessed, the sensitivity for the two assays (cPLI and spec-CPL) increased to 100%, as compared to 62.5% and 50% for amylase and lipase respectively. If using a 3 times- upper reference interval for lipase as indicated in a previous study (Mansfield and Jones, 2000), the sensitivity for total lipase decreased (14%). Again, it must be emphasised that there is no proven direct correlation between the histological index and clinical disease.

A study from a different research group has just become available for review that assessed 70 dogs consecutively presented for post-mortem at a tertiary referral centre (Trivedi et al., 2011). Sixty-three of those were found to have pancreatic inflammation on histology (56 mild, 7 moderate), whilst 7 had no histological evidence of pancreatic inflammation. The estimated sensitivity of canine pancreatic lipase was 21% for mild disease, and 71% for moderate disease. This was a lower sensitivity than total lipase (54 and 71% respectively) in the same cohort. Although only 7 dogs were classified as having normal pancreatic histology, they published a specificity of 86% for cPL as compared to 43% for total lipase. Again, this study has limitations due to the small number of dogs classified as true negatives, and the lack of correlation between clinically significant disease and the histological grading.

A recent paper was published to assess the specificity of pancreatic lipase in dogs (Neilson-Carley et al., 2011). In this study they assessed a total of 64 dogs, 20 from the previously mentioned study by Steiner et al with gross evidence of pancreatitis on post-mortem, and 44 other dogs that were euthanased and submitted for post-mortem analysis. Out of those 44 dogs, 27 were from a shelter and 26 of them were clinically healthy, whilst one emaciated dog was found to have diabetes mellitus.
Seventeen dogs from a referral centre with a variety of diseases (trauma 5, dystocia 2, osteosarcoma 1, osteoarthritis 1, diabetes ketoacidosis 1, adrenal tumour 1, metastatic sarcoma 1, pancreatic carcinoma 1 and pancreatitis 2) were also included. The pancreas from each dog was sectioned and inflammation scored as previously described (Newman et al., 2006). The authors classified 40 dogs as having no pancreatic disease due to an absence of clinical signs and no inflammation on histology. Thirty-eight of those 40 dogs had a SpecCPL value below 200 µg/L, and 39 had values below 400 µg/L. This resulted in a specificity using the lower cut-off value of 95% (95% CI 83.1-99.4), and using the higher cut-off value a specificity of 97.5% (95% CI 86.8-99.9).

The major drawback in this study is that the majority of animals without pancreatitis were clinically healthy, and so this specificity does not reflect a sick population of dogs in which pancreatitis would be considered as a differential. Additionally, there were no dogs with acute renal failure in this group, and decreased clearance of total lipase through the kidneys has been demonstrated in dogs (Mansfield and Jones, 2000; Polzin et al., 1983). It has not been demonstrated that the clearance of pancreas-specific lipase differs in any substantive manner from that of total lipase, except in one study that assessed dogs with experimentally induced chronic renal failure (Steiner et al., 2010). It is difficult to determine if this could be extrapolated to a clinical situation, with acute onset of decreased glomerular filtration rate.

To summarise, the three studies currently published that assess sensitivity of cPL (and cPLI) with pancreatic histology as the gold standard were combined for analysis (Steiner et al., 2001; Steiner et al., 2008a; Trivedi et al., 2011). Using the diagnostic cut-off of > 400 µg/L, a sensitivity of 41% (39/96) overall was determined, albeit with an increasing sensitivity for more severe inflammation. This result is lower than that quoted for three times the upper reference interval of lipase (64%) (Mansfield and Jones, 2000). Conversely, cPL does appear to be highly specific when considering
pancreatic inflammation, although in the face of acute renal failure this is unknown. Additionally, a histological diagnosis of pancreatic inflammation may not be clinically relevant, if there is another life-threatening reason for the animal’s clinical signs. This is demonstrated in an as yet unpublished study that has been supplied in the Appendix. This study showed a poor correlation between a positive cPL test and a primary presentation of acute pancreatitis in an emergency care setting.

1.3.5 Serum Pancreatic Elastase

Pancreatic elastase (PE-1) came to the attention of researchers and clinicians alike in the late 1960s, when elastase was shown to be involved in the pathogenesis of haemorrhagic pancreatitis in experimental models. This was later confirmed to occur at the same time or immediately after trypsin activation (Hartwig et al., 2007). Studies have shown that when macrophages are exposed to pancreatic elastase they up-regulate the expression of TNF-α, and so this supports the role of elastase in the systemic response to pancreatic inflammation (Zhang et al., 2003). Additionally, elastase has proteolytic effects, hydrolyses scleroprotein elastin, is fibrinolytic and increases the oxidative activity of neutrophils. It has been closely linked to the development of adult respiratory distress syndrome in severe pancreatitis (Wereszczynska-Siemiatkowska et al., 2003).

The most widely used application of pancreatic elastase in human medicine is measurement of the enzyme in faeces as a determinant of exocrine pancreatic function (Pezzilli et al., 2009; Tran et al., 2008). Faecal cPE-1 appears to be of limited use in dogs for the diagnosis of exocrine pancreatic insufficiency (Wiberg et al., 2000). In an early study in people, one group determined a sensitivity of 100% and specificity of 96% for measurement of serum PE-1 in diagnosing acute pancreatitis (Malfertheiner et al., 1987). They used a polyclonal RIA that measures the 1-α-antitrypsin complex (not α-macroglobulin), and all people were assessed within 72 hours of
onset of clinical signs. Overall the specificity of the test was 77%, and there was a large amount of overlap especially between people with non-pancreatic gastrointestinal disease and chronic pancreatitis. The authors concluded this was most likely due to other stimuli of pancreatic secretion; however a lack of gold standard for diagnosis of pancreatitis may have led to misclassification of the control group. The same group of researchers found no prognostic role in this test (Buchler et al., 1986). These results were similar to a larger study, which found that people with acute pancreatitis had significantly increased concentrations (Lesi et al., 1988). However, it remained measurable in some people that had had a complete pancreatectomy and was increased in acute ileo-colic disease.

It is important to put any early human papers published on PE-1 in context, as they are all based on an RIA. The RIA detects polyclonal elastase (as bound to 1-α-anti-trypsin complex) and has a half-life of 2.2 days. The ELISA that has been more recently developed detects free or unbound elastase, and has a half-life of 0.4 days (Millson et al., 1998). This means that the advantages of a prolonged increase in serum elastase cited by authors cannot hold for the ELISA (Buchler et al., 1986).

Additionally, there is a reasonable variation in the reference interval in healthy people, with some authors recommending an upper reference interval >2 ng/mL, whilst the manufacturers recommend a cut-off >3.5 ng/mL. The canine PE-1 assay is an ELISA (Schebo Biotech®).

Later human studies assessing PE-1 measured by ELISA found that elastase was not more sensitive at diagnosing acute pancreatitis than lipase (Millson et al., 1998; Scheefers-Borchel et al., 1992; Wereszczynska-Siemiatkowska et al., 2003; Wilson et al., 2005).

There has been a report in dogs that supports serum PE-1 as a diagnostic marker for pancreatitis, with a range of 32.1- 659.3 ng/mL (median 55.8) in 16 healthy dogs,
24-1720 ng/mL (median 160) in 14 dogs with pancreatitis and 5-182 ng/mL (median 43.3) in 6 dogs with renal disease (Spillman et al., 2002). When only dogs with severe pancreatitis (n=9) were analysed, there was a significant difference from healthy and renal dogs. A further study from the same authors assessed the measurement of serum cPE-1, along with amylase, lipase and TLI in seven healthy Beagles after endoscopic retrograde cholangiopancreatography (ERCP), a procedure which in people is commonly associated with the development of pancreatic inflammation (Spillman et al., 2004). There was no difference between the baseline and any subsequent measurement of cPE-1 in any dog, although there was in the other 3 enzymes. Additionally the range at baseline for the seven healthy dogs was quite wide (0.1-411.6 ng/mL) with a median of 5.5 ng/mL. This study calculated an appropriate intra-assay CV (range 2.1-11.6%) and inter-assay CV (range 3.4-10.8%) for the assay. These are to date the only data available about the serum measurement of cPE-1 in dogs.

There is a strong suggestion that serum elastase is not affected by renal clearance, as compared to many other pancreatic enzymes. In one study of 24 healthy people and 47 people with various degrees of renal insufficiency (but no known pancreatic disease) the measurements of elastase, lipase, amylase, pancreas-specific amylase, PLA-2 and trypsin were compared (Seno et al., 1995). Elastase was the least affected of all the enzymes, only increasing to a significant amount when the creatinine clearance reduced below 10 mL/minute. All other enzymes were increased when creatinine clearance was below 40 mL/min, although lipase tended to be less so. The proposed reason for this is that elastase circulates in the serum bound to inhibitor proteins such as α-macroglobulin, and is too large to pass through the glomeruli, relying on extra-renal metabolic pathways for clearance. There was also a suggestion that there could be an age-related decline in non-renal clearance.
1.3.6 Diagnostic Imaging

1.3.6.1 Radiology

The radiological findings in dogs with acute pancreatitis are not particularly sensitive (Schaer, 1979). Changes that may be present include decreased contrast and lack of detail in the cranial abdomen due to the surrounding peritonitis. As can be appreciated, this is often difficult to detect and is also not specific for pancreatitis, as it may occur in a number of conditions such as septic peritonitis. Other changes include a widened pyloric-duodenal angle, with a lateral shift of the descending duodenum towards the abdominal wall (Williams, 1994). These changes have been reported in only 22% of dogs with severe pancreatitis (Hess et al., 1998), whilst in another earlier study 76% of radiographs were considered abnormal (Schaer, 1979).

Despite these limitations, abdominal radiography is still a very important part of the diagnostic work-up for a dog with acute onset of vomiting or abdominal pain. This is mainly due to the ability to evaluate for differential diagnoses, such as intestinal obstruction or other changes such as free gas within the abdomen or a distended uterus.

1.3.6.2 Ultrasound

Abdominal ultrasound is increasingly performed in veterinary practice, and aids substantially in the diagnosis of acute pancreatitis in dogs. Changes in pancreatic echogenicity and development of focal lesions can be detected (Lamb and Simpson, 1995; Murtaugh et al., 1985; Nyland et al., 1983). Acute necrotizing pancreatitis is frequently associated with an enlarged, hypoechoic pancreas and peri-pancreatic necrosis (manifested as hyperechogenicity surrounding the pancreas), as demonstrated in Figure 1.8, and is relatively easy to identify, although gas and ingesta in the gastrointestinal tract may hamper visibility (Hecht and Henry, 2007).

It is extremely difficult to elucidate a sensitivity or specificity for this diagnostic modality. The diagnostic utility of ultrasound is highly operator dependent, and also
requires equipment that is of high standard. In the reviews from the 1990s, operator skill and equipment were not described in detail and there was a quoted sensitivity of about 68% (Hess et al., 1998; Mansfield and Jones 2000). It is likely that this sensitivity is now inaccurate. Expertise and training in this area has improved greatly in the past two decades, so it would be logical to assume the sensitivity is greater now than previously. It is also likely that this modality is much better at detecting acute necrotising pancreatitis due to the peri-pancreatic necrosis that results in an obvious hyperechoic area surrounding the pancreas. In one recent study, ultrasound was reported to have a sensitivity of 66% (Steiner et al., 2008a). However, this was only an assessment of nine dogs. On further analysis, the 3 dogs that had a negative ultrasound would not have been considered to have severe disease using many of the established criteria (as discussed in section 1.3.8). Of the 6 dogs that did have a positive ultrasound diagnosis, 4 of them were severely and 2 were moderately affected. In many ways this study demonstrated that in severe pancreatitis abdominal ultrasound is probably just as, if not more, sensitive in the diagnosis of canine pancreatitis as any available serum marker. This was also suggested with a 100% sensitivity and specificity (albeit in only 8 dogs) from another study (Trivedi et al., 2011). Specificity of this modality is virtually impossible to determine, as histology would need to be performed in order to establish this. It certainly remains an important component of a diagnostic work-up to evaluate for other abdominal disease.
Ultrasound can identify hypoechoic areas within the parenchyma, but cannot be used to differentiate between pancreatic neoplasia, inflammation, necrosis, true pseudocysts, infected necrosis or acute fluid collections (Hecht and Henry, 2007).

1.3.6.3 Magnetic resonance imaging (MRI) and computed tomography (CT)

CT scanning is the imaging modality of choice in people for detecting necrosis and infected necrosis of the pancreas (Dervenis et al., 1999). However, it is not recommended for use as a first line imaging test due to the radiation exposure and expense (Al Mofleh, 2008). Similarly, in veterinary patients it is not frequently used due to expense and availability. One report of two dogs used contrast-enhanced CT in combination with ultrasound (Jaeger et al., 2003). Both of the dogs had a diagnosis made on combination of clinical and laboratory findings, and also had consistent findings on abdominal ultrasound, suggesting no great clinical advantage in veterinary medicine in using CT. It was suggested it could be useful for detecting infected
necrosis, but as previously discussed this is not an important consideration in dogs, and diagnostic confirmation still requires sampling that is best guided by ultrasound.

MRI is also used, although not frequently, in people as it is considered a sensitive indicator of necrosis and provides greater detail of the pancreatic ducts than CT (Gosset et al., 2004). This again is a seldom used modality in veterinary patients due to the prolonged anaesthetic time required (which may be detrimental in a hypoperfused pancreas) and the expense. Additionally, the incidence of duct strictures or stones causing pancreatitis in dogs is much less than in man so its use is not justified.

1.3.6.4 Endoscopic ultrasonography

The use of an ultrasound probe inserted endoscopically theoretically reduces the interference from gas, ingesta and abdominal fat in obtaining clear ultrasound images of the pancreas (Barthel, 2005). This modality has not been described in dogs with acute pancreatitis, and again may have limited application in veterinary medicine due to the equipment and expertise required, along with a need for general anaesthesia.

1.3.7 Histopathology

Histological grading schemes have been developed for diagnosing pancreatitis in dogs, and to assist in assessing the sensitivity and specificity of diagnostic tests. The failing of these schemes is a lack of correlation with clinical severity, and therefore an understanding of the clinical significance of those grading systems. It has been established that pancreatic histological changes can be unevenly distributed throughout the pancreas, necessitating frequent sectioning along the organ in order to be able to rule out pancreatic inflammation (Newman et al., 2004). Chronic pancreatitis (lymphocytic inflammation) appears to be the most common inflammatory change, and less than 10% of dogs examined at post-mortem had no pancreatic abnormalities in one referral-centre based study (Newman et al., 2006). A slightly altered scoring system
was also adapted by another group, and focused on the classification of the chronic changes particularly (Watson et al., 2007). Both of these schemes describe grading of the severity of both inflammation and fibrosis.

The finding of hyperplastic nodules in the pancreatic parenchyma is thought to be incidental, and increase with age (Newman et al., 2005). These nodules can be both grossly and microscopically evident, and are very common. Approximately 96% of dogs with fatal pancreatitis were shown to have necrosis on post-mortem examination (Hess et al., 1998). This would suggest that dogs with more severe presentations will have some degree of necrosis within the pancreas or surrounding peri-pancreatic fat, although infected necrosis is extremely unlikely. Peri-pancreatic fat necrosis is demonstrated in Figure 1.9.

Although technically the gold standard, in cases of acute pancreatitis, it is extremely unlikely in practice that biopsy samples will be obtained in these animals due to the level of invasiveness required, along with potential detriment of a general anaesthesia. A less invasive modality such as laparoscopic biopsies may be of some use, as it has been shown to have a higher diagnostic yield than ultrasound (Webb and Trott, 2008). However, this is not a technique in widespread use and still requires general anaesthesia.

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**Figure 1.9** Haematoxylin and eosin stain (x100) section of an area of peripancreatic fat illustrating prominent neutrophil cell infiltration and necrosis of fat. Image courtesy M O’Hara.
1.3.8 Assessing severity

In order to be able to assess the usefulness of a diagnostic test, provide prognostic information or to evaluate individual treatment in research studies, it is ideal to have a method of stratifying severity of disease. This is unlikely to be one single laboratory test, or indeed be dependent on histological evaluation.

In people, early detection of severe pancreatitis is considered particularly important as this enables rapid transfer to intensive care units and improves the outcome (Al Mofleh, 2008). There is a plethora of studies assessing various clinical staging systems, in too large a number to be explored in this thesis, so they have been briefly summarised.

The most common system referenced in the medical literature is the Atlanta criteria produced in 1992, where severe pancreatitis was defined as the presence of local complications, organ failure, or death (Bradley, 1993). This certainly seems a self-evident and arbitrary classification scheme. There are several other clinical methods available for predicting the severity of pancreatitis in people. There are multifactorial scoring systems such as the Ranson, Glasgow and APACHE II (Acute Physiology and Chronic Health Evaluation) scores that take a large number of variables into account (Blum et al., 2001; Leese and Shaw, 1988; Muddana et al., 2009; Ranson et al., 1974); a system assessing obesity, age and aetiology (Dervenis et al., 1999; Karimgani et al., 1992); and other methods assessing blood markers in combination with the presence of pancreatic necrosis or age or respiratory status (Halonen et al., 2003; Ueda et al., 2007).

There has also been an explosion in measuring various blood markers, such as IL-6, -8, -18, PLA-2, CRP, PMN-elastase, matrix metalloproteinases (MMP)-9, serum amyloid A (SAA), trypsinogen-2, TAP and procalcitonin (Aufenanger et al., 2002; Gross et al., 1990; Leser et al., 1991; Mayer et al., 2002; Modrau et al., 2005;
The multifactorial systems appear effective for predicting severity and the presence of necrosis, but require assessment of dynamic changes and highly specialised assessments. This makes them most useful in tertiary-level intensive care units (Frossard et al., 2001). The APACHE system is considered the best of these systems, but although the negative predictive value (NPV) is very high the positive predictive value (PPV) or ability to predict a fatal outcome is low (Blum et al., 2001). It appears that currently the best method to predict disease severity in people is an APACHE II score $\geq 8$ and fulfilment of more than two Ranson Criteria. This results in a PPV of 75-89% for predicting severe disease within the first 48 hours of admission (Ioannidis et al., 2008). The added assessment of obesity is only of relevance in populations where obesity is common (Muddana et al., 2009; Yeung et al., 2006). A survival prediction index has been described in dogs admitted to critical care facilities, but faces similar problems in its ability to be applied in non-referral centres and does not consider body condition score (King et al., 2001).

Serum markers of inflammation as mentioned above are effective at early detection of severity but seem to be particularly associated with the risk of acute lung injury rather than mortality per se (Chen et al., 1999a; Gross et al., 1992; Leser et al., 1991; Pooran et al., 2003). Of these, IL-6 seems to have the most clinical relevance (Frossard et al., 2001; Leser et al., 1991). TNF-α has been investigated in dogs with presumed pancreatitis (Ruaux et al., 1999). There was no difference in dogs with a severe classification of pancreatitis compared to those with mild disease. This study suggests that this is not a viable method to predict prognosis in dogs.

C-reactive protein (CRP) is an acute phase protein that changes rapidly in the circulation when there is inflammation, or tissue damage, and is the blood marker most
commonly used in human medicine. SAA, which is also an acute phase protein though shows promise of increasing to a greater degree in severe pancreatitis at an early stage, and may replace CRP in the future (Mayer et al., 2002). CRP has been measured in dogs, and has been shown to be increased in a number of inflammatory conditions, including pancreatitis (Holm et al., 2004; Nakamura et al., 2008). Although the values are increased within 24 hours of an insult, there is often variation between dogs, so a change in CRP from day to day may be more relevant in dogs for predicting outcome (Otabe et al., 1998).

TLI and α-macroglobulin were reported to be poorly correlated with survival in dogs, with presumptive pancreatitis (Ruaux and Atwell, 1999). Serum lipase appears to be loosely associated with clinical severity in dogs, along with creatinine and phosphate concentration, but the most closely correlated measurement was urinary TAP-creatinine ratio in one study (Mansfield et al., 2003). This mirrors findings in people, but unfortunately due to technical difficulties with the assay it is unlikely to become available in the mainstream veterinary practice (Neoptolemos et al., 2000).

In contrast to using scoring systems or single markers, the persistence of organ failure or the presence of obesity, pleural effusion, necrosis (infected or sterile), SIRS and MODS appear to be the major determinants of mortality in people (Al Mofleh, 2008; Brown et al., 2007; Buter et al., 2002; Isenmann et al., 1999; Singh et al., 2009). The presence of SIRS has been shown to be associated with a high mortality rate in a study of dogs admitted to an intensive care unit, but this has not been specifically assessed in dogs with pancreatitis (Okano et al., 2002). The use of obesity as a poor prognostic indicator is not something that has been evaluated prospectively in dogs, but it is an interesting concept as obesity may contribute to inflammation via production of adipokines (Papachristou et al., 2006). Obese dogs are more commonly reported in
series of pancreatitis, but it is unclear whether this reflects the canine population of the centres, or is a real bias (Cook et al., 1993; Hess et al., 1999).

The biggest and most recent study in people assessed over 18,000 patients with acute pancreatitis in over 200 centres (Wu et al., 2008). This study used classification and regression tree (CART) analysis to predict in-hospital mortality. They determined five factors that contributed to prognosis including azotaemia, impaired mental status, the presence of SIRS, age > 60 years and the presence of pleural effusion. The mortality rate was significantly greater with a higher number of abnormalities present. This is a similar concept to a severity score developed in dogs, where assigned points were correlated to mortality rate (Ruaux and Atwell, 1998b). This scheme is discussed in detail in Chapter 4, but in that study the diagnosis was not definitive, the treatment was not standardised and death due to euthanasia on non-medical grounds was not taken into account.

1.4 Pancreatitis: Treatment

There is a paucity of controlled studies evaluating the optimal treatment of acute pancreatitis in the dog. In fact using multiple search engines (PubMed, Ovid, Web of Science, CAB abstracts) to search for prospective clinical trials, only one published article written in the course of this project was identified (Mansfield et al., 2011a). As such, the current treatment recommendations rely on extrapolated experimental studies, or general advice relating to treatment of critically ill dogs with other diseases.

1.4.1 Intravenous (IV) fluid therapy

Dogs that develop acute pancreatitis are typically dehydrated, and often hypovolaemic. In severe cases there may be substantial third space losses. As such, basic first aid principles would predicate the use of resuscitative intravenous fluid. The
major goals of fluid therapy are to maintain cardiac output, improve pancreatic and splanchnic perfusion and correct acid-base abnormalities.

The blood flow to and capillary structure of the pancreas is complex, as discussed in the first section of this review. This is in order to allow integration between the endocrine and exocrine functions of the pancreas, and requires a relatively high blood flow to maintain function (Gardner et al., 2008). One of the major factors that progresses mild pancreatitis to severe pancreatitis is disturbed pancreatic microcirculation (Bassi et al., 1994; Borodin et al., 2006; Knoefel et al., 1994). This disturbance is usually multi-factorial in origin and can occur as a result of increased vascular permeability resulting from inflammatory cytokines, and microthrombi formation resulting from hypercoagulability (Gardner et al., 2008). The increased capillary permeability leads to oedematous changes in the acinar cells, and further migration of inflammatory cells. In necrotising pancreatitis there is a progressive reduction in capillaries after acinar cell injury, which cannot be reversed by fluid resuscitation (Bassi et al., 1994). In rodent models, vasoconstriction within the pancreas appears to be an early event (Kusterer et al., 1993; Schröder et al., 1985). In fact, in people, early onset spasm of large pancreatic vessels has been shown to correlate with poorly perfused areas of the pancreas, and subsequently high mortality rates (Takeda et al., 2005).

As stated earlier in this chapter, there is substantial splanchnic hypoperfusion and regional mucosal acidosis that develops with acute pancreatitis. Reperfusion injury is hypothesised to occur when the splanchnic flow is restored. The initial ischaemia causes accumulation of hypoxanthine and depletion of ATP. Xanthine oxidase (XO) is produced upon reperfusion, which converts hypoxanthine to xanthine, and subsequently leads to production of free radicals (O$_2^-$). XO is present in large quantities in gut epithelial cells (Fink, 1991). Free radicals are chemoattractants for neutrophils and
inflammatory cytokines, which in turn amplify the inflammatory response. Therefore theoretically, the return of splanchnic circulation with fluid resuscitation may potentially be detrimental. However, delaying or reducing fluid resuscitation to avoid this theoretical problem is neither supported by the literature, nor indeed by common sense. One study in people has shown that early fluid resuscitation (as opposed to at 24 and 72 hours after onset of pain) leads to a better clinical outcome (Warndorf et al., 2011). This benefit was most apparent in people with milder forms of pancreatitis, and therefore may be a suitable comparative study for the majority of dogs hospitalised for pancreatitis.

In the veterinary literature there is no current recommended preference for either the use of Lactated Ringer’s (LRS) or saline solution as the initial crystalloid of choice. LRS is an isotonic fluid, containing the following electrolyte concentrations: sodium 130 mmol/L, chloride 109 mmol/L, lactate 28 mmol/L, potassium 4 mmol/L and calcium 1.5 mmol/L. It has a pH of 6.5, but is considered an alkalinising solution. Normal (0.9%) saline, on the other hand, contains 154 mmol/L of both sodium and chloride, and has a pH of 5.5. This fluid is considered a safe and first line fluid choice for resuscitation, but studies in people have shown that hyperchloraemic metabolic acidosis develops when it is used (Prough and Bidani, 1999).

In a landmark experimental study evaluating fluid resuscitation in rats with septic shock (induced by intravenous endotoxin administration), Kellum demonstrated that there was a higher mortality rate in rats treated with IV normal saline or LRS, as compared to hetastarch (Kellum, 2002). The premise for this study was that although hyperchloraemic metabolic acidosis invariably resulted from saline resuscitation, it was not proven that this was in fact detrimental. Survival in this study was significantly associated with higher arterial pH and hyperchloraemia, but not with lower lactate concentration. It is possible that acidosis directly contributes to the systemic
inflammatory state by stimulation of cytokine production, especially NF-κB, the precursor of many inflammatory cytokines.

Experiments using rodent models have also identified that high acinar pH protected against secretagogue induction of pancreatitis (Bhoomagoud et al., 2009; Noble et al., 2008). Possible reasons for this include the tendency for lysosomal proteins and zymogen granules to co-localise in the face of an acidic environment; and the presence of proton pumps on acinar cells, which help regulation of zymogen secretion and activation (Bhoomagoud et al., 2009; Waterford et al., 2005).

One experimental study in dogs (using a bile-trypsin induced method) showed that low rate infusion of LRS (1.75 ml/kg/hour) resulted in diminished pancreatic blood flow compared to a high rate LRS infusion (6.5 mL/kg/hour) over a 4-hour period (Knol et al., 1987). Whether this experimental finding correlates to a clinical benefit in naturally occurring disease in dogs is unknown. Initial work in people suggests that there may be an advantage in aggressive fluid resuscitation with LRS (Wu et al., 2011). Patients receiving LRS showed significant reductions in serum CRP concentration and reduction in the severity of SIRS at 24 hours compared to those receiving normal saline, regardless of the rate protocol used.

Another experimental study that induced acute pancreatitis in dogs via a bile infusion measured the pulmonary arterial pressures in dogs resuscitated with 4 mL/kg of hypertonic saline-dextran solution and LRS to effect compared to dogs resuscitated to effect with LRS alone (Horton et al., 1989). They identified that the dogs resuscitated with LRS alone required approximately 5L more fluid during resuscitation to maintain systemic pressures, and this resulted in pulmonary hypertension and pulmonary oedema. There are multiple rodent experimental models that also show dextrans has a beneficial effect in acute pancreatitis (Donaldson and Schenck, 1979; Knol et al., 1983; Schmidt et al., 1993; Hotz et al., 1996). The benefit appears to be
independent of the molecular weight, concentration (6% or 10%), or combination with hypertonic saline.

There is no current evidence that any particular type of fluid resuscitation protocol or fluid type is advantageous in severe canine pancreatitis. However, extrapolation of the experimental and human studies would suggest that LRS is preferable to normal saline. It may also be wise to formulate a similar rate protocol to that used in the most recent human paper (Wu et al., 2011), and to use LRS as the initial resuscitative crystalloid fluid in dogs with severe pancreatitis. In this protocol, fluid was administered as a bolus, with 8-hourly checkpoints to determine if this had been successful in correcting fluid imbalances. A potential fluid algorithm for dogs is detailed in Figure 1.10. Further evaluation of colloid therapy (hetastarch or dextrans) would also be of great clinical benefit.

**Figure 1.10** Extrapolated fluid therapy algorithm (using crystalloid therapy) from Wu et al (2011).

### 1.4.2 Plasma

The use of plasma in dogs with acute pancreatitis is widely reported in review papers and textbooks (Heinrich et al., 2006; Logan et al., 2001; Snow et al., 2010; Williams and Steiner, 2005; Wu and Conwell, 2010a). Administration of plasma was
shown to be superior to both crystalloid and colloid administration in a rat experimental model of pancreatitis (Leese et al., 1988). Purported benefits include replacement of circulating α-macroglobulins, replacement of coagulation factors and treatment of SIRS with anti-inflammatory factors (Weatherton and Streeter, 2009). Depletion of α-macroglobulin has been documented in an experimental model of canine pancreatitis, and correlated with severity of pancreatic inflammation (Murtaugh and Jacobs, 1985). Systemic effects of the pancreatic inflammation were not assessed in that study, nor whether there would have been amelioration with administration of plasma. Another experimental study in dogs with pancreatitis induced by a trypsin infusion showed that death occurred when α-macroglobulins were consumed (Balldin et al., 1978). One study of sick dogs with increased lipase and probable pancreatitis showed that serum concentration of α-macroglobulins did not correlate with the severity of disease or clinical outcome (Ruaux and Atwell, 1999). It is unlikely that any benefit seen with plasma will be due to colloid like properties, as fresh frozen plasma (FFP) has only about 20-30% of the oncotic properties of colloids. However, haemostatic proteins in FFP are well preserved regardless of whether freeze-thawed or not (Iazbik et al., 2001; Yaxley et al., 2010).

There are no controlled studies that prove the benefit (or lack thereof) of plasma transfusion in dogs with naturally occurring pancreatitis. The use of plasma for treating acute pancreatitis in dogs has been declining over the past 5 years (Snow et al., 2010). One retrospective veterinary study analysed data from a 10-year period, and identified 77 dogs with pancreatitis that were admitted for treatment during that time (Weatherton and Streeter, 2009). Of these cases, 20 (approximately 26%) were given plasma at a mean infused volume of 16 mL/kg, with the volume given not differing between survivors and non-survivors. There was a significantly higher mortality (P= 0.008) in dogs that received plasma (7/20) compared to those that did not (6/57). There appeared
to be no difference in the choice to administer plasma based on the presence of SIRS or a documented coagulopathy, however there was insufficient data to allow for meaningful analysis of this. This study is inherently flawed due to its retrospective nature, and the lack of stratification of disease severity or standardisation of other treatments. The biggest bias is likely to be that the clinically more severely affected dogs may be administered plasma due to the perceived benefit. Thus, those dogs that received FFP by inference would be more severely affected and inherently were more likely to die as a result of their disease. However, the lack of benefit seen in this study does reflect much of the human literature on this same subject (Leese et al., 1987).

In one randomised prospective study of 200 people assessing low-dose FFP administration over 2 consecutive days, there was no survival benefit observed. A reduction in α-macroglobulin over three consecutive days was present, which suggested the volume of FFP may also play a role. The same group of researchers then assessed the benefit of high dose FFP therapy in a group of 72 patients predicted to have severe pancreatitis (Leese et al., 1991). These were randomly assigned to receive either FFP (8 units daily for 3 days) or colloids. Again, there was no difference in survival between the two groups. This is despite a documented increase in serum anti-proteases in the treatment versus control group. This again highlights the difficulties in extrapolating experimental benefits to clinical settings.

Although there is little evidence either supporting or refuting the use of FFP, it remains an expensive treatment for veterinary patients. None of the studies in people or dogs, although limited, have shown a clinical benefit and in fact suggest it may not be useful. In the light of these findings, administration of FFP should probably be reserved for those dogs with documented coagulation abnormalities, or where overt disseminated intravascular coagulation (DIC) is present.
1.4.3 Anti-emetics

Anti-emetics are a commonly used group of drugs in the management of gastrointestinal disease in dogs. The overall aim of anti-emetic therapy is to reduce volume loss when there is refractory vomiting, and therefore also reduce subsequent electrolyte and fluid imbalances. Vomiting in dogs with pancreatitis is likely to be both centrally mediated due to the presence of circulating emetic agents, and peripherally mediated due to ileus, peritonitis, and pancreatic distension (Elwood et al., 2010).

There are no studies published on the efficacy of individual anti-emetic drugs in canine pancreatitis. Due to the large number of possible drugs that could be discussed, this review will concentrate on the most commonly used anti-emetics in veterinary practice: the dopamine-antagonistic (and weakly 5-HT$_3$ antagonistic) agent metoclopramide; the NK1-receptor antagonist maropitant; and ondansetron, a 5-HT$_3$ selective antagonist that is being increasingly used in veterinary practice (Elwood et al., 2010).

Metoclopramide has been used in veterinary medicine for a long period of time, and in the acute setting can be given as intermittent boluses (intravenously or intramuscularly), or as a constant rate intravenous infusion (Elwood et al., 2010). In one veterinary study assessing the efficacy of this drug in dogs with parvovirus, the number of emetic episodes was not reduced when metoclopramide was given as an intermittent bolus (Mantione and Otto, 2005). Additionally, there was no correlation between a positive outcome and the number of emetic events.

There is some anecdotal advice that suggests because metoclopramide is a dopaminergic antagonist, it should not be used in acute pancreatitis (Williams, 1994). Experimental animal models have shown that dopamine infusion improves the outcome in acute pancreatitis, and ameliorates the inflammatory severity of the disease (Karanjia et al., 1990; Karanjia et al., 1991). This does not appear to be related to blood flow to
the pancreas, and is instead postulated to be due to reduction of pancreatic microvascular permeability (Karanjia et al., 1991). Low-dose infusion of dopamine may be more effective at preventing distant lung injury than reducing pancreatic inflammation per se (Kaya et al., 2005). There is therefore a theoretical disadvantage in giving metoclopramide to dogs with acute pancreatitis, although this is unproven.

