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# THE ULTRASONOGRAPHIC APPEARANCE OF THE GASTROINTESTINAL TRACT IN NORMAL AND PARVOVIRAL INFECTED PUPPIES

by

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**PROMOTER**

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To Braham

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# SUMMARY

The normal sonographic appearance of the adult canine gastrointestinal tract has been well described. Interpretation of ultrasonographic findings in puppies presented for gastrointestinal evaluation is difficult due to the lack of information on normal ultrasonographic findings. The gastrointestinal tract, jejunal lymph node size and appearance as well as the presence or absence of peritoneal fluid were prospectively investigated in a group of 23 normal, 7 – 12 week old Beagle puppies. The duodenal wall thickness was significantly greater than other parts of the gastrointestinal tract (mean 3.8 mm). The mean stomach wall thickness was 2.7 mm, mean jejunal wall thickness 2.5 mm and mean colonic wall thickness 1.3 mm. In addition, the mean thickness of the duodenal mucosal layer (2.7 mm) was significantly thicker than that of the jejunal mucosal layer (1.5 mm). The mucosa was isoechoic to the muscularis layer and had a crisp luminal-mucosal interface in all puppies. There were no intestinal corrugations observed and wall layering was distinct in all gastrointestinal segments. The homogenous, hypoechoic jejunal lymph nodes were easily found and their mean thickness measured 7.1 mm (± SD 2.2 mm). A mild amount of anechoic free peritoneal fluid was seen in all puppies. Conclusions drawn from this study were that prominent jejunal lymph nodes and a mild amount of anechoic free peritoneal fluid can be considered normal findings in puppies.

Information from the above study was utilised to interpret findings of a prospective clinical study on the ultrasonographic appearance of the gastrointestinal tract of puppies suffering from parvoviral enteritis. Forty puppies between six and 24 weeks of age were examined ultrasonographically within 24 hours of admission for canine parvoviral enteritis confirmed on faecal transmission electron microscopy. A clinical score (assessing habitus, appetite, vomiting, faecal consistency, mucous membranes, abdominal palpation and borborygmi) was attributed to each puppy prior to the ultrasonographic examination. Sonographic findings included fluid filled small intestines in 92.5% of cases, and stomach and colon in 80% and 62.5% of cases respectively. Generalised atony was seen in 30 cases and weak peristaltic contractions indicative of functional ileus observed in the remaining 10 cases. The duodenal and jejunal mucosal

layer thicknesses were significantly reduced when compared to values obtained in the normal Beagle puppies with mean duodenal mucosal layer measuring 1.7 mm and jejunal mucosal layer 1.0 mm. Additionally, a mucosal layer with diffuse hyperechoic speckles was seen in the duodenum (15% of cases) and the jejunum (50% of cases). The luminal surface of the duodenal mucosa was irregular in 22.5% of cases and the jejunal mucosa in 42.5% of cases. In all of these puppies, changes were accompanied by generalised indistinct wall layering. Small intestinal corrugations were seen within the duodenum in 35% of cases and within the jejunum in 7.5%. A mild amount of anechoic free peritoneal fluid was observed in 26 cases and was considered within normal limits for puppies and a moderate amount of anechoic free peritoneal fluid was observed in six cases. The jejunal lymph node size was within normal limits for puppies and thus parvoviral enteritis does not appear to be associated with ultrasonographic evidence of regional lymphadenopathy. There was a tendency for animals with the most dramatic ultrasonographic changes to be in poor condition clinically i.e. they had a low clinical score.

Each of the above described changes cannot be considered pathognomonic for canine parvoviral enteritis but in combination, are suggestive of the disease. It is hoped that information from this study may alert the clinician as to the possibility of underlying parvoviral enteritis in puppies presented for abdominal ultrasound for investigation of gastrointestinal disease. Further studies are needed to document the ultrasonographic appearance of other paediatric gastrointestinal diseases such as severe verminosis, giardiasis, coccidiosis and distemper etc. before further conclusions can be drawn from this study. Daily ultrasonographic examinations of puppies suffering from canine parvoviral enteritis are needed to further understand the progression of this disease over time as well as the possible ultrasonographic indicators of clinical improvement or deterioration.

# CHAPTER 1 INTRODUCTION

## 1.1 Background

Ultrasonography is a popular modality in the assessment of gastrointestinal disease. Although the ultrasonographic appearance of the adult canine gastrointestinal tract has been well described, interpretation of ultrasonographic findings in puppies presented for gastrointestinal evaluation is difficult due to the lack of information on normal ultrasonographic findings. At the Onderstepoort Veterinary Academic Hospital (OVAH), a large number of puppies suffering from canine parvoviral enteritis are admitted to the isolation ward for intensive treatment. During their hospitalisation, these puppies are frequently presented for abdominal ultrasonographic examinations, primarily to rule out intussusceptions. During such examinations a number of changes related to the gastrointestinal tract have been noted. Prominent jejunal lymph nodes and a mild amount of anechoic free peritoneal fluid have also frequently been observed. Interpretation of ultrasonographic findings in puppies suffering from parvoviral enteritis is hampered by the lack of knowledge on normal ultrasonographic findings.

## 1.2 Problem statement

Interpretation of ultrasonographic findings associated with canine parvoviral enteritis is hampered by the lack of information on normal ultrasonographic findings in puppies.

## 1.3 Hypotheses

- Normal puppies
  Large, prominent jejunal lymph nodes and a mild amount of anechoic fluid within the abdomen are normal findings in healthy puppies.

- Puppies with parvoviral enteritis
  Acute canine parvovirus infection causes characteristic ultrasonographic gastrointestinal changes such as generalised atonic, fluid filled intestines, thinning or echogenicity changes within the intestinal mucosal layer, corrugated small intestines, jejunal lymphadenopathy and a moderate amount of free peritoneal fluid.

## 1.3 Objectives

- The purpose of the first part of the study was to:
  - Describe the normal ultrasonographic appearance of the gastrointestinal tract in puppies up to 12 weeks of age
  - Establish reference ranges for this age group
  - Compare findings to the documented ultrasonographic appearance of the gastrointestinal tract in adult dogs
  - Assess the effect of age and weight on the gastrointestinal wall thickness and jejunal lymph node size
  - Improve ultrasonographic assessment of gastrointestinal disease in puppies by documenting normal findings

- The purpose of the second part of the study was to describe the gastrointestinal ultrasonographic changes associated with canine parvoviral enteritis such as:
  - Generalised fluid filled intestines
  - Decreased motility or atony of the gastrointestinal tract

- Thinning, collapse or echogenic changes of the mucosal layer
- Corrugated small intestinal appearance
- Increased jejunal lymph node size
- Moderate peritoneal effusion

The first part of the study involving normal puppies was completed before the second part of the investigation on parvoviral infected puppies.

## 1.4 Benefits

- To provide information on the normal ultrasonographic appearance of a puppy's gastrointestinal tract.
- To document the gastrointestinal ultrasonographic changes observed with canine parvoviral enteritis.
- The research conducted serves as partial fulfilment of the investigator's MMedVet (DiagIm) degree.

# CHAPTER 2 LITERATURE REVIEW

## 2.1 Ultrasonography of the normal canine gastrointestinal tract

The normal ultrasonographic appearance of the adult canine gastrointestinal tract has been well described.[1,2] It is possible to identify five ultrasonographic wall layers in a normal gastrointestinal segment: the hyperechoic lumen-mucosal interface, the thick hypoechoic mucosa, the thin hyperechoic submucosal layer, the thin hypoechoic muscle layer and then the thin outer hyperechoic serosal layer. Measurements of the intestinal and stomach wall thickness are taken from the inner hyperechoic luminal interface to the outer hyperechoic serosal surface with electronic callipers.[2] Measurements are usually made on frozen images with the intestinal segment in long-axis orientation.[1,2]

The stomach is easily recognised by its shape, size and position caudal to the liver. The presence of rugae and regular peristalsis further aid identification of the stomach.[2] Upper gastrointestinal segments are observed for approximately 3 minutes to evaluate peristaltic activity.[2] The mean number of gastric peristaltic contractions observed by ultrasound examination are 4-5 per minute.[2] Administration of water to fasted dogs has been found to increase gastrointestinal motility by an average of 1 contraction per minute.[1] A wide range of gastric distension can be observed.[2] Stomach wall thickness is measured at an interval between rugal folds. In normal dogs, the stomach wall averages 3-5 mm in thickness when measured ultrasonographically.[2] In adult humans, the values are the same and the wall is 3 mm thick in children.[3] To the author's knowledge there is no specific information evaluating normal stomach wall thickness in adult dogs *vs.* puppies.

The appearance of the small and large intestinal wall varies with the degree of distension and the nature of the gastrointestinal contents. Four luminal patterns have been described on the basis of the contents namely, mucous, fluid, gas and

alimentary pattern.[2] The mucous pattern is the appearance of the gastrointestinal tract in the collapsed state and is characterised by echogenic contents (mucous) without acoustic shadowing and ultrasonographic wall layers are easily identified. The fluid pattern is characterised by anechoic luminal contents and the gas pattern has an intraluminal, highly hyperechoic reflective interface with dirty acoustic shadowing. Gas acts as an acoustic barrier thereby preventing evaluation of deeper structures. The appearance of the alimentary pattern depends on the type of food and the amount of fluid and gas swallowed. Food particles appear as discrete echogenic structures within the gastrointestinal lumen.[2]

Normal peristalsis has been reported to be 4-5 contractions per minute for the proximal duodenum, 3-5 contractions per minute for the rest of the small intestine and no contractions in the descending colon.[1] In the fasted state, gastrointestinal peristaltic activity has been demonstrated to be decreased with intervals of little or no activity lasting for an hour between peristaltic waves.[4] In a study using pulsed Doppler to evaluate intestinal motility in dogs, peristaltic waves were found to be decreased in starved animals. In fed animals, the number of peristaltic waves increased immediately after feeding and peristalsis was observed to gradually decrease thereafter.[4]

In humans, a study investigating differences in intestinal wall thickness between children, adolescents and young adults showed a significant increase in jejunal, ileal and colonic wall thickness with increase in age.[5] There are no age-specific normal values for intestinal wall measurements in dogs and very little research has been conducted in this field: Initial veterinary gastrointestinal research documented the sonographic small and large bowel thickness in normal dogs to range from 2-3 mm.[1] This study showed no significant effect of age between beagle puppies of 13 weeks, 29 weeks and 40 weeks on gastrointestinal wall measurements. It was subsequently observed that dogs with no clinical signs of gastrointestinal disease often had ultrasonographic measurements greater than the previously published norms and this prompted additional clinical research in this area.[6] A study documenting the body

weight, breed, jejunal thickness and duodenal thickness was done on 231 dogs with no evidence of gastrointestinal disease. A trend towards increased jejunal and duodenal thickness with increasing weight was observed. The duodenal wall thickness was significantly greater than jejunal wall thickness in the dogs of this study. Unfortunately, no mention was made of the age of the dogs studied. This more recent data indicates that the norms for the jejunum are ≤ 4.1 mm for dogs up to 20 kg, ≤ 4.4 mm for dogs 20 - 39.9 kg and ≤ 4.7 mm for dogs over 40 kg. Norms for the duodenum are ≤ 5.1 mm for dogs up to 20 kg, ≤ 5.3 mm for dogs 20 - 29.9 kg and ≤ 6.0 mm for dogs over 30 kg.[6]

Slight differences in thickness can be observed between distended and contracted intestinal segments.[1,2,3,7,8] Previous studies in people have demonstrated a difference of 2 mm between the intestinal wall thickness measured in a distended or non-distended state.[1,3]

Typically, normal abdominal lymph nodes are slightly hypoechoic or fairly isoechoic to the surrounding mesenteric fat, which may make them difficult to image.[9] When seen, they are usually homogenous structures, varying in size and shape from round or oval to more elongate and fusiform. Lymph nodes are easier to detect in younger or thinner animals due to less mesenteric fat.[9,10] The jejunal (often called mesenteric) lymph nodes are a group of fusiform lymph nodes aligned with the cranial mesenteric artery and vein. They belong to the cranial mesenteric lymphocenter and are the largest of the visceral abdominal lymph nodes draining lymph from the jejunum, ileum and pancreas.[11] Historically, jejunal lymph nodes have been reported to reach up to 5 mm in thickness in normal dogs[10] with thicknesses of more than 5 mm[12,13] considered abnormal. A recent study documented a median maximum jejunal lymph node thickness of 3.9 mm and median maximum width of 7.5 mm in dogs without clinical signs of gastrointestinal disease.[14] In humans lymph node size is evaluated by measuring the maximum short axis diameter, which is usually < 10 mm in normal jejunal lymph nodes.[15] An overview of canine paediatric abdominal ultrasonography described jejunal lymph nodes as enlarged and not necessarily abnormal in

paediatric patients.[16] A tendency towards higher vascularity and maximum thickness of jejunal lymph nodes in young normal canines has also been reported.[14] To date, no specific measurements for jejunal lymph nodes in puppies have been published.

