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Refinement of Canine Pancreatitis Model: Inducing Pancreatitis by Using Endoscopic Retrograde Cholangiopancreatography

Dawn S Ruben,* Diana G Scorpio, and Jonathan M Buscaglia

The causes and treatments of pancreatitis have been studied in diverse species, but the canine pancreatitis model has been used most often due to its similarities to the condition in humans. Although pancreatitis in dogs can be induced readily by numerous methods, managing these dogs can be difficult because they often develop severe abdominal pain, vomiting, inappetance, and lethargy. In an effort to study pancreatitis, we performed a pilot study to determine whether an endoscopic pancreatic procedure would be possible in a dog and whether, through various manipulations, a new method of inducing pancreatitis could be developed. The model uses endoscopic retrograde cholangiopancreatography (ERCP), a common procedure in human gastroenterology that has been associated with postprocedural pancreatitis. Although all 8 dogs used in developing the ERCP model had both biochemical and histologic changes consistent with pancreatitis, 7 of the 8 dogs remained free of classic clinical signs of the disease. This method is presented as a refinement of a canine model and presents an alternative method of inducing pancreatitis, with decreased risk of developing associated clinical signs.

Abbreviation: ERCP, endoscopic retrograde cholangiopancreatography.

Pancreatitis is a potentially devastating, life-threatening condition that affects as many as 80,000 people in the United States annually.[6] Cases of pancreatitis can be acute, which last for a short period of time and resolve, or chronic, which progresses and does not resolve. Acute pancreatitis is a relatively common clinical condition hallmarked by unregulated trypsin activity within the pancreatic acinar cell, leading to pancreatic autodigestion and parenchymal inflammation.[24] Acute pancreatitis has multiple causes, but gall stones and alcoholism account for as many as 90% of cases.[2]

In contrast, chronic pancreatitis is a progressive fibroinflammatory disease characterized by irreversible loss of the pancreatic parenchyma and subsequent functional insufficiency. Chronic pancreatitis is most often associated with excess alcohol consumption, and these patients frequently have clinical episodes of acute pancreatitis.[24]

Animal research investigating methods of prevention and treatment of pancreatitis has proven invaluable since the first published report of an animal model of pancreatitis in 1856. At that time, bile and olive oil were injected into the pancreatic duct of rabbits, inducing pancreatitis.[6] Over the subsequent 150 y, multiple species and multiple techniques have been used in the induction and treatment of pancreatitis. Nonhuman primates are the ideal model but are expensive.[13] Dogs are used more often, for various reasons. The canine pancreas closely mimics the human pancreas in size, facilitating manipulations.[4,13,15,20] The canine pancreas is freely mobile, suspended in the duodenal mesentery,[13] and both the major and minor ducts enter the duodenum separately from the bile duct.[4,13] Other species that have been used include mice, rats, rabbits, pigs, possums, and cats.[6, 7,8,11,20-22]

In addition to a variety of species, numerous techniques have been used to induce acute pancreatitis. Some of the noninvasive methods include administration of caerulein, alcohol, or L-arginine and feeding a choline-deficient diet.[2,6,19,21] Invasive methods include closed duodenal loop, biliopancreatic duct ligation, pancreatic duct infusion, and pancreatic vascular ligation.[2,6,19,21] Each method has its own advantages and disadvantages, but all result in clinical signs of pancreatitis (abdominal pain, vomiting, lethargy, and others). Here we describe a new method of inducing pancreatitis that does not lead to overt clinical signs.

In humans, endoscopic retrograde cholangiopancreatography (ERCP) is a procedure that is performed to help diagnose various pancreatic and biliary diseases. Postprocedural acute pancreatitis is 1 complication of ERCP.[12] Depending on the patient's underlying disease, procedural indications, and technical difficulties of an individual case, the incidence of pancreatitis after ERCP ranges between 1% and 22%.[3] The exact cause of postERCP pancreatitis is unclear.