Maropitant, which blocks the Neurokinin 1 (NK1)-receptor and therefore the actions of Substance P is an effective anti-emetic agent that blocks centrally and peripherally mediated emesis (Benchaoui et al., 2007; Conder et al., 2008; De la Puente-Redondo, 2007a; De la Puente-Redondo, 2007b; Rau et al., 2010; Sedlacek et al., 2008). In one experimental study in dogs, it was shown to be more effective than metoclopramide, but not ondansetron, in controlling emetic episodes due to peripheral emetic stimulation (Sedlacek et al., 2008). It was more effective than ondansetron, but not metoclopramide, in controlling emetic episodes due to centrally-mediated emesis (Sedlacek et al., 2008). In another study, this time prospectively assessing dogs with vomiting due to naturally occurring disease in Europe, maropitant was superior to metoclopramide, both within the first 24 hours, and then for every day up to 5 days, when the trial was stopped (De la Puente-Redondo, 2007). Therefore as emesis in pancreatitis is mediated both centrally and peripherally there is a theoretical advantage in using maropitant compared to either metoclopramide or ondansetron.

As well as being effective in controlling emesis, there is another theoretical benefit to NK1-receptor antagonism, via blocked production of Substance P. Substance P is a mediator that is produced by nerve endings throughout the body. It has been shown to be up-regulated in mouse models of acute pancreatitis (Frossard and Pastor, 2002). Substance P mediates capillary permeability and is produced when NK1 receptors are stimulated. Substance P has also been shown to be up-regulated in acinar cells during murine experimental pancreatitis (Frossard and Pastor, 2002). When the
NK1 receptor was blocked in a genetic mouse model, there was no difference in the amount of pancreatic inflammation produced, but distantly-mediated lung injury was reduced (Pastor and Frossard, 2001). It is this possible effect that may be of benefit in dogs with severe pancreatitis, as they are at risk to develop acute lung injury in addition to the potential to reduce pain.

Apart from one study experimentally inducing emesis, there have been no direct comparisons of ondansetron to maropitant in dogs. Anecdotally, many veterinary surgeons will give ondansetron either in conjunction with or instead of maropitant to control nausea. In people assessed post-operatively, there is evidence that NK-1 receptor antagonists are equal or superior to ondansetron in controlling nausea, and result in fewer emetic episodes (Diemunsch et al., 2009). Although there is again a danger of direct extrapolation, the NK1-receptor function is considered the same in dogs as in people (Leffler et al., 2009).

Consequently, current levels of knowledge suggest maropitant should be used as the first line treatment for controlling vomiting and nausea in dogs with acute pancreatitis. Although there is no evidence of any adjunctive benefits, it is possible that combination with ondansetron may improve nausea control.

1.4.4 Antibiotics

Antibiotic therapy is commonly used in the management of canine pancreatitis, despite the necrosis invariably being sterile, and a lack of data suggesting that antibiotics improve survival or outcome.

There has been a degree of controversy over the past few years regarding the use of prophylactic antibiotics in people with acute pancreatitis. The main purpose of antimicrobial use in people is to treat or prevent the infection of necrotic pancreatic tissue or ascending cholecystitis (Sainio et al., 1995). Many people with pancreatitis either have gall stones or alcohol-induced disease, so have different susceptibilities to
infections than dogs. Additionally, there is a high rate of hospital-introduced infections in people with acute pancreatitis (Sainio et al., 1995). This may also be the case in the veterinary population, but this has not been determined.

There have been a number of studies assessing antibiotics in people (Delcenserie et al., 1996; Isenmann et al., 2004; Luiten et al., 1995; Nordback et al., 2001; Sainio et al., 1995; Schwarz et al., 1997), as well as numerous meta-analyses (Golub et al., 1998; Sharma and Howden, 2001; Villatoro et al., 2006; Yao et al., 2010). These all have quite conflicting results, with the initial recommendation being that there should be prophylactic use of a drug with good tissue penetration, namely imipenem (Heinrich et al., 2006; Hirota et al., 2010; Villatoro et al., 2006). The basis for this recommendation was that although the numbers of infections are not reduced when people are treated with β-lactams compared to fluoroquinolones, the mortality rate is (Villatoro et al., 2006). This parallels the observations that carbapenems, some cephalosporins, trimethoprim-sulphonamide and enrofloxacin all have good pancreatic tissue penetration in people and dogs (Bradley, 1989; De Waele, 2010). However, in the clinical studies additional management protocols were used, so the beneficial effects could not be credited to antibiotic therapy alone (Luiten et al., 1995; Sainio et al., 1995).

To add confusion to the issue, there have also been studies that imipenem reduces infection rate, but has no effect on mortality (Pederzoli et al., 1993). This study was open labelled, and so some bias is present. Additionally, an increasing incidence of resistant organisms and fungal infections has been noted in people with pancreatitis pre-treated with antibiotics, and reflects an emerging trend (Behrman, 2011).

Overall, with more recent multi-centre double-blinded prospective studies (Dellinger et al., 2007; Garcia-Barrasa et al., 2009; Isenmann et al., 2004) and multiple meta-analyses the overall suggestion is that there is no benefit in prescribing
prophylactic antibiotics in people, regardless whether necrosis is present or not (Charbonney and Nathens, 2008; De Waele, 2010; Nathens et al., 2004; Nicholson, 2011). Instead, antibiotics should only be given when a documented infection is present, and should be given early in the course of the disease (De Waele, 2011; Pezzilli, 2009).

The documented organisms isolated from the pancreas in most people originate from extra-pancreatic sites, and may result from seeding during surgical resection, the overuse of prophylactic antibiotics and concurrent central intravenous lines to provide total parenteral nutrition (Behrman, 2011). The lower use of surgical interventions in dogs, along with the differences in aetiologies may well account for the lower numbers of documented pancreatic infection in dogs. In a canine induced-pancreatitis model, plasmid labelling showed that organisms detected in the mesenteric lymph nodes and pancreas were the same that colonised the intestine, indicating bacterial translocation (Liu et al., 2000).

Unpublished observations have identified the presence of bacteria in the pancreas of cats with no overt signs of pancreatitis using culture independent methods (K Simpson, personal communication). The true clinical significance of that in cats is yet to be determined. Cats have a common bile and pancreatic duct, in comparison to dogs. It is likely that this predisposes cats to bacteria being present in the pancreas, but it is unlikely such an incident occurs in dogs.

As true pancreatic infection is extremely rare in dogs with acute pancreatitis, the use of antimicrobials must be questioned. The biggest indication for their use would be signs consistent with a high risk of bacterial translocation along with a documented pro-inflammatory state (SIRS). Signs consistent with a high risk of bacterial translocation include bloody diarrhoea, prolonged fasting and haematemesis. The
penetration of antibiotics into the pancreatic tissue would therefore be of lesser importance in antibiotic selection than a spectrum effective against gut pathogens.

The standard use of prophylactic antibiotics in dogs without signs of concern may lead to increased community resistance to antibiotics. The use of fluoroquinolones or vancomycin is strongly discouraged without documented evidence for their use.

1.4.5 Analgesia

It is commonly accepted in the veterinary (Watson, 2004; Williams, 1994; Williams and Steiner, 2005) and human literature (Pezzilli, 2009; Wu and Conwell, 2010a) that analgesia is a vital component of managing acute pancreatitis. It should be presumed that dogs with pancreatitis severe enough to require hospitalisation also require analgesia (Williams and Steiner, 2005). Recently, the use of patient controlled analgesia in human gastroenterology has improved outcomes and reduced hospitalisation times (Hirota et al., 2010; Wu and Conwell, 2010a). Additionally, in rodent experimental models, the use of thoracic epidural analgesia has been shown to improve pancreatic blood supply and decrease mortality (Freise et al., 2006).

Opioids are the first line therapy in the management of pain. Buprenorphine does not cause contraction of the sphincter of Oddi, and therefore theoretically could be preferable as first line therapy (Jakobs et al., 2000). That being said, there is no indication that the stimulation of the sphincter of Oddi that this class of drug causes is detrimental in pancreatitis.

If opioid analgesia is considered insufficient, then other multi-modal delivery of analgesia can be used. It is important if the pain in an individual animal appears to be increasing that a complication such as acute fluid collection is ruled out and treated as necessary (discussed in section 1.4.7). An alternative analgesic agent is ketamine, given as a continuous rate infusion. This modality is attractive due to the lack of detrimental effect on gastrointestinal motility (Bares et al., 1995). Other alternative modalities
include epidural analgesia (morphine with or without gabapentin), inter-pleural blocks and lignocaine continuous rate infusion (Dravid and Paul, 2007; Smiley et al., 2004). None of these have been evaluated in canine acute pancreatitis, and modification of the analgesia plan should be performed on an individual basis.

The use of pancreatic enzyme supplements to provide analgesia when feeding animals with pancreatitis has been recommended, but there have been no studies to validate this (Williams, 1994). Only one randomised placebo-controlled trial in people has been undertaken, and this showed no analgesic benefit when given early in the course of the disease (Patankar et al., 1995). Additionally, only 52% of people assessed in a meta-analysis actually preferred oral enzymes (Gachago and Draganov, 2008). This is despite some earlier studies showing a significant advantage in people with idiopathic, mild disease (Isaksson and Ihse, 1983; Slaff et al., 1984). One possible reason for this discordance of results is the small number of people in each trial, the difference in enzymes (enteric vs. non-enteric coated preparations), a high placebo rate and also the possibility of inactivation within the stomach or duodenum. It is also highly likely that different sub-sets of patients will have different responses to this treatment. Currently, there is insufficient evidence to recommend their use in dogs, unless there is pain directly associated with feeding.

1.4.6 Gastric acid suppression

Reduction of gastric acidity medically or via physical suctioning of gastric contents is recommended in many veterinary summaries (Kalli et al., 2009; Stewart, 1994; Watson, 2004; Williams, 1994). The rationale these summaries have provided for this include that reduction of gastric acid production will lead to decreased pancreatic exocrine stimulation and that acute pancreatitis predisposes to the development of gastric mucosal ulceration due to hypovolaemia and local peritonitis.
There have been no studies that report on the efficacy of gastric acid suppression in dogs with acute pancreatitis. However, a lack of proven benefit does not negate the treatment if no adverse effects occur. Thus it remains ambiguous as to the recommendation for their use. The physical presence of a feeding tube across the gastro-oesophageal sphincter may well predispose to the development of severe oesophagitis due to gastro-oesophageal reflux, and so should not be routinely used.

In people with mild to moderate disease, there have been randomised clinical trials assessing nasogastric suctioning. None of these have shown any benefit of this treatment in reducing pain or hospitalisation duration (Field et al., 1979; Fuller et al., 1981; Levant et al., 1974; Loiudice et al., 1984; Naeije et al., 1978; Navarro et al., 1984; Sarr et al., 1986). In fact, some of these have actually shown prolongation of pain and nausea (Fuller et al., 1981; Loiudice et al., 1984; Navarro et al., 1984; Sarr et al., 1986). A meta-analysis of cimetidine confirmed that its use had no clinical benefit, and actually tended to increase the incidence of complications (Morimoto et al., 2002).

There are no meta-analyses or well controlled studies on the use of proton-pump inhibitors in acute pancreatitis in people, and very few in any disease in dogs. Theoretically proton pump inhibitors may have directly beneficial effects by blocking the vacuolar ATPase pump on pancreatic acinar cells. This is based on the observation that lower pH within the acinar cell accelerates zymogen activation, and amplifies the subsequent inflammatory damage (Bhoomagoud et al., 2009; Waterford et al., 2005). One experimental study in rats showed pantoprazole reduced inflammatory changes and leakage of acinar cells (Hackert et al., 2010). This must be an indirect effect, as in vitro there appears to be no direct effect of proton pump inhibitors on pancreatic exocrine secretion (Cai et al., 2007).

Currently, there is no evidence that reducing gastric acidity improves outcome in dogs with acute pancreatitis. However, if there is evidence of gastric ulceration
(substantial haematemesis, melena) or oesophagitis, then gastric acid suppression is indicated. In human medicine it is recommended that the gastric pH is increased to more than 4 for approximately 16-22 hours per day in critically ill patients or patients with severe gastro-oesophageal reflux (Bersenas et al., 2005). Omeprazole, a proton pump inhibitor, has been shown to be the most effective at increasing gastric pH for the longest period of time in dogs compared to famotidine, pantoprazole, and ranitidine (Bersenas et al., 2005; Tolbert et al., 2011). Dogs and cats are often dosed with oral omeprazole at 0.7-1mg/kg daily, but recent work would suggest that doses should be increased up to 2.5 mg/kg day, in divided doses (Tolbert et al., 2011).

1.4.7 Treatment of complications

Complications that develop in acute pancreatitis are broadly divided into the local pancreatic complications and systemic complications. Prediction of dogs likely to be affected with systemic complications will help guide the level of monitoring. Based on extrapolation from human experience (Singh et al., 2009), dogs with SIRS, lack of enteral nutrition, hypotension and hypovolaemia would be at high risk of developing complications. A proportion of dogs that present with acute pancreatitis have diabetic ketoacidosis (DKA), and this also requires specific treatment (Watson, 2004; Williams and Steiner, 2005). Although there is an assumption that the pancreatic inflammation causes damage to the islet cells and therefore causes DKA, it is possible that the ketoacidosis actually precipitates pancreatitis due to the lowering of acinar pH (Bhoomagoud et al., 2009; Noble et al., 2008; Waterford et al., 2005).

Oxygen therapy is recommended in many human society guidelines to maintain arterial oxygen saturation > 95% (Hirota et al., 2010; Johnson, 2005; Nathens et al., 2004; Wu and Conwell, 2010a). This is partly due to the high rate of lung injury associated with acute pancreatitis in people (Wang et al., 2006), a decreased respiratory
drive due to narcotic use and pain (Muddana et al., 2009); and the risk of pulmonary oedema with fluid overload. No studies of this modality have been undertaken in dogs.

Surgery to treat pancreatic acute fluid collections in dogs has invariably resulted in a high mortality rate (> 50%), regardless of the technique used (Bellenger et al., 1989; Edwards et al., 1990; Johnson and Mann, 2006; Salisbury et al., 1988). There have been reported spontaneous resolutions of acute fluid collections in the veterinary literature, and good responses to percutaneous drainage, suggesting these are preferable methods for managing this particular complication (Smith and Biller, 1998; VanEnkevort et al., 1999).

Current human recommendations are not to debride sterile fluid collections, and if infection is documented then there should be treatment with antimicrobials for as long as possible prior to surgical debridement. (Heinrich et al., 2006; Johnson, 2005; Muddana et al., 2009; Nathens et al., 2004). As well, there is increasing evidence that ductal disruption is more commonly associated with progression of local pancreatic complications, such as pancreatic fistulae, whereas normal ductal anatomy usually results in spontaneous resolution (Wu and Conwell, 2010b). Therefore if ductal disruption is not present, even in the face of documented infection, percutaneous or endoscopic guided drainage may be sufficient for treatment. However, surgical necrosectomy is still the preferred method in advanced cases of infected pancreatic necrosis (Heinrich et al., 2006; Hirota et al., 2010; Johnson, 2005).

Various surgical techniques have been described to treat extra-hepatic bile duct obstruction in dogs, including choleduodenectomy (Matthiesen and Rosin, 1986) and cholecystectomy (Mehler et al., 2004). These are invariably associated with a high mortality rate. Percutaneous drainage of the gall bladder has been described, and is a relatively safe and minimally invasive procedure (Herman et al., 2005). The benefits of
this approach appear to outweigh the risks, but there are no strict criteria indentified for when drainage of the gall bladder is required.

1.4.8 New therapeutic directions

There have been many studies in the past three decades to assess new medical treatments for acute pancreatitis based on experimental success. These have been drugs that target specific cytokines, counteract proteases or reduce pancreatic secretion along with novel treatments such as continuous haemodiafiltration and peritoneal dialysis (Pezzilli, 2009; Williams, 1994). None of the novel interventional treatments have been widely accepted or been shown to be particularly effective (Buchler et al., 1993; Dervenis et al., 1999; Johnson et al., 2001; Pezzilli, 2009), and as such don’t warrant extensive discussion in this review.

The most common drugs studied include the anti-proteases aprotinin and gabexate mesilate; and the anti-secretory drug octreotide. Initially, there was some promise for protease inhibitors in both reducing mortality or complications in rodent experimental models and naturally occurring disease in people (Andriulli et al., 1998; Buchler et al., 1993; Chen et al., 2000; Mikami et al., 2005). However, stringent analysis of multiple randomised and well controlled clinical trials has shown no benefit for these agents to reduce mortality or morbidity (Heinrich et al., 2006; Imrie et al., 1978; Uhl et al., 1999). Additionally, it is recommended that these drugs be given within 24 hours of onset of pain, and be continued for at least 7 days (Pezzilli, 2009). The lack of effect of gabexate mesilate in human clinical trials has been replicated in canine experimental models (Luh et al., 1999), with the greatest effect seen in prevention of lung injury prior to onset of acinar cell injury, but not following it. These findings, along with the lack of availability and prohibitive costs make this drug impractical in the treatment of acute pancreatitis in dogs.
Somatostatin and its long-acting analogue, octreotide, have been studied in people. They are potent inhibitors of pancreatic secretion, and also stimulate the reticuloendothelial system, and are considered cytoprotective to the pancreas (Pezzilli, 2009). However, this theoretical benefit is counterbalanced by the powerful splanchnic vasoconstrictive properties of these agents, something that contributes to and perpetuates pancreatic necrosis (Klar et al., 1991). They appear to be agents best used as prophylaxis for prevention of ERCP-associated pancreatitis, and not in treatment of already established pancreatitis (Bang et al., 2008). There was no amelioration of histological inflammation in a study of dogs with induced pancreatitis treated with a somatostatin analogue (Ko et al., 1992). Again, clinical trials and meta-analysis have failed to identify a benefit with using these agents in people, and there is little to support their investigation in dogs (Heinrich et al., 2006; Uhl et al., 1999).

In dogs, there have been no well-controlled studies of secretory inhibitors. There has been one case report of the use of chlorpromazine in a dog suffering pancreatitis as a result of organophosphate toxicity (Dalefield et al., 1999). This was an uncontrolled single observation, and as such lacks any level of evidence for efficacy. Despite interest in these agents beginning well over a decade ago, there is not sufficient basis to evaluate this further. Similarly, the use of glucagon cannot be recommended (Hirano and Hirano, 1999).

The effects of specific anti-cytokine therapies appear to be highly variable, as they are generally useful at ameliorating only one aspect of the inflammatory cascade. Along those lines, a TNF-α inhibitor showed no protective effect in dogs undergoing ERCP (Buscaglia et al., 2008). Neutrophil-elastase inhibition had no benefit in an experimental dog model, although survival was not assessed (Imamura et al., 1998). Platelet-activating factor antagonists such as lexipafant reduced lung-associated injury in rodent models when given prophylactically, but this did not translate to a clinical
benefit in well-powered human trials of spontaneous disease (Formela et al., 1994; Heinrich et al., 2006; Johnson et al., 2001; McKay and Imrie, 2004). However, on close review of those studies, when people that were treated with lexipafant within 48 hours of onset of symptoms, there was a significant reduction in the development of pseudocysts, early onset sepsis and death compared to placebo (Pezzilli, 2009).

As there are multiple cytokine cascades stimulated with acute pancreatitis, it is not surprising that antagonism of just one cytokine is unlikely to provide substantial benefit when used later in the onset of disease. The decreased likelihood of dogs being presented for veterinary attention shortly after onset of signs, a lack of widespread availability of anti-cytokine drugs in veterinary medicine and prohibitive costs, makes this group of drugs an unappealing avenue to pursue in veterinary medicine.

1.4.9 Anti-inflammatory therapy

1.4.9.1 Non-steroidal anti-inflammatory drugs (NSAIDs)

Although NSAIDs have potent analgesic and anti-inflammatory effects (Bang et al., 2008), they cannot be recommended for use in critically ill dogs with acute pancreatitis. This is due to the dehydration that is generally present, and the potential effects on renal perfusion and gastric mucosal health that may result from their use (Bang et al., 2008). There have been some small studies in people that show small differences in outcome when using indomethacin or diclofenac, but only in prevention of ERCP-associated pancreatitis (Bang et al., 2008).

1.4.9.2 Glucocorticoids

It is ironic that glucocorticoids, previously feared as a potential cause of pancreatitis are beginning to be used and evaluated again for treatment of acute pancreatitis. Historical reluctance to use this drug in dogs, and to a lesser extent in people, probably resulted from the presumption that corticosteroids could lead to pancreatitis. The putative link between pancreatitis and glucocorticoids in dogs may be
attributed to early studies showing dexamethasone increased pancreatic enzyme concentrations, but had no effect on pancreatic tissue (Imahori et al., 1984; Parent, 1982; Strombeck et al., 1981). Whilst theoretically any drug can cause pancreatitis in any individual dog, corticosteroids are no longer considered to be high risk in people (Bang et al., 2008). Alongside this, glucocorticoids are the one group of drugs that are known to counteract virtually all pathways of inflammation.

Corticosteroids may have multiple benefits in acute pancreatitis because they inhibit release of pro-inflammatory mediators, decrease sequestration of neutrophils in the pulmonary vasculature, reduce adhesion of primed neutrophils to the endothelial surface of pulmonary vasculature, reduce release of elastase and free radicals from adherent neutrophils and reduce pulmonary vascular permeability (Sun et al., 2007; Williams, 1994). A specific role of corticosteroids in enhancing apoptosis, and increasing production of pancreatitis-associated protein (PAP), which confers a protective effect against inflammation has also been proposed (Heller et al., 1999; Iovanna et al., 1991; Ortiz et al., 1998; Zenilman et al., 1996). In addition to these effects, the concept of relative adrenal insufficiency as discussed below, also has potential applications to acute pancreatitis.

1.4.9.2.1 Relative adrenal insufficiency

It order to put into context the concept of relative adrenal insufficiency it is important to first know the interaction of the hypothalamic-pituitary-adrenal (HPA) axis in illness (Marik et al., 2008). Anti-diuretic hormone (ADH), also known as vasopressin, is a weak adrenocortical-stimulating hormone (ACTH) secretagogue and vasoactive peptide that acts synergistically with corticotrophic-releasing hormone (CRH) to increase secretion of ACTH (Marik et al., 2008). More than 90% of circulating cortisol is bound to corticosteroid-binding globulin (CRG), and during acute illness this reduces to as much as 50%, leading to a higher amount of circulating free
cortisol (Marik et al., 2008). Cortisol exerts systemic effects by binding to intracellular glucocorticoid receptors (GR). The cortisol-GR complex moves to the nucleus, where it binds to DNA sequences called glucocorticoid-responsive elements in the promoter regions of target genes, thus causing up or down regulation of genes involved in inflammation (Marik et al., 2008).

Cortisol may directly affect activity of NF-κB. Cortisol also increases blood glucose by activating hepatic gluconeogenesis and inhibiting peripheral glucose uptake by peripheral tissue; activates lipolysis to release free fatty acids into the circulation; and increases catecholamine and glucagon production (Marik et al., 2008).

Haemodynamic effects are mediated by an increase in sensitivity to vasopressors (ADH-1 and ADH-2 receptors) in vascular smooth muscle, resulting in increased blood pressure (Marik et al., 2008).

During acute illness the HPA axis is stimulated as shown in Figure 1.11, but in about 10-20% of critically ill people and 60% of people with septic shock, this pathway becomes impaired (Marik et al., 2008). Mechanisms that lead to this dysfunction are complex and poorly understood. Possible causes include decreased production of CRH or ACTH; dysfunction of peripheral glucocorticoid receptors; or dysfunction of the adrenal gland due to haemorrhage or thrombosis, all in the face of high circulating cortisol concentration (Marik et al., 2008).
Figure 1.11 Activation of the HPA axis in response to stress. TGF-β, transforming growth factor β; POMC, pro-opiomelanocortin; LIF, leukaemia inhibitory factor; CRH, corticotrophin-releasing hormone; ADH, anti-diuretic hormone; ACTH, adrenocorticotrophic hormone.

The latest recommendation from the American College of Critical Care Medicine is to use the term critical illness-related corticosteroid insufficiency (CIRCI) to describe the adrenal dysfunction that occurs during critical illness in people (Marik et al., 2008). Effectively, CIRCI occurs when there is an adrenal insufficiency along with tissue resistance to the effects of corticosteroids, due to a prolonged and severe proinflammatory state. In particular, it causes hypotension and a poor response to fluid or vasopressor therapy. It is in the sub-group of people with poor response to resuscitative measures (fluid and vasopressor therapy) and those with acute lung injury, where cortisone replacement appears to be the most effective (Marik et al., 2008).

Low-dose hydrocortisone is the current recommended treatment for those people with septic shock and CIRCI, whilst methylprednisolone is recommended for those with acute lung injury. Continuous rate infusion therapy may result in better glycaemic control, and is advocated in septic shock, and abrupt cessation is not
recommended (Marik et al., 2008). Dexamethasone administration is not recommended due to prolonged suppression of the HPA axis. These recommendations have not been extended to people with acute pancreatitis to date.

These recommendations are based on a number of papers where low doses of hydrocortisone appeared to improve survival and haemodynamic status in people with septic shock poorly responsive to vasopressor treatment (Bollaert et al., 1998). Another trial in people with documented CIRCI showed that 50 mg hydrocortisone (combined with fludrocortisone) resulted in greater survival and no increase in adverse effects (Annane et al., 2002). However, a recent double-blind, placebo controlled, randomized trial of 499 people showed that there was no difference in survival between those treated with placebo or those treated with hydrocortisone, irrespective of their adrenal function testing (Sprung et al., 2008). Those people that had reversal of their shock had a quicker response when given hydrocortisone than those given placebo. However, there was a greater rate of superinfections and new septic episodes with hydrocortisone treatment. They were administered a higher dose of hydrocortisone (100 mg) than in the other studies.

1.4.9.2.2 Cortisol metabolism in people with acute pancreatitis

The potential role of CIRCI in spontaneous pancreatitis in people has begun to be explored (Muller et al., 2006). One study assessing 109 people with acute pancreatitis showed initially high free and total cortisol, low ACTH and CBG. Over time however, the ACTH concentration increased, whilst the cortisol concentrations decreased. In people with necrotising pancreatitis the ACTH increase occurred at day 5, whilst in oedematous pancreatitis it occurred at day 3 and there was no overall difference between the two groups at day 6.

Another study of severely ill people with acute pancreatitis identified a relatively low circulating cortisol response to ACTH in non-survivors (Peng et al.,
As well as correlating to survival, CIRCI was also related to rates of pancreatic necrosis, bacteraemia and degree of organ dysfunction, especially cardiovascular dysfunction. However, another study found no difference in mortality or rate of necrosis between people with or without CIRCI (De Waele et al., 2007). The testing in the latter study was not as early as the study by Peng et al (2009). This timing discrepancy and therefore potential for treatments to influence results, the low numbers in the studies, the different doses of synthetic ACTH used, and the different causes of pancreatitis may account for the differences between the studies. Regardless, there is a moderate level of evidence that CIRCI may exist in people with severe acute pancreatitis.

1.4.9.2.3 Corticosteroid therapy in animal models of pancreatitis

There have been multiple animal experimental models assessing the effects of glucocorticoids in pancreatitis. In short, they have shown a reduction in the treated groups in pro-inflammatory cytokines, arachidonic acid metabolites, serum amylase concentration, necrosis, histological indices, PLA-2 concentration, incidence of leucopenia and mortality (Gloor et al., 2001; Kilic et al., 2010; Kimura et al., 1998; Muller et al., 2008; Osman et al., 1999; Yubero et al., 2009a; Yubero et al., 2009b; Zhang et al., 2008b; Zhang et al., 2010). These effects appeared to be greater in necrotizing forms of the disease than in milder, oedematous forms. Additionally, reduced expression of P-selectin protein which is a marker for white cell adhesion to endothelial cells has been shown in dexamethasone treated animals (Zhang et al., 2010). Similarly, ICAM-1 has reduced expression in rodents pre-treated with hydrocortisone before induction of pancreatitis (Sun et al., 2007).

In another study, *in vitro* and *in vivo* rodent models showed that dexamethasone up regulates the expression of pancreatitis-associated protein (PAP) (Kandil et al., 2006). PAP is thought to have multiple protective effects, including anti-bacterial, anti-
inflammatory and amplifying pancreatic response to injury (Heller et al., 1999; Iovanna et al., 1991; Ortiz et al., 1998; Zenilman et al., 1996). It has been demonstrated that PAP decreases expression of NF-κB in an *in vitro* line of epithelial cells (Folch Puy et al., 2006), and that altered gene expression of PAP exacerbates the severity of pancreatitis (Zhang et al., 2004).

Not all studies have shown such a benefit in mortality, even though there was decreased ascites production and increased apoptotic index (Zhang et al., 2007a; Zhang et al., 2007c). It may well be that the short duration of the study (12 hours) prevented a difference in survival being able to be determined. Interestingly, there was no relationship between the apoptotic index and any particular inflammatory mediator, suggesting there may be a direct as well as indirect effect on apoptosis by corticosteroids.

1.4.9.2.4 Extrapolation to clinical cases (people and dogs)

As has been proven with the other forms of potential treatment, a theoretical basis and experimental evidence does not always correlate to a clinical benefit. Currently there are a number of prospective trials being undertaken to evaluate the potential benefit of glucocorticoids in people with severe acute pancreatitis. A recent meta-analysis showed no benefit in giving corticosteroids as prophylaxis for ERCP induced pancreatitis (Zheng et al., 2008). This is perhaps not surprising considering that necrosis is not a common feature of this disease, and therefore the potential benefits are not applicable to this form.

Before any further work is done assessing this treatment modality in dogs, it must be very carefully planned and used strictly only once the diagnosis of pancreatitis has been made. This is because the use of hydrocortisone in people has been associated with masking of oesophageal or intestinal perforation (Böhrer et al., 1996). The
relevance to veterinary medicine is great, in that intestinal foreign body obstruction or septic peritonitis are major differential diagnoses for acute pancreatitis.

There is no documented evidence that CIRCI occurs in dogs with acute pancreatitis, although it has been documented in dogs with hypotension and sepsis (Burkitt et al., 2007). This requires further evaluation, along with a standardised protocol for administering the adrenal function tests in affected dogs prior to evaluating efficacy of cortisone treatment. There is evidence that standard treatments given in an ICU setting could influence the ACTH stimulation results in dogs, and this influence should be accounted for in future studies (Sweeney et al., 2010).

There may be a potential for the use of low-dose hydrocortisone infusion prophylactically during procedures where there is a high risk of pancreatitis developing, such as adrenal surgery (Mehler et al., 2004; Schwartz et al., 2008). Experimentally, administration of glucocorticoids early after interventional procedures (within 1 hour) showed the highest benefit (Marik et al., 2008). In addition to prophylactic usage, there may be a potential role for using low-dose hydrocortisone in dogs with severe acute pancreatitis that have poor systolic pressures with minimal response to fluid resuscitation. However, optimisation of the other aspects of management of canine pancreatitis should be completed before stringent analysis of corticosteroid therapy is undertaken.

1.4.10 Follow-up management

There is no defined management protocol following a bout of acute pancreatitis in dogs. Extrapolating from human gastroenterology, and applying common sense, it would be logical to treat any underlying conditions that may predispose to recurrent bouts of pancreatitis. Recurrent bouts of acute pancreatitis may lead to fibrosis, chronic pancreatitis and in some cases ultimately exocrine pancreatic insufficiency and diabetes mellitus (Tran et al., 2008; Watson, 2003; Watson et al., 2007; Wu and Conwell,
The exact factors or mechanisms by which this occurs are not fully elucidated in dogs.

Although in most instances the initiating cause of pancreatitis is not known, in a certain percentage of dogs there may be an underlying lipid disorder and therefore it may be prudent to check serum triglyceride and cholesterol 1-2 weeks after recovery from a bout of acute pancreatitis. (Hess et al., 1999; Williams and Steiner, 2005; Xenoulis et al., 2011). If drug administration is implicated in the aetiology, such as azathioprine or potassium bromide, then withdrawal of that drug should be undertaken.

There is no evidence that dogs with dietary indiscretion will be predisposed to recurrent bouts of acute pancreatitis (Lem et al., 2008). However, the current consensus is that in those dogs where dietary factors are considered likely triggers, that they are fed low-fat and low-protein diets for an undetermined duration (Williams and Steiner, 2005).

A proportion of people following acute pancreatitis will have exocrine insufficiency that is manifested as steatorrhoea and diarrhoea (Wu and Conwell, 2010b). One study has shown that the degree of exocrine insufficiency following a bout of acute pancreatitis in people is directly correlated with the amount of necrosis, as is the presence of pancreatic pseudocysts (Boreham and Ammori, 2003; Niederau et al., 1990). Sub-clinical exocrine pancreatic insufficiency has been diagnosed in dogs, and is not always manifested by overt steatorrhoea (Wiberg and Westermark, 2002). Based on this, in severely affected dogs, treatment with exocrine pancreatic enzymes for 3-4 weeks following a bout of acute pancreatitis may be beneficial in selected instances.

1.4.11 Nutrition

1.4.11.1 Hypotheses and historical background

Some of the nutritional challenges seen with acute pancreatitis in people are equally applicable to dogs. These challenges are namely that acute pancreatitis is a catabolic
disease with significant nitrogen losses strongly associated with mortality (Ioannidis et al., 2008); ileus often complicates feeding; the sub-group of patients with necrosis have greater nutritional requirements, but are more difficult to effectively treat than those without necrosis and acute pancreatitis is diabetogenic (Thomson, 2006).