## 2.2 Ultrasonography of gastrointestinal disease

Ultrasonography is frequently utilised to investigate gastrointestinal diseases. Intestinal wall thickening, length of affected intestine, integrity and echogenicity of the wall layers, regional lymph node enlargement and regional motility have all been used in the evaluation of gastrointestinal disease.[12] The ultrasonographic features of many gastrointestinal diseases such as neoplasia, foreign bodies, enteropathies, intussusceptions and ileus have been well characterised.[17-23] Considerably less information is available on the ultrasonographic appearance of acute inflammatory intestinal lesions in dogs. Enteritis has been found to result in mild to moderate intestinal wall thickening without loss of layering throughout most of the length of the intestines.[12] In some severe inflammatory conditions, the wall layers can be significantly affected or even completely lost.[22,23] Altered motility is often present in diseased gastrointestinal segments. Decreased gastrointestinal motility is often associated with fluid and mucous bowel patterns and has been noted in paralytic and mechanical ileus.[18]

Regional lymphadenopathy has been reported in both neoplastic and inflammatory conditions. Lymph node changes suggestive of malignancy include: a rounder shape, irregular outline, presence of peripheral tissue oedema or modification of the internal architecture of the lymph node.[24] Mild to moderate lymph node enlargement can be seen in inflammatory or infectious disease.[12] The abnormal lymph nodes usually enlarge and become more hypoechoic, sometimes even anechoic but often maintain smooth margins and a homogenous echopattern. Jejunal lymphadenopathy has been reported in a variety of medical and surgical conditions in symptomatic children but is also occasionally seen in asymptomatic children.[25]

Only a small amount of fluid is normally present in the body cavities of dogs and cats.[26] This fluid provides lubrication that allows nearly frictionless movement of adjacent organ surfaces and the body cavity walls.[26] No values for the normal amount of fluid present in the peritoneal cavity could be found. The presence of a peritoneal effusion or ascites can be as a result of trauma, neoplasia, cardiovascular compromise, chronic liver failure and more specific to this study, ascites can also be seen in infectious or inflammatory diseases.[26] Ultrasound can detect as little as 1-2 ml/kg of peritoneal fluid in the dog.[27] Small amounts of free fluid can be detected in the region of the apex of the urinary bladder as well as between the liver lobes, between the diaphragm and the liver, and between the body wall and spleen.[10] Moderate amounts of free fluid are easily recognised by the appearance of intra-abdominal organs separated by large anechoic spaces. Moderate amounts of fluid may also aid in the visualisation of intra-abdominal organs, by making them appear more echoic than they would be without the presence of fluid, the acoustic enhancement phenomenon. Serosal surfaces become readily visible and the small intestine will appear to be floating in the fluid. The presence of ascites may help outline the intestinal wall.[3] Large amounts of free fluid may hamper ultrasonographic examinations, as abdominal organs are a greater distance from the transducer. When free peritoneal fluid is detected on abdominal ultrasound in adult dogs it is generally considered indicative of an underlying disease condition. It is however the author's impression that the majority of puppies presented for abdominal ultrasound examination normally have a mild amount of anechoic peritoneal fluid present.

The sonographic appearance of peritoneal effusion can aid in determining the fluid character and probable aetiology. Ultrasonographically, the fluid can be classified as anechoic, or echogenic. Low cellularity fluid (such as transudates) result in anechoic fluid. As the cellularity increases the fluid generally contains more and larger reflectors and becomes more echogenic e.g. exudates.[28]

## 2.3 Ultrasonography in paediatric patients

The paucity of intra-abdominal fat in paediatric patients results in less informative abdominal radiography but improves ultrasonographic imaging. In human medicine, paediatric abdominal ultrasonography differs slightly from adult ultrasonography, which bears an impact on the practical application of ultrasound in the diagnostic setting. Infants and children are physiologically different to adults: heart and respiratory rate are faster; tissue composition differs; maturation of organs has not yet been completed and the size and topographic relations of abdominal organs may vary.[29,30] The disease patterns expected or searched for on ultrasonographic examination, are often different from those seen in adults. Furthermore the small size of paediatric patients necessitates the use of high-resolution transducers preferably with small footprints and minor adjustments of technical settings may be needed. Nevertheless ultrasound has become the first line imaging procedure for many gastrointestinal conditions in paediatric diagnostics[31] such as Crohn's disease,[32] intussusception, necrotising enterocolitis, intestinal obstruction, ileus and viral enteritis.[33]

In human medicine, rotavirus is the most important cause of paediatric dehydrating gastroenteritis in the world. A study on the intestinal imaging of children < 2 years old with acute rotavirus gastroenteritis has been performed.[33] Patients were scanned during the acute (within 5 days of onset of gastrointestinal symptoms) and convalescent stage (5-9 weeks later) of the disease. Ileal wall measurements were performed from maximally distended intestinal loops. In addition jejunal lymph nodes were assessed quantitatively for number and size. As there are no standards for ileal wall thickness in patients of this age group, subjects were used as their own controls after recovery from the gastroenteritis. There was a trend towards increased ileal wall thickness and increased number and size of jejunal lymph nodes in the acute phase of rotavirus infection.[33]

To the author's knowledge, there are no studies detailing the normal ultrasonographic appearance of abdominal structures in puppies. To date, the only information available on small animal paediatric ultrasonography can be found in a fairly recent article broadly discussing abdominal ultrasound in paediatric patients.[16] However, this article was a general overview and hence contains no measurements or specific parameters. The following comments, pertaining to paediatric gastrointestinal ultrasonography are worth noting from this article:

- Jejunal lymph nodes often appear enlarged in the paediatric patient. This is common and not necessarily abnormal.
- Mild to moderate thickening of the gastrointestinal wall with preservation of wall layering and moderately enlarged jejunal lymph nodes are the most common ultrasonographic findings with non-specific paediatric gastroenteritis such as that resulting from dietary indiscretion.
- More severe pathologic findings such as extensive gastrointestinal oedema or haemorrhage accompanying infectious gastroenteritis can be associated with changes in fluid volume within the gastrointestinal lumen, wall thickening and loss of normal layering. These changes can be regional or diffuse.
- Fluid distension of the bowel with generalised decreased motility can be seen with functional ileus accompanying gastroenteritis.

## 2.4 Canine parvoviral infection

Canine parvovirus (CPV) is caused by type 2 canine parvovirus (CPV-2), a small (18-26 nm in diameter), non-enveloped, single stranded DNA viral particle,[34] of which two pathogenic variants, types 2a and 2b are commonly recognized. A third variant, type 2c, has recently been found in several parts of the world. More than 80% of the isolated cases of CPV in the United States today are CPV-2b.[36,37] The disease was first reported in South Africa in December 1979[38] with enteric CPV reaching epidemic proportions towards the end of 1980.[39] During 1988 – 1993, approximately 2.8% of all sick dogs presented to the OVAH were admitted for treatment of parvoviral enteritis.[40] Seasonal variation in the number of cases admitted to the OVAH has

clearly been demonstrated with a peak incidence in the summer months (September to January) and a trough in winter (May to July).[40]

The virus is transmitted primarily by the faecal-oral route after exposure of susceptible animals to contaminated faeces. After exposure, CPV replicates in lymphoid cells of the oropharynx, jejunal lymph nodes, and thymus, spreading haematogenously to crypt cells of the small intestine and epithelial cells of the oral cavity, tongue, and oesophagus within 3 to 5 days. Canine parvovirus typically targets tissues with rapid cell turnover such as the lymphoid tissues, intestinal epithelium, bone marrow and heart. Parvoviral induced myocarditis is rare and seen in puppies of less than 2 weeks of age during the period of rapid myocardial cell proliferation.[41] Virus excretion begins shortly after infection of intestinal epithelial cells and can occur as soon as 3 to 4 days after exposure; virus e and lasts for 1 to 2 weeks. In the intestinal tract, necrosis of infected crypt cells leads to villus collapse and loss of intestinal epithelial integrity.[42]

Clinical signs typically occur 4 to 7 days after infection. Anorexia, depression, fever, vomiting, diarrhoea (often profuse and haemorrhagic), and dehydration are common.[41] The haemorrhagic diarrhoea that is characteristic of the clinical disease results from a combination of direct villous damage, increased intestinal permeability and malassimilation from abnormal mucosal function.[42] Breakdown in the intestinal epithelial barrier predisposes to translocation of intestinal bacteria and absorption of bacterial endotoxins into the systemic circulation. Death is usually attributed to dehydration, electrolyte imbalances, hypercoagulability, endotoxic shock, or overwhelming bacterial sepsis related to mucosal barrier disruption.[42,43] Susceptible breeds include rottweilers, dobermans, English springer spaniels, and American pit bull terriers.[44] Dogs of any age can be affected, but the incidence of clinical disease is highest in puppies between weaning and 6 months. Puppies younger than 6 weeks usually are protected by maternal antibodies. [35]

## 2.5 Diagnostic imaging of canine parvoviral infection

A radiographic study in the early 1980's involved a series of upper gastrointestinal contrast studies on 60 dogs (age not mentioned) with serologically proven parvovirus infection.[45] Contrast radiographic studies were normal in the early stages of the disease but became abnormal as the disease progressed. Scalloping and corrugation of the bowel wall were described and attributed to irritability and associated spasm of the muscle layer secondary to the inflammatory effects of canine parvoviral enteritis. It was concluded that contrast radiography was not only highly specific for parvoviral enteritis but furthermore helped to rule out clinically similar disorders as well as intestinal obstructions.[45]

Small intestinal corrugations, defined as an undulating or rippled bowel wall, have retrospectively been evaluated in an ultrasonographic study of 18 dogs (1-16 years old).[20] Of all the dogs examined, only one dog (of unknown age) was confirmed to be positive for parvoviral enteritis. The presence of intestinal wall corrugation on ultrasonographic examination was found to be a sensitive but non-specific finding and can be seen in a number of conditions, such as enteritis, peritonitis, neoplasia and most commonly, pancreatitis.[20]

In another study, ultrasonographic findings associated with gastrointestinal disease, were reviewed in a clinical study of 18 dogs, one of which was a 2-month-old dog with parvoviral enteritis in which generalised fluid distension of the small intestine and lack of motility but presumed normal intestinal thickness (3 mm) and appearance was described.[18] The generalised decreased gastrointestinal motility was attributed to paralytic ileus. This finding was non-specific and was also noted in other diseases causing paralytic ileus such as duodenitis and pancreatitis. Mechanical ileus, as such due to an intestinal obstruction or intussusception can also result in proximal (oral) fluid accumulation and decreased intestinal motility.[18] An intussusception is a recognised complication of severe enteritis and appears as multiple concentric rings or a target like lesion in cross section through the intussuscepted segment on

ultrasonographic examination.[17] Intussusceptions associated with viral enteritis or gastroenteritis (parvovirus or distemper) have been reported to have an increased morbidity and mortality and carry a guarded prognosis.[46,47]

The histological structure of a normal canine duodenal wall has been correlated to the wall layers seen on ultrasound images.[17]