Given the prevalence of postERCP pancreatitis, clinical research efforts have focused on various methods of prevention, such as types of contrast used and pharmacologic agents used as prophylaxis before or during the procedure. To further investigate postERCP pancreatitis, we performed a pilot study to determine whether ERCP could be performed in the dog and whether pancreatitis could be induced through several different manipulations. All 8 dogs used in this study developed pancreatitis, and 7 of the 8 had no clinical signs. Why the incidence of postERCP pancreatitis is lower in humans than dogs is unclear. Because we

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Johns Hopkins University School of Medicine, Baltimore, Maryland.
**Corresponding author. Email: druben1@jhmi.edu*

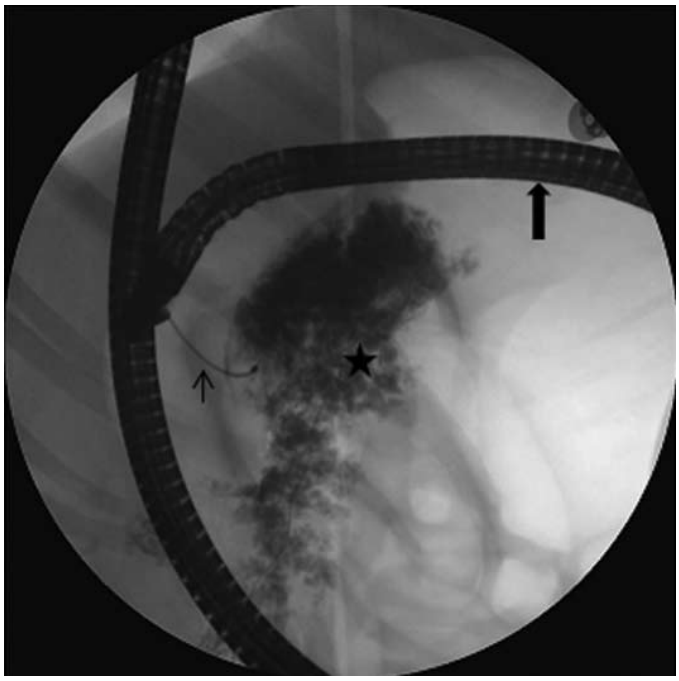

Figure 1. ERCP showing pancreatic acinarization. Fluoroscopic image demonstrating placement of the endoscope (thick arrow) and sphincterotome (thin arrow) and acinarization (star) of the pancreatic parenchyma.

were unsure whether pancreatitis would develop, several groups of animals were used in the study. During the course of this project, all 8 dogs developed biochemical and histologic evidence of mild to severe pancreatitis, with only 1 dog showing any of the classic clinical signs of severe abdominal pain, vomiting, inappetance, and lethargy.

Materials and Methods

This study was approved by the Johns Hopkins University Institutional Animal Care and Use Committee and is in compliance with the *Guide for the Care and Use of Laboratory Animals*[9]and the Animal Welfare Act.

Eight adult class B (Chestnut Grove, PA) male mongrel dogs (weight, 20.4 to 25,0 kg; *Canis lupus familiaris*) were used in the study. The dogs were housed individually under 30% to 70% relative humidity and a 12:12-h light:dark cycle. They were fed dry kibble daily (Teklad Global 25% Protein Dog Diet 2025, Harlan Teklad, Madison, WI) and free-choice reverse-osmosis water. The exact ages of the dogs were unknown, but all were adults and 2 to 5 y of age based on veterinary dental examination. The dogs were negative after testing for *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi,* and *Anaplasma phagocytophilum* (Snap 4DX, IDEXX Laboratories, Westbrook, ME) and healthy based on physical examination.

Prior to the procedure, all dogs were held off food for 12 h but allowed free access to water. The morning of the procedure, all dogs received 0.03 mg/kg acepromazine IM (10 mg/mL, Fort Dodge, Ames, IA). Thirty minutes later, a cephalic intravenous catheter was placed and lactated Ringers solution was administered at a maintenance rate of 2.5 to 3 mL/kg/h. Each dog was sedated with 2.2 mg/kg Telazol IV (50 mg tiletamine HCl and 50 mg zolazepam HCl; Fort Dodge, Ames, IA) and received 0.01 mg/kg buprenorphine IM (0.3 mg/mL, Abbott Laboratories, Chicago, IL). Each dog then was intubated and maintained on 1.5% to 2% isoflurane. End-tidal CO_2, electrocardiogram, heart rate, and reflexes were monitored throughout the procedure. Blood was obtained for baseline amylase and lipase values. Endoscopic retrograde pancreatography then was performed on each dog by using a standard adult-sized side-viewing duodenoscope. The duodenoscope was passed into the duodenum and the major papilla cannulated with a 7-French endoscopic sphincterotome with a 0.035-in. guide wire (Figure 1).