In human and animal (pig, rodent, canine) experimental models it has been shown that fasting leads to intestinal mucosal atrophy (Hernandez et al., 1999; King and Kudsk, 1997), an increased rate of enterocyte apoptosis in the intestine (Fukuyama et al., 2001), changes in mucin composition of goblet and deep crypt cells (Sharma and Schumacher, 1995), and decreased glutamine and arginine transport (Sarac et al., 1994). Overall, these changes result in a breakdown of the intestinal barrier and increased intestinal permeability, potentially leading to bacterial translocation (Deitch et al., 1987; Flint and Windsor, 2003; King and Kudsk, 1997).

However, the initial model put forward by researchers of bacterial translocation (Wolochow et al., 1966) has been superseded as perhaps being too simplistic. The gastrointestinal tract itself is now thought to be a major contributor to the systemic inflammatory state during acute pancreatitis, particularly if it is not supplied topical nutrients. These theories of the gut starter or gut motor model in perpetuation of acute pancreatitis were discussed in section 1.2.2.9, and are depicted in Figure 1.12.
Initially in human (and veterinary) gastroenterology the idea was to ‘rest’ the pancreas and therefore provide no exocrine stimulation during bouts of acute pancreatitis. These recommendations were based on the assumption that ongoing pancreatic secretion could be harmful by perpetuating the inflammation (Stewart, 1994; Williams, 1994). Two experimental studies in dogs paved the way for complete intestinal rest to be recommended in dogs with acute pancreatitis (Konturek et al., 1972; Vidon et al., 1978). These showed that despite pancreatic secretion being less when nutrients were delivered to the jejunum than to the duodenum in both people and dogs, it still occurred to some extent. As such, the authors proposed that in order to prevent pancreatic secretion, complete intestinal rest would be the only way to achieve this.

There is also a differing rate and content of pancreatic secretion in response to different food types. This is exemplified in one experimental canine model that showed dispersed oleate is a more effective stimulus of pancreatic secretion than the non-dispersed (oil phase), and when the intestinal pH is ≥ 7 only fatty acids longer than 8 carbons stimulate pancreatic secretion (Meyer and Jones, 1974). Additionally, when
triglycerides are placed directly into the duodenal lumen there is no pancreatic stimulation, rather this occurs only when the products of lipolysis are present. The clinical relevance of this study is difficult to establish, but it emphasises that it may be the molecular structure of a diet that may be as important as the overall nutrient profile.

1.4.11.2 The shift from parenteral to enteral nutrition in acute pancreatitis

Although total parenteral nutrition (TPN) was used to treat acute pancreatitis for a considerable length of time, in the 1990s the role of the intestine in initiating or perpetuating systemic inflammation began to receive attention and enteral nutrition was preferentially used in people. However, feeding was delivered into the jejunum rather than into the duodenum. The presumption for this was that jejunal administration of nutrients stimulated exocrine pancreatic production of digestive enzymes to a lesser degree, as shown in healthy people (Kaushik et al., 2005; Ledeboer et al., 1998). It had previously been shown in dogs that intra-jejunal administration of neutral pH elemental nutrition did not lead to pancreatic secretion, and glucose into the jejunum actually had a negative feedback effect (Ragins et al., 1973).

There were also a number of studies that supported jejunal feeding compared to TPN by suggesting trypsin secretion is minimal to none in jejunal feeding, although trypsin may still be produced by the pancreas (Bodoky et al., 1991; O'Keefe et al., 2006; O'Keefe et al., 2005). As well, in 4 rodent experimental models inducing pancreatitis of variable severity (oedematous, necrotic, haemorrhagic) it was demonstrated that exocrine pancreatic function was actually decreased during experimental pancreatitis (Niederau et al., 1990).

Running in parallel to these studies, vigorous assessment of TPN was also beginning. A study by Alverdy et al (1988) looked at bacterial translocation in rats (Alverdy et al., 1988). Two thirds of the rats receiving TPN had culture-positive mesenteric lymph nodes, compared to 1/3 of the orally fed group and none of the
controls. This could have clinical ramifications for dogs with pancreatitis, as decreased gut permeability may worsen this bacterial translocation. TPN was also associated in other experimental studies with increased mortality, and showed no proven benefit in people with pancreatitis (King and Kudsk, 1997; Sax et al., 1987).

Studies of TPN compared to no nutritional intervention for acute pancreatitis support the notion that TPN before volume resuscitation worsens prognosis, but may actually improve it afterwards (He et al., 2004; McClave et al., 2006; Sahin et al., 2007). This effect was even more apparent when TPN was supplemented with glutamine, although the studies were under-powered (He et al., 2004; Ockenga et al., 2002). This is based on the fact that glutamine is the preferred respiratory fuel for rapidly growing cells like enterocytes and lymphocytes, so potentially has a role to play both in gut health and lymphocyte function (Furst et al., 1997). The pancreas is also considered to have the highest turnover of glutamine in the body (Furst et al., 1997). Meta-analysis shows that TPN has a higher relative risk compared to enteral nutrition (EN) for hyperglycaemia and insulin requirements in people with acute pancreatitis, worsening prognosis (Petrov and Zagainov, 2007). The significance of this phenomenon is based on work in critically ill people that has shown tight glycaemic control reduces the risk of mortality and infectious complications (Van den Berghe et al., 2001).

Pancreatitis is one of the conditions where TPN is recommended as the first line nutritional support in dogs (Chan and Freeman, 2006; Stewart, 1994; Williams, 1994). TPN solution is a combination of dextrose, an amino acid solution and lipid solution. There is no evidence that lipid content will be detrimental to dogs with pancreatitis, and the current recommendation is only to reduce the lipid content when hypertriglyceridaemia is present (Chan and Freeman, 2006; McClave et al., 2006).
Interestingly, in dogs both protein and fat are potent stimulants of pancreatic secretion, even when given intravenously (Konturek et al., 1979).

Despite the recommendations, TPN in dogs is poorly evaluated. It has been experimentally demonstrated in dogs that TPN reduces stomach, duodenal and gall bladder motility (Kaji et al., 2002). This could perpetuate ongoing vomiting due to ileus, and increase the risk of extra-hepatic bile duct obstruction due to diminished gall bladder contractility. There have been few reviews on the clinical efficacy, or not, of TPN in any specific condition in dogs. One study identified a mortality rate of nearly 50% in over 200 dogs that received TPN, along with a high rate of metabolic (70%), mechanical (25%) and septic (5%) complications (Reuter et al., 1998). Despite a similar rate of complications, a more recent paper reported that complications of parenteral nutrition had no relationship with outcome (Queau et al., 2011). This study assessed both peripheral and centrally administered parenteral nutrition and a risk factor for death was a prolonged period of anorexia prior to the initiation of parenteral nutrition. Due to the retrospective nature of the study, it was not possible to specifically evaluate dogs with pancreatitis.

1.4.11.3 Animal experimental models supporting enteral nutrition

A number of studies to assess enteral nutrition in experimental models have been performed. In one rodent model study, dietary protein depletion and endotoxin administration caused a greater degree of bacterial translocation than with normal dietary protein intake (Deitch et al., 1987). Further studies have also confirmed increased gut permeability is present in people with acute pancreatitis, and the degree of increased permeability correlates to the severity of disease in rat models (Ryan et al., 1993; Wang et al., 1996).

One rodent study showed that enteral feeding maintained immune responsiveness and reduced villous atrophy compared to TPN (Kotani et al., 1999).
Despite these positive findings, there was no difference in outcome (survival) or rate of pancreatic healing, although the model appeared to induce mild disease. In a series of studies by the same group of researchers inducing disease in dogs, enteral and parenteral nutrition was compared (Qin et al., 2002a; Qin et al., 2002b, 2003, 2007). Dogs receiving enteral nutrition did so via a jejunal feeding tube 24 hours after induction of disease. Dogs receiving TPN were given an iso-caloric and iso-nitrogenic diet via a jugular catheter. Dogs that were enterally fed had lower serum glucose concentrations, and decreased bacterial translocation and endotoxin concentration. There was no difference in severity of disease as assessed by blood markers and histological analysis at the end of the study at Day 7. It was not possible to compare survival or distant organ complications in this study.

1.4.11.4 Clinical evidence for enteral nutrition in people with acute pancreatitis

The theoretical (and experimental) benefit of enteral nutrition by maintaining host immune responsiveness and preservation of intestinal integrity does not ensure a tangible clinical benefit, and so further clinical studies were undertaken. Despite the difficulty in interpreting these studies due to low numbers of patients, some selection bias, and failure to standardise disease severity or other treatments, there is still a moderate amount of evidence that enteral feeding is preferable to TPN in people with severe acute pancreatitis. All of the studies mentioned in this section used naso-jejunal (NJ) delivery of nutrients to the intestine.

Perhaps the biggest failing of the majority of studies is their failure to compare enteral nutrition to fasting (or full pancreatic rest), instead comparing to TPN. Meta-analysis that supports the use of early enteral nutrition in severe pancreatitis does this in the face of knowledge that TPN is probably harmful (McClave et al., 2006; Meier et al., 2006; Petrov et al., 2008b; Petrov et al., 2008c). In this way the studies are only comparing a treatment of unknown efficacy to one with harmful side effects (Marik and
Zaloga, 2004). TPN, as previously discussed, has been shown to impair humoral and cell-mediated immunity, magnify the pro-inflammatory response, increase bacterial translocation and infection rate in critically ill patients (Marik and Pinsky, 2003).

In an early study, enteral feeding was well tolerated, had fewer complications overall and a smaller rate of septic complications compared to TPN (Kalfarentzos et al., 1997). There was no difference in hospital duration or outcome. Other studies comparing enteral feeding to TPN have been performed, and showed a global reduction of CRP concentration, APACHE score, development of SIRS, organ failure and length of stay in an ICU ward in the enteral group (Windsor et al., 1998). As the feeding regimen was continued for 7 days, it is possible that within the TPN group many patients may have voluntarily eaten, and the persistence of a central line may have actually increased the inflammatory response in and of itself. It was not clear from the published data if there was a difference in response between the severely and mildly affected patients. Similar findings were present in other studies, with a faster reduction in serum CRP concentration in fed patients, however only small numbers were included (Louie et al., 2005; Doley et al., 2009).

One of the better constructed studies compared enteral nutrition to TPN in patients with predicted severe pancreatitis (Petrov et al., 2006). This trial only included patients with onset of clinical signs within 72 hours prior to admission, and all had serum CRP concentrations > 150 mg/L and APACHE II scores > 10, with no differences between groups at the start of the project. There was no difference in the reduction of serum CRP or APACHE-II scoring over the first 4-7 days of the study. However, overall there were significantly lower rates of pancreatic infectious complications (7 vs. 16), multi-organ failure in week 2 (7 vs. 17) and mortality (2 vs. 12) in the enteral nutrition group compared to the TPN group. Interestingly there was no difference in early single-organ failure between the groups, suggesting that the
benefit may be delayed and correspond to nutritional effects as well as immune functions.

This was reflected in another non-randomised, prospective study that demonstrated single or multi-organ failure occurred in 79% of the TPN group (vs. 31% in the enterally fed group), infected pancreatic necrosis in 74% (vs. 20%) and death in 35% (vs. 5%) (Modena et al., 2006). As this study was not randomised it is unclear to what degree selection bias played a role, by selecting more severely affected people to receive TPN. Indeed, a randomised clinical trial failed to show any difference in inflammatory markers, oxidative stress or endotoxin antibodies in people receiving enteral feeding versus TPN (Gupta et al., 2003). This particular study assessed mild disease only.

The major problems when assessing the above-mentioned studies of acute pancreatitis is that they are typically under-powered, many are not randomised, the remainder of the treatment protocol is not consistent (therefore questioning the effect of the nutrition alone), and it is difficult to determine which patients are truly severe based on the description in the papers.

1.4.11.5 Is enteral feeding better than resting the pancreas?

There are very few human studies comparing enteral nutrition to complete intestinal rest, all of which contain critical design flaws. The conclusions of one study (Pupelis et al., 2001) was that enteral feeding via a naso-jejunal (NJ) tube decreased mortality and the rate of septic complications compared to IV fluid therapy only, with no difference in hospitalisation duration or the rate of non-septic complications. The flaws of the study are so marked however, that these conclusions cannot be strongly supported. All 60 patients recruited for the study had surgery for their disease, and secondary peritonitis cases as well as pancreatitis cases were included in each treatment group but not analysed individually. There was the potential for significant selection
bias, and the patients in the NJ group received different dietary formulations at different rates. Additionally, the NJ tubes were inserted intra-operatively, and extrapolation of this to non-surgical patients is dangerous, as insertion of a NJ tube otherwise requires an additional anaesthetic procedure. The author of a meta-analysis (McClave et al., 2006) contacted the principal author of this study and obtained data that excluded secondary pancreatitis, and included subjects from an earlier study of 29 people by the same group (Pupelis et al., 2000). Assessing this data together there were overall 71 (out of the original 89) patients that could be assessed. Analysis showed a trend to decreased mortality but no other difference.

There is only one study that was well constructed comparing enteral feeding to fasting published in the human literature, but this was limited by the small number of patients in the study (Powell et al., 2000). This study prospectively administered 21% of total caloric requirements via NJ tube to 13 people in the first four days of hospitalisation, compared to 14 people that were given bowel rest. The authors showed that intestinal permeability actually increased in the feeding group, which may be due to increased mucosal blood supply rather than functional changes in the gut. There was no difference in inflammatory markers between the two groups over 4 days, but it failed to assess differences in length of stay, outcome, infective and late complications between the two groups. This reiterates that there is a lack of prospective well constructed studies comparing standard treatment to enteral feeding in the medical literature. There are none in the veterinary literature.

1.4.11.6 Is feeding proximal to the jejunum well tolerated?

Although enteral nutrition in acute pancreatitis has gained popularity over the past decade, feeding was preferentially delivered into the jejunum rather than proximally into the stomach or duodenum. The cost of feeding via a nasogastric (NG)
tube or by mouth is less than via an NJ tube, as well as being easier to insert. This is
ture in both human and veterinary medicine.

One study tried to assess whether there was pain when people were fed orally
compared to via an NJ tube (Pandey et al., 2004). This looked at consecutive
admissions of people with acute pancreatitis, so the severity of disease was not
uniform. However, there was no significant difference in recurrence of pain between
the two groups, although numbers were low.

One of the first studies assessing NG feeding was published in 2005 (Eatock et
al., 2005). This was a randomised controlled trial of 49 people with severe pancreatitis
comparing NG to NJ feeding. There was no difference in mortality hospitalisation
duration, severity scores or CRP reduction between groups. There was also no
difference in pain scores between groups, and NG feeding was very well tolerated.

Another study in 2006 supported the idea that NG feeding was as well tolerated
as NJ feeding, and there was no increase in pain upon feeding (Kumar et al., 2006).
There was no difference in survival or duration of hospitalisation between the two
groups, but there were potential flaws with the study. Conversely, a study that
compared EN via an NG tube to TPN within 24 hours of admission suggested that early
NG feeding may actually be detrimental as it might increase mucosal blood supply, and
lead to increased permeability (Eckerwell et al., 2006). This conclusion is difficult to
fully support as there were low numbers, and outcome was not fully evaluated. A meta-
analysis of NG tube feeding concluded that it did not result in different clinical
outcomes from NJ feeding, and was well tolerated in 80% of people (Petrov et al.,
2008a).

1.4.11.7 Consensus statements and meta-analysis of EN in acute pancreatitis

The most current consensus statement recommends that although in mild
pancreatitis there is no impact on disease with supplemental nutrition until after the first
5 days, there is indication to provide assisted (tube) feeding in people with severe pancreatitis. They also concluded that additional TPN may be indicated if nutritional requirements are not met, and that naso-gastric feeding should be attempted first, with naso-jejunal feeding started only if it is not tolerated (Meier et al., 2006). Other consensus statements are along similar lines, albeit not always with robust support (Hirota et al., 2010; Johnson, 2005; Muddana et al., 2009).

Multiple meta-analyses have also been performed. Meta-analysis is highly dependent on the number of studies included, as well as the design of those studies, so cannot always be given absolute credence. An example is one review which was primarily based on Powell’s study in 2000, and stated that EN decreased mortality when initiated within 48 but not 24 hours of admission (Petrov et al., 2008c). The original study cited did not actually compare or even detail survival characteristics, and was limited to 25 patients, making the conclusions of the meta-analysis questionable (Powell et al., 2000). It has been suggested that although there is no difference in mortality between EN and TPN, the lower cost and infectious complication rate associated with the former lends itself to be recommended at Level A in evidence-based medicine (Heinrich et al., 2006).

1.4.11.8 What to feed?

Even if the notion that enteral nutrition is well tolerated and perhaps beneficial is supported, it is still unclear as to what diet to feed. The preponderance of poorly controlled reports in the medical literature make this a confusing area as well. One study assessed the administration of n-3 polyunsaturated fats at 3.3 grams per day in people with moderate to severe pancreatitis via a NJ tube (Lasztity et al., 2005). This was not a blinded or placebo-controlled study. There was no difference between groups in total antioxidant status or CRP concentrations, but there was a significantly decreased feeding duration and hospitalisation in the treated group. Enteral feeding
supplemented with glutamine, arginine, tributyrin and n-3 fatty acids made no difference in CRP concentration in another study (Pearce et al., 2006). Clinical outcome wasn’t assessed however, and it is possible that arginine was detrimental as it has been shown to damage the pancreatic acini in a rat model (Lasztity et al., 2005).

The use of probiotics is also controversial. One double-blinded, randomised prospective clinical trial initially suggested a benefit, as there was a significant reduction in infection of pancreatic tissue and the need for surgical intervention (Olah et al., 2002). However, overall length of stay, outcome, incidence of systemic inflammatory response syndrome and multi-organ failure were not different between the groups. The same group of researchers found no benefit when a larger cohort was assessed (Olah et al., 2007). A later and larger randomised, double-blind, placebo-controlled study from another group also refuted the original findings (Besselink et al., 2008). In fact, the mortality rate was significantly greater in the probiotic group compared to placebo. The biggest cause of death was MODS, although 3 people died of bowel ischaemia. Potential reasons proposed for this include perpetuation of bowel ischaemia by increasing requirement for blood mucosal supply, or increasing gut-associated inflammation due to increased bacterial load (Besselink et al., 2008). At this point in time there is no support for the use of probiotics in critically ill people with pancreatitis, nor a valid set of evidence for their use in dogs.

1.4.11.9 What about enteral nutrition in dogs with pancreatitis?

There have been very few studies of nutritional intervention in dogs with pancreatitis. Most of these detail parenteral nutrition and the incidence of complications, without specifically assessing pancreatitis (Chan and Freeman, 2006; Chan et al., 2002; Queau et al., 2011; Reuter et al., 1998). Generally the rate of complications with parenteral nutrition is similar in all reports, although there was a
tendency for a better outcome in dogs treated concurrently with enteral nutrition (Chan et al., 2002).

In the only randomised, prospective study of enteral nutrition in dogs, there was a more rapid clinical improvement in dogs with parvovirus that were fed compared to those that were fasted (Mohr et al., 2003). There was no significant difference in survival, but the EN dogs gained more weight, and potentially showed improved gut barrier function.

In veterinary medicine, when enteral nutrition has been recommended as potentially beneficial in dogs with acute pancreatitis, it is usually advocated that NJ tubes be used (Watson, 2004; Williams and Steiner, 2005). Insertion of feeding tubes into the jejunum requires a prolonged general anaesthetic in dogs already critically ill (Swann et al., 1997). As well, the catheter lumen is often very small, which prevents the administration of many veterinary diets. A technique to insert a feeding tube via a percutaneous endoscopic technique has been described (Jergens et al., 2007). This technique is technically challenging and requires specialised equipment. In addition 5 of the 12 dogs reported in the initial study had complications with bolus feeding. A less invasive endoscopic assisted naso-jejunal insertion technique has also been described (Papa et al., 2009). This has not been evaluated in a sick population of dogs. Despite not requiring invasive surgery, the length of time required along with the expertise and equipment (endoscopy and ideally fluoroscopy), make these unlikely techniques to be used routinely in veterinary practice.

1.5 Conclusions from the literature

Despite a large number of publications and improved understanding of the cellular events that occur in pancreatitis, there has been a singular lack of effective translation to clinical medicine, let alone clinical veterinary medicine. There is a lack of
robust evidence for the current diagnostic modalities in veterinary medicine. Much of this results from a rarely attainable gold standard for diagnosis, and a lack of studies assessing the clinical utility of the test modalities. In human gastroenterology, understanding of the pathophysiology of the disease has not significantly altered the mortality rate of acute pancreatitis. Many novel treatment options did not result in clinical benefit, perhaps due to the disease in people differing from experimental models, or the disease process being established for longer than in experimental models. There are similar challenges facing veterinary medicine, with expense and availability of certain treatments contributing to this challenge as well. Many difficulties with clinical trials were also highlighted in this literature review. Namely, when trying to assess one intervention, the study subjects have to have the same form and general level of severity of disease, receive the exact same treatment apart from the intervention, and be randomly assigned to treatment groups.
1.6 Hypotheses

In the course of the background analysis, the following hypotheses were formulated:

Pancreatic Elastase-1 will be a specific and sensitive indicator of pancreatic inflammation

Pancreatic specific lipase concentration is correlated to the severity of pancreatic inflammation, but may be increased in other clinical conditions

Analysis of data will identify clinical data that could help to stratify severity of disease in dogs with acute pancreatitis

Plasma administration is not effective in the treatment of canine acute pancreatitis

Dogs with severe acute pancreatitis will tolerate early enteral nutrition
1.7 Aims and objectives

The specific aims and objectives of this thesis are:

- To determine the sensitivity and specificity of canine pancreatic elastase-1 in a group of dogs presenting with similar clinical signs to acute pancreatitis.

- To determine the clinical specificity and sensitivity of canine pancreatic lipase by correlating to level of pancreatic inflammation.

- Develop a clinical severity index in dogs with acute pancreatitis that correlates to outcome.

- To determine (stratifying according to clinical severity) if the administration of plasma or minimal enteral nutrition has any potential benefits in the treatment of acute pancreatitis in dogs.

- To determine if differing fat content of diet causes differing responses of the canine pancreas.

- To determine if early interventional enteral nutrition delivered proximal to the pylorus is well tolerated by dogs with acute pancreatitis.
Chapter 2: Specificity and sensitivity of serum canine pancreatic elastase-1 concentration in the diagnosis of pancreatitis

The following is a modified version of the published paper: Caroline S Mansfield, Penny D Watson, Boyd R Jones (2011) Journal of Veterinary Diagnostic Investigation 23(4):691-697

2.1 Introduction

Pancreatitis in dogs provides a diagnostic challenge for veterinarians. The difficulty in diagnosis is due to the lack of reliable laboratory testing for all types of pancreatitis. Acute pancreatitis is defined as inflammation and/or necrosis in the pancreatic or peri-pancreatic tissues, with an absence of permanent histological changes such as fibrosis and acinar atrophy (Xenoulis et al., 2008). Histological findings alone cannot determine the severity of acute pancreatitis, therefore clinical criteria are also used (Mansfield et al., 2008). Chronic pancreatitis is defined as a continuing inflammatory disease of the pancreas, with the presence of irreversible fibrosis and atrophy (Watson et al., 2007). When there is concurrent necrosis along with evidence of irreversible histological change, then this form of the disease can be termed recurrent acute or ‘acute-on-chronic’ pancreatitis (Watson et al., 2007). The term recurrent pancreatitis is preferred by the author and used throughout this chapter. It is possible that recurrent pancreatitis is indeed common in dogs, and this may compound the difficulties in diagnosis due to a lack of functional pancreatic mass able to produce circulating enzymes during times of inflammation.

Serum amylase, lipase and trypsin-like immunoreactivity have been shown to have poor sensitivities and specificities even in severe pancreatitis in dogs (Hess et al., 1998; Mansfield and Jones, 2000). Canine pancreas-specific lipase (previously measured as canine pancreatic lipase immunoreactivity) was initially thought to be highly sensitive as published in an abstract (Steiner et al., 2001). A more recent study has shown a sensitivity of 63.6% for this test in the diagnosis of pancreatitis, although,
when only severe cases of pancreatitis were included, this figure increased considerably (Steiner et al., 2008a). Canine pancreas-specific lipase on the other hand, has been shown to be specific (Trivedi et al., 2011).

Pancreatic Elastase-1 (PE-1) is synthesised in pancreatic acinar cells, and during active pancreatic inflammation it is released into the blood stream at the same time or immediately after trypsin (Hartwig et al., 2007). PE-1 contributes to the ongoing inflammatory state during acute pancreatitis by enhancing neutrophil-mediated tissue injury, and has been shown experimentally to induce pancreatic microcirculatory failure in the presence of sera co-factors (Keck et al., 2005). There have been inconclusive assessments about the clinical utility of serum PE-1 in people for diagnosing acute pancreatitis (Buchler et al., 1986; Malfertheiner et al., 1987; Wilson et al., 2005). In chronic pancreatitis, the exocrine function of the pancreas may become diminished, and in people with chronic pancreatitis serum and faecal PE-1 is often reduced, reflecting sub-clinical exocrine pancreatic insufficiency (Lesi et al., 1988). PE-1 has been measured in the serum of dogs undergoing endoscopic retrograde pancreatography (Spillmann et al., 2004), in healthy dogs, dogs with renal failure, and dogs with pancreatitis (Spillman et al., 2002). In the latter study, there was a significant difference in the median values of dogs with severe pancreatitis compared to healthy dogs or dogs with renal failure. Measurement of serum cPE1 is not affected by lipaemia according to the assay manufacturers, and is less affected by impaired renal function than most other pancreatic enzymes in people (Malfertheiner et al., 1987; Seno et al., 1995).

This part of the project was designed as a prospective study to assess the clinical utility of serum serum cPE1 in the diagnosis and differentiation of pancreatic disease in dogs.
2.2 Materials and Methods

Dogs presenting to three university veterinary referral centres (Murdoch University Veterinary Teaching Hospital, Murdoch, Western Australia; University College, Dublin, Ireland; Queen’s Mother Hospital, University of Cambridge, United Kingdom) with clinical signs that could be attributed to pancreatic inflammation, including both vomiting and abdominal pain, were recruited into the study. All centres operate under NHMRC equivalent Animal Use codes, and the study was approved by the relevant institutional ethics committee.

2.2.1 Animal Selection

Dogs were designated to have primary pancreatic disease on the basis of abdominal ultrasound and/or histological findings. They were further sub-divided into the following groupings:

- **Severe acute pancreatitis**: Dogs had marked pancreatic and peri-pancreatic fat necrosis or neutrophilic inflammation present histologically (obtained at exploratory laparotomy or post-mortem), or marked peri-pancreatic hyperechogenicity combined with abnormal pancreatic echogenicity as determined by ultrasonography, (Hess et al., 1998; Lamb and Simpson, 1995; Steiner, 2003) in addition to either a severe clinical severity index ≥ 2 as detailed in chapter 4 (Mansfield et al., 2008), or the need for intensive management.

- **Mild acute pancreatitis**: Dogs had pancreatic necrosis on histological evaluation (obtained at exploratory laparotomy or post-mortem), but did not satisfy the clinical or ultrasonographical criteria for having severe disease.

- **Recurrent acute pancreatitis**: Dogs had histological evidence of both acute (necrosis) and chronic (fibrosis or atrophy) pancreatitis; and blood and
pancreatic samples were taken during a time of active clinical signs. Clinical severity was not determined for dogs in this group.

- **Chronic pancreatitis**: Histological evaluation of the pancreas obtained at post-mortem or exploratory laparotomy showed evidence of chronicity (fibrosis and atrophy), with no active inflammation or necrosis.

- **Pancreatic neoplasia**: Primary pancreatic carcinoma was diagnosed via histological evaluation of biopsies obtained at exploratory laparotomy or post-mortem, with no concurrent inflammation. All histological analysis was performed by board-certified veterinary pathologists, and all ultrasound examinations by board-certified veterinary radiologists in the respective centres in a prospective manner. Dogs that were classified as having non-pancreatic disease had a confirmed primary diagnosis with no to minimal pancreatic changes observed histologically, in multiple sections (minimum 4) of the pancreas. Additionally, dogs did not have exogenous glucocorticoids within three days of admission. One dog that underwent pancreatic surgery for excision of an insulinoma had serial blood samples taken prior to surgery and 24, 48 and 72 hours after surgery.

### 2.2.2 Assays

Blood was taken from all dogs recruited into the study within 12 hours of admission to the university referral centre. Separated serum was stored at -20°C, then shipped frozen to Giessen, Germany for analysis by operators blinded to the disease classification of the samples. A species-specific sandwich enzyme immunoassay based on two monoclonal antibodies highly specific for canine PE-1 (cPE-1) was performed. This assay has been shown to have an intra-assay co-efficient of variation (CV) ranging from 2.1 to 11.6% and an inter-assay CV ranging from 3.4 to 10.8% in dogs (Spillman et al., 2004). An earlier abstract of 16 healthy dogs had a minimum value of 32.1 ng/mL, and a maximum value of 659.3 ng/mL, with a median concentration of 55.8
ng/mL (Spillman et al., 2002). Unfortunately the mean values were not reported in that study.

2.2.3 Statistical analysis

Statistical software package GraphPad® Prism 5 was used for calculations. The sensitivity and specificity of serum cPE-1 was calculated using Receiver-Operating Characteristic (ROC) curves to determine the optimal cut-off value for diagnosis, and 95% confidence intervals, positive, and negative likelihood ratios were also determined. Statistical difference between groups was determined using Mann Whitney U-test at confidence interval of 95%, with significance being a P value < 0.05.

2.3 Results

2.3.1 Animal Information

A total of 107 dogs were initially recruited into the study, but there was only sufficient information obtained for 61 dogs to be included. Forty-nine had pancreatic disease (severe acute pancreatitis n=23, mild acute pancreatitis n=11, recurrent acute pancreatitis n=7, chronic pancreatitis n=3 and pancreatic carcinoma n=5). All dogs with acute pancreatitis had mean follow-up of 9 months (range 6-36 months). A further 12 dogs with non-pancreatic inflammatory disease were evaluated: 4 dogs with inflammatory bowel disease, 2 dogs with small intestinal foreign bodies, 2 dogs with renal failure, and 1 each of immune-mediated thrombocytopenia, gastric carcinoma, multi-centric lymphoma, and insulinoma.

Eight of the samples were haemolysed on analysis (2 each from severe acute pancreatitis, mild acute pancreatitis, recurrent acute pancreatitis and non-pancreatic disease).
2.3.2 Median and intervals

The median, mean and intervals for serum cPE-1 in all groups are presented in Table 2.1, and graphically in Figure 2.1. Overall, the median for all dogs with pancreatic disease was 19.3 ng/mL, and the mean 47.46 ng/mL. For dogs with acute (mild, severe, and recurrent acute) pancreatitis, the median was 20.85 ng/mL, and the mean 55.04 ng/mL. Dogs with non-pancreatic disease had a median of 5.81 ng/mL and a mean of 8.19 ng/mL. There was a significant difference in serum cPE-1 concentrations between all pancreatic disease groups and the non-pancreatic disease group (P= 0.005) as well between the acute pancreatitis group (including mild, severe and acute-on-chronic) versus the non-pancreatic disease group (P= 0.002), but not between the acute pancreatitis groups versus the pancreatic neoplasia group (P=0.08). The difference between severe and mild acute pancreatitis was also significant (P= 0.04), and there was a strongly significant difference between severe acute pancreatitis when compared to non-pancreatic disease (P=0.002).

There was no change in significance when haemolysed samples were excluded from the analysis.
Table 2.1

Minimum, maximum, median and mean concentrations of serum cPE1 in the nominated disease categories

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Canine Pancreatic Elastase-1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic Group</td>
<td>Number of dogs</td>
</tr>
<tr>
<td>Severe acute pancreatitis</td>
<td>23</td>
</tr>
<tr>
<td>Mild acute pancreatitis</td>
<td>11</td>
</tr>
<tr>
<td>Acute-on-chronic pancreatitis</td>
<td>7</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>3</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Non-pancreatic disease</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure 2.1 Serum Canine Pancreatic Elastase-1 concentrations for all disease groups. There is a significant difference between the means of all groups except when comparing all dogs with acute pancreatitis (mild, severe and acute-on-chronic) to dogs with pancreatic carcinoma.
Table 2.2

<table>
<thead>
<tr>
<th></th>
<th>Severe Acute Pancreatitis vs. NPD</th>
<th>All Pancreatic inflammation vs. NPD</th>
<th>Acute Panreatitis vs. NPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPE-1 cut-off &gt; 17.24 ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>78.26%</td>
<td>61.36%</td>
<td>65.85%</td>
</tr>
<tr>
<td></td>
<td>58.1-93.04</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>Specificity</td>
<td>91.67%</td>
<td>91.67%</td>
<td>91.67%</td>
</tr>
<tr>
<td></td>
<td>64.61-98.51</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>PLR</td>
<td>9.391</td>
<td>7.364</td>
<td>7.92</td>
</tr>
<tr>
<td></td>
<td>1.42-62.09</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>NLR</td>
<td>0.237</td>
<td>0.421</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td>0.11-0.53</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>CPE-1 cut-off &gt; 5.5 ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>82.61%</td>
<td>81.82%</td>
<td>85.37%</td>
</tr>
<tr>
<td></td>
<td>62.86-93.02</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>Specificity</td>
<td>50%</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>25.38-74.62</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>PLR</td>
<td>1.652</td>
<td>1.636</td>
<td>1.707</td>
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<tr>
<td></td>
<td>0.91-3.0</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
<tr>
<td>NLR</td>
<td>0.348</td>
<td>0.364</td>
<td>0.293</td>
</tr>
<tr>
<td></td>
<td>0.12-1.0</td>
<td>95% CI</td>
<td>95% CI</td>
</tr>
</tbody>
</table>

The sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR), with 95% confidence intervals (CI), were calculated for diagnosis of severe acute pancreatitis (as compared to non-pancreatic disease [NPD], diagnosis of all pancreatitis (severe acute, mild acute, recurrent acute and chronic), and diagnosis of dogs with all forms of acute pancreatitis (severe, mild and recurrent acute). The calculations were done using two cut-off values; 17.24 ng/mL, as calculated by ROC analysis and 5.5 ng/mL, the median determined in healthy dogs in another study (Spillmann et al., 2004).
2.3.3 Sensitivity and specificity cPE-1

Using ROC analysis, an optimal diagnostic cut-off value of 17.24 ng/mL for serum cPE-1 was calculated, when comparing all groups (Figure 2.2). The 95% confidence intervals (CI) for the sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were calculated with this cut-off value of 17.24 ng/mL, as well as with the previously reported median concentration of 5.5 ng/mL for healthy dogs (Spillman et al., 2004). This is presented in Table 2.2. There was a high specificity for the diagnosis of severe acute pancreatitis (91.7%) using a cut-off value of 17.24 ng/mL, resulting in a high positive likelihood ratio, but a lower sensitivity (78.3%) with wide confidence intervals. The sensitivity also decreased when assessing all types of pancreatic disease (61.4%), and all cases of acute pancreatitis (65.9%), but the specificity remained the same. When the value 5.5 ng/mL was used, the specificity greatly reduced, (50%) with an increased sensitivity for severe acute pancreatitis (82.61%), all types of acute pancreatitis (85.37%) and all pancreatic disease (81.62%). There was overall a low negative likelihood ratio, which was lower when assessing acute pancreatitis.