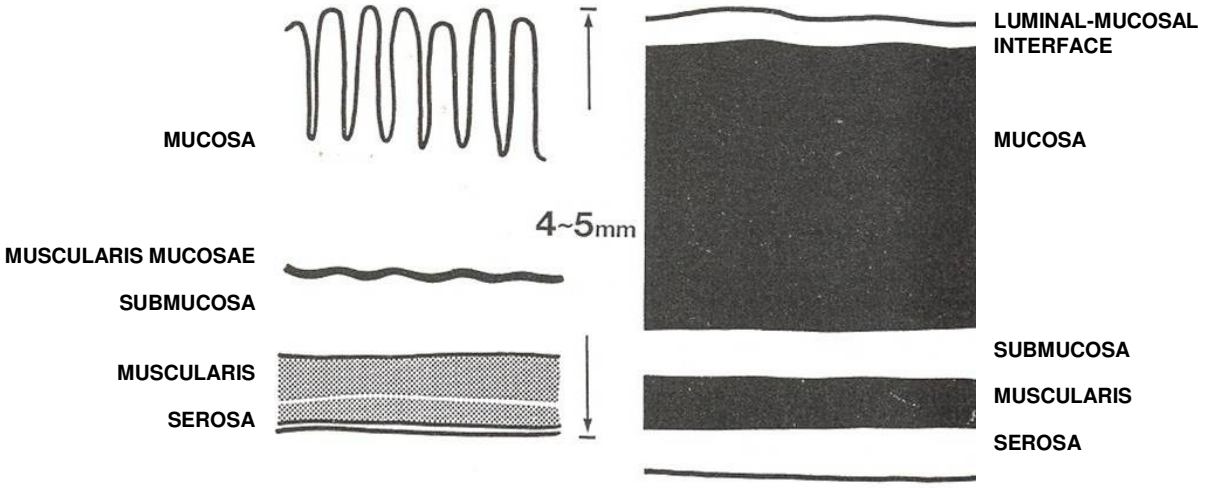


**FIGURE 1.** Correlation between *ex vivo* histological structure of the normal canine duodenal wall (*left*) and the layers seen on ultrasound images obtained *in vivo* (*right*). Reproduced with permission[17]

Necropsy findings characteristic of canine parvovirus may provide information on the possible ultrasonographic findings that can be expected. Macroscopic changes in the small intestine are most apparent during the phase of severe enteric disease and consist of diffuse thickening and congestion of the small intestines.[41] The mucosa is usually congested and may be covered by a fibrinous exudate.[34] The stomach often contains large quantities of thick mucous with normal underlying mucosa.[48] The colon is rarely affected. The jejunal lymph nodes may be enlarged, congested or reduced in size.[34] Histopathologically, destruction of crypt epithelial cells with resultant loss of villi and dilation of the remaining crypts with necrotic debris is seen. In extreme cases, collapse of the intestinal mucosa can be seen.[48] Electron microscopic features are similar but a thick sheet of mucous has been described covering the intestinal mucosal surface of 10-week-old experimentally infected canine parvovirus puppies.[49]

## 2.6 Conclusions drawn from the literature review

The normal ultrasonographic appearance of the adult canine gastrointestinal tract has been well described and reference ranges for the normal wall thicknesses of the different segments of the gastrointestinal tract have been established. This published data includes body weight correlated reference values for canine duodenal and jejunal wall thicknesses, but does not take into account differences in age or maturity. Interpretation of ultrasonographic findings in puppies presented for the investigation of gastrointestinal disease is difficult due to the lack of information on normal ultrasonographic findings. Parameters such as enlarged regional lymph nodes and the presence of peritoneal effusion which are often seen in conjunction with gastrointestinal disease in adults may be normal findings in puppies. To date, there are no age-specific normal values for gastrointestinal wall measurements in dogs nor have specific measurements of jejunal lymph node size in puppies been published.

Canine parvoviral enteritis, a disease of high prevalence in South Africa, is an important and potentially lethal cause of enteritis in puppies. Aside from a brief mention in the literature, the ultrasonographic appearance of canine parvoviral enteritis has not been investigated and a comparison of these gastrointestinal changes to normal puppy gastrointestinal ultrasonography has not been made.

# CHAPTER 3 MATERIALS AND METHODS

## 3.1 Experimental design

### 3.1.1 Normal puppies

A prospective study was conducted utilising normal Beagle puppies of up to 12 weeks of age from a research colony (Onderstepoort Teaching Academic Unit). Compulsory re-homing of the puppies by 12 weeks of age prohibited recruitment of older puppies. Puppies were considered for the study if they had:

- no history of previous gastrointestinal disease or clinical signs of gastrointestinal disease
- no blood-borne parasites or evidence of inflammatory conditions on peripheral blood smear evaluation conducted by a clinical pathologist
- no evidence of verminosis, giardiosis or coccidiosis on faecal examination

Once selected, puppies could still be excluded if any abnormalities were detected upon clinical examination (see 3.2.1) or ultrasonographic examination (see 3.2.3).
The puppies were fasted for 16 hours prior to the ultrasonographic examination. Note: the puppies were routinely fed at 4pm and then at 8am thus inadvertently resulting in a 16 hour fast prior to the early morning ultrasound examinations.

### 3.1.2 Puppies with parvoviral enteritis

A prospective clinical study was performed. Puppies admitted to the OVAH Isolation Unit with clinical signs of parvoviral infection (such as anorexia, depression, fever, vomiting, haemorrhagic diarrhoea and dehydration) were considered for the study.
Additional inclusion criteria were:

- puppies of less than 6 months of age
- any sex or weight; however, breeds of comparable size to Beagle puppies were preferentially selected
- free of intestinal giardiasis or coccidiosis on fresh saline faecal ‘wet prep’ examinations

- confirmed canine parvovirus *positive* by faecal electron microscopy (EM) within 12-24 hours after admission
- able to be examined ultrasonographically within 24 hours of admission and fasted for 4 hours prior to such examination
- owner consent provided for participation in this study (Appendix A) having read the client information sheet (Appendix B)

Puppies were excluded from the study if any of the following were noted:

- pathology of abdominal organs not under investigation (i.e. liver, spleen, urinary tract, pancreas, peritoneal cavity other than fluid, etc) as detected during the general abdominal ultrasound examination
- concurrent gastrointestinal foreign body or intussusception detected during abdominal palpation or ultrasound examination
- detection of distemper- or corona virus particles on faecal EM

## 3.2 Experimental procedure

### 3.2.1 Normal puppies

The Beagle puppies had a clinical examination conducted by the primary investigator and a clinical score assigned (as per 3.2.3) prior to the abdominal ultrasound examination. An ultrasonographic examination was conducted as per 3.2.4 below.

### 3.2.2 Puppies with parvoviral infection

All patients admitted into the study were managed according to the treatment guidelines for CPV enteritis set out by the OVAH (Appendix C). A faecal sample was collected at the time of admission (a lubricated 1 ml syringe inserted into the rectum was used to aspirate at least 1 ml of faeces). The faecal sample was submitted to the EM unit of the Department of Anatomy and Physiology for examination by direct transmission electron microscopy. The samples were refrigerated immediately after collection and submitted to the EM unit within 12-24 hours and examined for the

presence of parvo, distemper or corona virus particles. A clinical examination was conducted on each patient as per 3.2.3. Furthermore, the patient's most recent potassium, glucose, haematocrit and total serum protein readings (the latter by means of a refractometer) were noted as per Appendix D, immediately prior to the ultrasonographic examination. Drug dosages and administration times were also recorded.

Data regarding the outcome of hospitalisation i.e. death (natural or euthanasia requested by the owner or the duty clinician) or discharge were also recorded (Appendix D).

### 3.2.3 Clinical score

The clinical scoring system was adapted from a previously described clinical scoring system[50] utilised at the OVAH and allowed objective assessment of each puppy's clinical status immediately prior to the ultrasound examination. The clinical scoring system involved assessment of eight parameters, namely mentation, appetite, vomiting, faecal consistency, mucous membrane colour, capillary refill time, abdominal palpation and borborygmi. A clinical score was then assigned to each puppy as outlined in the clinical scoring assessment sheet (Appendix E). A maximum score of 29 was attainable in healthy puppies. Based on the clinical scores, the puppies were categorised as below:

| Clinical score | Category |
|---|---|
| 26-29 | Healthy |
| 20-26 | Mildly ill |
| 15-20 | Moderately ill |
| ≤ 14 | Moribund |

### 3.2.4 Ultrasonographic examination

The puppies were conscious during the ultrasonographic examinations. Tranquilisation was avoided due to its uncertain effect on gastrointestinal motility.

Puppies were positioned in dorsal recumbency, the ventral abdominal hair clipped and acoustic coupling gel applied. All sonographic examinations were performed by the author, using a Sonoline Omnia (Siemens, Berlin, Germany) ultrasound unit. The images were acquired by means of a 5 MHz – 9 MHz multi-frequency linear array transducer operated at 7.5 MHz – 9 MHz and technical settings were adjusted for optimal image quality. A general abdominal ultrasound examination was conducted to rule out obvious pathology in unrelated organs followed by a detailed examination focusing on the gastrointestinal tract, jejunal lymph nodes and presence or absence of free peritoneal fluid. Ultrasonographic findings were documented on the ultrasound examination form (Appendix F).

The amount of free peritoneal fluid present was subjectively categorised as none, mild (single or multiple small fluid collections of up to 5 mm wide)[13], moderate (larger fluid accumulations sufficient to separate abdominal structures e.g. liver lobes)[13] or severe amount (intestines freely floating within fluid). The echogenicity of the fluid was categorised as anechoic, anechoic with echogenic specks or echogenic (hyperechoic). The degree of difficulty in locating the jejunal lymph nodes was described as not found, difficult to find or easily seen and their echogenicity relative to the mesenteric fat was subjectively assessed and recorded as anechoic, hypoechoic, hyperechoic or isoechoic. The lymph node echopattern was documented as homogenous or heterogenous. The jejunal lymph nodes were assessed in short and long axis orientation and the maximum short axis diameter (thickness) of the largest jejunal lymph node measured in millimetres.

Gastrointestinal measurements were taken by means of electronic callipers from the inner hyperechoic luminal mucosal interface to the outer hyperechoic serosal surface.[2] Stomach wall measurements were taken at the straightest portion of the greater curvature between rugal folds. Intestinal wall measurements were made on frozen images with the intestinal segment in long-axis orientation.[1,2] The thickness of the entire intestinal loop was maximally visualised prior to measurement of mural thickness to prevent erroneous measurement of an obliquely positioned intestine.

Measurements were taken from the near or far wall depending on which one had the clearest visibility. A single measurement was made of the proximal descending duodenum. Two measurements of the jejunum were made choosing a random loop from each of the left and right cranial abdominal quadrants. Results from the two jejunal measurements were averaged. The descending colon was measured in the region of the urinary bladder. Time constraints precluded assessment of the ileum which is a difficult gastrointestinal segment to rapidly localise in dogs. Additionally the jejunal and duodenal mucosal widths were measured and the echogenicity of the mucosal layer noted as isoechoic or hyperechoic relative to the adjacent muscularis layer. Wall layering was categorised as normal (all layers identified and with normal echogenicity), altered (layers were identified but had changes in echogenicity or relative thickness) or lost (layers were not visible).[12] Additionally, for puppies with parvoviral enteritis the distribution of these changes was categorized as focal (limited to one bowel segment), multisegmental (multiple regions of bowel affected) and diffuse (all visible bowel affected).[13] In cases where all the wall layers were identified but had lost their normal crisp distinction, overall wall layering for each segment was additionally categorized as crisp or indistinct (hazy). The luminal-mucosal interface was classified as normal (distinct horizontal line) or irregular and undulating. The mean sum of each of the duodenal and jejunal submucosa, muscularis and serosal layer thicknesses were calculated by subtracting the mucosal thickness from the total wall thickness.

The luminal pattern for each gastrointestinal segment was described as fluid, gas, mucus (collapsed) or alimentary.[2] Small intestinal corrugation, defined as an undulating or rippled bowel wall,[20] was recorded as present or absent. Measurements of corrugated intestines were taken from sections between the ridges formed by corrugations wherever possible. Each gastrointestinal segment, excluding the colon, was observed for a 90 second period. The number of peristaltic contractions was recorded and the average number of peristaltic contractions per minute determined. The quality of the gastrointestinal contractions were classified as normal (strong contraction with intestinal walls contacting each other during

contraction and luminal content actively moved aborally) or weak (intestinal walls not in contact and luminal contents slopping backwards and forwards).

### 3.2.5 Histopathology

Histopathological assessment of diseased organs in parvoviral infected puppies was not the primary objective of the study. However, subject to owner consent (Appendix A), patients that died naturally or those that were euthenased were submitted to the Pathology Unit, Faculty of Veterinary Science, Onderstepoort for post mortem examination. During the necropsy, lesions were described and the following tissues were collected in 10% formalin for routine haematoxylin and eosin (H&E) processing: stomach, duodenum, jejunum, ileum, colon and jejunal lymph node.

## 3.3 Data and statistical analysis

Statistical analysis was performed by means of a commercial statistical package STATA 10.1 (StataCorp, College Station, Texas). The mean, SD and range were calculated for each variable measured.