The dogs were assigned randomly to 1 of 4 groups by the investigator. Group 1 (dogs 1 and 2) had 20 mL of nonionic, low-osmolarity iodinated contrast medium (Omnipaque, Amersham Health, Princeton, NJ) injected into the pancreatic duct; group 2 (dogs 3 and 4) had 30 mL of contrast medium injected into the pancreatic duct; group 3 (dogs 5 and 6) received 30 mL of contrast medium plus balloon occlusion of the papillary orifice for 5 min and endoscopic pancreatic sphincterotomy (cutting of the sphincter that lies at the juncture of the intestine with both the bile and pancreatic ducts). Group 4 (dogs 7 and 8) had 30 mL of contrast medium plus 3 g of ursodeoxycholic acid infused into the pancreatic duct, balloon occlusion, and endoscopic pancreatic sphincterotomy.

After the procedure, each dog recovered uneventfully and was returned to its home cage. The dogs then were examined twice daily by a veterinarian and once daily by a member of the laboratory for 5 d, and no additional analgesics were needed for 7 of the dogs. Amylase and lipase serum levels were evaluated immediately prior to the procedure to be used as a control for each individual; 2 h after the procedure; and on days 1, 2, and 5 after the procedure. Five days after the procedure, 7 of the dogs were euthanized with 100 mg/kg pentobarbital IV (Virbac AH, Fort Worth, TX). The remaining dog was euthanized on day 1 due to the development of clinical signs. For all dogs, the pancreas was harvested and histology performed.

Each pancreas was fixed in 10% formalin within 30 min of euthanasia and divided into 6 sections. The individual sections then were evaluated by 2 veterinary pathologists blinded as to treatment group. Each pathologist evaluated the pancreatic sections for neutrophilic inflammation, mononuclear inflammation, acinar cell necrosis, fibrosis, acinar cell atrophy, fat necrosis, edema, and hemorrhage. Based on the percentage of the section affected by each of the above listed lesions, an injury severity score was assigned to each type of lesion for each section. For example, each section of experimental pancreas 1 was evaluated separately. An injury score was assigned based on the amount of neutrophilic inflammation in section 1; separate injury scores were assigned based on the amount of mononuclear inflammation, necrosis, and so on for section 1. The remaining 5 sections of pancreas 1 were evaluated in the same way as section 1, and the remaining pancreata were evaluated as was pancreas 1. After each section was evaluated for all 8 lesions, the 6 values for each lesion were averaged for that pancreas. The severity of each lesion was graded on a scale of 0 to 4 by using a previously described scoring system.[10] Severity scores were defined as grade 0 (no lesion seen), 1 (less than 10% of the section affected by abnormal lesion), 2 (10% to 33%), 3 (33% to 66%), and 4 (more than 66% of the section affected). For each pancreas the lowest score possible was 0, and the highest score was 32 (if all lesions in all 6 sections were grade 4).