The dog that underwent pancreatic surgery showed an increase in serum elastase concentration after the day of the surgery, but the measured elastase value had reduced to less than baseline by Day 3 (Figure 2.3).
Figure 2.2 An area under the curve of 0.8261 was determined using ROC analysis comparing severe acute pancreatitis to non-pancreatic disease in measurement of serum PE-1, with a calculated optimal cut-off value of 17.24 ng/mL.

Figure 2.3 Changes in serum PE-1 in a dog that underwent partial pancreatectomy

2.4 Discussion

Serum cPE1 had less than optimal sensitivities when assessing all dogs with pancreatic disease (61.4%) in this study. However, when only dogs with severe acute pancreatitis were considered, the sensitivity increased to 78.3%, and there was a high positive likelihood ratio. Of significant interest was the finding of a specificity of 91.7%.
This combined with a high positive likelihood ratio and low negative likelihood ratio would suggest that a high cPE1 is likely to be due to pancreatitis. This benefit was greatest when using a cut-off value of 17.24 ng/mL, and when assessing severe acute pancreatitis.

Our study did not assess healthy dogs, as the aim was to determine the clinical utility by comparing groups of dogs that could be considered to have acute pancreatitis on presentation. As such, the cut-off value determined in this study by comparing dogs with similar clinical presentations is more clinically relevant than comparison to healthy dogs. Additionally, the authors did not compare this test modality to clinical suspicion (based on clinical signs at presentation), as 46 dogs were excluded due to an unknown final diagnosis.

Serum cPE-1 appears to be less sensitive than current modalities such as abdominal ultrasound for the overall diagnosis of pancreatic disease in dogs (61.4% versus 66%). This finding is contrary to initial studies in people where good correlation with disease was demonstrated, although overlap between groups was observed (Buchler et al., 1986; Lesi et al., 1988; Malfertheiner et al., 1987). Later studies did not show such a convincing correlation (Millson et al., 1998; Wereszczynska-Siemiatkowska et al., 2003; Wilson et al., 2005). The major reason for this discrepancy is that the earlier studies used a radioimmunoassay (RIA) which detects polyclonal elastase (bound to 1-α-anti-trypsin complex), with a half-life of 2.2 days. As a result the RIA detects greater amounts for a longer period. The ELISA, as used in this study, is a monoclonal assay that detects free or unbound elastase with a half-life of 0.4 days (Millson et al., 1998), thus decreasing the sensitivity of the test after a short period of time has elapsed since onset of clinical signs. Conversely, this makes a positive result more likely to be a true positive if there is persistently an increased value.
Most human studies suggest that serum cPE-1 is most clinically useful for diagnosis of severe acute pancreatitis. This is also supported by our findings, as well as previous reports in dogs. One abstract from 2002 describes a reference interval of elastase in 16 healthy dogs of 32.1-659.3 ng/mL (median 55.8); 14 dogs with pancreatitis had a range of 24-1720 (median 160) and 6 dogs with renal disease had a range of 5-182 (median 43.3) (Spillman et al., 2002). When the dogs with pancreatitis were classified into severe (n=9) or mild (n=6) forms based on histological and clinical features, there was a significant difference in serum cPE-1 between healthy dogs and dogs with renal failure compared to severe pancreatitis.

It is uncertain why so many of the healthy dogs in that study had such high serum cPE-1 results compared to those measured in our study. However, a later publication from the same author also reported a wide-ranging baseline cPE-1 concentration in 7 healthy dogs (0.1-411.6 ng/mL) but with a median value of 5.5 ng/mL, similar to dogs with non-pancreatic disease in our study (Spillman et al., 2004). This initially wide reference interval raises the question of whether sample handling or haemolysis may have contributed to the variability of measured values. The manufacturers of the assay report that haemolysis or storage above 8°C for more than 72 hours can artefactually increase serum cPE-1 measurements. Haemolysis was recorded in only 8 of the 61 samples measured in our study. The results of these 8 samples were consistent with their diagnosis, and statistical analysis was unaffected by their inclusion or exclusion, so to mimic conditions expected in clinical practice the results were retained. In this light, more weight is lent to the premise that serum cPE-1 is highly specific.

It is also possible that the high results observed in the study of healthy animals may be outliers, have sub-clinical pancreatic disease or that serum cPE-1 excretion may decrease with age, as non-renal excretory mechanisms may diminish (Seno et al., 1995).
Laboratory error is another potential reason for the outliers. It is not possible from the available data of that study to further evaluate the most likely of these reasons.

Each of the three dogs in this study that had severe renal failure and no pancreatic inflammation (urea 70.4 mmol/L, Reference < 12; creatinine 804 µmol/L, Reference < 120, urine specific gravity <1.030) had serum cPE1 below the calculated value. There is a strong suggestion that serum elastase is not affected by renal clearance, as compared to many other pancreatic enzymes. In one study of 24 healthy people and 47 people with various degrees of renal insufficiency (but no known pancreatic disease) the measurements of elastase, lipase, amylase, pancreas-specific amylase, PLA-2 and trypsin were compared (Seno et al., 1995). Elastase was less affected than the other enzymes, only increasing to a significant amount in the serum when the creatinine clearance reduced below 10 mL/minute. All other enzymes were increased when creatinine clearance fell below 40 mL/min, although lipase tended to be less so.

An interesting finding in our study was that serum cPE1 was generally lower in the chronic pancreatitis group than the other pancreatic disease groups, as well as being below the reference interval in many dogs with acute pancreatitis. This would suggest that these dogs may have reduced functional exocrine pancreatic mass.

No information is available regarding the time interval between onset of clinical signs and the blood sample collection for the patients included in this study, although all samples were collected within 12 hours of admission to the veterinary referral centres. It was therefore unable to be determined if there was a relationship between the concentration of serum PE-1 and time after onset of clinical signs. However, in the dog that underwent pancreatic surgery there was a rapid decline in PE-1, confirming the short half-life for ELISA-measured PE-1. In general, dogs with severe pancreatitis will likely be presented much sooner after the onset of clinical signs than those with chronic disease. It
may well be possible that cPE-1 is more useful when measured in the first 24-36 hours after the onset of clinical signs, but this strategy may not be a practical suggestion for clinical veterinary practice.

No attempt was made to characterise the clinical severity of the recurrent acute pancreatitis group, as this was a histologically-based classification. It is possible that the sensitivity of serum cPE-1 for the diagnosis of severe disease could be altered if animals in this group were re-classified. The fact that all of the dogs in the non-pancreatic disease group had pancreatic histopathology performed increases the clinical significance of findings in this study. Although not all dogs in the severe acute pancreatitis group had histopathology performed the combination of clinical and ultrasonographical changes were strongly supportive of a diagnosis of pancreatitis, although the presence of necrosis could not be confirmed. It is possible that some of these dogs had ‘acute-on-chronic’ disease, and if they were excluded from the analysis then the sensitivity of the assay may be even higher. However, in veterinary practice pancreatic biopsies are seldom performed in dogs that have severe clinical signs of disease and concurrent abnormalities on abdominal imaging.

In the human literature it has been proposed that increased serum cPE-1 may be specific for pancreatic carcinoma (Ito et al., 1991), but this specificity is not supported in other studies (Buchler et al., 1986). Similarly, in our study, none of the dogs with pancreatic carcinoma had serum cPE-1 concentrations greater than the calculated cut-off value of 17.24 ng/mL, which may be related to the short serum half-life, reduced functional pancreatic mass or the lack of concurrent pancreatic inflammation. Conversely, all dogs in the severe acute pancreatitis group had follow-up of sufficient duration (> 9 months) to rule out the presence of concurrent pancreatic neoplasia.
Abdominal imaging is often relied upon for confirmation of the diagnosis of acute pancreatitis. This modality has a reported sensitivity of 66 to 68% (Hess et al., 1998; Mansfield and Jones, 2000; Steiner et al., 2008a). Although the sensitivity of ultrasound is reported as being low, these studies are not recent, and are assessing animals with chronic and mild disease as well as severe disease, decreasing the sensitivity of ultrasound as a result (Lamb and Simpson, 1995). Additionally, the specificity of ultrasound is actually quite high, meaning it is unlikely that any false positives were included in our patient group with severe disease. In a recently published paper, all dogs with severe pancreatitis had a positive ultrasound diagnosis (Steiner et al., 2008a). The presence of hyperechogenicity surrounding the pancreas on ultrasound reflects peri-pancreatic fat necrosis in dogs, and necrosis is considered the most significant indicator of severe disease in people (Charbonney and Nathens, 2008; Jaeger et al., 2003).

Serum cPE1 and cPLI appear to have similar sensitivities in the diagnosis of severe acute pancreatitis (Steiner et al., 2008a). The use of blood tests alone to diagnose pancreatitis should not be relied upon in clinical practice, and combination with abdominal ultrasound may well prove to have the highest sensitivity and specificity for diagnosis of severe acute pancreatitis in dogs. Additionally, abdominal imaging is very important to rule out non pancreatic causes of ‘acute abdomen’ that would require immediate surgical management, such as intestinal foreign bodies.

2.5 Conclusion

Serum cPE-1 is useful for the diagnosis of severe acute pancreatitis, but less so for milder forms of the disease. The diagnosis of milder or more chronic forms of the disease remains dependent on histological confirmation for definitive diagnosis. The usefulness of
serum cPE-1 in assessing residual exocrine pancreatic function following acute pancreatitis should be further evaluated.
Chapter 3: Association between canine specific pancreatic lipase (Spec-
cPL™) and histological exocrine pancreatic inflammation in dogs:
assessing specificity

The following is a modified version of the paper accepted for publication November 2011:
Veterinary Diagnostic Investigation

3.1 Introduction

Pancreatitis in dogs is a commonly diagnosed condition in veterinary clinics
worldwide (Cook et al., 1993; Hess et al., 1998; Mansfield and Jones, 2000; Watson,
2004). Clinically, pancreatitis in dogs results from an acute inflammatory or necrotising
process centred on the pancreas, with variable systemic complications (Kalli et al., 2009).
Histologically, acute pancreatitis is defined as a neutrophilic inflammation, without fibrosis
or exocrine atrophy; and usually is present within the body of the pancreas and/or peri-
pancreatic fat (Newman et al., 2006). The pathological distinction between acute
pancreatic necrosis, acute pancreatitis, acute peripancreatic necrosis and acute
peripancreatic steatitis, may reflect a different pathogenesis, however the clinical signs of
the disease will be similar, as the systemic effects can occur with any of these (Mansfield
et al., 2008). Chronic pancreatitis is defined as a mononuclear (often lymphocytic)
inflammation, with disruption of the pancreatic architecture due to concurrent fibrosis
(Watson et al., 2007). The presence of nodular hyperplasia in the pancreas of dogs is now
considered an incidental finding, with an increased presence in older dogs (Newman et al.,
2005). It has also been shown that pancreatic inflammation is present variably throughout
the pancreas, and so single biopsies may miss the true extent of the disease (Newman et al.,
2004). Histological grading schemes have been published that take into account the uneven
distribution of the inflammatory changes in categorising exocrine pancreatic disease
(Newman et al., 2006; Watson et al., 2007).
Recent evidence would suggest that chronic pancreatic inflammation is more common than previously thought, but diagnosis is difficult due to a failure of laboratory testing or imaging modalities to reliably detect it (Steiner et al., 2008a; Watson, 2004). Acute pancreatitis is considered easier to diagnose, but this diagnosis is often reliant on ultrasound imaging, something not always available to veterinary surgeons. Acute pancreatitis usually presents with acute vomiting and/or diarrhoea, in combination with abdominal pain and anorexia. This combination of clinical signs is not pathognomonic for acute pancreatitis, and other conditions such as septic peritonitis, acute renal failure, intestinal obstruction and others, are also associated with similar clinical signs.

Spec-cPL™ is a monoclonal enzyme-based assay that was developed from the original ELISA test for canine pancreatic lipase immunoreactivity (Steiner et al., 2003) (cPLI), and results of each assay are closely correlated (Steiner et al., 2008b). Spec-cPL™ has been shown to be virtually undetectable in dogs with exocrine pancreatic insufficiency (Steiner et al., 2006), and immunochemical studies have determined it is localized in the exocrine pancreas (Steiner et al., 2002). Spec-cPL™ (or originally cPLI) has been reported to have a sensitivity ranging from 21-82% for the diagnosis of pancreatitis (Steiner et al., 2001; Steiner et al., 2008a; Trivedi et al., 2011). The sensitivity appears to be higher in studies where the disease is more severe in nature. This sensitivity is comparable to a reported sensitivity ranging from less than 50% up to 78% for total lipase, using three times the upper reference interval as the diagnostic cut-off point (Cook et al., 1993; Hess et al., 1998; Mansfield and Jones, 2000). One of these studies established a specificity of 55% for total lipase (Mansfield and Jones, 2000). Dogs with acute renal failure, intestinal foreign bodies, acute enteritis and liver disease have all been shown to have increased total serum lipase concentration (Mansfield and Jones, 2000; Quigley et al., 2001; Rallis et al., 1996; Strombeck et al., 1981). A recent paper assessing dogs with no clinical or
histological signs of pancreatitis (essentially healthy dogs) determined a specificity of > 95% for Spec-cPL™ (Neilson-Carley et al., 2011). Another study showed specificity of 100% using the upper cut-off reference interval, however this was only in 7 dogs (Trivedi et al., 2011).

The aim of this study was to determine the specificity, and to a lesser degree the sensitivity, of Spec-cPL™ in a population of sick dogs using histological assessment of the pancreas as the gold standard for diagnosis.

3.2 Materials and Methods

Dogs that were submitted for post-mortem pancreatic examination, regardless of the cause of death or ante-mortem diagnosis, at Murdoch University, Perth, Western Australia during the period from September 2008 until May 2010, were initially recruited for the study. Blood samples were obtained within 6 hours of death, or immediately post mortem, and then serum harvested and stored at -20˚C. Informed owner consent was obtained for all post-mortem examinations, and if additional serum was needed to be obtained for this study, specific consent was also sought. Some owners consented only to the pancreas being removed, and not full post-mortem evaluation. The project was approved by the Murdoch University Animal Ethics Committee, following National Health and Medical Research Council guidelines.

Post-mortem evaluation when performed was as standard, and in all dogs, samples were obtained from the left lobe, right lobe and body of the pancreas and fixed in 10% formaldehyde for a minimum of 24 hours. These were then processed routinely through graded alcohol into paraffin before sectioning at 5μm and staining with haematoxylin and eosin (H&E) for later analysis by the two investigators (CSM and AO’H). Both investigators were blinded to the final diagnosis and results of serum testing when
reviewing the histopathology. Samples that were too autolysed for histological interpretation were excluded, as were cases where there were not 3 pancreatic samples available for assessment.

Histological grading of the pancreas was adapted from two previous studies (Newman et al., 2006; Watson et al., 2007). Inflammation was assessed and assigned a score for each of the three pancreatic sections, with a total cumulative score of 12 possible:

0- No inflammation present, or <2 small foci mononuclear cells with no disruption of the architecture
1- <5% neutrophilic or lymphoplasmacytic inflammation
2- 5-50% neutrophilic or lymphoplasmacytic inflammation
3- >50% neutrophilic or lymphoplasmacytic inflammation
4- Necrosis of pancreatic tissue, or peri-pancreatic necrosis/steatitis

Additionally, the presence of fibrosis was defined as mature fibrous connective tissue replacing the acinar structures or expanding the interstitium. The severity of the fibrosis in each section was scaled according to the following numerical system, with a maximum cumulative score of 9:

0- None evident
1- <20% of each section effaced by mature connective tissue
2- 20-50% of each section effaced by mature connective tissue
3- >50% of each section effaced by mature connective tissue

If fibrosis could not be definitely differentiated on routine H&E sections, additional staining with Sirius Red was performed to characterize this more clearly (Watson et al., 2007). Other pancreatic changes (in addition to the above) were recorded if present, but not
assigned severity scores: haemorrhage, oedema, hyperaemia, neoplasia and exocrine nodular hyperplasia.

Sera from every dog that was assessed in the study was sent as a batch for measurement of Spec-cPL™ at a commercial laboratory. For the purposes of the study, a result < 30 µg/L was recorded as 30; and results > 1000 µg/L were recorded as 1000 to reflect the limit of quantification of the assay. This assay has previously been shown to have good reproducibility with both inter- and intra-assay coefficient of variation < 12% (Huth et al., 2010). Current laboratory recommendations are that a result > 400 µg/L is consistent with pancreatitis, and a result in the 200-399 µg/L interval should be considered to possibly signify pancreatitis (Steiner et al., 2008a,b).

For the purposes of analysis, dogs with a cumulative inflammatory score of ≤ 3 were considered to have sub-clinical pancreatitis of little to no significance; dogs with a cumulative inflammatory score of ≥ 4 had pancreatic inflammation of increasing degrees. Concurrently, a cumulative score of ≤ 2 for fibrosis indicated a mild to minimal degree of chronicity, whilst ≥ 3 indicated moderate to severe fibrosis.

Statistical analysis and graphical representation were done using a statistical software package (MedCalc 11.6.1, MedCalc software, Mariakerke, Belgium). Statistical difference between groups was determined using the Mann-Whitney U test. Spearman’s rank correlation coefficient was calculated to determine the association between Spec-cPL™ concentration and the inflammatory and fibrosis scores, respectively. A two-tailed P-value ≤ 0.05 was considered to be statistically significant.

3.3 Results

There were a total of 54 dogs initially included in the study, but only 32 had all the required data and were analysed. Twenty dogs had a pancreatic inflammation score ≤ 3, 5
dogs had a score of 4-5; 4 dogs had a score 6-8 and 3 dogs had a score ≥ 9. Pictorial examples of histological sections from each of these categories are shown in Figure 3.1.

In the 20 dogs with a pancreatic inflammatory score of ≤ 3, the cause of death or euthanasia was determined in 17 and included dog attack (2), and 1 each of the following: vasculitis/coagulopathy, disseminated intravascular coagulation due to anaphylaxis, congestive heart failure, hepatic necrosis, intestinal infarct secondary to NSAID overdose, osteosarcoma, heart base mass, multiple endocrine neoplasia, lung carcinoma, septic peritonitis (stick foreign body), splenic haemangiosarcoma, metastatic malignant melanoma, snake envenomation, islet cell neoplasia, and metastatic nephroblastoma. In the 12 dogs with a pancreatic inflammatory score > 3, the primary cause of death was identified in 11, and included acute pancreatitis (2), and 1 each of splenic haemangiosarcoma, haemabdomen secondary to splenic haematoma, small intestinal foreign body, multicentric lymphoma, metastatic carcinoma, pituitary tumour (non-functional), pneumonia, septic peritonitis (secondary to hepatic abscessation) and extra-hepatic bile duct obstruction due to chronic pancreatitis.

The descriptive statistics for Spec-cPL™ are shown in Table 3.1. There was a statistical difference in Spec-cPL™ concentration between dogs with a pancreatic inflammation score of ≤ 3 and those with a score of > 3 (P=0.011), as demonstrated in Figure 3.2.

Seventeen dogs had a fibrosis score of ≤ 2, 10 of which had no observed fibrosis. Thirteen dogs had a moderate (score 3-6) degree of fibrosis, and 2 had severe (score ≥ 7) fibrosis (Table 3.1). There was no statistical difference in Spec-cPL™ concentration between dogs with a pancreatic fibrosis score of ≤ 2 and those > 2 (P=0.055). Haemorrhage was observed in samples from 8 dogs, all with pancreatic inflammation.
scores \leq 3. Haemorrhage was not observed uniformly through all sections in each dog. Additionally nodular exocrine hyperplasia was identified in 2 dogs. No oedema was noted.

Spearman’s rank correlation coefficient between pancreatic inflammation score and Spec-cPL™ was significant ($r_s = 0.47; 95\% \text{ CI } 0.15 - 0.71; P = 0.0060$), as demonstrated in Figure 3.3, while there was no correlation between pancreatic fibrosis and Spec-cPL™ ($r_s = 0.30; 95\% \text{ CI } -0.05 \text{ to } 0.59; P = 0.091$).

When using 400 µg/L as a diagnostic cut-off for Spec-cPL™, and a pancreatic inflammation score of $\leq 3$ to indicate no histological pancreatitis, there were 2 false positives. These 2 dogs had Spec-cPL™ concentrations of 914 and 568 µg/L, both with pancreatic inflammation and fibrosis scores of 1 and 3 respectively. No other pancreatic abnormalities were observed. When using a diagnostic cut-off for Spec-cPL™ of 200 µg/L, there were 4 false positives. The additional 2 dogs had Spec-cPL™ concentrations of 270 and 255 µg/L, with pancreatic inflammation and fibrosis scores of 3,3 and 1,3 respectively. This correlates to a specificity of 90% (95% CI 68 - 99%) using a Spec-cPL™ cut-off value of 400 µg/L and 80% (95% CI 56 - 94%) using a cut-off value of 200 µg/L. The area under the receiver operating characteristic (ROC) curve was 0.77 (95% CI 0.59 – 0.90).

There were 12 dogs in this study that had a histological diagnosis of pancreatitis (a pancreatic inflammation score $\geq 4$). By using 400 µg/L as a diagnostic cut-off concentration for Spec-cPL™ there were 4 true positives (4/12, sensitivity of 33%, 95% CI 10 - 65%), whilst using the diagnostic cut-off value of 200 µg/L, there were 7 true positives (7/12, sensitivity 58.3%, 95% CI 28 - 85%). Three of these dogs had peri-pancreatic fat necrosis present, but minimal to no inflammation present within the pancreatic interstitium. On analysis of these 11 dogs with histological pancreatitis and a known cause of death, only 3 had pancreatitis as the primary cause of their clinical signs.
Figure 3.1 Haematoxylin & Eosin stained sections from four dogs with variable pancreatic inflammatory scores (PIS); A: nil or minimal pancreatic inflammation (PIS < 3; Fibrosis < 3; 200x magnification); B: Mild to moderate pancreatic inflammation (PIS 4-5; fibrosis 3; 200x magnification); C: Moderate to severe pancreatic inflammation (PIS 8; Fibrosis 3; 200x magnification); D: Severe pancreatic inflammation, including peri-pancreatic necrosis (PIS ≥ 9; 40x magnification).

Table 3.1

<table>
<thead>
<tr>
<th></th>
<th>Pancreatic Inflammation Score</th>
<th>Pancreatic Fibrosis Score</th>
</tr>
</thead>
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<td>Grading</td>
<td>≤ 3</td>
<td>4+</td>
</tr>
<tr>
<td>Number</td>
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<td>12</td>
</tr>
<tr>
<td>Mean</td>
<td>144.1</td>
<td>347.5</td>
</tr>
<tr>
<td>Median</td>
<td>39</td>
<td>327</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>225.3</td>
<td>290.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Maximum</td>
<td>914</td>
<td>1000</td>
</tr>
</tbody>
</table>

Statistical summary of Spec-cPL™ results (µg/L) for each pancreatic inflammatory and fibrosis group.
Figure 3.2  
Results of Spec-cPL™ concentrations in 20 dogs with minimal to no pancreatic inflammation (Pancreatic inflammation score ≤3) and in 12 dogs with scores of 4+. The median concentration is represented by the short horizontal bar.
Figure 3.3  Scatterplot of pancreatic inflammation score and Spec-cPL™ concentrations in 32 dogs. Spearman’s rank correlation coefficient 0.47 \((P=0.0060)\).

3.4 Discussion

The pathogenesis of clinical pancreatitis is complex and multi-factorial. The clinical signs from acute pancreatitis may result purely from the local effects of the pancreatic digestive enzymes released into the cranial abdomen when trypsinogen is activated to trypsin within the pancreatic acinar cell (Rinderknecht, 1986). Pancreatic safeguards such as pancreatic secretory trypsin inhibitor (PSTI) can become overwhelmed, allowing trypsin activation to spill into the interstitium (Fernandez-del-Castillo et al., 1994). Circulating anti-proteases (such as α-macroglobulin or α₁-protease inhibitor) are responsible for binding to proteases and clearing them through the reticuloendothelial system, but these too may become overwhelmed in individual animals (Lasson and
Circulating pancreatic proteases are then capable of inciting inflammation systemically via activation of free radical, coagulation, complement and kinin cascades (Ruaux, 2000). The generation of chemokines and cytokines leads to further tissue inflammation and injury, which in turn may cause multi-organ failure and death. As such, determination of histological severity alone cannot conclusively determine if an animal has clinically severe pancreatitis. Additionally, it is possible that mild pancreatic inflammation may be along the spectrum of normal in dogs, and it is the way the body responds to that inflammation that determines if clinical disease is manifested or not.

Along those lines, this study demonstrates the difficulty in truly defining clinical pancreatitis based on histological grading alone. One post-mortem survey identified 92% of dogs in a referral institution to have pancreatic inflammation (Newman et al., 2006), whilst another study found approximately 34% of dogs surveyed in a first opinion clinic to have chronic pancreatitis (Watson et al., 2007). As it seems highly unlikely that 90% of sick dogs will be sick due entirely to pancreatitis, it can be surmised that histological pancreatitis does not always translate to clinically important pancreatitis. It has not been established what percentage of the pancreas needs to have inflammation present before it can be truly categorised as having clinically important pancreatitis. It is interesting to note that in our study, of the 11 dogs with histological pancreatitis (an inflammation score > 3) and a known cause of death, only 3 had pancreatitis as the primary cause of their clinical presentation. This highlights the need to consider the pancreas as a participant in many presentations, rather than always as an instigator.

The use of the current scheme was an attempt by the authors to correct for this potential discrepancy between histological and clinical pancreatitis. Whilst it is possible that a dog with a pancreatic inflammation score ≥ 4 in this study did not have clinical pancreatitis, the 8 dogs that were designated to be false negatives all had inflammation
scores ≥ 5, and had clinical signs of pancreatitis. The possible reason for this discrepancy is that 4 of the 8 had predominantly peri-pancreatic fat necrosis, with little to no inflammation or necrosis of the pancreatic interstitium. One possible explanation for this is that the disease may have initiated in the peri-pancreatic fat, and inflammation centred on the pancreas itself had yet to develop. This may have resulted in less stimulation of the pancreatic enzyme cascade than if it had involved the interstitium. This also raises an interesting alternative conjecture in that this sub-group of dogs may not have true pancreatitis as is currently understood (where trypsinogen is activated within the acinar cell as the initiating event). Rather, the disease may actually be initiated in fat within the abdomen and extend towards the pancreas. The role of adipokines in initiating inflammation is increasingly being recognised in the human and veterinary field (Martínez-Clemente et al., 2011; Radin et al., 2009a). Clinically, these 2 possibilities would be impossible to differentiate. As 3 of these 4 dogs had clinical signs (abdominal pain, vomiting) for more than 3 days, lack of opportunity for inflammation to develop seems unlikely.

When using a cut-off value of 400 µg/L, the specificity of Spec-cPL™ in this study was 90%, and when using a cut-off value of 200 µg/L, the specificity was 80%. Caution should be taken when interpreting these results however, as the post-mortem nature of this study biased the selection of cases. By the very nature of requiring the pancreas in its entirety, the study is pre-selecting for dogs with severe disease. Additionally, this study only assessed dogs presented for post-mortem at a referral institution. Dogs with diseases that have similar clinical signs to pancreatitis (intestinal foreign body, acute gastroenteritis) are more likely to be treated at a general veterinary practice, or survive their illness. This, combined with a lack of a non-invasive gold standard, makes it extremely difficult to
assess the true clinical specificity of Spec-cPL™ in a large population of dogs with acute gastrointestinal signs.

The dogs in this study with false positive results had a number of underlying causes of death or euthanasia, and none was considered likely to have pancreatitis as a primary problem. That being said, it is possible that the inflammation score determined in this study did not accurately reflect changes throughout the pancreas. Previous studies have identified that histological changes in the pancreas are not uniformly distributed throughout the organ (Newman et al., 2004). This study assessed samples from the left and right lobe, as well as the body of the pancreas. Although the entire pancreas was not sectioned, the authors felt this was representative of an overall inflammatory assessment. Other possible reasons for the false positive results includes cross-reactivity with other lipases (which seems unlikely based on previous work), excessive production of pancreas-specific lipase by the pancreas in the absence of pancreatic inflammation, or production of this isoenzyme of lipase in an organ(s) other than the pancreas. It is not possible from the current information to postulate on the most likely cause.

This study did not include any dogs with renal failure, and increased total lipase has been identified in dogs with renal failure (Mansfield and Jones, 2000; Strombeck et al., 1981). Further work assessing Spec-cPL™ and pancreatic inflammation in dogs with renal failure is required before conclusions can be made regarding this particular aspect.

A recent study has established similar sensitivities for measurement of serum canine pancreatic-elastase-1(cPE-1) (Mansfield et al., 2011b, Chapter 2) to what has previously been reported for Spec-cPL™ (Steiner et al., 2008a). Again, with both analytes the sensitivity increases when more severe forms of pancreatitis are assessed. This current study actually reported a much lower sensitivity for Spec-cPL™ of 33-58% than what had previously been reported, however caution should be had in over interpreting this aspect
due to the very small numbers of animals with true disease. The dogs in this study were presented for post-mortem evaluation, and were not necessarily considered likely to have pancreatitis. The specificity for cPE-1 was also reported to be approximately 92% (Mansfield et al., 2011b, Chapter 2), again similar to the result reported for Spec-cPL™ in this study.

The specificity identified in this study is similar, but slightly lower than another study published assessing the histological and gross appearance of the pancreas (Neilson-Carley et al., 2011). The slightly lower value in our study may be due to only sick dogs being assessed, whilst almost two thirds of the dogs without pancreatitis were clinically healthy. To date, the current sensitivity and specificity for clinical suspicion combined with imaging findings to diagnose pancreatitis in dogs is not known. It can certainly be extrapolated that no single laboratory test will replace good clinical acumen and imaging of the abdomen, but rather can serve to enhance it.

3.5 Conclusion

In this study of pancreatic histology obtained post-mortem in dogs, Spec-cPL™ had a specificity ranging from 80-90%, dependent on which value (200 µg/L and 400 µg/L respectively) was used as the lower limit in the analysis. The sensitivity for this test ranged from 33-58% (using 400 µg/L and 200 µg/L respectively), which is less than previously reported. The authors recommend additional testing, especially abdominal imaging, should aid in the diagnosis of the primary problem in dogs presenting with vomiting and/or abdominal pain.

Although both pancreatic elastase and pancreatic lipase are released by the pancreas in response to inflammation, they appear to be unable to predict the severity of clinical disease. The next section of this thesis relates to the development of a clinical
severity scoring index that would be useful in dogs to allow assessment of treatment modalities.
Chapter 4: Development of a clinical severity index for dogs with acute pancreatitis


4.1 Introduction

Pancreatitis is a major disease of dogs, and acute necrotising inflammation is the most common form of pancreatitis that is diagnosed in dogs (Hess et al., 1998; Watson, 2004). Pancreatitis may result in a wide range of clinical signs of differing severity and cause multisystem inflammation in dogs (Ruaux, 2000). The mortality rate among dogs with acute pancreatitis is often high because of the systemic effects of the disease, and surviving animals usually require intensive treatment and hospitalization (Mansfield et al., 2003; Ruaux and Atwell, 1998a; Ruaux and Atwell, 1998b; Williams, 1994). Studies in people with acute pancreatitis have identified that the optimal therapeutic window is approximately 48 to 72 hours after the first onset of pain (Norman, 1998). Prediction of which dogs with acute pancreatitis will develop fatal complications is problematic, although a significant association between acute pancreatitis-related death and concurrent disease, such as diabetes mellitus, epilepsy, or hyperadrenocorticism, has been reported (Cook et al., 1993; Hess et al., 1998; Strombeck et al., 1981).