*Normal puppies*

Direct comparisons of gastrointestinal wall thickness between each of the various segments as well as comparisons between the duodenal and jejunal mucosal thicknesses were made by means of a paired student's t-test. Significance was set at $p < 0.05$. Linear regression models were used to assess the effect of age and weight on each of the variables.

*Puppies with parvoviral enteritis*

Comparisons between gastrointestinal and jejunal lymph node measurements between normal and parvovirus infected puppies were conducted by means of multiple regression analysis, adjusting for age, weight and sex. Significance was set at $p < 0.05$. For descriptive variables, the proportion of the study population affected was assessed, and 95% binomial exact confidence intervals determined.

## 3.4 Ethical considerations

Only puppies for which owners had given their written consent were used for the study. Treatment of puppies included in the trial was in no way prejudiced and the OVAH standard treatment protocols were applied in all puppies (Appendix F). The study was approved by the Animal Use and Care Committee of the University of Pretoria. (V041/07).

# CHAPTER 4 RESULTS

## 4.1 Normal puppies

Twenty-three Beagle puppies met the inclusion criteria. The puppies were between 7 and 12 weeks of age (mean 8.8 ± SD 1.8 weeks) and their body weight ranged from 2.3 - 5 kg (mean 3.0 ± SD 0.7 kg). There were eight females and 15 males. The age, weight, sex and clinical findings of the 23 Beagle puppies examined are summarised in Appendix G.

### 4.1.1 Ultrasonographic findings

A mild amount of anechoic free peritoneal fluid was observed in all puppies and was most frequently seen as a triangular focus of anechoic fluid cranial to the bladder apex or between intestinal loops in the caudal abdomen (Fig. 2).

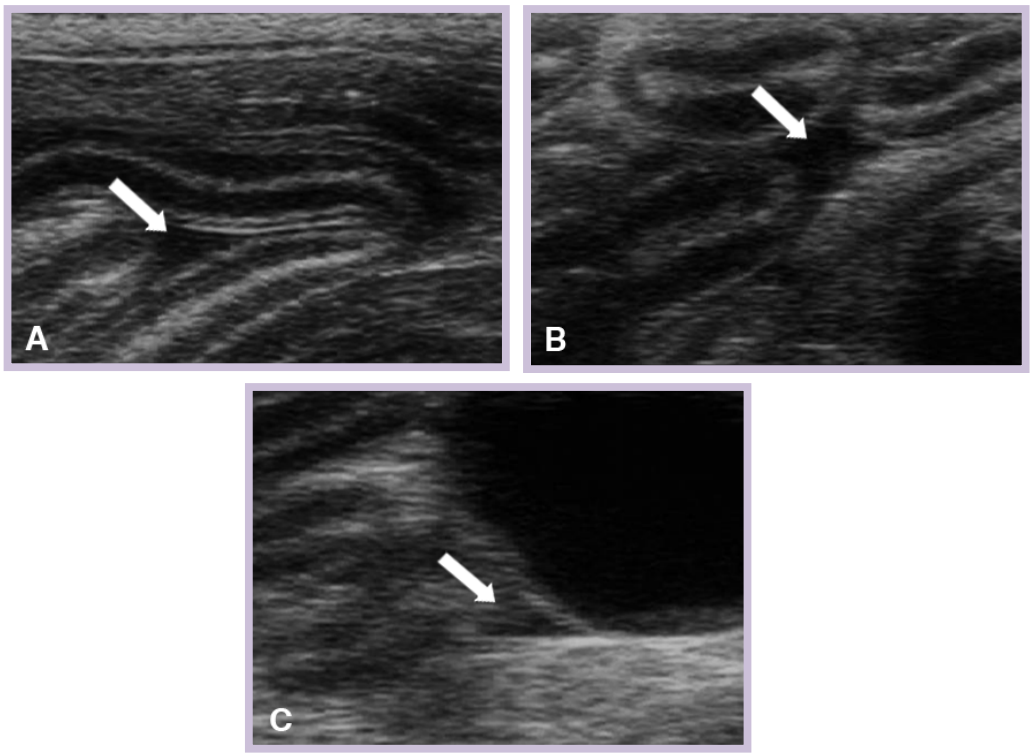


**FIGURE 2.** A mild amount of anechoic free peritoneal fluid was noted in all puppies (white arrows). (A) and (B) fluid is seen between the intestines. (C) Triangular fluid accumulation cranial to the bladder.

In all puppies, the jejunal lymph nodes were easily found (Fig. 3). They were markedly hypoechoic to the surrounding tissue with a mean thickness of 7.1 ± SD 2.2 mm (range 1.5 - 12.5 mm). There was no effect of age or weight on jejunal lymph node size in this study population ($p = 0.462$).

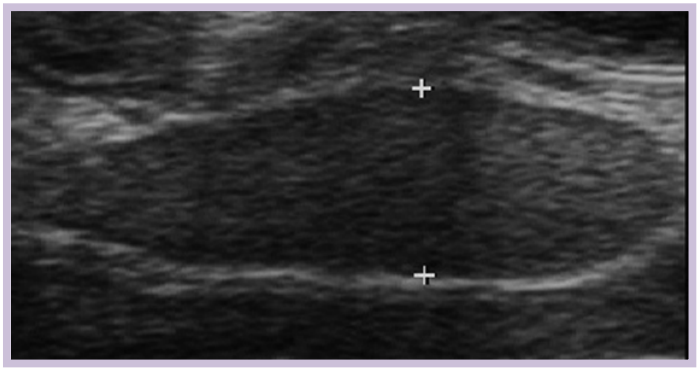

**FIGURE 3.** Jejunal lymph node (in long axis orientation) of a puppy. These lymph nodes are typically easy to find and hypoechoic to the surrounding mesenteric fat. The distance between the callipers measures 9.2 mm.

Gastrointestinal wall layering was categorized as normal in all puppies and the duodenal and jejunal mucosal and muscularis layers were isoechoic throughout. The mean thicknesses measured for each of the various regions of the gastrointestinal tract are presented in Table 1. Duodenal and jejunal mucosal thicknesses are also indicated. A list of the gastrointestinal measurements for each puppy is provided in Appendix H.

**TABLE 1. ULTRASONOGRAPHIC GASTROINTESTINAL MEASUREMENTS IN NORMAL PUPPIES**

| Anatomical site | Mean ± SD (mm) | Range (mm) | Number measured |
|---|---|---|---|
| Stomach wall | 2.7 ± 0.4 | 2.2 - 3.7 | 19/23 (93%)* |
| Duodenal wall | 3.8 ± 0.5 | 3.2 - 4.8 | 23/23 (100%) |
| Duodenal mucosa | 2.7 ± 0.5 | 2.0 - 3.8 | 23/23 (100%) |
| Duodenal wall-mucosa× | 1.1 ± 0.2 | 0.8 - 1.6 | 23/23 (100%) |
| Jejunal wall | 2.5 ± 0.5 | 1.2 - 3.4 | 23/23 (100%) |
| Jejunal mucosa | 1.5 ± 0.4 | 0.6 - 2.5 | 23/23 (100%) |
| Jejunal wall-mucosa× | 0.9 ± 0.2 | 0.5 - 1.3 | 23/23 (100%) |
| Colon wall | 1.3 ± 0.3 | 0.7 - 2.0 | 19/23 (93%)* |

* Stomach and colonic wall measurements were not obtained in all the puppies due to non-compliance
× Duodenal wall – (minus) mucosa thickness = sum of submucosa, muscularis and serosa thicknesses

The duodenal wall was significantly thicker than that of the stomach, jejunum and colon ($p < 0.0001$) and similarly the duodenal mucosal thickness was significantly thicker than the jejunal mucosal thickness ($p < 0.0001$). There was no significant effect of age or weight on jejunal or colonic wall thickness ($p > 0.05$), nor was there any effect of age or weight on jejunal or duodenal mucosal thicknesses ($p > 0.05$). There was a significant increase in duodenal and stomach wall thickness with increase in age ($p = 0.042$ and $p = 0.045$ respectively) as well as an increase in stomach wall thickness with increasing weight ($p = 0.03$). The ultrasonographic appearances of the various gastrointestinal segments are presented in Fig. 4 and their thicknesses relative to each other in Fig. 5.

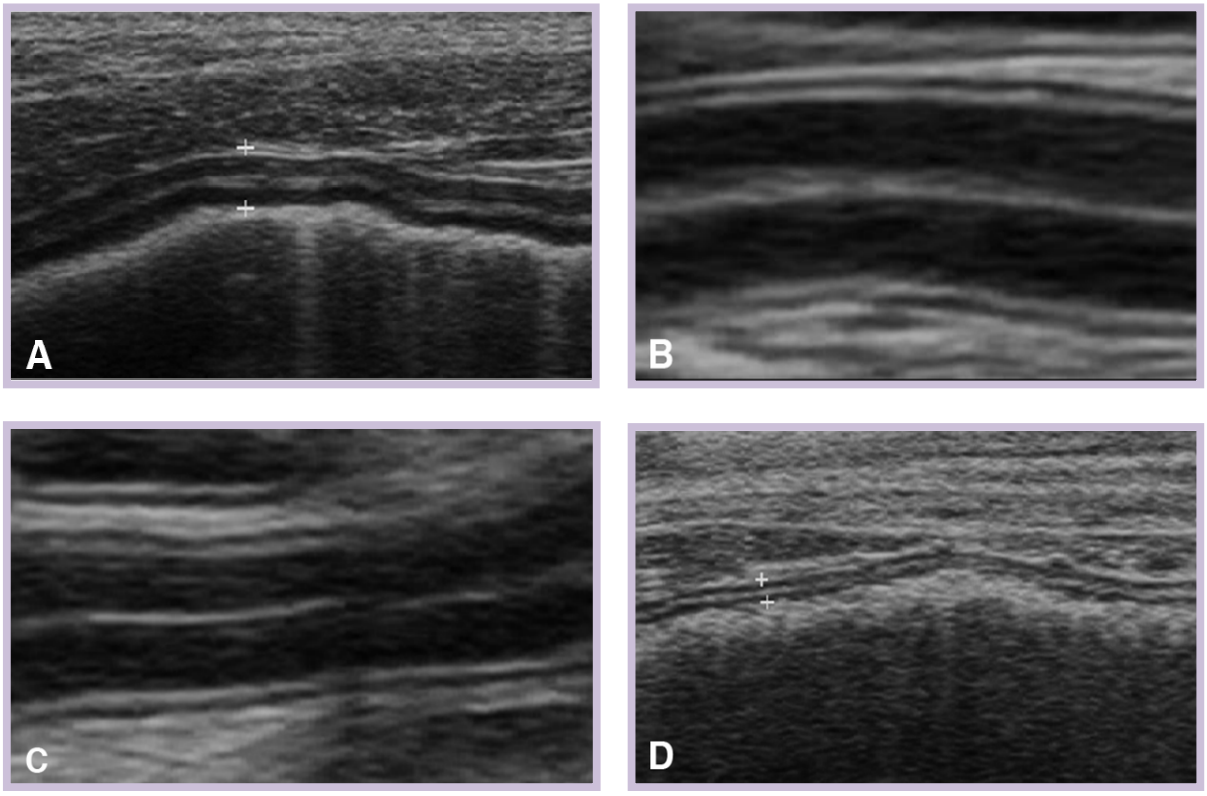


**FIGURE 4.** Appearance of wall layering in the various gastrointestinal segments in long-axis orientation (A) stomach (B) descending duodenum (C) jejunum (D) descending colon