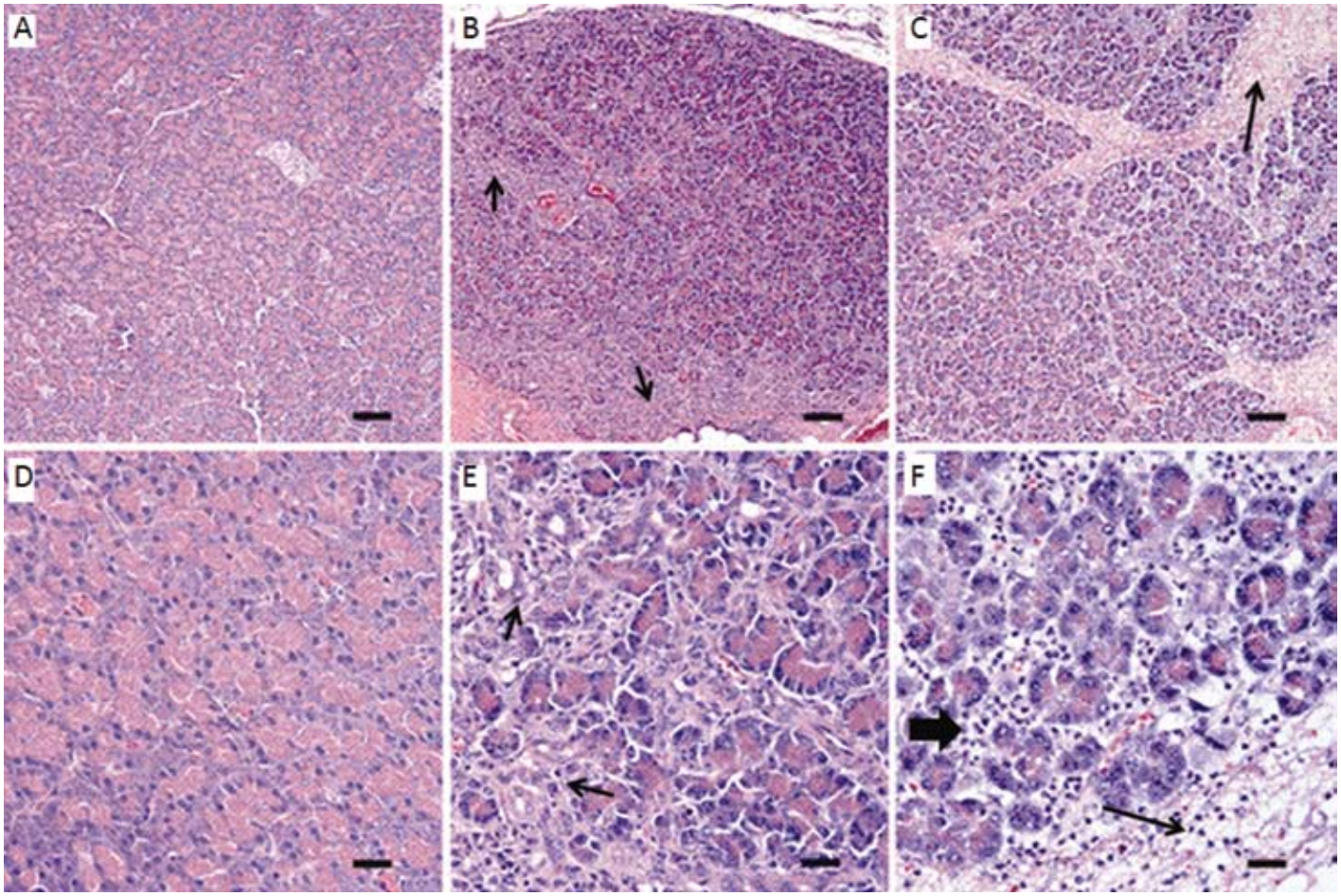

Figure 2. Histologic analysis of control dogs and 2 study dogs. (A, D) Pancreas of control dog 3, showing normal lobular architecture with tightly associated acinar cells. (B, E) Representative sections of pancreas from dog 1, showing mild leukocytic infiltration and acinar cell atrophy (arrows). (C, F) Representative sections of pancreas from dog 4, showing inter- and intralobular edema (narrow arrow) and marked acute inflammation (large arrow). Hematoxylin and eosin stain. Scale bar, 100 µm (A through C); 25 µm (D through F).

The sum of the averages for all lesions and sections resulted in the final pancreatic injury score. For histologic controls, the pancreas was obtained from 3 class B dogs used as controls in a separate cardiac study. These dogs had no experimental manipulations prior to pancreatic harvest and were healthy based on veterinary physical exam. Bloodwork was not performed on dogs used for control pancreatic histology.

Results

Pancreatic acinarization (fluoroscopically observed contrast in the pancreatic parenchyma) was accomplished in each dog without any intraprocedural complications. Dog 7 (group 4) did develop severe pancreatitis with marked abdominal pain, tachycardia, and vomiting the day after the procedure. The dog was treated with intravenous lactated Ringers solution, buprenorphine (0.02 mg/kg IV), and cefazolin (22 mg/kg IV). Due to a lack of response to treatment, the dog was euthanized with 100mg/kg pentobarbital IV 2 h after the onset of clinical signs the day following the procedure. The pancreas was harvested and used in the overall comparison.