To date, no readily available blood test has been shown to differentiate mild from severe disease in dogs with acute pancreatitis (Hess et al., 1998; Mansfield et al., 2003; Ruaux and Atwell, 1998b; Strombeck et al., 1981; Williams, 1994). Thus, there is a singular lack of objective criteria that correlate with the severity of acute pancreatitis in affected dogs. Traditional biochemical methods of diagnosing pancreatitis, such as high serum amylase and lipase activities, are poor predictors of death (Mansfield et al., 2003; Strombeck et al., 1981). Much attention in veterinary medicine has been focused on
measurement of circulating concentrations of acute-phase proteins, especially CRP, in the
diagnosis and prognostication of acute and chronic inflammatory conditions. Acute-phase
proteins are part of a complex and nonspecific reaction that occurs immediately after tissue
injury to restore homeostasis and remove the cause of its disturbance (Ceron et al., 2005).
Production of CRP is stimulated by inflammatory cytokines such as IL-6, IL-1, and tumor
necrosis factor-α (Ceron et al., 2005). The main biologic functions of CRP appear to be
promotion of bacterial phagocytosis, induction of other cytokines, inhibition of
chemotaxis, and modulation of neutrophil function (Ceron et al., 2005). C-reactive protein
is stable and increases in association with several inflammatory conditions in dogs,
including pancreatitis (Berghoff et al., 2006; Chan et al., 2006; Holm et al., 2004; Merlo et
al., 2006; Spillman et al., 2002).

Extrapolation of a clinical findings-based classification scheme for acute
pancreatitis in humans (Bradley, 1993), would mean a severe pancreatitis in dogs requires
2 or more of the following findings: necrosis of pancreatic acinar tissue, systemic
complications (e.g., disseminated intravascular coagulation or acute respiratory distress
syndrome), severe clinical signs causing profound obtundation or death, or development of
abdominal complications. This classification scheme relies heavily on histological
confirmation of pancreatic necrosis; such confirmation is seldom obtained ante-mortem in
most dogs with acute pancreatitis, and the system is therefore not easily applied to canine
patients. Among dogs with pancreatic necrosis, there is widespread degree of severity of
disease, dependent on the dogs’ systemic response to pancreatic inflammation and
activation of pancreatic proteases (Mansfield and Jones, 2000; Ruaux, 2000; Watson,
2004). The use of such a classification scheme is also highly subjective and does not allow
for standardization of less objective criteria.
A clinicopathological abnormality-based scheme for classification of pancreatitis in dogs has also been described (Ruaux and Atwell, 1998b); on the basis of that scheme, classification of severity correlates with outcome. However, the devised scoring scheme did not assess definitively confirmed cases of pancreatitis and did not take into account non-clinicopathological abnormalities. Development of a clinical severity index for acute pancreatitis in dogs which take into account multiple body systems, clinicopathological abnormalities, and local pancreatic complications will allow comparisons of data among practices and objective assessment of various treatments of this disease. The purpose of the study reported here was to establish a clinical severity index that correlates severity of body system abnormalities with outcome in dogs with acute pancreatitis as well as determines the usefulness of serum CRP concentration as an objective measure of acute pancreatitis severity. The wider context was to provide a method of ensuring dogs with acute pancreatitis assessed in later studies had similar degrees of disease severity.

4.2 Materials and Methods

4.2.1 Inclusion criteria

Records of dogs that were evaluated at a primary emergency center (Murdoch Pet Emergency Centre) and a referral veterinary teaching hospital (Murdoch University Veterinary Teaching Hospital) between 1999 and 2006 and for which a diagnosis of pancreatitis was recorded were reviewed. As no clients were contacted, or interventional studies performed, no institutional animal ethics approval was required for the retrospective study. Client permission was obtained when extra blood sampling was required, under approval of Murdoch University Animal Ethics Committee, for the prospective study. Inclusion criteria included evidence of acute pancreatitis detected via abdominal ultrasonography or histological examination of surgical or necropsy tissue
samples, admission to hospital for treatment, complete daily clinical records (physical examination findings), and results of full clinicopathological evaluation (haematological and serum biochemical analyses and urinalysis as minimum) performed within 12 hours of admission. Ultrasonography was performed by a board-certified (or equivalent Australian qualification) radiologist, using a curvilinear 8 MHz or linear 15 MHz probe (Sequioa 512, Acuson, Munich, Germany). A diagnosis of acute pancreatitis was made if there was evidence of a large pancreas with patchy echogenicity or hyperechoic mesentery. Dogs were excluded if they were not admitted for treatment or were euthanatized for nonmedical reasons. Signalment, interval between onset of clinical signs and evaluation, and interval from hospital admission until outcome (considered to be death or discharge from the hospital; designated as days until outcome) were recorded for each dog that fulfilled the inclusion criteria.

4.2.2 Development of the severity scoring index

Several body systems were evaluated in the study animals to develop an appropriate clinical severity index scheme (Table 4.1). Abnormalities of the endocrine system (e.g. diabetes mellitus or diabetic ketoacidosis) and hepatic system (e.g. high serum activities of hepatocellular and cholestatic enzymes) were included and graded as part of the index on the basis of their inclusion in a previously published severity scheme (Ruaux and Atwell, 1998b). Other systems were included on the basis of criteria developed for critically ill patients (King et al., 2001); these included the haematopoietic system to evaluate for signs of inflammation (high WBC count and moderate left-shift), development of systemic inflammatory syndrome (severe leucocytosis or leucopenia), or development of disseminated intravascular coagulation. Coagulation abnormalities included prolongation of prothrombin time, activated partial thromboplastin time, or activated
clotting time and high plasma concentration of fibrinogen degradation products. Other critical care factors evaluated were the presence of cardiac arrhythmias (graded in ascending order according to whether relatively benign or potentially malignant), respiratory complications (graded in ascending order to reflect possible development of acute respiratory distress syndrome), renal disease (severe azotaemia or anuria), and altered vascular forces (graded in ascending order to reflect changes in serum albumin concentration and systolic arterial blood pressure). On the basis of the human and veterinary medical literature, local pancreatic complications were also considered and included peritonitis that extended beyond the peripancreatic region and presence of a pancreatic acute fluid collection (also known as a pseudocyst), or pancreatic abscess. Poor enteral health or altered intestinal integrity was also graded if there was poor intestinal motility (defined as an absence of intestinal sounds during ≥ 3 auscultations of the left and right sides of the abdomen during a 24-hour period), development of regurgitation (presumed to be the result of reflux oesophagitis), altered intestinal integrity (evidence of bleeding into gastrointestinal tract, such as melena or haematochezia), or a prolonged period of anorexia (> 3 days), regardless of whether this was prior to or during hospitalisation.

Each of these scored factors was evaluated for significance (i.e. whether it contributed to survival) by use of a Pearson $\chi^2$ and Fisher exact test; a value of $P < 0.05$ was considered significant. Factors were retained for evaluation if they were significant. A clinical severity index based on the significant factors was determined for each dog by assessment of the medical record data collected within the first 24-hour period after admission to the hospital. An organ score based on clinicopathological variables was also calculated for each dog from laboratory information obtained within the first 24-hour
period after admission to the hospital, according to a previously published method (Ruaux and Atwell, 1998b). In that organ scoring scheme, 1 point is allocated for abnormalities associated with the leucogram (> 10% band neutrophils or WBC > 24 X 10^9 cells/L); kidneys (serum urea concentration > 14 mmol/L [39.2 mg/dL] and serum creatinine concentration > 300 μmol/L [3.3 mg/dL]); liver (serum alanine transferase activity > 240 U/L, serum aspartate aminotransferase activity > 240 U/L, or serum alkaline phosphatase activity > 420 U/L); acid-base buffering (blood bicarbonate concentration > 26 mmol/L or < 13 mmol/L or anion gap < 15 mmol/L or > 38 mmol/L); and the endocrine pancreas (blood glucose concentration > 13 mmol/L [234 mg/dL] or serum β-hydroxybutyrate concentration > 1 mmol/L).

4.2.3 Serum CRP concentration measurement

During a 3-month period, dogs admitted to Murdoch University Veterinary Teaching Hospital that had a diagnosis of acute pancreatitis (confirmed via ultrasonographic or histological evaluation of the pancreas as described) were also evaluated prospectively. The dogs were admitted and treated as considered appropriate by attending veterinarians and none were euthanatized for nonmedical reasons. Severity scores were determined as described and treatment data were recorded. From each dog, a blood sample (2 mL) was obtained via jugular or cephalic venipuncture within 24 hours of admission, with informed owner consent. Serum was harvested within 30 to 60 minutes of blood collection and frozen at -20°C. Samples were then transported on dry ice and CRP concentration was measured by use of a solid-phase sandwich immunoassay (Tridelta Phase Canine CRP Assay, Tridelta Diagnostics Inc, Morris Plains, NJ). The reference interval for this assay in healthy dogs has been determined as 0 to 7.6 mg/L (Berghoff et al., 2006).
4.2.4 Statistical analysis

Correlation between the 2 severity scoring systems (the clinical severity index and clinicopathological variable-based organ scoring scheme) and survival in all dogs was determined via Spearman rho calculation (a value of $P < 0.05$ was considered significant) and an ANOVA. Correlation between the 2 severity scoring systems and days until outcome in all cases was also determined by use of the Spearman rho (non-parametric) analysis, because data were not distributed normally. Spearman rho analysis was also used to determine the correlation between serum CRP concentration and each of the severity scoring schemes, between serum CRP concentration determined within 2 days of onset of clinical signs and outcome (survival to discharge from the hospital or death), and between serum CRP concentration determined at any time and outcome (survival to discharge from the hospital or death). For all analyses, a value of $P < 0.05$ was considered significant.
<table>
<thead>
<tr>
<th>System</th>
<th>Finding</th>
<th>Point allocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pre-existing diabetes mellitus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Diabetes ketoacidosis (glycosuria/hyperglycaemia+ ketonuria or increased beta-hydroxy butyrate)</td>
<td>2</td>
</tr>
<tr>
<td>Hepatic</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>≥ 2.5-fold increase (compared with upper limit of reference range) in at least 2 of the following: serum alkaline phosphatase, alanine transferase, and aspartate aminotransferase activities</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≥ 5-fold increase (compared with upper limit of reference range) in at least 2 of the following: serum alkaline phosphatase, alanine transferase, and aspartate aminotransferase activities</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Extra-hepatic bile duct obstruction</td>
<td>3</td>
</tr>
<tr>
<td>Renal</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Azotaemia (≤ 1.5-fold increase [compared with upper limit of reference range] in serum urea and creatinine concentration)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Anuria or azotaemia (≥ 1.5-fold increase [compared with upper limit of reference range] in serum urea and creatinine concentration)</td>
<td>2</td>
</tr>
<tr>
<td>Haematopoietic</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>WBC count ≥ 20.0 X 10^9 cells/L or ≤ 4.0 X 10^9 cells/L, with ≤ 10% band neutrophils</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WBC count ≥ 20.0 X 10^9 cells/L or ≤ 4.0 X 10^9 cells/L, or neutrophil count ≤ 1.0 X 10^9 cells/L, or ≥ 10% band neutrophils</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Clinicopathological evidence of hypercoagulability or coagulation abnormalities</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Clinical evidence of disseminated intravascular coagulation or bleeding diathesis</td>
<td>4</td>
</tr>
<tr>
<td>Local complications</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Peritonitis extending beyond peripancreatic area</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pseudocyst or other acute fluid accumulation</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Pancreatic abscess</td>
<td>3</td>
</tr>
<tr>
<td>Cardiac</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&lt; 60 ventricular premature complexes/24-hour period or heart rate &gt; 180 beats/min</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Paroxysmal or sustained ventricular tachycardia</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Clinical evidence of dyspnoea or tachypnoea (&gt; 40 breaths/min)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Clinical evidence of pneumonia or acute respiratory distress syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Intestinal integrity</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Intestinal sounds not detected during &gt; 3 auscultations in 24-hour period</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Haematochezia, melena, or regurgitation</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No enteral food intake for &gt; 3 days</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No enteral food intake for &gt; 3 days and at least 2 of the following: haematochezia, melena, and regurgitation</td>
<td>4</td>
</tr>
<tr>
<td>Vascular forces</td>
<td>No abnormalities</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Systolic arterial blood pressure &lt; 60 or &gt; 180 mm Hg or serum albumin concentration &lt; 18 g/L</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Systolic arterial blood pressure &lt; 60 or &gt; 180 mm Hg and serum albumin concentration &lt; 18 g/L</td>
<td>2</td>
</tr>
</tbody>
</table>

*Full details of all aspects reviewed and points allocated when initially determining which factors would correlate to survival.*
4.3 Results

4.3.1 Animals

From the medical records, 68 dogs for which a diagnosis of pancreatitis had been made during the period of 1999 to 2006 were identified. Sixty-one dogs fulfilled the inclusion criteria and were admitted for treatment; in 12 of these dogs, serum CRP concentration was measured. Seven dogs were excluded because they were euthanatized due to financial reasons alone or had incomplete medical records. In the study group, there were 36 females (of which 35 were spayed and 1 was sexually intact) and 25 males (of which 17 were neutered an 8 were sexually intact). Mean age of all dogs was 8.5 years (range, 1 to 16 years). Breeds included Terrier-breeds (n = 12), Australian Kelpie or Australian Kelpie crossbreeds (8), Border collie or Border collie crossbreeds (7), Australian cattle dog (6), Bull terrier (4), Miniature schnauzer (3), Miniature poodle (3), Rottweiler (2), Corgi (2), Golden retriever (2), and Lhasa Apso (2). The other 10 dogs were mixed breeds.

4.3.2 Clinical severity index and organ scoring scheme

The medical records for each dog were reviewed and the system factors used in the clinical severity index were assessed (Table 4.1). Statistical analysis of those data revealed an association between outcome and each of 4 body system classifications: the cardiac and respiratory system factors ($P \leq 0.044$ and $P \leq 0.035$, respectively), intestinal integrity ($P \leq 0.009$), and vascular forces ($P \leq 0.003$) (Table 4.2). There was no statistically significant association between outcome and the renal, haematopoietic, hepatic, endocrine, or local complication factors. Therefore, those classifications were excluded from the clinical severity index. Further evaluation of the local complication and haematopoietic systems also failed to identify a significant association between outcome and scores of 2 or 3 and 3
or 4, respectively. Therefore, in the final clinical severity index, the total maximum score was 10 points (maximum score of 2 for the cardiac, respiratory, and vascular forces systems and a maximum score of 4 for the intestinal integrity system).

Of the 61 dogs, 47 survived to discharge from hospital and 14 died or were euthanatized for pancreatitis-associated medical reasons; the overall mortality rate was 23%. On the basis of severity scores (maximum of 10 points) derived by use of the clinical severity index, dogs were grouped into categories of 0, 1, 2, 3, 4, 5, and ≥ 6 scores (no score was > 6) and the outcomes for each category were examined (Figure 4.1a). The numbers of dogs in each score category were 15 (24.6%), 15 (24.6%), 6 (9.8%), 8 (13.1%), 12 (19.7%), 4 (6.5%), and 1 (1.7%) respectively. The mortality rate for dogs that had a clinical severity index ≥ 4 was 53%, compared with the overall mortality rate among all dogs of 23%. On the basis of the organ scoring scheme, dogs were grouped into categories of 0, 1, 2, 3, or 4 scores and the outcomes for each category were also examined. The numbers of dogs in each score category were 15 (24.6%), 17 (27.9%), 19 (31.1%), 7 (11.5%), and 3 (4.9%), respectively (Figure 4.1b).
Number and percentage in brackets of dogs with the various point allocation in the severity index calculation. The factors considered significant on analysis and included in the clinical severity index are highlighted. Comparison between survival for each factor is also demonstrated in the 4th column.

The score derived by use of the clinical severity index had a significantly greater correlation with survival (Spearman correlation coefficient = −0.437; \( P = 0.000 \)) than the score derived by use of the organ scoring scheme (Figure 4.2). Scores using the latter system had no significant correlation with outcome in the cohort of the present study (Spearman correlation coefficient = −0.096; \( P = 0.460 \)). The mean score derived by use of the clinical severity index for the 47 survivors (1.62) was significantly lower than the mean score for the 14 dogs that died or were euthanatized (3.4). The ANOVA revealed that death

<table>
<thead>
<tr>
<th>System</th>
<th>Point allocation</th>
<th>No. of dogs (%)</th>
<th>No. of Survivors (Non-Survivors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine</td>
<td>0</td>
<td>49 (80.3%)</td>
<td>39 (10)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4 (6.6%)</td>
<td>3 (1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8 (13.1%)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Hepatic</td>
<td>0</td>
<td>22 (36.3%)</td>
<td>19 (3)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14 (22.9%)</td>
<td>10 (4)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9 (14.8%)</td>
<td>6 (3)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16 (26.2%)</td>
<td>12 (4)</td>
</tr>
<tr>
<td>Renal</td>
<td>0</td>
<td>51 (83.6%)</td>
<td>39 (12)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7 (11.5%)</td>
<td>6 (1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3 (4.9%)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Haematopoietic</td>
<td>0</td>
<td>17 (27.9%)</td>
<td>15 (2)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>19 (31.1%)</td>
<td>14 (5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18 (29.5%)</td>
<td>14 (4)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5 (8.2%)</td>
<td>4 (1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2 (3.3%)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Local complications</td>
<td>0</td>
<td>19 (31.1%)</td>
<td>14 (5)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>36 (59%)</td>
<td>29 (7)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5 (8.2%)</td>
<td>4 (1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1 (1.7%)</td>
<td>0 (1)</td>
</tr>
<tr>
<td>Cardiac</td>
<td>0</td>
<td>47 (77%)</td>
<td>38 (9)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>14 (23%)</td>
<td>9 (5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Respiratory</td>
<td>0</td>
<td>57 (93.4%)</td>
<td>45 (12)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4 (6.6%)</td>
<td>2 (2)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Intestinal integrity</td>
<td>0</td>
<td>22 (36.1%)</td>
<td>21 (1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>13 (21.3%)</td>
<td>9 (4)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5 (8.2%)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20 (32.7%)</td>
<td>13 (7)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1 (1.7%)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Vascular forces</td>
<td>0</td>
<td>45 (73.7%)</td>
<td>41 (4)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>15 (24.6%)</td>
<td>8 (7)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1 (1.7%)</td>
<td>0 (1)</td>
</tr>
</tbody>
</table>
was more likely to occur in dogs with higher clinical severity index scores ($P = 0.008$).

There was generally a fair degree of correlation within individuals between scores derived by both schemes; however, there were some outliers with poor correlation (Table 4.3). Six dogs had significantly lower organ scores, compared with their clinical severity index scores ($P < 0.05$); 5 of these dogs died or were euthanatized as a result of their disease, and the dog that survived had a prolonged hospitalisation period. There was a high rate of abnormalities in vascular forces or evidence of severely altered intestinal integrity in all 6 dogs (Table 4.4).

Among all 61 dogs, the mean ± SD interval from onset of clinical signs until outcome (i.e. days until outcome, namely death or discharge from the hospital) was $5.7 \pm 3.12$ (range, 1 to 15 days). Results of the ANOVA indicated that there was no significant ($P = 0.329$) difference in days until outcome between survivors (mean, $5.9 \pm 3.23$, range 2-15; n = 47) and non-survivors (mean, $5.0 \pm 2.69$, range 1-10; 14). However, there was a highly significant correlation (Spearman correlation coefficient = 0.554; $P = 0.000$) between the days until outcome and the clinical severity index score. There was also a significant correlation (Spearman correlation coefficient = 0.278; $P = 0.03$) between days until outcome and the organ score.
Figure 4.1 Distribution of dogs with acute pancreatitis that survived to discharge from the hospital (black bars; n = 47) versus those that died or were euthanatized for reasons related to acute pancreatitis (grey bars; 14) according to scores derived by use of a clinical severity index (A) or an organ scoring scheme (B).
Figure 4.2  Individual scatter plot comparing results for each individual dog using the laboratory organ scoring system and the clinical severity index (numbers above crosses indicate number of animals with that combination of results).

Table 4.3

<table>
<thead>
<tr>
<th>Organ score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>0</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1*</td>
<td>0</td>
<td>1*</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1*</td>
<td>2*</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>1*</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Comparison of severity scores for individual dogs assessing the organ score and clinical severity index evaluated in this chapter.
*The correlation between the two scoring systems for these dogs was poor (i.e., organ score was significantly [P < 0.05] lower than the clinical severity index score) using Spearman rho analysis.
### Table 4.4

<table>
<thead>
<tr>
<th>Dog</th>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Signalment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Breed</strong></td>
<td>Australian Cattle Dog</td>
<td>Border Collie</td>
<td>Corgi</td>
<td>Australian Cattle Dog</td>
<td>Border Collie</td>
<td>Tibetan Terrier</td>
</tr>
<tr>
<td></td>
<td><strong>Sex (reproductive status)</strong></td>
<td>Male (N)</td>
<td>Female (N)</td>
<td>Male (N)</td>
<td>Male (SI)</td>
<td>Female (N)</td>
<td>Female (N)</td>
</tr>
<tr>
<td></td>
<td><strong>Age (y)</strong></td>
<td>9</td>
<td>7</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td><strong>Clinical severity index point allocations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Cardiac</strong></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><strong>Respiratory</strong></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><strong>Intestinal integrity</strong></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><strong>Vascular forces</strong></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><strong>Total clinical severity index score</strong></td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><strong>Laboratory organ score</strong></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><strong>Serum CRP concentration (mg/L)</strong></td>
<td>48.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td><strong>Outcome</strong></td>
<td>ERD</td>
<td>ERD</td>
<td>Died</td>
<td>Recovered</td>
<td>Died</td>
<td>ERD</td>
</tr>
<tr>
<td></td>
<td><strong>Days until outcome from initial onset of signs (from hospitalization at study centre)</strong></td>
<td>10 (6)</td>
<td>6 (5)</td>
<td>10 (8)</td>
<td>12 (10)</td>
<td>3 (1)</td>
<td>5 (3)</td>
</tr>
<tr>
<td></td>
<td><strong>Potential underlying cause</strong></td>
<td>Post surgery complication</td>
<td>Suspected dietary indiscretion</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Data obtained from 6 dogs with acute pancreatitis for which the scores derived by use of the final clinical severity index and organ scoring scheme were poorly correlated. N = Neutered. SI = Sexually intact. ND = Not done. ERD = Euthanatized as a result of disease.

### 4.3.3 Assessment of serum CRP concentration

In 12 dogs with acute pancreatitis, serum CRP concentration was prospectively evaluated within 24 hours of hospital admission. Three of these dogs had hyperlipidaemia at the time of sample collection. Concurrent diseases in this group included idiopathic epilepsy (n = 2), suspected hyperadrenocorticism (2), and atopic dermatitis that was
controlled with prednisolone (n = 2). All 12 dogs had high serum C-RP concentration; the mean ± SD value was 111.02 ± 103.93 mg/L (range, 24.2 to 374.51 mg/L [reference interval, 0 to 7.6 mg/L]). There was poor correlation between serum CRP concentration and scores derived by use of the clinical severity index (Spearman correlation coefficient = –0.057; P = 0.859) or organ scoring scheme (Spearman correlation coefficient = 0.026; P = 0.936). The mean CRP concentration for survivors (89.2 mg/L ± 71.8, range 24.2-200.32 mg/mL; n = 9) did not differ significantly from the value for non-survivors (176.4 mg/L ± 173.9, range 48.8-374.51 mg/mL; n = 3). There was no significant correlation (Spearman correlation coefficient = –0.362; P = 0.247) between outcome and serum CRP concentration. When serum CRP concentration measured within 2 days of the onset of clinical signs and outcome were analyzed, findings for survivors and non-survivors differed significantly (mean serum CRP concentration, 90.6 mg/L ± 75.2, range 26.77-200.32 [n = 7] and 374.5 mg/L [n = 1]; P = 0.012) but this difference did not represent a significant correlation between serum CRP concentration and outcome (Spearman correlation coefficient = –0.577; P = 0.134).

4.4 Discussion

In dogs, the morbidity and mortality rates associated with acute pancreatitis are high (Watson, 2004). In affected dogs, circulating pancreatic proteases are capable of inciting inflammation systemically and, subsequently, various free-radical, coagulation, complement, and kinin cascades can become activated (Ruaux, 2000). The generation of chemokines and cytokines leads to further tissue inflammation and injury, which in turn may cause multi-organ failure and death. In particular, IL-6, -8, tumor necrosis factor-α, and CRP have been associated with a poor prognosis in people with pancreatitis, whereas platelet-activating factor has been specifically implicated in the development of lung-
associated injury (Chen et al., 1999a; Formela et al., 1994; Mentula et al., 2005). Of these cytokines, it would appear that the circulating concentration of IL-6 has the best predictive value for organ failure in humans with severe pancreatitis (Chen et al., 1999a; Sathyanarayan et al., 2007).

In the present study, serum CRP concentration was measured as previously described (Berghoff et al., 2006; Kjelgaard-Hansen et al., 2003), by use of an ELISA that is specific for canine CRP and accurate for samples stored at temperatures less than –10°C for 3 months. For analysis of canine serum samples, the inter- and intra-assay coefficients of variation of the ELISA have been reported by the manufacturer (Tridelta Phase Canine CRP Assay, Tridelta Diagnostics Inc, Morris Plains, NJ) as 8% and 6.7%, respectively, well within accepted limits for an ELISA. All 12 dogs in which serum CRP concentration was measured in our study had values that were markedly greater than the upper limit of the established reference interval. This finding is consistent with other data reported for other dogs with acute and chronic illnesses, including dogs with acute pancreatitis (Berghoff et al., 2006; Burton et al., 1994; Chan et al., 2006; Holm et al., 2004; Merlo et al., 2006; Ohno et al., 2006; Rush et al., 2006; Spillman et al., 2002; Tecles et al., 2005).

None of the 12 dogs evaluated for CRP in our study had evidence of concurrent inflammatory or neoplastic disease with the exception of mild atopic dermatitis, and it would seem unlikely that atopic dermatitis would have contributed to the high serum CRP concentration in the 2 affected dogs. Serum CRP concentration is unaffected by treatment with prednisolone (Martinez-Subiela et al., 2004; Merlo et al., 2006); thus, the administration of that drug to the 2 dogs with atopic dermatitis was unlikely to have altered the concentrations. Lipaemia was detected present in 3 of the 12 dogs in which serum CRP concentration was assessed. Although lipaemia can artefactually increase serum CRP
concentration, it has minimal impact on clinical interpretation of those values (Ceron et al., 2005; Kjelgaard-Hansen et al., 2003).

In the present study, we failed to identify a correlation between serum CRP concentration and outcome or clinical severity index score. This is in contrast to results of a study in which a difference in serum CRP concentration between dogs with pancreatic necrosis (n = 9) and dogs with oedematous pancreatic inflammation (5) was detected (Spillman et al., 2002). A likely explanation for this disparity is that dogs in our study were probably more severely affected than the dogs evaluated in the previous study, and would all likely have fallen into the pancreatic necrosis group. In another recent study, findings were similar to results of our study, in that although serum CRP concentration was relatively increased in dogs with pancreatitis, all dogs that were critically ill had similar concentrations and it was not possible to differentiate between survivors and non-survivors on the basis of a single CRP value (Chan et al., 2006). The reason that serum CRP concentration may not correlate strongly to outcome may be dependent on the stage of the disease process during which the assessment was made. Serum concentration of CRP sequentially decreases in dogs during treatment for pancreatitis and in humans with acute pancreatitis after 2 days from the onset of clinical signs (Chen et al., 1999a; Holm et al., 2004).

Serum CRP concentration in samples that were obtained within 2 days of the onset of clinical signs had a greater correlation with outcome in the present study than serum CRP measured after 2 days of clinical signs developing, although fewer dogs were assessed. The delay between onset of clinical signs and admission to the hospital in our study appeared to be attributable to the facts that some dogs were treated by their veterinarians before being hospitalised and the owners of others did not seek veterinary care immediately. This period of delay probably provides a true representation of the
attendant times of admission and diagnosis of acute pancreatitis at a veterinary hospital in the general dog population.

Although the number of dogs in which serum CRP concentration was evaluated was small, the results of the present study suggest that assessment of this variable in dogs with acute pancreatitis at the earliest possible time should be considered in conjunction with application of a clinical severity index for acute pancreatitis in dogs, rather than as a stand-alone test to determine disease severity. However, if a serum sample is obtained within 2 days of the onset of clinical signs and is greatly increased, then a poorer prognosis could be given. Sequential assessment of serum CRP concentration may also be an effective and objective method for monitoring response to treatment.

Many clinical classification schemes for acute pancreatitis in humans have been assessed. The acute physiology, age, chronic health evaluation (APACHE) scheme, Ranson scheme, and Balthazar computed tomography severity index have been used with mixed results (Chatzicostas et al., 2003; Eachempati et al., 2002; Williams and Simms, 1999; Yeung et al., 2006). In people, the development of pancreatic infection is considered the worst prognostic indicator, but such infections do not appear to develop in dogs with acute pancreatitis (Bradley, 1993; Isenmann et al., 1999; Williams, 1994). Clinical severity scoring schemes have been developed for assessment of inflammatory bowel disease in dogs and as a survival prediction index in dogs admitted to intensive care units but the latter fails to take into account complications specific to pancreatitis (Jergens et al., 2003; King et al., 2001). A previously published severity score for pancreatitis was calculated on clinicopathological abnormalities determined from sample submissions to a clinical pathology laboratory (Ruaux and Atwell, 1998b). For purposes of that scheme, a diagnosis of pancreatitis was made solely on the basis of whether dogs had increased serum lipase or amylase activity (Ruaux and Atwell, 1998b). As such, that scheme may have included dogs
that did not truly have pancreatitis, because the rate of false-positive diagnosis associated
with assessment of those biochemical variables alone is fairly high (Mansfield and Jones,
2000). The mortality rate for our cohort of dogs that scored 3 and 4 using this laboratory-
based organ score were much lower than those reported in the original study (Ruaux and
Atwell, 1998b). The reported mortality rate in the study involving the clinicopathological
scheme may also be inaccurate because of the possibility that dogs from which samples
were derived did not have pancreatitis, as well the possibility that some dogs that did have
pancreatitis were excluded because the samples did not contain high serum lipase and
amylase activities. Additionally, dogs that were euthanatized as a result of nonmedical
reasons or that did not receive an optimal standard of care may have been included (Ruaux
and Atwell, 1998b). In the present study, scores derived by use of the organ scoring
scheme that was based on clinicopathological variables were not significantly correlated
with outcome.

Interestingly, scores for all but one of the organ systems evaluated for the organ
score (i.e. the endocrine pancreas, leucogram, renal, and hepatic systems) were not
significantly associated with outcome in our study. The remaining system, acid-base
buffering, was not thoroughly assessed in our study because of a lack of consistent blood
gas analysis among dogs during the study period.

The clinical severity index score had a significant correlation with outcome,
although days until outcome were equally associated with severity by use of either
classification scheme. When considering this association in a retrospective manner, it is
difficult to determine if the days until discharge were determined by factors other than
medical reasons. It may be that some dogs were discharged prior to full recovery due to
financial reasons, or that client factors may have precluded prompt discharge. In future
studies, the determination of level of illness (i.e. able to be cared for in a ward situation or in an intensive care unit is likely to be a better assessment than duration of hospitalisation.

In people, the development of severe peritonitis or infected pancreatic necroses is strongly indicative of a poor prognosis, whereas in dogs, pancreatic lesions (abscesses, acute fluid accumulations, or pseudocysts) have been similarly implicated (Edwards et al., 1990; Johnson and Mann, 2006; Salisbury et al., 1988). Interestingly, among the 61 dogs with acute pancreatitis in the present study, local complications, even those considered severe, did not have an impact on outcome. This may reflect the large number of dogs (both survivors and non-survivors) that were recorded as having widespread peritonitis. Six dogs were recorded as having a score ≥ 2 in this category, and 5 of those dogs survived to discharge from the hospital; there was no significant association of scores ≥ 2 with outcome. This probably reflects both the low incidence of pancreatic abscess formation in dogs with acute pancreatitis and the recent trend in human gastroenterology toward conservative treatment of uninfected pancreatic lesions instead of surgical correction (Isenmann et al., 1999). The 5 dogs with pancreatic pseudocysts or acute fluid accumulations that survived were all conservatively treated. Follow-up information was available for 3 of those dogs; in all, the original lesion had resolved within 4 to 6 months.

The development of systemic inflammatory response syndrome has also been identified as a strong indicator of poor prognosis in dogs with any illness, and diagnosis is often made on the basis of WBC abnormalities and high respiratory or heart rates (Okano et al., 2002). Coagulation abnormalities were also included in our initial body-system based score because disseminated intravascular coagulation and thromboembolic complications are potentially devastating consequences of severe acute pancreatitis (Ruaux, 2000). However, the haematopoietic complications initially considered (development of systemic inflammatory response syndrome or thromboembolic disease)
were not correlated with survival in our study. This is despite strong links between these conditions and prognosis for other critical illnesses in dogs (King et al., 2001; Okano et al., 2002). A large percentage of dogs in our study had abnormalities in the haematopoietic category, supporting the notion that virtually all dogs with acute pancreatitis have some degree of inherent systemic inflammation. Stratification within the haematopoietic system category was also assessed, and thromboembolic disease (either overt or subclinical) was not significantly correlated with prognosis. Seven dogs had scores ≥ 3 in this category, and 5 of those dogs recovered. Causes of death of the 2 dogs that died were not recorded as potentially being attributable to thromboembolic complications; therefore, thromboembolic disease does not appear to play an important role in determination of acute pancreatitis severity in dogs. It was not possible to determine the effect of treatment on these haematopoietic factors, but no anti-coagulant therapy was used.

Several factors may have contributed to the greater correlation of outcome with the scores derived by use of the clinical severity index developed in the present study, compared with findings in studies involving previously identified methods. What appears to be of most importance in dogs with acute pancreatitis is intestinal health, in particular during the period in which direct enteral nutrition is lacking, whether this is an intentional occurrence as part of the treatment or a disease-related complication. During starvation, inflammatory cytokines are produced by enterocytes and there is an increased incidence of bacterial translocation as a result of altered intestinal permeability, both of which have been shown to occur in naturally occurring acute pancreatitis in humans and experimentally induced acute pancreatitis in dogs (Abou-Assi et al., 2002; Ammori et al., 1999; Kalfarentzos et al., 1997; Mohr et al., 2003; Powell et al., 2000; Qin et al., 2002a; Windsor et al., 1998). Restoration or maintenance of intestinal health is of potentially great importance when developing treatment strategies for dogs with acute pancreatitis, and
provision of enteral nutrition early in the disease process may prove to be a prime factor in treatment of this condition.