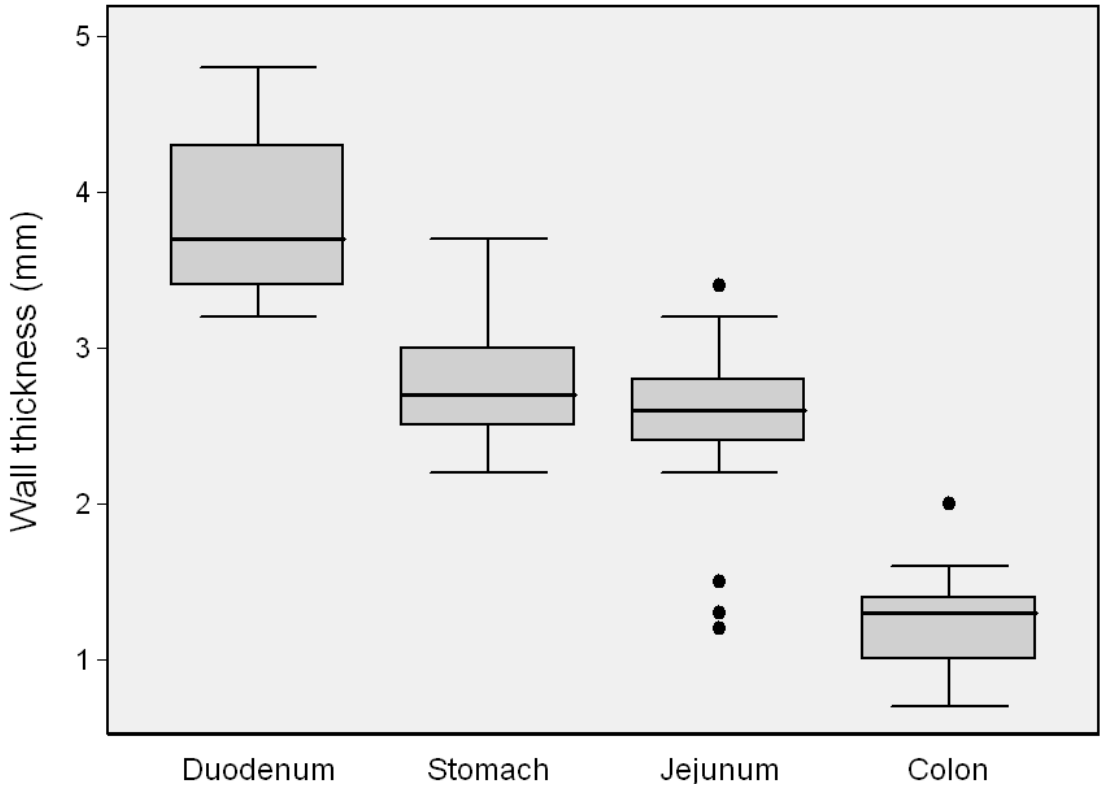


**FIGURE 5.** Box and whisker illustrations representing the wall thicknesses in millimetres of the various gastrointestinal segments relative to each other. The box extends from the 25th to the 75th percentile; the centre line represents the mean; the whiskers represent the upper and lower adjacent values (the largest observation that is less than or equal to the 75th percentile plus 1.5 times the interquartile range (IQR) and the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times the IQR). The dots represent outliers.

A mucosal (collapsed) pattern with mild amounts of gas was the predominant luminal pattern observed in the stomach, duodenum and jejunum in all puppies. Gas and faeces were observed within the colonic lumen in all puppies. There was no peristaltic activity observed in 17/23 puppies. Six puppies could not be evaluated due to non-compliance. No jejunal or duodenal corrugations were observed

## 4.2 Puppies with parvoviral infection

### 4.2.1 Ultrasonographic findings

A total of 40 puppies were included in the study (Appendix I). The puppies were between six and 24 weeks of age (mean 11 ± SD 4.7 weeks) and their body weight ranged from 1.4 – 12.5 kg (mean 4.3 ± SD 2.9 kg). There were 31 males and nine females. Affected breeds were: German Shepherd Dog (9/40), mixed breed (9/40) Jack Russell terrier (7/40), boerboel (4/40), pitbull (2/40), dachshund (2/40), Bull terrier (2/40) and one of each of Irish terrier, rottweiler, boxer, dalmation and Rhodesian ridgeback.

The amount of peritoneal fluid identified was none (8/40), mild (26/40) and moderate (6/40). In the 15% of puppies in which a moderate amount of anechoic peritoneal fluid was observed, serum total protein levels were within normal limits (although serum albumin levels were not specifically measured). In all cases in which peritoneal fluid was observed, the echogenicity of the fluid was anechoic. In 39/40 puppies, the jejunal lymph nodes were easily found and they were hypoechoic to the surrounding tissue. In the remaining dog, a 12-week-old Jack Russel terrier, the jejunal lymph nodes could not be found. The mean jejunal lymph node thickness measured 7.3 ± SD 2.1 mm (range 4.6 – 12.2 mm).

The gastrointestinal measurements for each puppy are provided in Appendix K. The mean thicknesses measured for each of the various regions of the gastrointestinal tract in parvoviral infected puppies compared to the normal values obtained in the Beagle puppies are presented in Table 2.

The duodenal and jejunal mucosa thicknesses of parvoviral infected puppies were significantly thinner than that of normal puppies ($p < 0.001$ and $p < 0.003$ respectively) (Figs. 6 and 7.) There was, however, no significant overall difference in duodenal or jejunal wall thickness in parvoviral infected *vs*. normal puppies ($p = 0.71$ and $p = 0.397$ respectively). In fact, the mean sum of duodenal submucosa, muscularis and serosal thicknesses in parvoviral infected puppies (1.9 ± SD 0.5 mm)

was significantly thicker than that of normal puppies (1.1 ± SD 0.2 mm) ($p < 0.001$). Similarly, a significantly ($p < 0.001$) thicker mean jejunal submucosa, muscularis and serosal thickness was observed in parvovirus infected puppies (1.6 ± SD 0.4 mm) *vs.* normal puppies (0.9 ± 0.2 SD mm). No significant differences in gastric or colonic wall thicknesses between parvoviral infected and normal puppies was noted.

**TABLE 2. ULTRASONOGRAPHIC GASTROINTESTINAL MEASUREMENTS IN CANINE PARVOVIRAL INFECTED PUPPIES *VS.* NORMAL PUPPIES**

| | Parvoviral infected puppies | | Normal Beagle puppies | |
|---|---|---|---|---|
| Anatomical site | Mean ± SD (mm) | Range (mm) | Mean ± SD (mm) | Range (mm) |
| Stomach wall | 2.5 ± 0.8 | 1.6 - 5.3 | 2.7 ± 0.4 | 2.2 - 3.7 |
| Duodenal wall | 3.5 ± 0.6 | 2.5 - 5.3 | 3.8 ± 0.5 | 3.2 - 4.8 |
| Duodenal mucosa | 1.7 ± 0.6* | 0.1 - 3.4 | 2.7 ± 0.5 | 2.0 - 3.8 |
| Duodenal wall-mucosa× | 1.9 ± 0.5** | 0.3 - 3.0 | 1.1 ± 0.2 | 0.8 - 1.6 |
| Jejunal wall | 2.6 ± 0.5 | 1.7 - 3.7 | 2.5 ± 0.5 | 1.2 - 3.4 |
| Jejunal mucosa | 1.0 ± 0.3* | 0.7 - 2.1 | 1.5 ± 0.4 | 0.6 - 2.5 |
| Jejunal wall-mucosa× | 1.6 ± 0.4** | 0.7 - 2.4 | 0.9 ± 0.2 | 0.5 - 1.3 |
| Colon wall† | 1.0 ± 0.3 | 0.8 - 2.0 | 1.3 ± 0.3 | 0.7 - 2.0 |

* Significantly thinner than normal puppies
** Significantly thicker than normal puppies
† Measurements only obtained in 35/40 puppies
× Duodenal wall – (minus) mucosa thickness = sum of submucosa, muscularis and serosa thicknesses

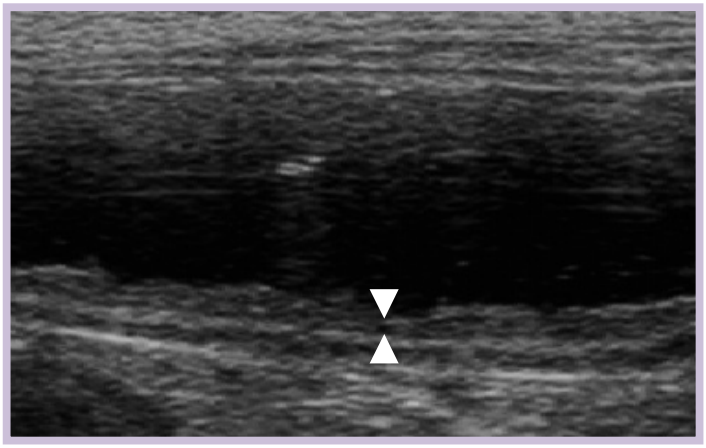

**FIGURE 6.** Longitudinal image of the duodenum of a puppy with parvoviral enteritis. Note the markedly thinner (sloughed) irregular mucosal layer indicated by the white arrow heads. The mucosal layer measured 1.3 mm in this puppy.

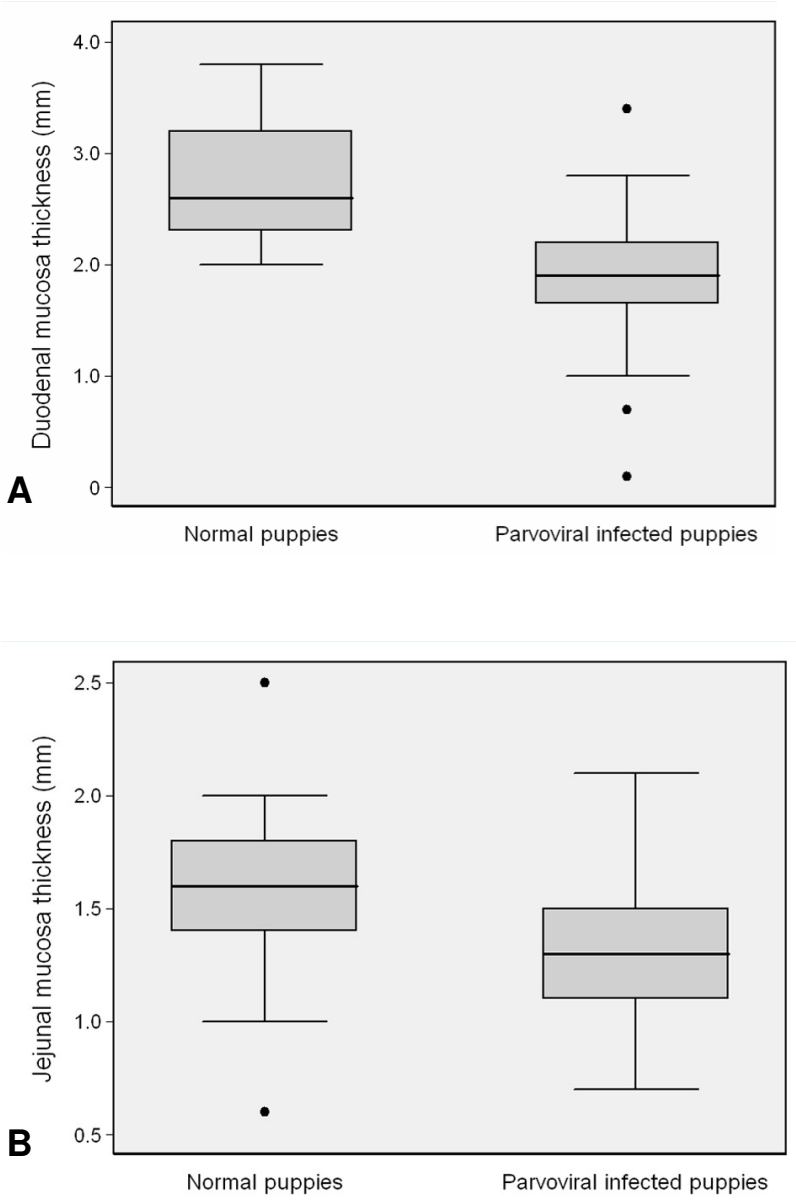


**FIGURE 7.** Box and whisker plots representing the mean thickness in millimetres of (A) duodenal and (B) jejunal mucosal layers in normal puppies compared to parvoviral infected puppies. The box extends from the 25th to the 75th percentile; the centre line represents the mean; the whiskers represent the upper and lower adjacent values (the largest observation that is less than or equal to the 75th percentile plus 1.5 times the interquartile range (IQR) and the smallest observation that is greater than or equal to the 25th percentile minus 1.5 times the IQR.) The dots represent outliers.

A fluid luminal pattern was observed in the stomach (32/40), duodenum (37/40), jejunum (38/40) (Fig. 8) and colon (25/40). In the remainder of the patients a gas or alimentary pattern was observed.