Pancreatic injury scores. The overall mean total injury score for the experimental dogs was 6.16 ± 1.73 (range, 4.01 to 8.83). For the control dogs, the overall mean total injury score was 1.06 ± 0.39 (range, 0.5 to 1.35). When compared with the controls by using a paired *t* test, each experimental pancreas had significantly ($P < 0.05$) elevated total injury scores. All experimental dogs had increased inflammation, atrophy, and edema (Figure 2).

Amylase and lipase. The amylase (Figure 3) and lipase (Figure 4) serum levels were evaluated by Antech Diagnostics (Lake Success, NY). Each dog's baseline value was used as a control value and was compared by using paired *t* tests with the postprocedural data. In comparison, amylase was significantly increased (mean: after procedure, 11,581U/l ± 5409; baseline, 702U/l ± 147; $P < 0.00012$). The peak amylase value was at 24 h in 6 of the dogs and at 48 h for the remaining 2 dogs. The peak lipase values were significantly ($P < 0.0035$) elevated using a paired *t* test (mean: after procedure, 3637U/l ± 2183; baseline, 246U/l ± 117). The peak lipase value was at 24 h for 6 of the dogs and at 48 h for the remaining 2 dogs.

Discussion

Despite over a century of research, the exact cause, methods of prevention, and effective therapies of acute pancreatitis still remain elusive. The prevalence and potential severity of the disease

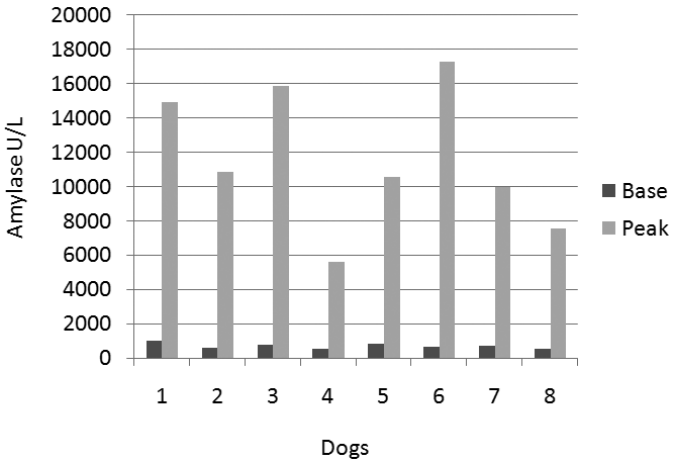

Figure 3. Baseline and peak amylase levels of each dog.

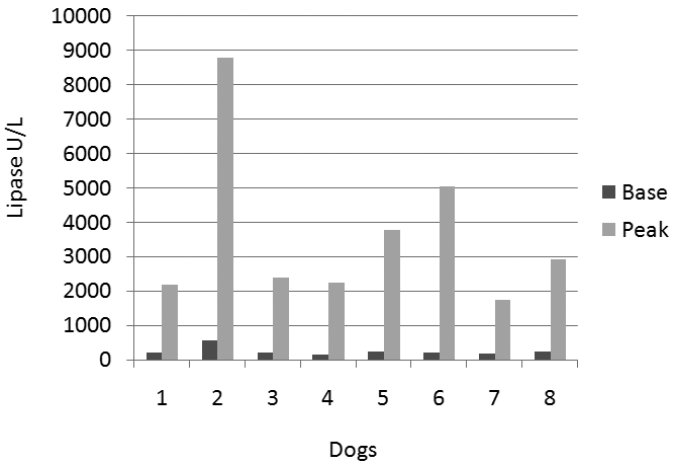

Figure 4. Baseline and peak lipase levels of each dog.

in humans make it an area of continued research focus; finding the best animal model and appropriate techniques is crucial. To date, no acute pancreatitis models are identical to human clinical acute pancreatitis. The majority of techniques used do not simulate the pathophysiology of acute pancreatitis.[15] In the present study, acute pancreatitis was induced by using a minimally invasive method: endoscopic retrograde cholangiopancreatography (ERCP). After confirming that the procedure could be performed in dogs, we used this technique to induce acute pancreatitis in much the same way that it would in humans, making this model an excellent tool for future pancreatitis research, including ERCP-related pancreatitis studies.[1]

We chose to try 4 different methods to induce pancreatitis with 2 dogs per method because of time and resource restraints. For the purpose of this manuscript, the experimental dogs were considered a single group. Differences between the groups were not compared nor conclusions drawn. All dogs in the experiment developed acute pancreatitis, regardless of the method used.