The clinical severity index established in the present study was developed to incorporate easily measurable variables that are most likely to contribute to the overall wellness of a dog with acute pancreatitis. Initial evaluation of this index revealed a significant negative correlation with outcome—as the clinical severity index score increased, the chance of survival decreased. A similar significant negative correlation with outcome was not identified for the organ scoring scheme. The strength of the clinical severity index, compared with the organ scoring scheme, is highlighted by the 6 dogs for which clinical severity index scores were high (mainly because of severely altered intestinal integrity or vascular forces) and poorly correlated with the organ scores. Without inclusion of factors relating to intestinal integrity or vascular forces, these dogs would have been assigned a low clinical severity index score indicative of mild disease. However, 5 of the 6 dogs died or were euthanatized as a result of their disease despite intensive treatment. Serum CRP concentration was measured in 1 of these dogs; and that assessment alone would not have correlated with acute pancreatitis severity or outcome. Further analysis of additional dogs with acute pancreatitis from other veterinary hospitals may result in ongoing modification of the clinical severity scoring index, eventually allowing development of the most robust system possible for severity assessment.

On the basis of sex and age distributions, the group of dogs evaluated in the present study was fairly similar to those evaluated in other studies (Cook et al., 1993; Hess et al., 1998; Mansfield and Jones, 2000) of pancreatitis in dogs, although the breed representation in our study may be geographically unique. Reported rates of mortality in dogs with acute pancreatitis range from 27% to 42%, (Cook et al., 1993; Hess et al., 1998; Mansfield and Jones, 2000; Mansfield et al., 2003; Ruaux and Atwell, 1998b) which suggests that the
group of dogs in our study was representative of the general population of dogs with acute pancreatitis, that treatment was essentially appropriate, and that there was no pre-selection for the most severe cases (no dogs with chronic pancreatitis were knowingly included). The inclusion criteria in our study allowed dogs with ultrasonographic evidence of pancreatitis (with supportive clinical findings and clinicopathological abnormalities) to be assessed. In 1 study, the sensitivity of ultrasonography for diagnosis of pancreatitis in dogs was approximately 62% (Mansfield and Jones, 2000). Although this sensitivity is relatively low, dogs with acute and chronic pancreatitis were evaluated in the study; thus the true diagnostic value of ultrasonography in dogs with acute pancreatitis is probably much higher. In dogs, the typical ultrasonographic changes associated with acute pancreatitis include a large pancreas with a hypoechoic appearance and cavitatory lesions, dilated pancreatic duct, and hyperechoic mesentery (Lamb, 1989; Lamb and Simpson, 1995; Murtaugh et al., 1985; Williams, 1994). In comparison to the situation in people, advanced imaging such as contrast-enhanced computed tomography may have no advantage over ultrasonography for the diagnosis of acute pancreatitis in dogs (Jaeger et al., 2003). This likely reflects the fact that infected pancreatic necrosis (for which computed tomography is considered the gold standard diagnostic tool) develop less frequently in dogs than in people (Isenmann et al., 1999). In the present study, it is possible that some dogs with pancreatitis were evaluated at the hospital but were not included in the analyses because the diagnosis was not confirmed ultrasonographically. The rate of false-positive diagnosis of pancreatitis via ultrasonography is likely to be low with experienced operators, so it was assumed that all dogs that were included in our study did in fact have acute pancreatitis. The classification of pancreatitis is confusing and problematic, but in dogs and people, it is assumed that the acute form involves some degree of necrosis (Bradley, 1993; Charles, 2007; Watson, 2004). Although ultrasonography cannot determine the cellular process
within the pancreas, pancreatic neoplasia is considered unlikely in the dogs included in the present study because the outcome for each individual is known. Often the presence of pancreatic neoplasia is accompanied by pancreatic inflammation and necrosis; thus, the definitive differentiation of neoplasia and inflammation in the short term would have little bearing on our study results (Williams, 1994). Ultrasonography is also considered a highly sensitive technique for detection of pancreatic complications such as pseudocysts, acute fluid accumulations, or abscesses (Johnson and Mann, 2006; Murtaugh et al., 1985; Rutgers et al., 1985; Schaer, 1979; Williams, 1994).

4.5 Conclusion

For dogs with acute pancreatitis, use of a clinical severity scoring index, such as that developed in the present study, along with sequential measurement of serum CRP concentration appears to be helpful in prognostic determination. Nevertheless, the clinical severity index score should not be used as the sole criterion on which treatment or prognosis in acute pancreatitis-affected dogs is determined at present, because some dogs in our study that had high scores survived to discharge from the hospital and others that had lower scores died or were euthanatized. The clinical severity index may be useful in allowing multicenter comparison of treatments of dogs with severe naturally occurring acute pancreatitis. As new treatments based on an improved understanding of the pathophysiology of this disease are developed, use of this index may allow objective assessments of the usefulness of particular treatment options to be made.
Chapter 5: Retrospective analysis of the effect of plasma and microenteral nutrition administration on mortality and morbidity in dogs with acute pancreatitis

This chapter has not been submitted for publication.

5.1 Introduction

Most treatment recommendations for canine acute pancreatitis are based on general supportive principles or extrapolated from human and experimental studies in species other than dogs. Plasma transfusion is currently recommended in the veterinary literature for the treatment of this disease (Logan et al., 2001; Snow et al., 2010; Williams and Steiner, 2005). Purported benefits include replacement of circulating α-macroglobulins, replacement of coagulation factors and treatment of systemic inflammatory response syndrome (SIRS) with anti-inflammatory factors (Weatherton and Streeter, 2009). Depletion of α-macroglobulin has been documented in an experimental model of canine pancreatitis, and correlated with severity of pancreatic inflammation (Murtaugh and Jacobs, 1985). Systemic effects of the pancreatic inflammation were not assessed in that study, nor whether there would have been amelioration with administration of plasma. It is unlikely that any benefit seen with plasma will be due to colloid like properties, as fresh frozen plasma (FFP) has only about 20-30% of the oncotic properties of colloids (Iazbik et al., 2001; Yaxley et al., 2010). However, haemostatic proteins are well preserved in FFP, regardless of whether freeze-thawed or not (Iazbik et al., 2001; Yaxley et al., 2010).

There are no published prospective studies in naturally occurring acute pancreatitis to support many of the current treatment recommendations such as plasma transfusion in dogs. One retrospective study identified a negative correlation between plasma administration and survival (Weatherton and Streeter, 2009). As the dogs included in that study were not stratified according to severity, it is difficult to elucidate whether more
severely affected dogs were selectively administered plasma to start with, contributing to the poor mortality.

Nutritional intervention in acute pancreatitis and other diseases necessitating critical care are beginning to be evaluated in the veterinary literature (Chan et al., 2006; Chan et al., 2002; Freeman et al., 1995; Mansfield et al., 2011a; Mansfield et al., 2011b; Chapter 7; Mohr et al., 2003; Queau et al., 2011). Historically, the recommendation in canine pancreatitis is to rest the pancreas, and provide no nutrition via the gut (Simpson and Lamb, 1995; Williams and Steiner, 2005). This paradigm is being challenged in human gastroenterology, where current consensus opinion is to feed in people with severe acute pancreatitis as early as possible (Johnson, 2005; Meier et al., 2006). This shift was based on experimental observations that fasting led to mucosal atrophy, changes in the goblet cell function within the intestine, and decreased glutamine transportation, potentially leading to oxidative stress (Hernandez et al., 1999; King and Kudsk, 1997; Sarac et al., 1994; Sharma and Schumacher, 1995).

Interventional nutrition in the form of feeding tubes is sometimes difficult to apply in veterinary practice. A relatively easy method by which to accomplish nutrition delivered into the gut is by insertion of a naso-oesophageal feeding tube, a procedure which does not require sedation or general anaesthesia. The drawback with this methodology is the tubes normally able to be inserted by this method are very narrow gauge and so only very liquid formulations can be delivered. In Australia, no veterinary liquid diet is available that is of the appropriate consistency, and so one potentially useful method is to trickle in a balanced electrolyte solution that contains glucose to try and go some way to overcoming the negative nitrogen balance that occurs in pancreatitis (Abou-Assi and O'Keefe, 2001). For the purposes of this study, this is referred to as microenteral nutrition (MEN). Whether there is potential for MEN to also directly counteract gut mucosal atrophy is unknown.
The aim of this project is to assess whether plasma administration or MEN administration is beneficial, when stratified according to the severity criteria developed in Chapter 4.

5.2 Materials and Methods

5.2.1 Animal selection

A database review of cases seen at Murdoch University (primary emergency accession centre and referral hospital) for the period between 1999 and 2006 was performed. Search terms included pancreatitis, acute pancreatitis, pancreatic necrosis or suspected pancreatitis. Inclusion criteria were evidence of acute pancreatitis detected via abdominal ultrasonography or histological examination of surgical or necropsy tissue samples, admission to hospital for treatment, complete daily clinical records (including all medical treatments), and known outcome, as per Chapter 4. Ultrasonography using a curvilinear 8 MHz or linear 15 MHz probe (Sequioa 512, Acuson, Munich, Germany) was performed by a board-certified (or equivalent Australian qualification) radiologist. A diagnosis of acute pancreatitis was made if there was evidence of a large pancreas with patchy echogenicity or hyperechoic mesentery. Dogs were excluded if they were not admitted for treatment, received TPN or full EN or were euthanatized for nonmedical reasons.

5.2.2 Data collection

For each dog that fulfilled the inclusion criteria, duration of hospitalisation and outcome (died, euthanatized due to medical reasons or survived to discharge) was recorded, along with the clinical severity index as previously described in Chapter 4 based on data collected within 12 hours of admission (Mansfield et al., 2008). The administration of plasma or MEN was also recorded, if given within 72 hours of the clinical severity
index calculation. For the purposes of this study, MEN was defined as a trickle electrolyte solution (Lectade®; Jurox Pharmaceuticals, Rutherford NSW, Australia) delivered via a naso-oesophageal tube at 0.5 mL/kg/hour.

5.2.3 Statistical Analysis

Association between plasma treatment and survival, and between use of MEN and survival in all cases was calculated using Fisher’s exact test. Association between plasma treatment or use of MEN and survival in cases with a modified clinical severity index greater than or equal to two was also calculated using Fisher’s exact test. A Mann-Whitney test was used to compare modified clinical severity scores between two groups. A $P$ value $< 0.05$ was considered significant. Stata v12.0 (StataCorp, Texas, USA) was used for statistical analyses.

5.3 Results

Analysing all data, there were 59 dogs that fulfilled the inclusion criteria. Two of the previously identified dogs in Chapter 4 were excluded due to administration of TPN.

The treatment group, outcome, duration of hospitalisation and clinical severity index on admission of these 59 dogs identified on retrospective review of medical records are detailed in Table 5.1. Of the dogs with a clinical severity index $\geq 2$, four received MEN plus plasma and supportive care, but no other form of interventional nutrition in the first 3 days of admission. All 4 dogs survived, with a range in duration of hospitalisation from 6-10 days (median 8 days). Six dogs received plasma treatment, but no MEN or other nutritional support. Three of these dogs died, with a hospitalisation duration ranging from 3-10 days (median 6 days). One dog had nutritional support in the form of MEN, but did not receive any plasma. This dog survived with a hospitalisation of 11 days.
Thirteen dogs were treated with plasma and 10 (76.9%) recovered, compared to 46 dogs that received no plasma, of which 37 (80.4%; P=0.72) recovered. When only dogs with a modified clinical severity index ≥ 2 were analysed, there was still no effect of plasma treatment on survival. Ten of these dogs were treated with plasma and 7 (70%) recovered, whilst 20 of these dogs were not treated with plasma, of which 12 (60%) recovered. The $P$ value using Fisher’s exact test was 0.70, indicating no statistically discernible benefit in giving plasma.

If dogs with a clinical severity index ≥ 2 that were administered plasma but also received MEN were excluded, there was no difference in survival (3/6 dogs given plasma survived vs. 11/19 dogs not given plasma survived; $P = 1.0$). It was not possible to determine from the retrospective case records the clinician’s reasoning for administering plasma to individual dogs. There was no individual factor of the clinical severity index that appeared to be related to this.

When all dogs that had received plasma transfusions (regardless of other treatments) were compared to all other treatment groups (excluding total parenteral nutrition) there was no difference in outcome ($P = 0.72$), however there was a difference in initial clinical severity index ($P = 0.0039$). The median (range) of the clinical severity index in the 13 dogs given plasma was 4 (0-6) and the median in the dogs not given plasma was 1 (0-5).

When all dogs that had received MEN (regardless of other treatments) were compared to all treatment groups there was no statistical difference in initial clinical severity index ($P = 0.078$). The difference in outcome between all dogs that received MEN (8/8; 100%) and those that didn’t (39/51; 76%) was not statistically significant ($P = 0.19$). Analysis of dogs with an initial clinical severity index ≥ 2 showed that all 5 dogs receiving
MEN survived and 14 of 25 (56%) dogs not receiving MEN survived (P = 0.13). MEN had only been instituted in 2001.

Assuming a survival rate of 70% without extra treatment such as nutrition or plasma, sample sizes required to prove a 10%, 20% and 25% improvement in mortality with each treatment are 313, 69 and 39 respectively. This is with 80% power and Type I error of 5%.
### Table 5.1

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Sex</th>
<th>CSI admission</th>
<th>LOH</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN + plasma</td>
<td>Bull terrier X</td>
<td>9</td>
<td>MN</td>
<td>4</td>
<td>6</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Australian cattle dog X</td>
<td>7</td>
<td>FN</td>
<td>4</td>
<td>10</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Border collie</td>
<td>9</td>
<td>MN</td>
<td>4</td>
<td>8</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Jack Russell terrier</td>
<td>3</td>
<td>FN</td>
<td>5</td>
<td>8</td>
<td>Survived</td>
</tr>
<tr>
<td>MEN only</td>
<td>Miniature poodle</td>
<td>9</td>
<td>FN</td>
<td>4</td>
<td>11</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Miniature schnauzer</td>
<td>9</td>
<td>FE</td>
<td>1</td>
<td>5</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Miniature schnauzer</td>
<td>11</td>
<td>MN</td>
<td>1</td>
<td>5</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Bull terrier X</td>
<td>4</td>
<td>FN</td>
<td>1</td>
<td>4</td>
<td>Survived</td>
</tr>
<tr>
<td>Plasma only</td>
<td>Kelpie X</td>
<td>3</td>
<td>ME</td>
<td>4</td>
<td>6</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Border collie</td>
<td>7</td>
<td>FN</td>
<td>5</td>
<td>6</td>
<td>Died</td>
</tr>
<tr>
<td></td>
<td>Corgi</td>
<td>8</td>
<td>MN</td>
<td>6</td>
<td>10</td>
<td>Died</td>
</tr>
<tr>
<td></td>
<td>Collie X</td>
<td>6</td>
<td>FN</td>
<td>1</td>
<td>7</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Shih Tzu</td>
<td>5</td>
<td>MN</td>
<td>3</td>
<td>8</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>Bull terrier</td>
<td>8</td>
<td>FN</td>
<td>4</td>
<td>3</td>
<td>Died</td>
</tr>
<tr>
<td></td>
<td>Flat Coat retriever</td>
<td>9</td>
<td>ME</td>
<td>1</td>
<td>5</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>FN</td>
<td>3</td>
<td>6</td>
<td>Survived</td>
</tr>
<tr>
<td></td>
<td>West Highland White terrier</td>
<td>9</td>
<td>FN</td>
<td>0</td>
<td>3</td>
<td>Survived</td>
</tr>
</tbody>
</table>

Details of dogs that were retrospectively identified to have received micro-ental nutrition (MEN) and/or plasma transfusion for the treatment of severe pancreatitis, but no other nutritional intervention, at Murdoch University Veterinary Hospital in the period 1999-2006. CSI = clinical severity index; LOH = length of hospitalisation (days).

### 5.4 Discussion

Plasma treatment alone was shown to have no beneficial treatment in all dogs and in all dogs with a clinical severity index \( \geq 2 \) in our study. Controlled studies in people would also suggest that there is no benefit of fresh frozen plasma over albumin infusion, at either a high or low dose rate (Leese et al., 1991; Leese et al., 1988). The main clinical rationale for the use of fresh frozen plasma in our study population appears to have been for prevention of complications such as disseminated intravascular coagulation or other
inflammatory-mediated syndromes rather than to provide oncotic support. Although this study only evaluated a small numbers of dogs, and therefore only had power to detect large improvements in mortality, it is suggestive that plasma administration may not actually be beneficial in canine acute pancreatitis. This reflects the current trends in veterinary medicine, and also a recent retrospective report (Snow et al., 2010; Weatherton and Streeter, 2009).

All cases that received plasma transfusion also received concurrent treatment such as analgesia, intravenous crystalloid fluid therapy and anti-emetic treatment considered standard for acute pancreatitis, and thus do not reflect a sub-group of the population that received sub-optimal treatment in other regards. However, the treatment was not standardised amongst dogs, which along with the low numbers is a major limitation of this type of study. It is these flaws that limit the clinical conclusions that can be drawn from this study, but do suggest large scale studies (with 319 dogs in the treatment group) would be necessary to show a 10% improvement in survival.

The most recent new treatment protocol introduced into the hospital during the period of analysis (in 2001) was the introduction of early MEN in all cases of acute pancreatitis. Eight dogs with acute pancreatitis received MEN, whilst 4 of these also received a plasma transfusion. Potential reasons for benefit of MEN include improvement of enterocyte health, general reversal of a catabolic state, decreasing incidence of bacterial translocation and reduction of systemically circulating inflammatory cytokines, although these are purely speculative.

The potential benefit of MEN did not translate to a discernible statistical benefit in our analysis. Effectively, this type of ‘nutrition’ doesn’t supply any direct nutrients except glucose and electrolytes, and so is unlikely to contribute to caloric intake to any discernible degree. It is unknown what direct effect (if any) these would have on the enterocytes. It
may be more relevant to study trickle nutrition containing glutamine, due to the potential for glutamine to be directly beneficial to the enterocytes (De Beaux et al., 1998, Furst et al., 1997). Further studies may prove MEN to be of no benefit, and full enteral nutrition may provide the only discernible impact on this disease. There was however a very low number of dogs given MEN in this study.

There are obviously a large number of flaws in this study. Namely, the retrospective nature leads to selection bias, there is no control group, and the study size is small. By correlating treatment decision to the clinical severity index, this flaw became apparent as plasma administration was given to dogs with more severe disease overall. This is likely to reflect the views of clinicians who felt that plasma would be of most benefit in severe animals. Despite this flaw, when comparing severely affected dogs only, no benefit of plasma on survival was discernible. Prospective, well controlled trials are necessary to further investigate the potential benefit, or lack thereof, for plasma administration in dogs.

5.5 Conclusion

Currently, given the high cost of plasma it would not appear that there is strong clinical evidence for its use in canine acute pancreatitis. However, large multi-center studies are required before this can be conclusively shown, with a minimum number of 313 in each treatment group required to show a 10% improvement in survival. To be truly clinically significant, such a trial would need to be randomized and controlled. Additionally, it would appear that MEN without other interventional nutrition has little to no effect, albeit this is based on very small numbers and only assessed a solution containing electrolytes and glucose, not amino acids like glutamine that may be directly beneficial to the gut.
Chapter 6: Pancreatic response in healthy dogs fed diets of various fat compositions

The following is a modified version of the paper: Fleur E. James, Caroline S. Mansfield, Jörg M. Steiner, David A. Williams & Ian D. Robertson (2009) Journal of the American Veterinary Medical Association 70: 614-618. The author of this thesis designed the study, as well as contributed to sample and data collection, data analysis, and equally to the writing of the paper. It is included in this thesis to give a clear understanding of the reasoning behind the nutritional intervention in the following chapter.

6.1 Introduction

Canine acute pancreatitis can be a challenging disease to manage and may be associated with high morbidity and mortality rates. Part of the traditional treatment recommendation in the management of this disease has been to withhold food from dogs followed by feeding an ultra–low-fat diet. Results of recent studies in humans and experimental studies in dogs indicate that provision of enteral nutrition early in the course of the disease improves survival and decreases complication rates, but the ideal diet has never been determined (Meier and Beglinger, 2006; Qin et al., 2002a; Weber and Adler, 2003).

Serum canine trypsin-like immunoreactivity (cTLI) concentration is a specific marker of exocrine pancreatic mass; assessment of this variable has a relatively low sensitivity for detection of pancreatitis yet is the most sensitive and specific test for the diagnosis of exocrine pancreatic insufficiency in dogs (Hess et al., 1998; Mansfield and Jones, 2000; Williams and Batt, 1988). Because measurement of serum cTLI concentration includes all circulating cationic trypsinogen and approximately 80% of cationic trypsin, it may also be used to assess pancreatic adaptation or function (Watson, 2004). Compared with total serum lipase activity, assessment of serum canine pancreatic lipase immunoreactivity (cPLI) activity appears to have improved sensitivity and specificity as a commercially available laboratory test for diagnosis of pancreatitis in dogs; moreover, serum cPLI concentration is unaffected by prednisolone administration or
concurrent renal failure (Steiner, 2003; Steiner et al., 2001; Steiner et al., 2003). Serum cPLI concentration is reduced in dogs with exocrine pancreatic insufficiency and may also be a useful marker to indirectly determine the degree of pancreatic adaptation or response within an individual dog (Steiner et al., 2006).

In the gastric antrum, G-type cells secrete gastrin in response to gastric distension and ingestion of protein (Williams, 1996). The main forms of gastrin secreted in dogs are gastrin 34, 17, and 14; collectively, they have a short circulating half-life of 3 to 9 minutes (Williams, 1996). The presence of gastrin, other gastrointestinal hormones such as cholecystokinin (CCK), and enteric neuropeptides stimulate pancreatic acinar cells to release lysosomes and zymogens in response to food (Williams, 1996). This response to food occurs both by the anticipation and smell of food, mediated via neural pathways, as well as the presence of food in the stomach and small intestine, mediated via hormonal pathways (Williams, 1996). Thus, measurement of serum gastrin concentration may serve as an indirect measure of one aspect of pancreatic stimulation.

The purpose of the study reported was to determine whether amounts of dietary fat or addition of pancreatic enzymes and MCTs to diets alters concentrations of cTLI, cPLI, and gastrin in healthy dogs.

6.2 Materials and Methods

6.2.1 Analytical validation of an assay for gastrin

An automated chemiluminescent, enzyme-labeled immunometric assay (Immunolite 2000, Diagnostic Products Corporation, Los Angeles, California, USA) was used. The assay was based on a ligand-labeled murine monoclonal capture-antibody that was specific for gastrin and involved separation by use of an anti–ligand-coated solid
phase (DPC, 2005). To analytically validate this assay for use in dogs, the interassay variability was assessed via measurement of the coefficient of variation (CV) and the intra-assay variability was assessed via measurement of the CV and linearity. A blood sample (3 mL) was collected via jugular venipuncture from 3 healthy dogs (designated as dogs A, B, and C; dogs B and C also participated in the analytical part of the study), that each weighed > 9 kg and all had body condition scores <6/9, and serum was obtained. For each dog, serum gastrin concentration was measured. On the basis of findings, dog A was classified as having low (< 8.0 pg/mL), dog C as medium (8.0 – 13.0 pg/mL), and dog B as high concentration of gastrin (> 13.0 pg/mL). A second blood sample (20 mL) was collected from dog A after food was withheld for 12 hours. Dogs B and C were each fed one of the experimental diets to stimulate gastrin release (diet A that contained 16% crude fat per dry matter) and the second blood sample (12.5 mL) was collected 5 to 10 minutes after feeding. Blood samples were placed on ice and allowed to clot, prior to centrifugation; serum was separated promptly by use of a refrigerated centrifuge at 10°C and immediately divided into aliquots of 100 to 250 μL that were stored at –18°C.

Aliquots of serum were used to prepare 3 batches for analysis (30 replicates per batch). Batch 1 consisted of 28 replicates of dog A serum (low-concentration control samples) and 2 replicates of dog B serum (high-concentration control samples). Batch 2 consisted of 2 replicates of dog A serum, 2 replicates of dog B serum, and 26 replicates of dog C serum (medium-concentration control samples). Batch 3 consisted of 3 replicates of dog A serum, 6 replicates of dog B serum, and 3 replicates of dog B serum for each of the following dilutions: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Serum from dog A was used as the diluent. Each batch was tested as an entire batch and separate from other batches.

The specificity of the assay for detection of gastrin in human serum had been determined by the manufacturer on the basis of results of assays of samples with high
concentrations of mini-gastrin; low, medium, and high concentrations of sulphated gastrin G-17 (type II); low, medium, and high concentrations of both sulphated (type II) and non-sulphated (type I) gastrin G-34; medium and high concentrations of non-sulphated (type I) gastrin G 1-13; low, medium, and high concentrations of pentagastrin; and low, medium, and high concentrations of cerulein (DPC, 2005). The assay antibody interacts predominantly with G-17, with lesser interaction with G-34, mini-gastrin, and cerulein (DPC, 2005). Recovery studies were performed by the manufacturer and results indicated mean recoveries for G-17 type II and G-17 type I of 119% and 116%, respectively (DPC, 2005). Haemolysis did not appear to interfere with the test (DPC, 2005). It was reported that bilirubin at concentrations > 85.5 µmol/L may interfere with the test, and that serum triglyceride concentrations > 11 mmol/L results in degradation of values (DPC, 2005). The assay range was 5 to 1,000 pg/mL, and results < 5 pg/mL were recorded as 0 pg/mL.

6.2.2 Study protocol

The study was approved by the Animal Ethics Committee at Murdoch University, fulfilling requirements of the National Health and Medical Research Council. Healthy staff-owned dogs that weighed > 9 kg were recruited into the study with owner consent. The dogs had no prior history of pancreatitis or clinically important gastrointestinal disease. All dogs were determined to be healthy on the basis of results of physical examination, haematology, serum biochemical analyses, urinalysis, and assessments of serum amylase and lipase activities. No dogs were considered to be more than 10% overweight.

Four experimental diets were used in the study, and each dog was fed all 4 diets once in random order using a Latin square design at 1-week intervals. Between dietary treatments, dogs were fed their normal diet, which did not exceed 24% dry matter in any
individual dog. Diet A was a maintenance commercial dog food (PurinaONE, adult dog chicken and rice formula, Nestle Purina PetCare Company, St Louis, Missouri, USA) that had a crude fat content of 16% dry matter (as stated by the manufacturer). Diet B was a low-fat commercial dog food (Royal Canin digestive low fat Canine Veterinary Diet, Royal Canin, Aimargues, France) that had a crude fat content of 5% dry matter as stated by the manufacturer. Diet C was composed of diet A with supplemental pancreatic enzymes (Creon 5000, Creon 10000 and Creon Forte, Solvay Pharmaceuticals, Pymble, NSW) containing lipase, 526 to 1,667 British Pharmacopoeia units/kg; amylase, 421 to 1,200 British Pharmacopoeia units/kg; and protease, 31 to 67 European Pharmacopoeia units/kg. Diet D was composed of diet B with supplemental pancreatic enzymes (as described for diet C) and medium chain triglyceride (MCT) oil (SHS International Ltd, Liverpool, UK) containing 0.5 mL/kg; C₈ and C₁₀ fatty acids composition > 95%. Food was withheld from the dogs for at least 12 hours prior to the feeding of each diet on the test dat. For each dog, the amount fed of each diet was based on half the daily calculated maintenance energy requirement (resting energy requirement x 1.4). A blood sample was collected from each dog via jugular venipuncture before (0 hours [baseline]; 12.5mL) and at 5 to 10 minutes (2.5mL), 1 to 2 hours (3mL), and 6 hours (3mL) after feeding each experimental diet. Blood samples were placed on ice and allowed to clot, prior to centrifugation; serum was separated promptly by use of a refrigerated centrifuge at 10°C and stored at −18°C. Serum cTLI (commercial RIA, Diagnostic Products Corporation, Los Angeles, California, USA) and cPLI (ELISA) concentrations were measured at 0, 1 to 2, and 6 hours after feeding. Both cTLI and cPLI were measured at the Gastrointestinal Laboratory, Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, Texas A & M University, College Station, Texas. Serum gastrin concentration was measured at 0 hours, 5 to 10 minutes, and 1 to 2 hours after feeding.
6.2.3 Data analysis

The results were analysed by use of a repeated-measures general ANOVA (SPSS, version 14.0; SPSS Inc, Chicago, USA). Differences in the dogs’ serum cTLI, cPLI, and gastrin concentrations among diets fed, among dogs, and over time were analysed with significance set at a value of $P \leq 0.05$. To assess variation in cTLI and cPLI concentrations from baseline (0 hours) at 1 to 2 and 6 hours after feeding, the percentage change was calculated. To assess variation in gastrin concentrations from baseline (0 hours) at 5 to 10 minutes and 1 to 2 hours after feeding, the actual change rather than percentage change was calculated to allow for zero values. The interassay CV was determined for serum gastrin concentrations when samples were assayed on different days.

6.3 Results

6.3.1 Dogs

Ten healthy dogs were used in the study. Their ages ranged from 1 to 12 years (mean ± SD age, 5.7 ± 3.68 years) and weights ranged from 9.8 to 40.8 kg (mean weight, 26.5 ± 9.4 kg). There were 2 sexually intact males, 4 castrated males, and 4 spayed females. Among the dogs, there were 2 Labrador retrievers, 1 Gordon setter, 1 Miniature schnauzer, 1 Dalmatian, 1 Australian cattle dog, 1 Greyhound, and 3 mixed-breed dogs.

6.3.2 Serum sample quality

No samples in the present study were grossly lipaemic, haemolysed, or icteric.

6.3.3 Gastrin assay validation

On the basis of the data obtained by use of the enzyme-labeled immunometric assay on serum samples obtained from 3 healthy dogs, the mean interassay CV was
11.56% and the mean intra-assay CV was 11.41%. Dilutional parallelism evaluations revealed acceptable linearity (Figure 6.1).

**Figure 6.1** Results of the dilutional parallelism evaluations of an enzyme-labeled immunometric assay for measurement of serum gastrin concentration in dogs. Serum samples were collected from 3 healthy dogs (designated as dogs A, B, and C). The assay was performed on 3 replicates of dog A serum, 6 replicates of dog B serum, and 3 replicates of dog B serum for each of the following dilutions: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. Serum from dog A (low gastrin concentration) was used as the diluent.
6.3.4 Serum cTLI concentration

Among the 10 dogs during the 4 experimental periods, baseline cTLI concentration ranged from 4.4 to 128.5 μg/L (mean ± SD, 13.6 ± 19.5 μg/L). Mean serum concentrations of cTLI at 1 to 2 hours and at 6 hours after feeding were 12.6 and 12.3 μg/L, respectively, in dogs fed diet A; 11.9 and 11.2 μg/L, respectively, in dogs fed diet B; 12.6 and 11.3 μg/L, respectively, in dogs fed diet C; and 12.8 and 11.4 μg/L, respectively, in dogs fed diet D. For each diet, the percentage change (from baseline) in serum cTLI concentration over time was calculated (Figure 6.2).

![Figure 6.2](image)

**Figure 6.2** Percentage change in mean serum cTLI concentrations in 10 healthy dogs before (0 hours [baseline]) and at 1 to 2 and 6 hours after consumption of 1 meal of each of 4 diets (1-week interval between diet treatments). Dogs were fed a maintenance commercial dog food that had a crude fat content of 16% (diet A [circles]); a low-fat commercial dog food that had a crude fat content of 5% (diet B [diamonds]); diet A with supplemental pancreatic enzymes (diet C [triangles]); and diet B with supplemental pancreatic enzymes and MCT oil (diet D [squares]). Food was withheld from the dogs for at least 12 hours prior to the feeding of each diet. Data are presented as mean ± SD percentage change from baseline values.
6.3.4 Serum cPLI concentration

Among the 10 dogs during the 4 experimental periods, the baseline cPLI concentration ranged from 5.3 to 169.6 μg/L (mean, 29.6 ± 31.6 μg/L). The mean serum concentration of cPLI at 1 to 2 hours and at 6 hours after feeding were 24.3 and 24.2 μg/L, respectively, in dogs fed diet A; 28.5 and 24.2 μg/L, respectively, in dogs fed diet B; 33.6 and 28.5 μg/L, respectively, in dogs fed diet C; and 59.2 and 42.6 μg/L, respectively, in dogs fed diet D. For each diet, the percentage change (from baseline) in serum cPLI concentration over time was calculated (Figure 6.3).

**Figure 6.3** Percentage change in mean serum cPLI concentrations in 10 healthy dogs before (0 hours [baseline]) and at 1 to 2 and 6 hours after consumption of 1 meal of each of 4 diets (1-week interval between diet treatments). Food was withheld from the dogs for at least 12 hours prior to the feeding of each diet. Data are presented as mean ± SD percentage change from baseline values. Dogs were fed a maintenance commercial dog food that had a crude fat content of 16% (diet A [circles]); a low-fat commercial dog food that had a crude fat content of 5% (diet B [diamonds]); diet A with supplemental pancreatic enzymes (diet C [triangles]); and diet B with supplemental pancreatic enzymes and MCT oil (diet D [squares]).
6.3.5 Serum gastrin concentration

Among the 10 dogs during the 4 experimental periods, the baseline gastrin concentration ranged from < 5 to 16 pg/mL (mean, 2.2 ± 4.3 pg/mL). The mean serum concentration of gastrin at 5 to 10 minutes and at 1 to 2 hours after feeding were 8.3 and 11.0 pg/mL, respectively, in dogs fed diet A; 10.9 and 8.2 pg/mL, respectively, in dogs fed diet B; 9.1 and 8.9 pg/mL, respectively, in dogs fed diet C; 8.0 and 12.7 pg/mL, respectively, in dogs fed diet D. For each diet, the actual change (from baseline) in serum gastrin concentration over time was calculated (Figure 6.4).