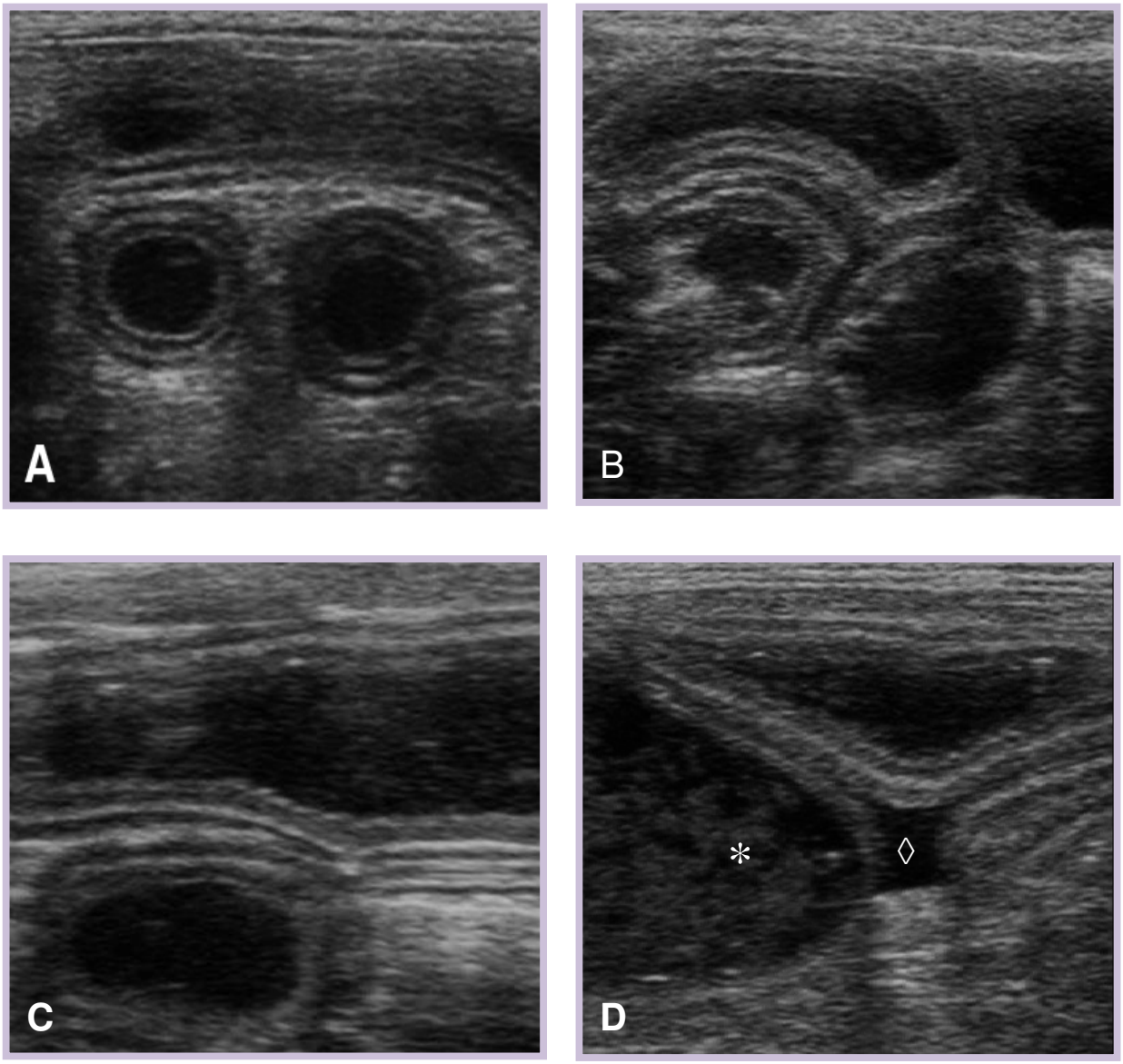


**FIGURE 8.** Images A – D demonstrate multiple fluid distended jejunal loops typically seen in canine parvoviral enteritis. Additionally, in (D) a fluid filled colon (*) with adjacent anechoic free peritoneal fluid (◊) is appreciable.

There was no gastric or duodenal peristaltic activity observed in 33/39 cases and no jejunal peristalsis in 30/39 cases. Peristaltic activity could not be assessed in 1 puppy due to non-compliance. In the remainder of the patients, 1 – 4 weak contractions per minute were observed in the stomach, duodenum and jejunum.

Results of analysis of the descriptive parameters are presented in Table 3 and comprehensive summary of the findings in each puppy in Appendix L. Duodenal corrugations (Fig. 9A) were observed in 14/40 of cases and jejunal corrugations (Fig. 9B) in 3/40 cases. Two of the cases with duodenal corrugations had concurrent jejunal corrugations. The corrugation predominantly involved the mucosal and submucosal layers. The duodenal mucosa was classified as hyperechoic in 6/40 of cases and isoechoic relative to the muscularis layer in the remaining cases (Fig. 10A). The jejunal mucosa was classified as hyperechoic to the muscularis in 20/40 of cases and isoechoic in the remaining cases. Five of the 6 cases with hyperechoic duodenal mucosal layers also had concurrent hyperechoic jejunal mucosal layers. An irregular, undulating luminal-mucosal interface (Fig. 10A) was seen in the duodenum in 9/40 cases and the jejunum in 17/40 cases. Duodenal and jejunal wall layering was classified as altered in 18/40 and 22/40 of the cases, respectively. In all of these cases, the wall layers were indistinct and had lost their crisp definition (Fig. 11). Altered wall layering was limited to the duodenum and jejunum. Gastric and colonic wall layering was intact and distinct in all cases. The extent of the duodenal lesions was classified as none (3/40), focal (2/40), multisegmental (3/40) and diffuse (32/40). The extent of the jejunal lesions was classified as none (1/40), focal (1/40), multisegmental (15/40) and diffuse (23/40).

| TABLE 3. RESULTS OF ANALYSIS OF ULTRASONOGRAPHIC DESCRIPTIVE PARAMETERS FOR CANINE PARVOVIRAL INFECTED PUPPIES | | | |
|---|---|---|---|
| Parameter | Number observed | Proportion (%) | 95% binomial exact confidence interval |
| Duodenal corrugation | 14/40 | 35 | 20.6 – 51.7 |
| Jejunal corrugation | 3/40 | 7.5 | 1.60 – 20.4 |
| Hyperechoic duodenal mucosa | 6/40 | 15 | 5.70 – 29.8 |
| Hyperechoic jejunal mucosa | 20/40 | 50 | 33.8 – 66.2 |
| Duodenum fluid filled | 37/40 | 92.5 | 79.6 – 98.4 |
| Jejunum fluid filled | 38/40 | 95 | 83.0 – 99.3 |
| Stomach fluid filled | 32/40 | 80 | 64.3 – 90.9 |
| Colon fluid filled | 25/40 | 62.5 | 45.8 – 77.0 |
| Irregular duodenal mucosa | 9/40 | 22.5 | 10.8 – 38.4 |
| Irregular jejunal mucosa | 17/40 | 42.5 | 27.0 – 59.1 |
| Indistinct duodenal wall layers | 18/40 | 45 | 29.2 – 61.5 |
| Indistinct jejunal wall layers | 22/40 | 55 | 38.4 – 70.7 |

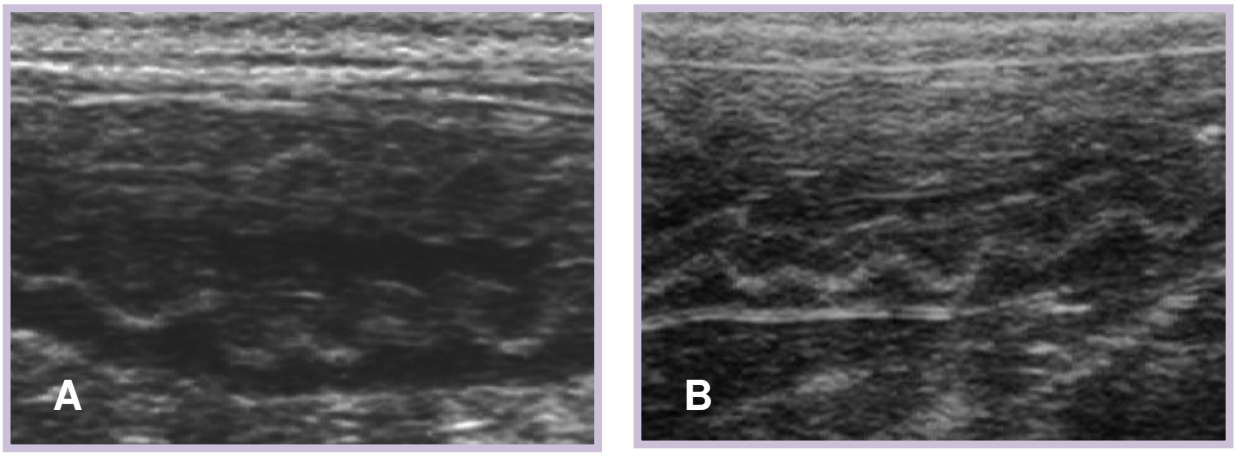


**FIGURE 9** Longitudinal image of a corrugated duodenum (A) and jejunum (B) in a puppy suffering from canine parvoviral enteritis. Note the corrugation is predominantly within the mucosa and submucosa.

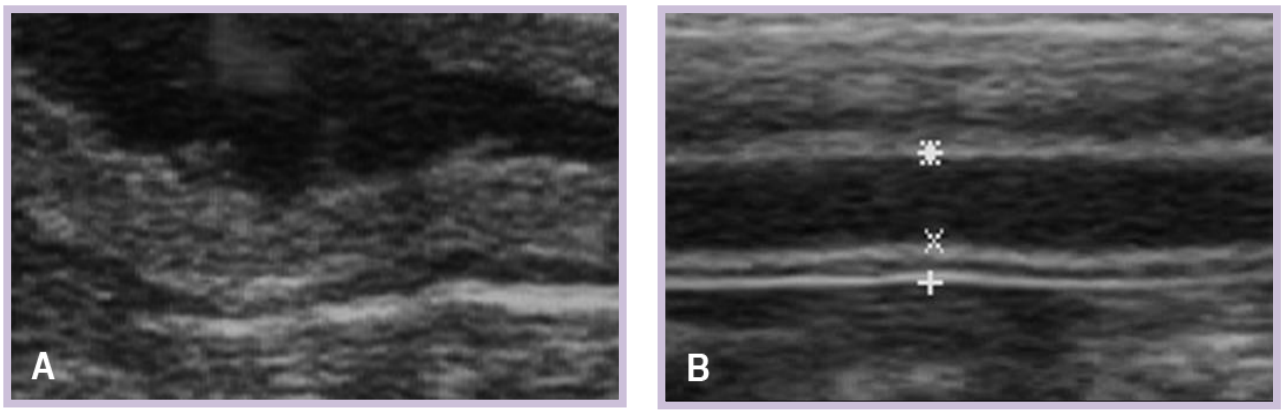


**FIGURE 10.** (A) Longitudinal image of the far wall of the descending duodenum of a 12-week-old puppy with parvoviral enteritis. The mucosal layer is markedly hyperechoic and there is irregularity and mild undulation of the luminal-mucosal interface. (B) Longitudinal image of the far wall of the descending duodenum of an 8-week-old normal beagle puppy for comparison. Note the smooth luminal-mucosal interface and the hypoechoic mucosal layer. Incidental callipers indicating (+) wall and (x) mucosal thicknesses.

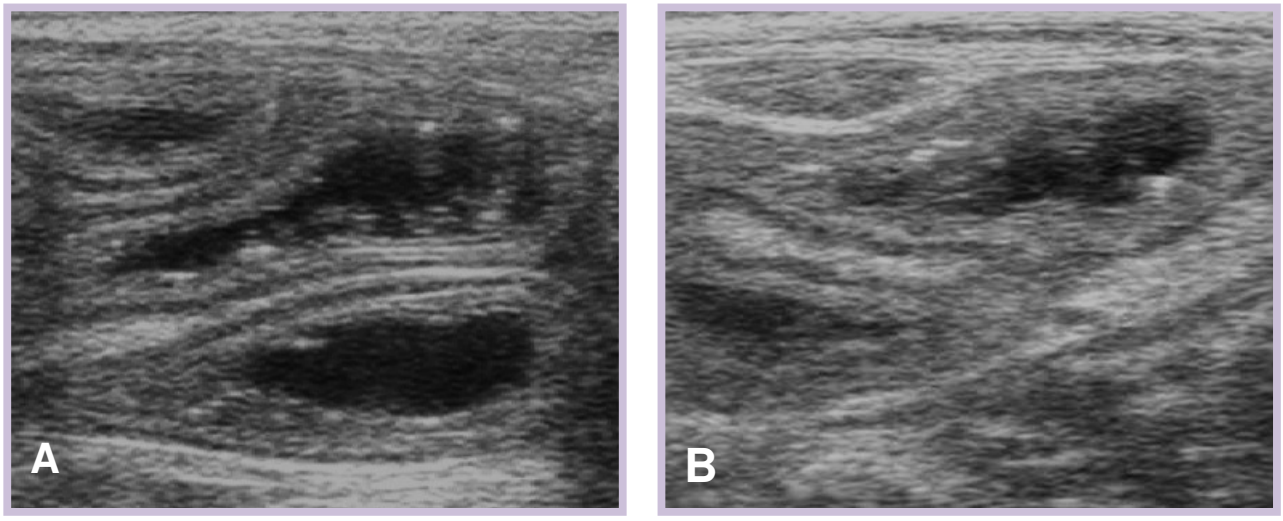


**FIGURE 11.** Fluid filled loops of jejunum displaying indistinct wall layering, thinner mucosal layers with extensive hyperechoic speckling and irregular luminal-mucosal surfaces seen in two puppies suffering from canine parvoviral enteritis. The wall layering is barely distinguishable in B.