For this study, acute pancreatitis was defined as a statistically significant elevation of pancreatic enzyme levels when compared with baseline levels for each dog as well as a significant increase in the total histologic pancreatic injury score when compared with controls. Despite being control dogs, they did have minor inflammation, atrophy, and so forth. The amount of inflammation encompassed by normal ranges for canine pancreata is unknown. In 1 study that examined the histology of over 100 pancreata from dogs that had clinical signs of pancreatitis, had other illness not related to pancreatitis, and were considered healthy, all dogs had pancreatic lesions.[10] More histology needs to be performed on dogs that are considered healthy to determine the acceptable range.

Amylase and lipase were the pancreatic enzyme levels evaluated for this study. In the veterinary field, elevated amylase and lipase levels do not correlate well with a clinical diagnosis of pancreatitis but can support the diagnosis with other diagnostic tests [14, 15, 16]. Currently, the most sensitive test for dogs is canine pancreatic lipase immunoreactivity [16, 17], which was not performed because it was not part of the original study[5,23] and no serum was stored for retrospective analysis. For the purpose of this pilot study, we used amylase and lipase with the understanding of its limitations. The laboratory data combined with the histology was used to diagnose acute pancreatitis. Based on the criteria of elevated amylase and lipase, in addition to histologic changes, pancreatitis was induced in all dogs involved in the study regardless of the technique used.

One great advantage of inducing acute pancreatitis through this method was that the majority (7 of 8) of the dogs in this study showed none of the classic symptoms of pancreatitis such as severe abdominal pain, anorexia, vomiting, diarrhea, and lethargy. All dogs except 1 remained clinically normal throughout the procedure. This dog was in the group that received the most invasive procedures: injection of 30 mL contrast material into the pancreatic duct, injection of 3 g of ursodeoxycholic acid, balloon occlusion of the papillary orifice, and endoscopic pancreatic sphincterotomy. The addition of ursodeoxycholic acid could explain the clinical signs, except that dog 8 received the same procedures with no clinical signs. Ursodeoxycholic acid, a secondary bile acid, is a caustic agent and has a direct toxic effect once it refluxes back into the pancreatic duct.[14] Why the other dog in this group did not develop clinical signs is unclear. Perhaps the ursodeoxycholic acid was delivered to dog 8 under lower pressure or less was given than the intended 3 g. Another possibility is an idiopathic reaction in dog 7. Determining the reason for the response in dog 7 is difficult given that there were only 2 dogs in this group. A larger study is necessary to make any conclusions.

For the remainder of the dogs, all had significantly elevated pancreatic injury scores and elevated amylase and lipase but remained symptom free for the 5 d after the induction of acute pancreatitis. Peak amylase and lipase levels occurred at 24 h for most dogs (6 of 8) and at 48 h for the others (2 of 8). Why there was a delay in 2 dogs is unclear. These dogs underwent different methods for inducing pancreatitis, and the method used did not correlate with injury scores. Because ERCP is a new procedure in a canine model, more research is necessary with more dogs per group to determine an average amount of time for peak enzyme levels.

Using ERCP to induce acute pancreatitis has several limitations, the most obvious of which is the specific equipment and skill needed. Another limitation is that this study lasted only 5 d. Whether ERCP induces chronic pancreatitis or other pancreatic abnormalities weeks to months later is unknown. Further studies evaluating long-term effects are needed. In addition, because no clinical symptoms developed in most of the dogs, this model would only be appropriate for studies investigating etiology and

methods of prevention of acute pancreatitis; ERCP would be less useful for studies involving acute pancreatitis therapy.

Only 8 animals were used in this pilot study due to time and resource constraints. The initial goal of this study was to determine whether ERCP could be performed in dogs and whether pancreatitis could be induced by using this method. These goals were achieved. Overall, ERCP-induced pancreatitis has the potential to be a reliable, reproducible animal model of acute pancreatitis without potentially severe procedure-associated complications that can be difficult to manage. By reducing or eliminating pain and distress associated with current pancreatitis models, ERCP can be a refinement of current methods of inducing acute pancreatitis.

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