![Figure 6.4](image)

**Figure 6.4** Change in mean serum gastrin concentrations in 10 healthy dogs before (0 hours [baseline]) and at 5 to 10 minutes and 1 to 2 hours after consumption of 1 meal of each of 4 diets (1-week interval between diet treatments). Food was withheld from the dogs for at least 12 hours prior to the feeding of each diet. Data are presented as mean ± SD actual change from baseline values. Dogs were fed a maintenance commercial dog food that had a crude fat content of 16% (diet A [circles]); a low-fat commercial dog food that had a crude fat content of 5% (diet B [diamonds]); diet A with supplemental pancreatic enzymes (diet C [triangles]); and diet B with supplemental pancreatic enzymes and MCT oil (diet D [squares]).

6.3.6 Data comparisons

In the study dogs, serum cPLI and cTLI concentrations did not differ significantly among diets fed, among dogs, or over time. Compared with the other diets, diet D was
associated with a higher mean baseline serum cPLI concentration, but this difference was not significant \((P = 0.2)\). Serum gastrin concentration at 0 hours was significantly different from values at the 2 later time points after feeding diet D. Serum gastrin concentration did not differ among diets fed; diet D induced the least amount of pancreatic response, compared with the effects of the other diets, although this difference was not significant \((P = 0.33)\). No significant deviation from this finding was apparent in data for any individual dog.

### 6.4 Discussion

Known triggers for pancreatic stimulation include gastric distension, dietary protein and fatty acid intake, emptying of gastric contents into the duodenum, and enteric neuropeptides (Williams, 1996). Results of the present study indicated no significant effect of the content of fat or the presence or absence of supplemental pancreatic enzymes and MCTs on the degree of pancreatic stimulation (assessed by measurement of serum cTLI, cPLI, and gastrin concentrations) in healthy dogs. Serum gastrin concentration was monitored to assess the immediate effects of gastric distension on pancreatic stimulation and serum cTLI concentration was monitored to assess overall pancreatic function. Assessment of serum cPLI concentration was also undertaken; this variable reflects the pancreatic response in relation to varying dietary fat content because co-lipase is activated and converted into lipase to enable fat digestion, and because cPLI is specific to the pancreas.

The variables measured in our study are considered indirect indices of pancreatic stimulation in dogs. Gastrin, cTLI, and cPLI were chosen as indicators because they remain stable in serum samples and because high-specificity assays for measurement of serum concentrations are commercially available. To determine the degree of pancreatic
response in healthy dogs in the study of this report, the percentage changes in serum cTLI and cPLI concentrations and the actual change in serum gastrin concentration were assessed over time and among the 4 diets fed. It is possible that the measurements of these serum markers of pancreatic function were not sufficiently sensitive to accurately assess exocrine pancreatic stimulation and instead more direct intraduodenal markers should be measured. However, the latter procedures are more technically difficult and invasive to perform. In our opinion, the variables measured in the present study are useful as indirect measures of pancreatic response. It was not possible to calculate the percentage change in serum gastrin concentration from baseline values for all dogs at all time points after feeding of the diets because some concentrations were less than the detection limit of the assay. The assessment of serum gastrin was still considered appropriate because none of the concentrations were less than the detection limit of the assay during the validation process. The fact that some of the study dogs had serum gastrin concentrations that were less than the detection limit of the assay at various time points during the 4 diet treatments is considered to reflect individual variation rather than inaccuracy of the test.

Because of the short half-life of gastrin, serum concentrations were assessed at 5 to 10 minutes after feeding and again at 1 to 2 hours after feeding. Mean serum concentrations of gastrin at these 2 time points after feeding did not differ significantly, which likely represents ongoing secretion because of the persistence of food within the stomach 2 hours after feeding. There was no significant difference in the dogs’ serum gastrin concentration over time, among the 4 diets fed, or among dogs; thus, the preferred sample collection time point cannot be determined.

The specificity of the gastrin assay used in the present study was assessed by the manufacturer. The antibody in the assay reacts predominantly with G-17 and to lesser extents with G-34, mini-gastrin, and cerulein (DPC, 2005). Cerulein is considered an
important pancreatic stimulant (DPC, 2005; Williams and Steiner, 2005). Haemolysis in serum samples does not interfere with the test (DPC, 2005). The validation procedure performed in the present study revealed acceptable intra- and inter-assay CVs for an enzyme-based assay; therefore, results obtained for the study dogs can be considered reliable. Bilirubin concentrations > 85.5 µmol/L may interfere with the test and serum triglyceride concentrations > 11 mmol/L has been associated with degradation of values (DPC, 2005). No samples in the present study were grossly lipaemic, haemolysed, or icteric.

In this study, indirect indicators of pancreatic adaptation or response were measured in 10 healthy dogs that had no previous history of pancreatitis or any other intestinal illness. It is unknown whether differences in diet composition, especially the percentage of crude fat, can markedly alter the degree of pancreatic stimulation or outcome in dogs with a history of naturally occurring pancreatitis. Standard enteral diets contain long-chain triglycerides and consumption of such diets increases cholecystokinin (CCK) secretion and subsequently pancreatic stimulation in people (Shea et al., 2003). Enteral administration of medium-chain triglycerides is thought to result in minimal CCK secretion, thereby reducing pancreatic stimulation (Shea et al., 2003). Type of dietary fat may play a more important role than fat content per se, but whether this is of benefit in treatment of pancreatitis is not known. Although there is no evidence that restriction of dietary fat alters pancreatic stimulation, restriction of dietary fat in dogs that have a confirmed history of pancreatitis is a current recommendation. Further studies would be required to adequately challenge the dogma of feeding low-fat diets to all dogs with pancreatitis, rather than feeding such diets only to those with hyperlipidaemia after food has been withheld.
Administration of supplemental pancreatic enzymes has been applied in the management of people and dogs with exocrine pancreatic insufficiency that is secondary to chronic pancreatitis (Meier and Beglinger, 2006; Watson, 2004). The provision of pancreatic enzymes appears to decrease postprandial pain in humans with chronic pancreatitis and has been suggested to decrease the risk of reoccurrence of acute pancreatitis via a negative feedback effect on pancreatic enzyme secretion (Meier and Beglinger, 2006). The reduction in the signs of pain may also be due to other unknown mechanisms.

6.5 Conclusion

In the present study, diets C and D included supplemental pancreatic enzymes; the reduced (albeit not significantly) pancreatic response associated with diet D was most likely attributable to the addition of MCTs. The effects of dietary supplements containing pancreatic enzymes may however be more appreciable in dogs with acute or chronic pancreatitis rather than in normal dogs. Nevertheless, results of the present study indicated that the fat content of diets or the addition of MCT oil or pancreatic enzymes to diets fed to healthy dogs did not have any significant effect on serum concentrations of cTLI, cPLI, or gastrin. Although this may have implications for the treatment of pancreatitis (both acute and chronic) in dogs, it is uncertain how much of this conclusion can be extrapolated to dogs with acute pancreatitis.
Chapter 7: A pilot study to assess tolerability of early enteral nutrition via oesophagostomy tube feeding in dogs with severe acute pancreatitis


7.1 Introduction

Acute pancreatitis has a high mortality rate in dogs, and surviving animals often require intensive treatment and hospitalisation (Simpson and Lamb, 1995; Watson, 2004; Williams and Steiner, 2005). Apart from non-specific recommendations regarding correction of fluid, electrolyte and acid-base abnormalities there remains a paucity of information regarding specific management of acute pancreatitis in dogs.

Nutritional management of acute pancreatitis in people is considered difficult due to the presence of substantial catabolism, pancreatic necrosis, increased metabolic demands and complications of ileus (Thomson, 2006). Historically, the nutritional recommendation for management of acute pancreatitis in dogs has been ‘strict pancreatic rest’, in the belief that fasting results in no feedback that will further stimulate exocrine pancreatic secretion, thus protecting against auto-digestion to some extent (Simpson and Lamb, 1995).

However, this theory has been questioned, because it has been extrapolated from studies of healthy people, and has not been proven in dogs. In fact, 3 experimental studies have suggested that there is minimal to no negative pancreatic feedback in dogs given nutrition in both the jejunum and duodenum (Imamura et al., 1993; Ragins et al., 1973; Sale et al., 1977). It also has been demonstrated in experimental models in rodents and in people with naturally-occurring disease that exocrine pancreatic secretion is in fact decreased during pancreatitis, and the decrease is most prominent in severe inflammation (Niederau et al., 1990; O’Keefe et al., 2005).
In people and in studies using animal (rodent and canine) experimental models, fasting leads to intestinal mucosal atrophy, increased rate of enterocyte apoptosis, decreased glutamine and arginine transport, changes in mucin composition of goblet and deep crypt cells and a breakdown in the intestinal barrier resulting in increased intestinal permeability (Deitch et al., 1987; Fukuyama et al., 2001; Hernandez et al., 1999; Sarac et al., 1994; Sharma and Schumacher, 1995). Additionally, the gut itself may either start or contribute to the systemic inflammatory response in acute pancreatitis (Flint and Windsor, 2003). Intra-luminal nutrition is the most potent stimulator of intestinal mucosal regeneration due to stimulation of growth factors and mucosal blood flow (Flint and Windsor, 2003). In addition, enteral nutrition may decrease splanchnic cytokine production, modulate the acute phase response, decrease catabolism and preserve protein (Ioannidis et al., 2008).

Experimental models of pancreatitis in dogs have shown a benefit of early intra-luminal nutrition compared to parenteral nutrition in decreasing bacterial translocation and down-regulating the severity of inflammation (Qin et al., 2002a; Qin et al., 2002b, 2003). There also have been several clinical trials in people demonstrating benefit in early enteral nutrition compared to parenteral nutrition for treatment of acute pancreatitis, with fewer infectious complications, and in severe cases improved outcome and decreased length of hospitalisation (Eatock et al., 2005; Eckerwell et al., 2006; Koretz, 2009; Kumar et al., 2006; Marik and Zaloga, 2004; McClave et al., 2006; Petrov et al., 2006; Petrov et al., 2009; Powell et al., 2000). However, meta-analysis of these studies does not show a clear benefit of early enteral nutrition compared to parenteral nutrition, and there are no well-constructed randomised controlled trials comparing enteral nutrition to nothing by mouth (Koretz, 2009; McClave et al., 2006). More recent studies in people also suggest that
gastric feeding (rather than jejunal) is well tolerated and safe, with no exacerbation of pain (Eatock et al., 2005; Eckerwell et al., 2006; Kumar et al., 2006).

A previous study (as detailed in chapter 6) determined that there was no statistical difference in the indirectly measurable pancreatic response to diets of variable fat content in healthy dogs (James et al., 2009). This, along with the changing paradigm of feeding in people with severe acute pancreatitis, formed the background to this pilot study evaluating the tolerability of giving enteral nutrition proximal to the pylorus in dogs with severe pancreatitis.

7.2 Materials & Methods

7.2.1 Animal selection

Ten dogs with a diagnosis of acute pancreatitis were recruited into the study within 12-24 hours of admission to Murdoch University Veterinary Hospital. The study was approved by the Murdoch University Animal Ethics Committee. Informed, signed owner consent was obtained.

The diagnosis of acute pancreatitis was based on a combination of clinical signs (abdominal pain, vomiting), serum canine pancreatic lipase > 200 µg/L and, in all dogs, the presence of appropriate pancreatic changes on abdominal ultrasonography performed by a board-certified radiologist using a curvilinear 8 MHz or linear 15 MHz probe (Sequioa 512, Acuson, Munich, Germany). These ultrasonographical changes included diffuse enlargement of the pancreas, hyperechoic mesentery surrounding the pancreas and variable pancreatic hypoechogenicity as previously published (Lamb and Simpson, 1995; Steiner, 2003; Williams and Steiner, 2005). Dogs were excluded if other disease that could cause secondary pancreatic inflammation, such as septic peritonitis or pancreatic neoplasia, were evident during or after completion of the study, with a follow-up period of 2-4 years. Dogs
had to weigh > 10 kg to comply with the volume of blood sampling required, and not have concurrent diabetic ketoacidosis. Only dogs that showed substantial consequences of their disease (e.g. dehydration, pain, hypovolaemia, persistent vomiting, other systemic effects) necessitating hospitalisation and intravenous fluid therapy were recruited.

### 7.2.2 Initial patient assessment and treatment

Historical and clinical information including signalment, potential inciting factors, previous medical history, physical examination findings, days of clinical illness preceding admission, days fasting and the clinical severity score as previously published were recorded for each dog on admission (Day 0) (Mansfield et al., 2008). After recruitment into the study, all dogs had baseline haematology, biochemistry (including glucose, cholesterol, lipase and amylase), electrolytes and urinalysis performed.

All dogs received intravenous fluid therapy (using crystalloids with appropriate electrolyte supplementation) calculated to correct dehydration and ongoing losses on an individual basis. Anti-emetic medication (metoclopramide; Metomide, Delvet Pty Ltd, Seven Hills, NSW at 1-2 mg/kg/day constant rate infusion) and analgesia (including fentanyl continuous rate infusion and patches, methadone, tramadol, lignocaine continuous rate infusion and morphine via epidural) also could be administered based on individual requirements. These treatments were initiated before inclusion into the study for all dogs. Within the first 12 hours of admission to the study, a naso-oesophageal feeding tube was inserted and a low-rate (0.5 mL/kg/hour) infusion of a balanced electrolyte solution (Lectade, Jurox Pharmaceuticals, Rutherford NSW, Australia) was started in all dogs. Fresh frozen plasma (10 mL/kg/day) was administered to each dog for the first 2 days of the study. Antibiotics (ampicillin; Ampicillin, Aspen Pharmcare Pty Ltd, St Leonard’s NSW at 25 mg/kg IV every 8 hours and metronidazole; Metronidazole, Baxter Healthcare,
Toongabbie, NSW at 10 mg/kg IV every 12 hours) were to be given if dogs had a neutrophil count < 2.0 x 10^9/L or a left shift in combination with pyrexia (rectal temperature > 39° C), unless another specific indication was present. Additional anti-emetic (prochlorperazine; Stemetil, Aventis Pharma, Lane Cove, NSW) therapy could be administered if clinically indicated.

7.2.3 Treatment groups

Dogs were consecutively alternatively assigned to 1 of 2 treatment groups, parenteral nutrition (PN) or enteral nutrition via oesophageal tube feeding (EN) within the first 12-24 hours of admission, as described below. The first 24 hours of the study protocol was designated Day 1, with continuation in the study until the end of Day 3. After that time, treatment decisions including altering nutritional intervention were based entirely on individual dog requirements and not on the basis of the treatment assignment during the study.

All dogs were anaesthetised using intravenous alfaxan (Alfaxan, Jurox Pharmaceuticals, Rutherford, NSW at 1-2 mg/kg) and inhalational isoflurane (Isoflurane, Veterinary Companies of Australia Pty Ltd, King’s Park, NSW) with full anaesthetic monitoring. A brief endoscopic examination of the oesophagus was performed to subjectively assess the presence of moderate to severe oesophagitis, to account for oesophagitis as a confounding factor for causing regurgitation or lack of tolerability for EN in individual dogs. This was done both at the time of the endoscopy and retrospectively with blinded review of the recordings.

The dogs receiving PN had double-lumen jugular 16G catheters inserted under sterile conditions and the dogs then were recovered from anaesthesia. Parenteral nutrition administration was started within 2 hours of anaesthetic recovery, at a rate of 50% resting
energy requirements (RER) on Day 1 and 100% on Day 2. For every 100 kcal of calculated RER, an additional 4 gms of protein (14 kcal) was added. This meant that 17.5% of the calories were supplied as lipid and 70.2% as 50% dextrose, along with 12.3% amino acid solution. Full asepsis was used when checking the jugular catheter site daily.

The dogs receiving EN had large gauge (14-16F) single lumen feeding tubes inserted in the oesophagus and secured externally before recovery from anaesthesia. Dogs were fed within 2 hours of recovery. A total of 1/3 of their daily calculated calories (RER x 1.25) was fed, divided every 6 hours on day 1, 2/3 on day 2 and 100% on Day 3. They were fed a low-fat commercial dog food (Digestive Low-Fat tinned diet, Royal Canin, Bristol, UK) with a fat content of 1.7% (1.9 g) per 100 kcal. This diet was supplemented with commercial enteric-coated pancreatic enzymes (Creon, Solvay Pharmaceuticals, Pymble, NSW) at 5000 units/feed if body weight < 15 kg, 10,000 units/feed body weight 15-30 kg or 25,000 units/feed if body weight > 30 kg. Medium chain triglyceride (MCT) oil (MCT oil, SHS International Ltd, Liverpool, UK; with C₈ and C₁₀ fatty acids composition > 95%) also was supplemented at 1.0 mL/kg/day in divided doses, as established in a previous study that showed this combination trended towards a minimal pancreatic response (James et al., 2009).

7.2.4 Monitoring

Full daily clinical records were kept for each dog, including results of physical examination, clinical severity score as previously published, presence of any complications, vomiting or regurgitation events (these were not differentiated as they were not always observed), baseline laboratory data and body weight (Mansfield et al., 2008). Daily pain score was recorded from admission until Day 3, and used to modulate analgesic therapy. The pain score ranged from 0-10, adapted from previously published criteria, with
a score of 2 indicating mild discomfort, 7 moderate to severe pain and 10 severe pain causing substantial alterations in mentation (Mathews, 2000).

Blood was collected into plain and EDTA containers daily via cephalic or jugular venipuncture before the start of nutritional support at admission into the study (Day 1) until completion of study (Day 3) in all dogs and on Day 1 at 5 to 10 minutes after the first feed in the enteral nutrition dogs. Blood was collected into chilled tubes, stored on ice to allow clotting and then centrifuged at 10°C at 5000 rpm for 5 minutes before harvesting sera or plasma and storing at -18°C.

Serum samples were shipped on dry ice for measurement of canine trypsin-like immunoreactivity (cTLI), canine pancreatic-specific lipase (s-CPL) and C-reactive protein (CRP), which all were measured at the Gastrointestinal Laboratory, Texas A&M University using enzyme-based assays that previously have been validated in dogs (Kjelgaard-Hansen et al., 2003; Steiner et al., 2006). Reference intervals for these markers in dogs are 5.7-45.2 µg/L, 0-200 µg/L and 0-7.6 mg/L, respectively. Frozen plasma and serum samples were transported for measurement of gastrin (Immunolite assay, DCP, Los Angeles) at a local laboratory. A previous study had established a reference interval for gastrin in healthy dogs of < 5 to 16 pg/mL, and results < 4.77 pg/mL were recorded as 4.77 (James et al., 2009). Additional aliquots of serum were shipped on dry ice for measurement of serum pancreatic elastase-1 (PE-1) at Schebo Biotech, Geissen, Germany. Gastrin, cTLI, and s-CPL, were measured at all time points in all dogs. CRP and elastase were measured at all time points, except the post-feed sample in dogs from the EN group.

### 7.2.5 Statistical analyses

Differences in clinical severity scores, oesophagitis, duration of clinical signs preceding hospitalisation, and blood marker concentrations (cTLI, s-CPL, gastrin, CRP,
and PE-1) between the 2 treatment groups at admission were assessed by one-way ANOVA analysis.

One-way ANOVA analysis was also used to compare the relative change in all marker concentrations from Day 1 to Day 3 between groups, and the duration of hospitalization between groups. Change in clinical severity score from Day 0 to Day 3 was compared between the treatment groups by non-parametric Mann Whitney test, as the data was not homogenous. The difference in marker concentrations at baseline (Day 1) and 5-10 minutes post the first feeding for Group O was assessed using Fisher’s T-test. Differences were considered statistically significant if P was < 0.05.

The number of vomiting or regurgitation episodes and the number of days each animal was hospitalised both were analysed by a Poisson model for an effect of treatment. Significance of the estimated beta coefficient was determined at P < 0.05. The beta estimate was exponentiated to determine the ratio of the incidence of events. The proportion of dogs surviving after each treatment was compared using Fisher’s exact test with significance determined at p<0.05 using a t2-sided probability. In the face of a non-significant result, the sample size required to prove this difference with 95% confidence at a statistical power of 80% was determined. PROC GENMOD and PROC FREQ were used for all analysis (SASv9.1, SAS Institute, Cary, North Carolina).

7.3 Results

There were 4 neutered females and 6 neutered males in the study. The median age was 7 years, with a range of 2 to 11. The median body weight was 26.1 kg, with a range of 14.8-48.6 kg. There were 3 cross breeds and 1 each of Siberian husky, Beagle, Bull terrier, Weimaraner, Golden retriever, Border collie and Rhodesian ridgeback (Table 7.1). All
dogs that survived this episode (n=9) were still alive at 2-year follow-up, some up to 4 years. No dog received PN after the initial 3-day period.

**Table 7.1**

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O tube feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Siberian Husky</td>
<td>F (N)</td>
<td>7</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Beagle</td>
<td>F (N)</td>
<td>6</td>
<td>14.8</td>
</tr>
<tr>
<td>3</td>
<td>Bull Terrier</td>
<td>F (N)</td>
<td>9</td>
<td>26.9</td>
</tr>
<tr>
<td>4</td>
<td>Staffordshire Bull Terrier X</td>
<td>M (N)</td>
<td>5</td>
<td>34.9</td>
</tr>
<tr>
<td>5</td>
<td>Weimeraner</td>
<td>M (N)</td>
<td>10</td>
<td>34.8</td>
</tr>
<tr>
<td>TPN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Standard Poodle</td>
<td>M (N)</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>Golden Retriever</td>
<td>M(N)</td>
<td>7</td>
<td>48.6</td>
</tr>
<tr>
<td>8</td>
<td>Border Collie</td>
<td>M (N)</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>Rhodesian Ridgeback</td>
<td>M (N)</td>
<td>11</td>
<td>42.5</td>
</tr>
<tr>
<td>10</td>
<td>Border Collie X</td>
<td>F (N)</td>
<td>2</td>
<td>25.4</td>
</tr>
</tbody>
</table>

*Animal Data of study dogs. M = Male, F= Female, N= neutered.*

One dog had hyperlipidaemia on admission that persisted after recovery, and required ongoing management, and may have contributed to the development of acute pancreatitis. This dog received EN. No underlying cause was definitively identified in the other 9 dogs, but dietary indiscretion was suspected in 2. No abnormalities in serum glucose concentration were present in any dog at any time point. All dogs had serum canine pancreatic lipase > 300 µg/L, with 8 having concentration > 800 µg/L. The median, mean and range for clinical severity score, duration of signs before admission into the study, serum CRP concentrations and concentrations for gastrin, cTLI, PE-1 and specCPL are presented in Table 7.2. There was no significant difference between treatment groups for any of these variables.

All dogs in the EN group survived, whereas 1 dog in the PN group died. The proportion surviving was not different between treatments (P = 1.0). Using this result of a binomial proportion of survival of 5/5 versus 4/5, the calculated sample size to conclude a difference with 95% confidence (at 80% power) would be 43 in each group. If the number
of controls (PN) versus treated (EN) was 2:1, the sample size required decreases to 35, with 70 controls.

Adverse reactions or complications occurred in 3 dogs in the EN group, but were considered mild or short-term. These included abdominal pain refractory to increasing analgesic modalities for the first 36 hours of the study (1 dog), urinary tract infection (1 dog), pyoderma (1 dog) and oesphagostomy tube site infection (the same 3 dogs). The tube site infections were considered mild except in the 1 dog that had pre-existing severe pyoderma. Discontinuation of tube feeding was not required in any dog. The 3 dogs in this group with documented infection all received cephalexin. The dog with refractory pain (pain scores were Day 0: 8, Day 1: 4, Day 2: 1) did not demonstrate any exacerbation of the pain after tube feeding, and the pain decreased substantially by Day 2. Analgesics used in this dog initially were buprenorphine, then methadone along with fentanyl continuous rate infusion, and then combined fentanyl and lignocaine continuous rate infusion. Analgesia was not necessary after Day 3. In the PN group, jugular phlebitis occurred in 4 dogs, with 1 severe enough to cause caval syndrome and require removal of the catheter on Day 3. One dog had refractory abdominal pain and a pancreatic pseudocyst that developed after the treatment trial had been completed (Day 4) and required percutaneous ultrasound-guided drainage. The dog in this group that died developed extra-hepatic bile duct obstruction and pleural effusion. One dog had no complications. The individual dog complications are detailed in Table 7.3.

In the EN group, only one dog had three episodes of vomiting or regurgitation over 7 days of hospitalisation, whereas in the PN group 3 dogs had 33 episodes (1 had 22 in 6 days of hospitalisation, 1 had 9 in 10 days and 1 had 2 in 5 days). There was a significant effect of treatment on the number of vomiting or regurgitation episodes (P < 0.001), with
the ratio of the incidence of episodes for parenteral versus enteral nutrition estimated at 11.0 (95% CI 3.4-35.8).

The median duration of hospitalisation for the EN group was 5 days (range 4-7) and for the TPN group 6 days (range 5-10) (Table 7.3). This was not statistically significant (P= 0.237).
Table 7.2

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Days of clinical signs before admission</th>
<th>Clinical severity index</th>
<th>Pain score</th>
<th>C-reactive protein (mg/L)</th>
<th>Spec-CPL(^\circ) (µg/L)</th>
<th>cTLI (µg/L)</th>
<th>Gastrin (pg/mL)</th>
<th>PE-1 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>45.3</td>
<td>316.0</td>
<td>9.9</td>
<td>4.77</td>
<td>0.9</td>
</tr>
<tr>
<td>Maximum</td>
<td>4</td>
<td>5</td>
<td>6.2</td>
<td>173.8</td>
<td>1001.0</td>
<td>33.0</td>
<td>21.8</td>
<td>31.7</td>
</tr>
<tr>
<td>Mean</td>
<td>1.9</td>
<td>3</td>
<td>3.2</td>
<td>79.3</td>
<td>158.2</td>
<td>18.7</td>
<td>4.77</td>
<td>7.7</td>
</tr>
<tr>
<td>Median</td>
<td>1.5</td>
<td>4</td>
<td>3.1</td>
<td>52.6</td>
<td>109.1</td>
<td>16.2</td>
<td>4.77</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Statistical summaries for blood markers and clinical severity on admission to the study. EN = enteral nutrition as supplied by oesophagostomy feeding tube; PN = parenteral nutrition; Spec-CPL = canine pancreatic specific lipase; cTLI = canine trypsin-like immunoreactivity; PE-1 = serum canine pancreatic elastase-1.
There were no differences in the change of any of the markers measured between the two treatment groups (Figure 7.1), although the change in CRP bordered on significant ($P = 0.057$). The change in clinical severity score between admission (Day 0) and the end of the study (Day 3) was significantly different between the two treatment groups (an increase of 1.2 for the TPN group compared to a decrease of 3.2 in the EN group) (Figure 7.2). Full laboratory results for all dogs are detailed in Table 7.4.

There was no difference in the measurement of any of the markers of pancreatic adaptation (cTLI, s-CPL, gastrin or haptoglobin) in the EN group at baseline (Day 1, Time 0) and 5-10 minutes after feeding, although s-CPL was almost significant ($P = 0.055$), Figure 7.3.
### Table 7.3

<table>
<thead>
<tr>
<th>Group</th>
<th>Dog #</th>
<th>Outcome</th>
<th>Length of Hospital (days)</th>
<th>Days clinical signs prior to admission</th>
<th>CSS (Day 0)</th>
<th>CSS (Day 1)</th>
<th>CSS (Day 2)</th>
<th>CSS (Day 3)</th>
<th>Complications</th>
<th>Vomiting/reg episodes</th>
<th>O score</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN</td>
<td>1</td>
<td>Survived</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>Refractory pain/O site infection</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Survived</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>UTI (pre-existing)/O site infection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Survived</td>
<td>5</td>
<td>1.5</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>Pyoderma/O site infection</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Survived</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>UTI/O site infection</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Survived</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>Nil</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TPN</td>
<td>6</td>
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<td>6</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td>22</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>Jugular phlebitis</td>
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<td>1</td>
</tr>
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<td></td>
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<td>10</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>EHBDO, jugular phlebitis, pleural effusion</td>
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<td>4</td>
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<td>1</td>
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<td>0</td>
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<td>Nil</td>
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Results of clinical severity scores (CSS), outcome, duration of hospitalisation and complications for each prospective study group (EN= enteral nutrition and TPN= total parenteral nutrition), with all dogs also receiving standard care, plasma and micro-entalal nutrition. O= oesophagus, EHBDO = extra-hepatic bile duct obstruction, UTI = urinary tract infection, O score = oesophagitis score.
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dog #</th>
<th>Time sampling</th>
<th>cTLI</th>
<th>SpecCPL</th>
<th>CRP</th>
<th>Gastrin</th>
<th>Haptoglobin</th>
<th>Elastase</th>
</tr>
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<td>EN</td>
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<td>27.8</td>
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<td>NM</td>
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<td></td>
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<td>NM</td>
<td>17.6</td>
<td>3.28</td>
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<td>Day 1, T= 1 - 2 hr</td>
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<td>334</td>
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<td>59.98</td>
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<td>2.27</td>
<td>3.35</td>
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Enzyme Concentrations for each dog in the prospective pilot study. EN = enteral nutrition as supplied by oesophasgostomy tube feeding; TPN = total parenteral nutrition; cTLI = canine trypsin-like immunoreactivity, SpecCPL = canine pancreatic specific lipase; CRP = C-reactive protein, NM = not measured.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dog #</th>
<th>Time sampling</th>
<th>cTLI</th>
<th>SpecCPL</th>
<th>CRP</th>
<th>Gastrin</th>
<th>Haptoglobin</th>
<th>Elastase</th>
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<td>2.81</td>
<td>2.84</td>
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</table>
Figure 7.1

Median values of concentrations of specific canine pancreatic lipase (1a); C-reactive protein (1b), serum trypsin-like immunoreactivity (1c); Serum pancreatic elastase (1d); gastrin (1e) and haptoglobin (1f) on Days 1-3 of the study for the enteral nutrition (EN) group compared to the total parenteral nutrition group (TPN). There is no significant difference in changes in any of the measurements, although the change in specific canine pancreatic lipase approaches significance.
Change in Clinical Severity score as represented by change in median between admission (Day 0) and completion of study (Day 3) between total parenteral nutrition (TPN) and enteral nutrition (EN).

Figure 7.3
Change in serum markers canine pancreatic lipase (Spec cPL), trypsin like immunoreactivity (cTLI) and gastrin in dogs one hour after first feeding through an oesophagogostomy tube. There is no statistical difference between the two readings for any markers, although Spec cPL approaches significance.
7.4 Discussion

This pilot study primarily aimed to demonstrate that early interventional nutrition was ethical, safe and well tolerated in dogs with severe pancreatitis. Additional aims were to refine the study design and calculate the numbers required to run a larger, prospective study. The number of dogs included in this pilot study is too small for meaningful analysis on the survival or rate of reduction in serum markers of general or pancreatic inflammation. Additionally, the development of inflammatory conditions as a result of the intervention (severe phlebitis in the PN group) may confound analysis of inflammatory markers. However, it is planned that in future larger studies analysis of these serum markers may help in determining the benefit of early enteral nutrition.

This pilot study supports the notion that early enteral nutrition delivered proximal to the jejunum is well tolerated in dogs with severe pancreatitis. It may also be considered that PN could be detrimental in dogs with acute pancreatitis due to the high number of complications, but this may be an institutional bias as there were fewer complications in the EN group than the PN group. The complications and adverse effects seen with PN were all related to catheter sepsis, and none with the composition of the infusion. None of the PN group had hyperglycemia, hyperlipidaemia, re-feeding syndrome or hypervolaemia at any point, all of which have been associated with a poor outcome in critical care settings in both people and animals (Chan et al., 2002; Freeman et al., 1995; Thomson, 2006). The 1 dog that had pre-existing hyperlipidaemia was in the EN group. Because of this, it would seem logical to assume that any benefits seen with EN are due to direct nutritional delivery to the intestinal lumen rather than total caloric input alone. The additional resting energy requirement administered to the PN group (14%) and the EN group (25% by Day 3) is unlikely to have had detrimental effects, because RER is only an estimate of daily calorie
requirements that may vary from measured direct calorimetric energy requirements as much as 25% in individual patients (Kleiber, 1961).

One major argument against pre-pyloric (or gastric) feeding in pancreatitis is that it will be poorly tolerated due to decreased intestinal motility. Certainly in our population of dogs this was not the case. There were significantly more vomiting or regurgitation episodes in the PN group than in the EN group. This could be attributable to differences in patient selection, however case allocation was consecutive, there was no difference identified between groups at onset of the study and no animal was selected for the study on the basis of the prospective treatment group. Additionally, all animals had decreased gut sounds on admission. EN may improve gut health to a point that minimizes ileus and vomiting, although this could not be conclusively determined from this study. Although pro-kinetic agents such as cisapride were allowed by the study protocol they were not needed in any animal. Initially, all animals had metoclopramide constant rate infusion as part of their treatment, and there may have been a pro-kinetic benefit conferred from this treatment. This pro-kinetic treatment was given to all dogs, and consequently is unlikely to have influenced the frequency of vomiting or regurgitation between groups. There were similar degrees of oesophagitis visible grossly in both groups, but no statistical comparison was made due to the poor sensitivity of detecting oesophageal inflammation on visual assessment.

Another argument against feeding proximal to the jejunum is the possible development of pancreatic pain. There was no temporally associated abdominal pain observed when feeding was commenced in any of the EN dogs. One dog in the EN group had substantial pain on admission that was initially refractory to increasing analgesia, but this declined rapidly after Day 2 while still receiving EN, and did not appear directly associated with feeding. All analgesia could be discontinued in this dog by Day 4. Also,
the dog that developed a pancreatic acute fluid collection (previously called a pseudocyst) was in the PN group. One possible hypothesis for acute fluid collection is increased and persistent pancreatic enzyme leakage, and so if stimulation of the pancreas in acute pancreatitis is increased with intra-luminal nutrition, development of acute fluid collections could be expected to be more likely in a dog given enteral nutrition.