### 4.2.2 Clinical outcome and correlation with ultrasonographic findings

Sixty percent of puppies (24/40 cases) had a clinical score of ≤ 14 out of 29. Table 4 indicates the correlation between the clinical score in these puppies compared to ultrasonographic findings of intestinal corrugation and mucosal changes. A comprehensive table can be found in Appendix M. Seventy-nine percent (11/14) of the duodenal corrugations and 100% (3/3) of the jejunal corrugations were seen in puppies with a clinical score of ≤ 14. Similarly 83% (5/6) of cases with hyperechoic duodenal mucosal layers, 70% (14/20) with hyperechoic jejunal mucosa, 78% (7/9) with irregular duodenal luminal-mucosal interface and 76% (13/17) of cases with irregular jejunal luminal-mucosal interfaces had a clinical score of ≤ 14.

**TABLE 4. CORRELATION BETWEEN CLINICAL SCORE, INTESTINAL CORRUGATION AND MUCOSAL CHANGES IN PARVOVIRAL INFECTED PUPPIES WITH CLINICAL SCORES ≤ 14**

| CLNICAL SCORE | Duodenal corrugation | Jejunal corrugation | Hyperechoic duodenal mucosa | Hyperechoic jejunal mucosa | Irregular duodenal mucosa | Irregular jejunal mucosa |
|---|---|---|---|---|---|---|
| 11 | X | | X | X | X | X |
| 11 | X | | | | | X |
| 11 | | | | X | X | X |
| 12 | | | X | X | X | X |
| 12 | | | | X | X | X |
| 12 | X | | | X | | X |
| 12 | X | | | X | | X |
| 12 | X | | | X | X | X |
| 13 | | | | X | | |
| 13 | | X | X | | | |
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| 14 | X | X | | X | | X |
| 14 | | | | | | |
| 14 | X | | | | X | X |
| 14 | X | | | X | | X |

In this study population, a mortality rate of 30% (12/40 cases) was observed. No correlation was found between the outcome of the patients and their clinical score or ultrasonographic findings. The average length of hospitalisation of the surviving puppies was 5 ± SD 2.4 days.

### 4.2.2 Histopathology findings in two puppies

Two of the puppies that died underwent a full post mortem and histopathological examination of the stomach, duodenum, jejunum, ileum, colon and jejunal lymph nodes was performed. Puppy 1 died 4 days after admission to the OVAH Isolation Unit and puppy 2 died 6 days after admission.

**Stomach**

Puppy 1 - moderate mucosal congestion

Puppy 2 - essentially normal (Fig. 12)

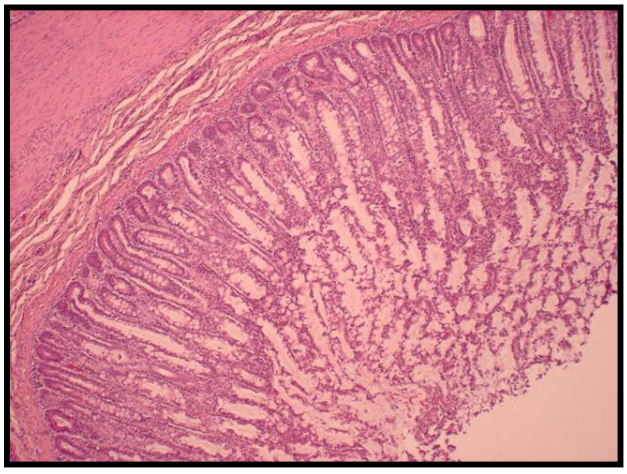

**FIGURE 12.** Essentially normal histopathology section of the stomach of a puppy suffering from canine parvoviral enteritis. (H & E processing; 10X objective was used)

**Duodenum**

Puppy 1 - multifocal crypt collapse/necrosis with severe transmural vascular congestion, villous necrosis with numerous bacteria invading the necrotic tissue. Relatively few lymphocytes were seen in the lamina propria, some of which showed necrosis.

Puppy 2 – loss of normal villous architecture, collapse of the mucosa and extensive blunting and fusion of villi with widespread crypt epithelial necrosis and multifocal complete crypt loss (Fig. 13). The denuded mucosa was extensively colonised by filamentous bacteria.

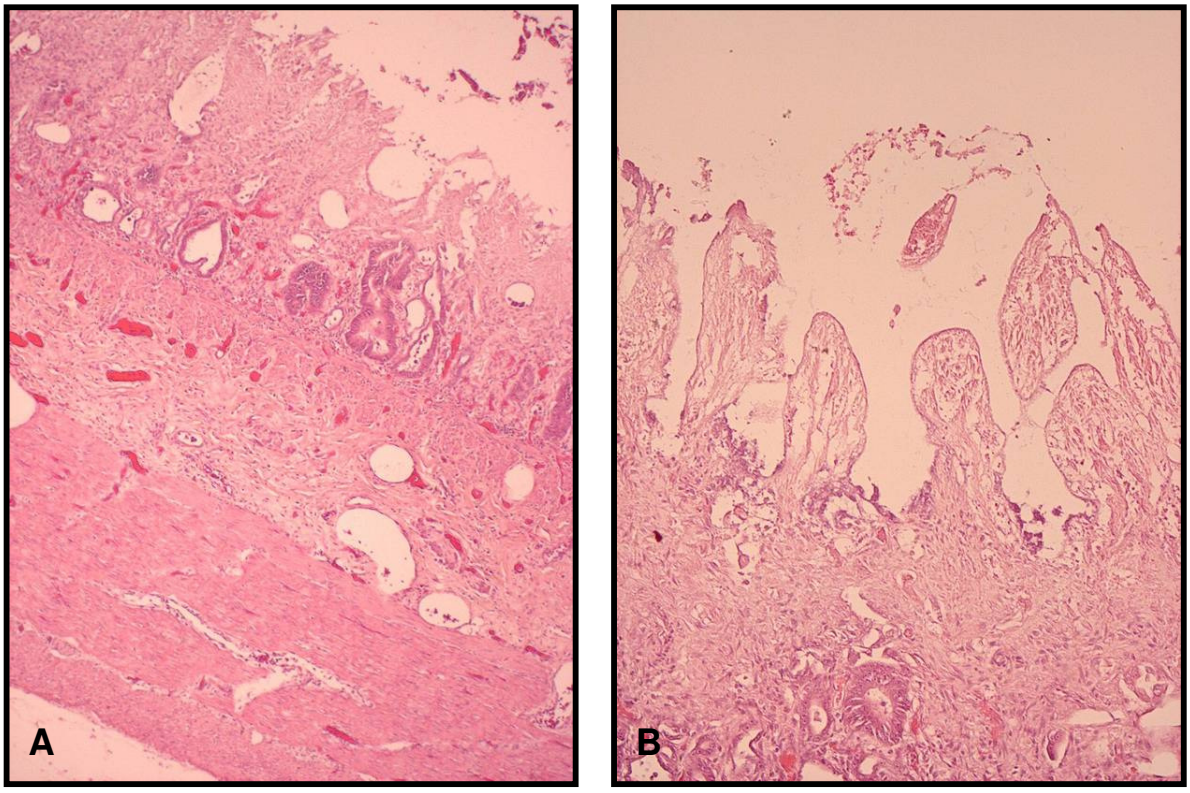


**FIGURE 13.** Histopathology sections of a duodenum affected by canine parvoviral enteritis. (A) Overview of duodenal wall layers with luminal surface at the top of the image. (B) Magnified section of mucosa displaying villous collapse and crypt necrosis. (H & E processing)

**Jejunum**

Puppy 1 - extensive, diffuse crypt necrosis with extreme vascular congestion. Necrotic villi were noted and numerous bacterial colonies were seen invading the necrotic tissue (Fig. 14).

Puppy 2 - as in the duodenum, there was loss of normal villous architecture, collapse of the mucosa and extensive blunting and fusion of villi with widespread crypt epithelial necrosis and multifocal complete crypt loss. The denuded mucosa was extensively colonised by filamentous bacteria.

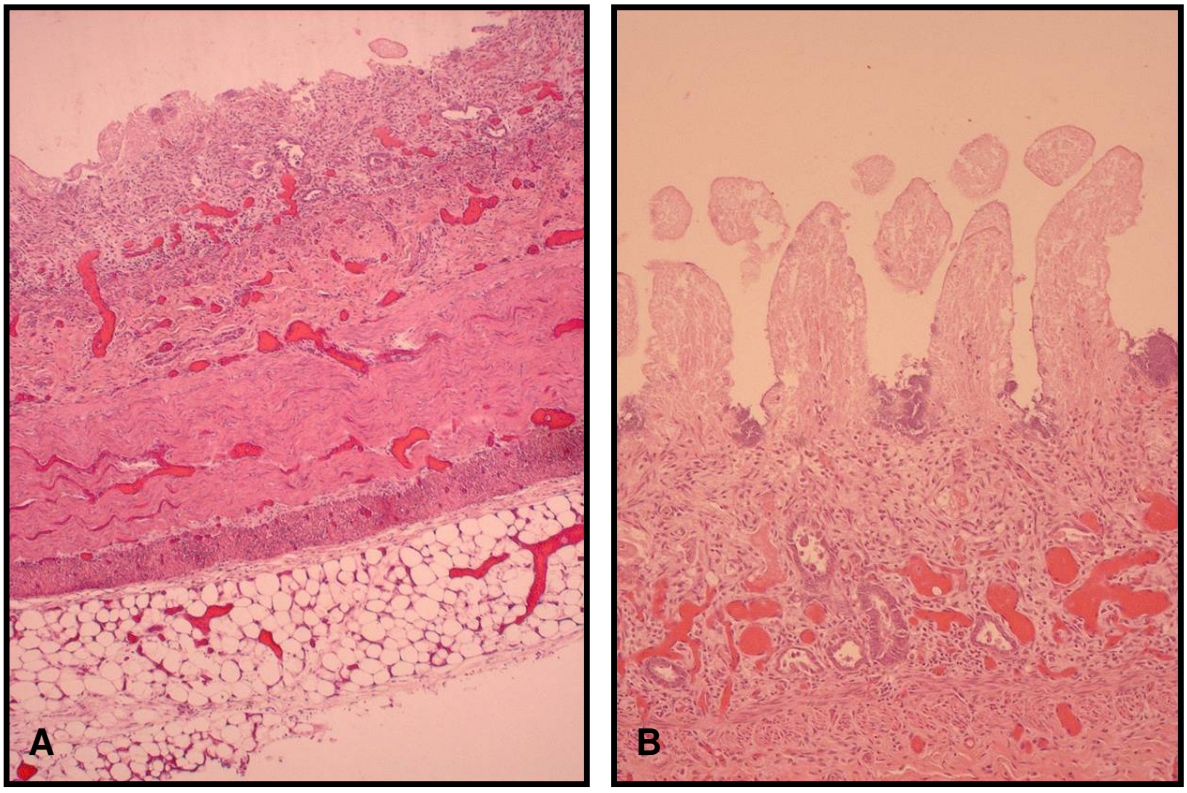


**FIGURE 14.** Histopathology sections of a jejunum affected by canine parvoviral enteritis. (A) Overview of jejunal wall layers with luminal surface at the top of the image – note the near complete absence of villi. (B) Magnified section of mucosa displaying villous blunting and extreme vascular congestion. (H & E processing)

**Ileum**

Puppy 1 - Severely depleted lymphoid tissue of Peyer's patches. Extreme transmural vascular congestions with multifocal crypt necrosis/collapse and villous necrosis of the mucosa overlying the Peyer's patches (Fig. 15). The rest of the mucosa appeared to be sloughing off strands of enterocytes.