Another argument against pre-pyloric feeding is that it will increase pancreatic secretion by food stimulus within the duodenum. None of the dogs in the EN group showed any adverse effects from feeding. The need to avoid stimulus of the duodenum in acute pancreatitis does not appear to be upheld by this study.

Currently, the recommendations of many gastroenterology societies in human medicine is to supply enteral nutrition early in severe (but not mild) pancreatitis as there is a reduction in mortality and infectious complications (Heinrich et al., 2006; Johnson, 2005; Meier et al., 2006; Muddana et al., 2009; Petrov et al., 2008b). There is still extensive debate about the advisability of these recommendations.

It has been demonstrated in people and animal experimental models that there is significant third space loss with acute pancreatitis, and consequently splanchnic circulation decreases as part of circulatory shock (Flint and Windsor, 2003). The body adjusts for this loss by increasing the oxygen extraction from the systemic circulation, leading to regional ischaemia. Intestinal ischaemia causes increased intestinal permeability and mucosal acidosis, which further increases the rate of apoptosis and decreases nutrient transport of epithelial cells, resulting in disruption of the lamina propria. Ischaemia also causes accumulation of hypoxanthine and depletion of ATP (Flint and Windsor, 2003). After fluid resuscitation (reperfusion is performed), xanthine oxidase is produced, which converts hypoxanthine to xanthine, with production of $O_2^-$. Free radicals are chemoattractants for neutrophils and other cytokines, which in turn amplify the
inflammatory response. Endothelial cells become stimulated by this, and express adhesion molecules that bind activated neutrophils, and thus further perpetuate the inflammatory process within the gut (Flint and Windsor, 2003).

In this study, we primarily wanted to evaluate 2 methods of interventional nutrition, and all dogs received a high level of baseline care. This care included plasma transfusion and trickle esophageal electrolyte administration, so that any difference between the 2 groups could be attributed to the interventional nutrition alone. It is questionable whether plasma transfusion actually confers a positive benefit, as was recently proposed in 1 retrospective study of dogs with pancreatitis (Weatherton and Streeter, 2009). However, a prospective comparison between animals with similar degrees of disease severity is needed before a definitive determination can be made about whether or not plasma administration provides any benefit in dogs with pancreatitis.

A limitation of the current study was the fact that study observers were not blinded to the treatment groups, leading to the possibility that bias may have influenced results. Given that the 2 treatment modalities were so different, it was impossible to adequately blind the clinicians involved. To overcome this in the future, it is suggested that treatment groups be randomly assigned and not known to the observer until the patient has been recruited into the study. This will remove the bias of sequential treatment group assignment, as a clinician may not actively recruit a dog into the study if he or she feels the treatment is not suitable for that animal.

Relying on ultrasound examination for diagnosis may have meant that some dogs with pancreatitis that were presented to the center were not recruited for this study. However, this study was aimed at assessing efficacy of treatment in severely affected dogs, and false positive diagnoses were unlikely in this group. All of the surviving 9 dogs were still alive 2-4 years later, and the non-surviving dog had a full post-mortem examination
that did not identify any changes unrelated to severe pancreatitis, and diseases such as pancreatic neoplasia or septic peritonitis seemed extremely unlikely.

The diet chosen in this study was based on a previous study that assessed which dietary combination led to minimal pancreatic stimulation in healthy dogs (James et al., 2009). Extrapolation of this diet to dogs with pancreatic disease is not supported, nor indeed is it known if dietary modification is even necessary if there is minimal pancreatic stimulation during acute pancreatitis. As there were no adverse effects associated with feeding, it can only be concluded that the diet used was not detrimental. There may be additional or unknown benefits from adding supplements such as fiber, pro-biotics, omega-3 fatty acids, or glutamine, as has been suggested in some studies in people (De Beaux et al., 1998; Karakan et al., 2007; Olah et al., 2002; Xu et al., 2006).

Certainly, the optimal treatment of pancreatitis cannot rely on a single modality, nor does this pilot study suggest that early enteral nutrition necessarily is beneficial in severe pancreatitis. Rather, this study is an initial step in trying to establish the best nutritional options for dogs with pancreatitis. It would seem logical from the data from humans and dogs to restrict the use of interventional enteral nutrition to dogs with severe disease, but the optimal timing and type of nutrition is yet to be established. It was calculated that 35 to 43 dogs would need to be treated with EN in a prospective study to determine if there is a statistical difference in survival or days between PN and early EN. Future studies also should assess the number of days intensive management is required or the number of days until voluntary food intake occurs between the 2 groups.

### 7.4 Conclusion

The authors feel that due to the high number of technical complications and adverse catheter effects that parenteral nutrition should be reserved for those dogs that cannot
support any form of enteral nutrition after a period of 4-5 days of anorexia. Because oesophagostomy tube feeding is well tolerated, a study comparing this method to minimal enteral nutrition in severely affected animals should be undertaken, with similar numbers of dogs to be recruited as calculated for this study.
Acute pancreatitis in dogs remains a challenging disease, with many still as yet answered questions regarding optimal diagnostic criteria and treatment options. A major part of this difficulty lies in the fact that pancreatitis has a spectrum of clinical presentations, various aetiologies and differing degrees of severity. The pathophysiology and current state of knowledge regarding this disease was explored in the first chapter of the thesis. In particular, the role of intra-acinar pH, inflammatory cascades and perpetuation of disease by the intestine were discussed. Each area of expanding knowledge regarding pathophysiology creates the potential for a new treatment strategy to be developed.

Human medicine faces similar problems to canine medicine in regards to determination of optimal treatment. Although there has been a large increase in the amount of work published in the medical literature exploring the pathophysiology of pancreatitis in relation to potential treatments, these experiments are predominantly performed on rodent models. This in itself may be problematic, as the type of naturally occurring disease in dogs and people may differ substantially from the experimental models. Even extrapolating from people to dogs is flawed, as the pancreatic anatomy is slightly different, the physiological control of pancreatic secretion is different, the aetiology of the disease is different, and the complications that develop are different. Apart from surgical intervention for infected disease however, standard treatment of the severe acute form of the disease is very similar between people and dogs.

Despite a plethora of new diagnostic tests and published studies, there remains a gap in the knowledge regarding the best way to diagnose acute pancreatitis in dogs. Currently, the gold standard is considered histological diagnosis. As this is seldom done in
clinical practice, it means that establishment of true clinical utility of testing is problematic. Additionally, histological evidence of pancreatic inflammation may not always reflect clinically relevant pancreatitis. This in and of itself creates many difficulties in evaluating modalities such as ultrasound as commonly used in veterinary practice, and histology only obtained in very severely affected animals that have necropsy performed. In Chapter 2, the sensitivity and specificity of pancreatic-elastase-1 was determined in dogs with clinical presentations consistent with acute pancreatitis. Histopathology was not obtained in all dogs that had a diagnosis made by ultrasound, but was obtained in all dogs that were classified as not having pancreatitis. This study determined that pancreatic elastase-1 had an overall sensitivity of 61%, comparable to published sensitivities for other pancreatic markers such as lipase and pancreatic lipase. If only dogs with severe acute pancreatitis were evaluated, this sensitivity increased. Specificity was high at 92%. This study deliberately tried to replicate the clinical situation, where histology is not routinely performed in dogs with suspected acute pancreatitis. As such, this method of assessment is more clinically relevant than one based entirely on absence or presence of pancreatic inflammation.

Along similar lines to other studies, in Chapter 3 we evaluated the specificity of canine pancreatic lipase in dogs that died or were euthanized for a variety of reasons. This study determined a specificity of 80% using the calculated diagnostic cut-off value, lower than other published reports for this test. This likely reflects the sick population of dogs assessed. It also likely results from an attempt to stratify the amount of pancreatic inflammation present in order to only assign a diagnosis of positive pancreatitis to dogs that had a minimum amount of histological pancreatitis present. Again, however this represented a sub-section of dogs that had severe disease, as evidenced by death or euthanasia. It also didn’t properly address the clinical utility of this assay, as there was no
way to correlate to primary cause of death. Although not included in the body of this thesis, a study was performed that tried to address the clinical utility of a cage-side version of this test (appendix) in dogs presenting with clinical signs consistent with acute pancreatitis. This showed there was very poor correlation between the primary diagnosis and the pancreatic lipase result (either the quantified ELISA or semi-quantified SNAP test). This, along with the literature reviews highlights the difficulties in using histology to assign a primary diagnosis of acute pancreatitis, as the dog may present with another disease such as intestinal obstruction but have bystander pancreatic inflammation.

The recent work aimed at understanding the pathophysiology of the disease in experimental models has been mirrored by an improved understanding of the systemic inflammatory response in naturally occurring critical illness. It is fairly well accepted that acute pancreatitis exerts its systemic effects by a complex and profound interaction of a variety of inflammatory pathways. This makes it likely that there may not be a single drug likely to counteract all aspects of this inflammation.

The role of the gut in starting and perpetuating systemic inflammation is gaining wide acceptance in the medical community. That this occurs in dogs with acute pancreatitis is probable, although not proven. This thesis aimed to establish some guidance regarding treatment of this common and complicated condition. Development of a severity index was considered essential for this, as comparing treatments between dogs with differing degrees of severity would lead to considerable bias. The severity index (as presented in Chapter 4) was developed from clinical and laboratory data that could easily be obtained in general practice within 24 hours of admission. This severity index then could allow for stratification of disease, and recruitment only of severely affected animals into treatment trials. The biggest contributor to this severity was fasting for 3 or more days, and highlighted the need for studies of nutritional intervention. Further analysis of cases given
plasma or a minimal form of enteral nutrition found no benefit in either of these modalities, as presented in Chapter 5. This conclusion is not robust due to the retrospective nature and small numbers of the study, but certainly raises some questions as to whether plasma, a costly and scarce resource, is truly of benefit in dogs with severe acute pancreatitis.

In Chapter 6, the role of dietary fat in relationship to pancreatic secretion was explored. This study was done in healthy dogs, and measured various markers of pancreatic adaptation. There was no significant difference in the markers measured to various fat contents of the diet. This also brings into question whether feeding of a low-fat diet is essential in the management of acute pancreatitis in the dog. To answer the question of whether early enteral feeding would be well tolerated, or potentially be detrimental, this was assessed in a pilot study, as presented in Chapter 7. This found that oesophageal feeding was very well tolerated, and resulted in significantly less vomiting and/or regurgitation than total parenteral nutrition. There were not enough dogs in the study to determine a difference in mortality or outcome, but dogs fed via an oesophagostomy tube appeared to get better sooner, as indicated by reduction in the clinical severity index and other markers over 3 days. Whether this was due to a benefit derived from the enteral feeding, or a detriment derived from the intravenous feeding could not be determined. It was possible to calculate the number of dogs required in larger studies to determine a survival benefit between standard intestinal rest and tube feeding.

In summary, these studies emphasise the difficulty in establishing clinical utility of diagnostic tests, when the gold standard (in this case pancreatic histopathology) is flawed, and difficult to do in general veterinary practice. Both pancreatic elastase and pancreatic lipase appear to have reasonably similar, and good, specificities. Pancreatic elastase has only been reviewed in this study, but this has higher sensitivity than pancreatic lipase when
combing all previous reports. Additionally, it would appear that the clinical utility of these tests may be less as the pancreas may have microscopic inflammation in a variety of other abdominal diseases that require specific intervention. It has also been established that large, multi-centre studies should be performed to determine how much of the potential treatment options (as determined in experimental rodent models and people) can be extrapolated to dogs. A starting point has now been made, in that a severity index has been developed and early feeding has been shown to be well tolerated. The author plans to initiate international, multi-centre randomised prospective trials evaluating multiple aspects of the management of this condition.
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Appendix

Clinical Utility of SNAP cPL™ in Dogs with Acute Abdominal Disease

A version of this chapter has been submitted to Journal of Veterinary Emergency and Critical Care; authors M Haworth, G Hosgood and C Mansfield. The author of this thesis designed the study, participated in data collection and analysis, and contributed equally to the writing. This has been included as an appendix in this thesis in order to demonstrate the difficult translation from sensitivity of diagnostic testing to clinical utility, something not fully appreciated in the current published literature. It is not to be considered as part of the thesis.

Introduction

Acute pancreatitis is an important disease of dogs and the clinical signs are variable and non-specific, including abdominal pain, vomiting and diarrhoea (Williams and Steiner 2005). These signs are indistinguishable from other diseases such as septic peritonitis or intestinal obstruction, which may require specific and timely treatment such as surgical intervention. The list of potential aetiologies that can cause pancreatitis in dogs includes nutritional excesses, hyperlipoproteinaemia, drugs, toxins, hypercalcaemia, duct obstruction, duodenal/biliary reflux, pancreatic trauma, ischaemia/reperfusion and others (Charles, 2007; Williams, 1996). The pancreas may also be involved in other abdominal disease, particularly septic peritonitis, where the pancreatic inflammation is secondary to the primary problem. Therefore, sensitivities established for diagnostic tests based on histology alone may not be clinically reproducible.

Accurate ante-mortem diagnosis of pancreatitis is challenging. Histological examination of pancreatic tissue is considered the gold standard for diagnosis (Charles, 2007; Watson et al., 2007). However, pancreatic biopsies are seldom obtained in critically ill dogs, and inflammation may be unevenly distributed throughout the pancreas (Newman et al., 2004). Multiple histological scoring systems have been developed, but it is unclear what relationship the histological activity has to clinical severity or presentation (Newman
et al., 2006; Watson et al., 2007). Abdominal radiography is an important part of investigation of acute abdominal disease, but the signs of pancreatitis are non-specific, and the modality is poorly sensitive (Hess et al., 1998; Schaer 1979). Abdominal ultrasound is used extensively in veterinary practice, with the primary lesion being peri-pancreatic fat necrosis seen in the acute necrotizing form (Hecht and Henry, 2007). Reported sensitivities for ultrasound are about 66-68%, but this is substantially greater in more severely affected dogs (Hess et al., 1998; Mansfield and Jones, 2000; Steiner et al., 2008a). Therefore, history, clinical signs, laboratory testing, and abdominal imaging are used to make a presumptive diagnosis. In an emergency situation, rapid and timely diagnosis is imperative.

An ELISA for the measurement of serum canine pancreatic lipase immunoreactivity (cPLI) was developed in 2002; with cPLI shown to be located solely in the pancreas (Steiner et al., 2002; Steiner et al., 2003). It was subsequently adapted to a commercial kit (spec-cPL; canine pancreas-specific lipase) with good reproducibility (Huth et al., 2010). Sensitivity for cPLI and spec-cPL ranges from 21-88%, as reported in a total of 96 dogs in 3 studies (Steiner et al., 2001; Steiner et al., 2008a; Trivedi et al., 2011). The sensitivity increases with increasing severity of pancreatic inflammation. The specificity for spec-cPL was reported to be high (86-95%) in two studies that assessed dogs at post-mortem (Neilson-Carley et al., 2011; Trivedi et al., 2011). The first mentioned of these studies assessed a substantial proportion of dogs that were young and healthy, and neither study evaluated the primary disease diagnosis. The specificity for dogs presenting with similar signs to pancreatitis is unknown.

The quantitative SNAP® cPL™ test has been developed as a cage-side semi-quantitative test utilizing the same ELISA technology as the spec cPL (Beall et al., 2011). A negative result correlates to a spec-cPL concentration < 200 µg/L; an
indeterminate result with concentrations between 200-399 µg/L, and a positive result with concentration ≥ 400 µg/L, which is considered consistent with pancreatitis. The SNAP- cPL™ test is reproducible and has good agreement with spec-cPL® in validation studies (Beall et al., 2011).

The primary objective of this study was to determine agreement of SNAP® cPL™ and a diagnosis of primary pancreatitis in dogs presenting with acute abdominal disease, in order to determine the clinical utility in an emergency setting. 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.

Materials and Methods

Animal Selection and Investigations

Client-owned dogs presenting to a first-opinion and referral emergency centre 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 diarrhoea.

Blood was collected via jugular, cephalic or saphenous venipuncture from dogs within 24 hours of admission. If blood was 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.

Diagnostics included a history, physical exam, haematology, serum biochemistry, and abdominal ultrasound by a specialist radiologist and other procedures as indicated for each individual dog to reach a definitive diagnosis. Dogs were diagnosed with pancreatitis if they had ultrasonographical or histopathological confirmation of pancreatic inflammation and/or necrosis with no other identifiable disease. Dogs that died had full post-mortem examination whenever possible, and the dogs surviving to discharge in this
group had no recurrence of clinical signs 6 months or greater on follow up, making exocrine pancreatic neoplasia unlikely. Supportive ultrasonographical evidence of acute pancreatitis was defined as enlarged, hypoechoic pancreatic tissue surrounded by hyperechoic peripancreatic mesentery or fat. These dogs may have also displayed peritoneal effusion, extra-hepatic biliary obstruction, and thickening of the duodenal wall. Dogs with primary disease other than acute pancreatitis had definitive diagnosis made either by histological examination or gross findings at surgical exploration.

Laboratory testing

The storage of the SNAP<sup>®</sup> cPL™ kits, sample handling and testing procedure was as per the manufacturer’s instructions. Two milliliters of patient blood was collected into plain serum tubes, centrifuged for 10 minutes, and allowed to equilibrate to room temperature for serum collection. Testing using SNAP<sup>®</sup> cPL™ was either performed at the time of collection and the remaining serum frozen at -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. All staff performing the test recorded a result of 1 (test spot lighter than control) or 2 (test spot equal or darker than control) and were blinded to the actual meaning of this result. A later batch analysis of spec-cPL<sup>®</sup> concentration was performed on the frozen serum which had been stored up to 18 months.

Data Analysis

For analysis, dogs were allocated to one of two groups:

- **Group 1:** Dogs with confirmed pancreatitis as their primary disease (criteria as above)
- **Group 2:** Dogs with confirmed disease other than acute pancreatitis as the primary cause of their clinical presentation. Microscopic pancreatic inflammation was only
ruled out when histological examination of the pancreas was obtained at exploratory laparotomy or post mortem examination. If pancreatic neoplasia was present with no histological evidence of inflammatory infiltrates, it was included in this group.

SNAP® cPL™ was recorded as positive or negative. A positive result was considered to have spec-cPL® ≥ 200 µg/L, and a negative was considered < 200 µg/L. The reliable limits of detection of spec cPL® are ≥ 36 µg/L and ≤ 954 µg/L (Huth et al., 2010). Concentrations ≤ 30 µg/L were recorded as 30 µg/L. Concentrations ≥ 1000 µg/L were recorded as 1000 µg/L. The agreement between positive / negative SNAP® cPL™ tests and spec-cPL® concentrations of greater than / less than 200 µg/L respectively, for all paired serum samples, was made using McNemar’s test and quantified by the kappa (κ) coefficient PROC FREQ (SAS v9.1, SAS Institute, Cary, NC). The kappa (κ) coefficient range between 0 and 1 represents no agreement other than chance and perfect agreement respectively. Data were then categorized for dogs according to the diagnosis as above.

Results

Samples were collected from 64 client-owned dogs but 26 dogs were excluded as no definitive diagnosis could be made. The remaining 38 dogs ranged in age from 17 weeks to 14.7 years (median 9.9 years, mean 9.4 years). There were 15 neutered females, 4 entire females, 15 neutered males and 4 entire males. Breeds included Labrador retriever (n=4), Siberian husky (n=3), Border collie (n=3), Jack Russell terrier (n=2), German shepherd (n=2), Rottweiler (n=2) and Australian cattle dog (n=2). The remaining 20 dogs were represented by a single or mixed-breeds.

Spec-cPL® was measured in 36 of the 38 dogs. One dog diagnosed with pancreatitis and one without pancreatitis had insufficient serum available and so were not included in the agreement assessment. The spec-cPL® concentrations was ≥ 200 µg/L in
14/36 dogs and < 200 µg/L in 22/36 dogs. SNAP® cPL™ was performed in all 38 dogs. Overall, SNAP® cPL™ was positive in 20 dogs and negative in 18 dogs. The agreement between a positive/negative SNAP® cPL™ test and a spec-cPL® concentration of ≥/ < 200 µg/L, respectively, for all 36 dogs resulted in κ = 0.78 (95% CI 0.58-0.98).

Eleven dogs were diagnosed with pancreatitis as their primary disease (Group 1), all by ultrasound with 1 also confirmed by histopathology performed after euthanasia. SNAP® cPL™ was positive in 9 of these dogs and negative in 2. All positive (9/9) SNAP® cPL™ tests had spec-cPL® concentrations ≥ 200 µg/L (range 320 to ≥ 1000 µg/L; median 800 µg/L; mean 748 µg/L). Both dogs from this group with negative SNAP® cPL™ tests had spec-cPL® concentrations ≤ 30 µg/L. The agreement for a positive/negative SNAP® cPL™ with spec-cPL® concentrations in dogs with primary pancreatitis resulted in κ =1.0 (or perfect agreement).

Primary disease other than pancreatitis was diagnosed in 27 dogs (Group 2). Histology of the pancreas was performed in 5 of these cases that confirmed no evidence of pancreatic inflammation or necrosis. The diagnoses included anaplastic, large, T-cell lymphoma of the liver (n=1); small intestinal infarction (n=1); erosive enterocolitis and pancreatic islet cell carcinoma (n=1); pancreatic islet cell carcinoma with hepatic metastasis (n=1); multicentric poorly differentiated carcinoma involving pancreas, liver, proximal duodenum, abdominal lymph nodes and lung (n=1). The other 22 dogs that did not have pancreatic histology may have had microscopic bystander pancreatic inflammation, but if present would have been considered inconsequential to the primary diagnosis. These diseases included small intestinal foreign bodies (n=7), haemoperitoneum due to splenic and/or concurrent hepatic masses (n= 3), pyometron (n=2), hepatic abscesses (n=2), emphysematous cholecystitis (n=2), a large abdominal mass that was unable to be associated with a definite organ by ultrasound with concurrent septic
peritonitis (n=1), hepatic neoplasia of unidentified origin (n=1), septic peritonitis due to a ruptured jejunal mass (n=1), prostatic abscessation (n=1), pericardial effusion (n=1), and hepatic lymphoma (n=1).

Eleven of these 27 dogs from group 2 had positive SNAP® cPL™ whilst 16 were negative. This results in a misleading diagnosis from this test in 41% of this group. Of the 10 dogs with a positive SNAP® cPL™ that also had serum available, spec-cPL® measured from < 30 to ≥ 1000 µg/L with a median of 477 µg/L and mean of 415 µg/L. Therefore, 6 dogs had spec-cPL® consistent with pancreatitis (≥ 400 µg/L) and 4 had normal spec-cPL® (< 200 µg/L). The remaining 16 dogs with negative SNAP® cPL™ had spec-cPL® concentrations from < 30 to 121 µg/L (median: <30 µg/L; mean: 51 µg/L).

The κ for agreement between a positive or negative SNAP® cPL™ and a spec-cPL® concentration of ≥ or < than 200 µg/L, respectively, for dogs in group 2 was 0.65 (95% CI: 0.35-0.94). The κ for agreement between a positive or negative SNAP® cPL™ and a spec-cPL® concentration of ≥ or < 200 µg/L respectively for all dogs overall was 0.78 (95% CI: 0.59-0.98). When considering all dogs, the agreement of SNAP® cPL™ with a diagnosis of primary pancreatitis resulted in a κ of 0.33 (95% CI 0.06-0.61). If dogs in group 1 testing negative on SNAP® cPL™ were moved to group 2 for analysis, agreement for diagnosis pancreatitis resulted in a κ of 0.44 (95% CI 0.20-0.67).
Table 1

<table>
<thead>
<tr>
<th>SNAP\textsuperscript{®} cPL\textsuperscript{™} positive</th>
<th>SNAP\textsuperscript{®} cPL\textsuperscript{™} negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient sample</td>
<td>&lt;30</td>
</tr>
<tr>
<td>320</td>
<td>&lt; 30</td>
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<tr>
<td>504</td>
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<tr>
<td>582</td>
<td></td>
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<td>612</td>
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<td>969</td>
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<td>≥ 1000</td>
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</tbody>
</table>

Dogs presenting for acute abdomen with a diagnosis of primary pancreatitis grouped by SNAP\textsuperscript{®} cPL\textsuperscript{™} result with Spec-cPL\textsuperscript{®} results (μg/L); n = 11.
Table 2

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Spec-cPL\textsuperscript{®} result (µg/L)</th>
<th>Diagnosis</th>
<th>Spec-cPL\textsuperscript{®} result (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI foreign body</td>
<td>Insufficient sample</td>
<td>SI foreign body</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>SI foreign body</td>
<td>&lt; 30</td>
<td>SI foreign body</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Pyometron and sepsis</td>
<td>&lt; 30</td>
<td>SI foreign body</td>
<td>&lt; 30</td>
</tr>
<tr>
<td><strong>Hepatic T-cell lymphoma</strong></td>
<td>68</td>
<td>Multicentric carcinoma</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>SI foreign body and septic peritonitis</td>
<td>105</td>
<td>Pyometron</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Hepatic mass</td>
<td>404</td>
<td>Haemabdomen / splenic mass</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Haemabdomen / septic peritonitis</td>
<td>550</td>
<td>Haemabdomen / splenic mass</td>
<td>&lt; 30</td>
</tr>
<tr>
<td><strong>Small intestinal infarction</strong></td>
<td>568</td>
<td>Hepatic lymphoma</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Hepatic masses with septic peritonitis</td>
<td>672</td>
<td>Emphysematous cholecystitis</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Haemabdomen with splenic and hepatic masses</td>
<td>720</td>
<td>Emphysematous cholecystitis</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>Hepatic abscess</td>
<td>≥ 1000</td>
<td>Pericardial effusion</td>
<td>&lt; 30</td>
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<td></td>
<td></td>
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<td>Prostatic abscess</td>
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<td></td>
<td>Jejunal mass / septic peritonitis</td>
<td>121</td>
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<tr>
<td><strong>Pancreatic and hepatic carcinoma with haemabdomen</strong></td>
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</table>

* Dogs presenting for acute abdomen where pancreatitis was not the primary cause grouped by SNAP\textsuperscript{®} cPL\textsuperscript{™} result. Those in bold had pancreatic histology performed, and there was a confirmed absence of pancreatic inflammation. SI, Small Intestine
Discussion

The agreement between a positive or negative SNAP® cPL™ and spec-cPL® concentration ≥ or < than 200 µg/L respectively is good. This is not unexpected as the two tests are designed to measure pancreatic lipase and are based on the same technique, with previous studies showing good reproducibility (Beall et al., 2011). Our results showed that if the SNAP® cPL™ test was negative, spec-cPL® concentration was correspondingly below the 200 µg/L cut-off value. However, when the SNAP® cPL™ test was positive, spec-cPL® concentrations were variable, with results at both the extremes of the detection limits. Further, the primary reason for presentation of many of these cases was not pancreatitis.

SNAP® cPL™ appears to perform well in dogs with primary pancreatitis. Previous studies assessing spec-cPL® have shown a sensitivity of between 21-71% based on gross or histological evidence of pancreatitis (Steiner et al., 2008a; Trivedi et al., 2011). However, despite this initially good performance this study suggests that SNAP® cPL™ should not be used as a single diagnostic test for primary pancreatitis. Failure to look beyond a positive result would have resulted in an erroneous and potentially fatal test result in approximately 29% of dogs presenting with acute abdominal disease in this study.

There are several causes for the positive results in dogs with acute abdominal disease other than pancreatitis. It is unlikely that other iso-forms of lipase were being measured, as spec-cPL® has been shown to detect only pancreatic lipase in dogs, and is not increased in dogs with exocrine pancreatic insufficiency (Steiner et al., 2002; Steiner et al., 2006). Bystander pancreatic inflammation from local extension of peritoneal inflammation is another possible explanation. Additionally, any condition that causes hypoperfusion of the pancreas, ischemia/reperfusion of the splanchnic circulation may also cause pancreatitis, as the pancreas is exquisitely sensitive to disturbances of microcirculation (Charles, 2007; Cuthbertson and Christophi, 2006; Williams and Steiner 2005). There were
no dogs in this cohort that had suffered blunt force trauma, although this could also be a potential cause of pancreatitis in addition to other soft tissue trauma or injuries.

Marked increases in total serum lipase activity have been reported in dogs with duodenal foreign bodies, dogs with acute gastroenteritis and dogs with renal failure (Mansfield and Jones, 2000; Rallis et al., 1996; Strombeck et al., 1981). This phenomenon may be due to poor renal excretion of lipase, production of lipase by organs other than the pancreas or due to duodenal reflux causing pancreatitis. It is unknown whether these same possibilities apply to pancreatic-specific lipase. One study has shown that spec-cPL® is not increased in experimentally induced renal failure, but this has not verified in animals where there may be an acute decline in glomerular filtration, and there were no dogs in this cohort with substantial azotaemia or anuria (Steiner et al., 2010). Specificity of pancreatitis has been reported to be high, but it cannot be determined from those studies if any dogs had septic peritonitis or intestinal foreign bodies (Neilson-Carley et al., 2011; Trivedi et al., 2011). As those studies were based on post-mortem analysis from referral centers, it is quite likely that dogs with intestinal foreign bodies weren’t assessed in them, as they would likely have been diagnosed and treated successfully.

Four dogs in this study had positive SNAP- cPL™ results but spec-cPL® concentrations well below the lower cut-off value of 200 µg/L. Instability of spec-cPL® in serum is unlikely to be the cause of this anomaly, as spec-cPL® has been shown to be stable for at least 21 days whether frozen at – 20°C, refrigerated, or stored at room temperature (Steiner et al., 2009). All samples from this study were either immediately frozen or refrigerated for less than a week and then frozen, and assayed within manufacturer recommended limits of storage. Haemolysis, icterus and lipaemia have been demonstrated to not interfere with the visual interpretation of SNAP- cPL™, so is unlikely to be a factor (Beall et al., 2011). Operator error is unlikely to have been the cause of the
discrepant results in those 4, as all personnel had received training on how to perform the test. It is possible a yet to be identified factor may have interfered with the test accuracy.

One limitation of this study is the use of ultrasound alone to diagnose acute pancreatitis. The diagnosis of acute pancreatitis by ultrasound has been reported to have a sensitivity ranging from 66 to 68%, although these cases were not limited to acute presentations (Hess et al., 1998; Mansfield and Jones, 2000; Steiner et al., 2008a). As well, diagnostic sensitivity is likely to be much higher now than the earlier studies due to improved equipment and operator expertise. In the more recent study, it is unclear whether there was other disease such as septic peritonitis, as only the pancreatic histology was reported (Steiner et al., 2008a). Regardless of the sensitivity of ultrasound, which will be extremely difficult to establish, the specificity of for diagnosis of acute pancreatitis and peri-pancreatic fat necrosis is well accepted (Hecht and Henry, 2007).

All dogs classified into group 1 were treated appropriately for pancreatitis, and had no recurrence of clinical signs within 6 months of discharge for all survivors, making concurrent pancreatic neoplasia unlikely. Of the 3 dogs that were euthanatized, 1 had a post-mortem performed that confirmed pancreatitis. If any of the dogs in group 1 had another clinically relevant disease as the cause for presentation that was not primary pancreatitis, then dogs testing positive on SNAP® cPL™ moving to group 2 would further deteriorate the overall agreement for pancreatitis. It was also demonstrated that if dogs testing negative were moved from Group 1 to Group 2, the best \( \kappa \) was 0.44, which still represents poor agreement between SNAP® cPL™ and a diagnosis of pancreatitis in the best case scenario.

A further limitation of this study was the small number of dogs analysed. Many dogs were excluded due to an absence of a final definitive diagnosis. Therefore, bias towards severe disease may have arisen in this study, as dogs with milder presentation may
not have received full investigation. Agreement of the SNAP® cPL™ in determining primary pancreatitis may have been enhanced if these cases were included, but it is just as likely that as many dogs with mild pancreatitis as those with non-pancreatic gastrointestinal disease did not reach a final diagnosis. Despite this potential limitation, this study reflects the general population of cases in an emergency veterinary facility that is both general access and referral in nature. Additionally, dogs that present with the level of severity presented here are the very population of dogs in which the SNAP® cPL™ test is likely to be used, and where rapid diagnostic accuracy is desired.

It has been suggested that the highest diagnostic sensitivity for diagnosing pancreatitis may be the combination of spec-cPL® or serum canine pancreatic-elastase-1 and abdominal ultrasonography by an experienced operator (Mansfield et al., 2011b; Steiner et al, 2008a). Certainly this study also supports additional testing such as abdominal imaging, along with stringent assessment of clinical and historical findings. The manufacturer also recommends performing a quantitative spec-cPL® assay following SNAP® cPL™. Our study shows there is a good concordance between SNAP® cPL™ and spec-cPL® concentration, making this less clinically necessary at initial presentation. Furthermore, measurement of spec-cPL® and serum canine pancreatic-elastase-1 incurs a delay in results and many dogs with acute abdominal disease will require immediate surgical intervention. There is no data to show that monitoring of pancreatic enzymes correlates to clinical improvement, or should be used to select treatment regimens.

**Conclusion**

This study indicates good agreement between SNAP® cPL™ and spec-cPL® concentration. SNAP® cPL™ performs poorly for discriminating pancreatitis from other primary causes of acute abdominal disease in dogs. Many of the dogs testing positive to SNAP® cPL™
without primary pancreatitis required timely surgical intervention to maximize the chances of a positive outcome. This is concerning as a possible temptation exists for clinicians to rely on the relatively inexpensive cage-side SNAP\textsuperscript{©} cPL\textsuperscript{TM} when access to ultrasonography is limited or financially prohibitive for clients, leading to a false sense of diagnostic security. A positive SNAP\textsuperscript{©} cPL\textsuperscript{TM} may indicate pancreatic inflammation is present, but cannot discriminate why it is present. Additional diagnostics, such as abdominal ultrasound or abdominal radiography are indicated to rule out these diseases.