Puppy 2 – Less marked villous collapse and crypt epithelial necrosis than noted in the duodenum and jejunum. Completely lymphoid depleted Peyer's patches.

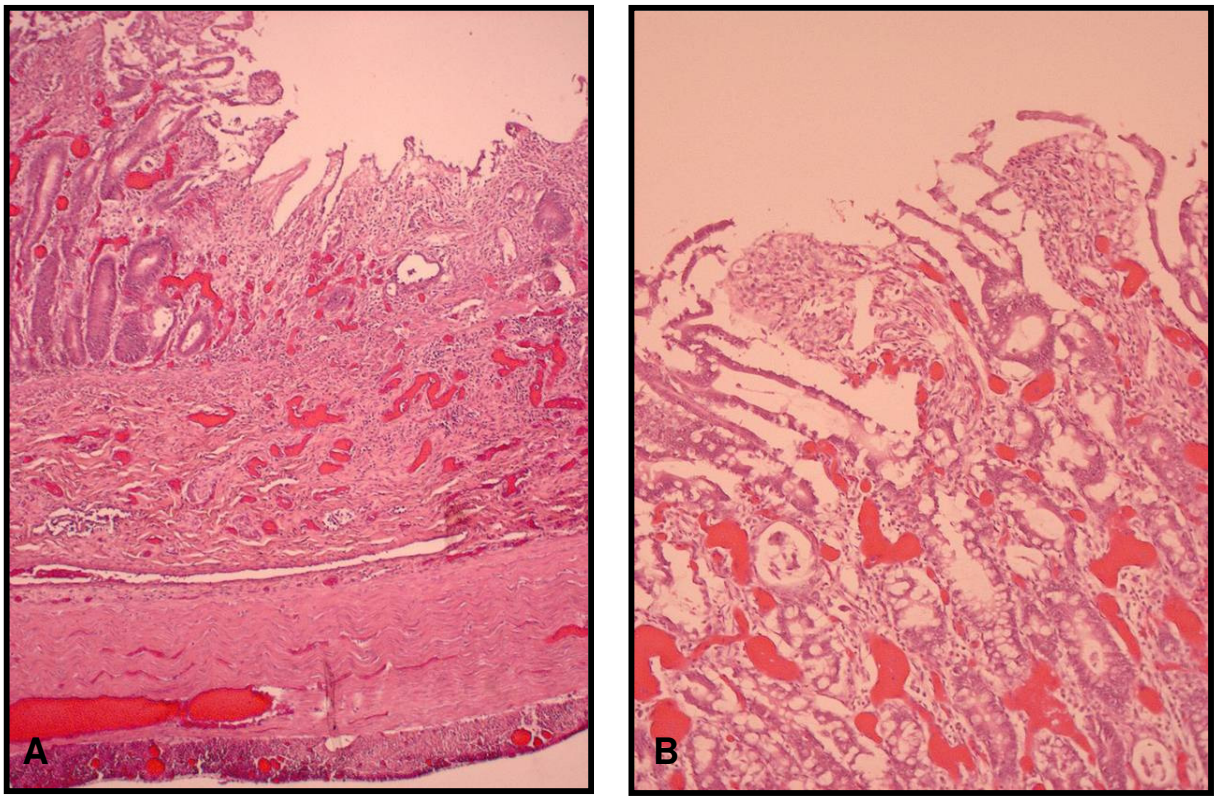


**FIGURE 15.** Histopathology sections of an ileum affected by canine parvoviral enteritis. (A) Overview of ileal wall layers with luminal surface at the top of the image. (B) Magnified section of mucosa displaying extreme vascular congestion with villous necrosis and sloughing. (H & E processing)

**Colon**

Puppy 1 - diffuse extreme congestion and multifocal mucosal haemorrhage and depleted gut associated lymphoid tissue.

Puppy 2 - essentially normal with overgrowth of bacterial rods noted.

**Jejunal lymph node**

Puppy 1 - all jejunal lymph nodes were found to be extremely congested with small lymphoid follicles and expanded interfollicular regions of lymphocytes.

Puppy 2 - severe diffuse lymphoid atrophy (Fig. 16).

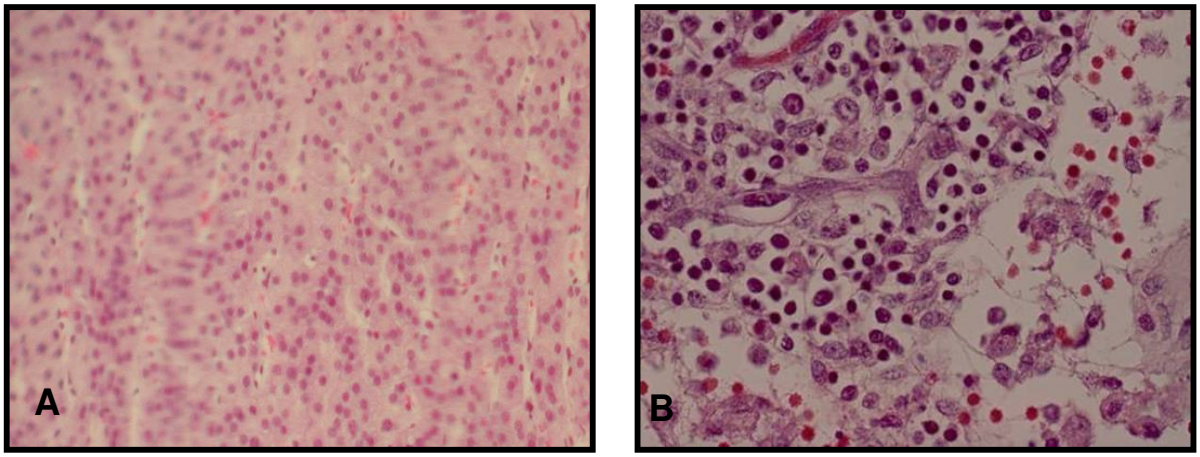


**FIGURE 16.** Histopathology sections of the cortex of a normal jejunal lymph node (A) and that of a jejunal lymph node affected by canine parvovirus (B). Note the cortical depletion of lymphocytes in the lymph node affected by CPV. (H & E processing)

# CHAPTER 5 DISCUSSION

## 5.1 Ultrasonography of normal puppies

Ultrasonography of canine paediatric patients is challenging due to poor patient compliance. The healthy puppies examined in this study were incredibly wriggly and reluctant to lie stationary for the 20 min ultrasonographic examination time needed to assess the various abdominal structures. As a result, attempts to accurately observe the number of peristaltic contractions over a 90 sec period for each gastrointestinal segment were largely unsuccessful in six puppies. Furthermore, the non-compliance led to progressive aerophagia and hampered assessment of abdominal structures due to overlying gastrointestinal luminal gas.

### 5.1.1 Gastrointestinal tract

In a previous study comprising a mixed population of dogs, ultrasonographic small and large intestinal wall measurements ranged from 2 - 3 mm and gastric wall measurements from 2 - 5 mm.[1] This study also included four Beagle dogs that were scanned at 13, 27 and 40 weeks of age. No significant differences were noted in gastrointestinal wall thickness of immature and mature dogs of the same breed, nor between dogs of small, medium or large breeds.[1] Results from our study indicate mean gastric wall measurements of 2.7 ± SD 0.4 mm, mean duodenal wall thickness of 3.8 ± SD 0.5 mm and mean jejunal wall thickness of 2.5 ± SD 0.5 mm. Furthermore, contrary to that study,[1] colonic wall measurements in our study population were significantly thinner than both duodenal and jejunal wall thicknesses with a mean value of 1.3 ± 0.3 mm. A trend towards increased jejunal and duodenal thickness with increasing body weight was documented in a study of 231 dogs[6] with no evidence of gastrointestinal disease. The study included 69 breeds with body weights ranging from 2.1 kg - 64 kg. No mention was made of the age of the dogs in the study population. Forty-one dogs had a body weight of < 10 kg and duodenal and

jejunal thicknesses for this group were 4.0 ± SD 0.7 mm and 3.2 ± SD 0.6 mm respectively. Increased thickness of the duodenal wall compared to the jejunal wall has been attributed to a thicker duodenal mucosal layer[48] and our results concur with these histological findings.

Ultrasonographic measurement of the canine duodenal and jejunal mucosal layer thickness has not been previously documented. Generally, the gastrointestinal mucosal layer has been described as being the thickest of the wall layers but the mucosa and muscularis layers can be of equal thickness during peristalsis.[51] In our study the duodenal and jejunal mucosal layers were by far the thickest of the wall layers, measuring 2.7 ± SD 0.5 mm and 1.5 ± SD 0.4 mm respectively. The mean duodenal mucosal thickness constituted 71% of the total wall thickness and the mean jejunal mucosal thickness 60% (Figs. 4B and 4C). The remainder of the duodenal and jejunal wall layers namely the submucosa, muscularis and serosa, subjectively appeared to be of equal thickness. Interestingly, the gastric mucosa was not the thickest of the layers, instead the mucosa, submucosa and muscularis all appeared to be of equal thickness with a thinner serosa noted (Fig. 4A). All colonic wall layers appeared to have comparable thickness (Fig. 4D). This is however a subjective opinion as no measurements were taken of the above mentioned wall layers due to the limited scan time tolerated by the puppies.

### 5.1.2 Peritoneal fluid

A mild amount of anechoic free peritoneal fluid was found in all puppies – a finding which in the author's opinion cannot be appreciated in adult canines. The nature of the fluid could not be determined as abdominocentesis was not performed. Based on previously described ultrasonographic characteristics of peritoneal fluid,[28] the anechoic fluid observed in the puppies was presumed to be a fluid of low cellularity.

### 5.1.3 Jejunal lymph nodes

Recently, the ultrasonographic appearance of jejunal lymph nodes in 57 dogs between 6 months and 14 years was described.[14] The jejunal lymph nodes were found to be slightly hypoechoic or isoechoic to the mesentery. Median maximum thickness was reported to be 3.9 mm and median maximum width 7.5 mm. No mean values were reported. Significantly thicker lymph nodes were found in younger dogs (exact numbers and age of dogs in this category were not stated) and attributed to higher immunological activity. Furthermore, a wide, hypoechoic rim of uneven, undulating diameter was described in normal jejunal lymph nodes of dogs less than 2 years of age. This echo pattern was not observed in our study population where jejunal lymph nodes were found to be homogenous and markedly hypoechoic to the surrounding mesenteric fat making them prominent and easy to locate. Results from our study indicate a mean jejunal lymph node thickness of 7.1 ± SD 2.2 mm (range 1.5 - 12.5 mm) in puppies up to 12 weeks of age.

### 5.1.4 Limitations of the study

There were several limitations to this study. Only Beagle dogs were examined and reference values for puppies of different breeds remain to be established. Secondly, although our study demonstrated a significant effect of age on duodenal and stomach wall thickness, the age range of our study population was too small to make further deductions. Further studies on dogs of the same breed but different ages are needed. In addition, power Doppler assessment of jejunal lymph node vascularity was not performed. Lastly, assessment of jejunal lymph node size and appearance within the study population over time was not conducted. Studies of the same animal at various time intervals are needed to further our understanding of the change in ultrasonographic appearance of the jejunal lymph nodes with increasing age.

## 5.2 Ultrasonography of puppies with parvoviral infection

### 5.2.1 Gastrointestinal tract

Infection with canine parvovirus is acquired by the faecal-oral route of transmission. Canine parvovirus exhibits a tropism for rapidly replicating cell populations of the intestinal crypt epithelium, lymphoid and haematopoeitic tissues.[52] The virus reaches the intestinal mucosa by means of the bloodstream, replicating in the germinal epithelium of the intestinal crypts. Small intestinal viral proliferation causes extensive epithelial necrosis with villus blunting and atrophy.[37,52] Loss of villi results in collapse of the intestinal epithelium, loss of absorptive capacity, and development of haemorrhagic diarrhoea 4 - 5 days after oral exposure. Characteristic microscopic intestinal lesions consist of necrosis of the epithelium, villus atrophy or collapse, and/or disruption of the lamina propria. Mucosal thinning, erosion and ulceration have been noted.[52] Dilated intestinal glands filled with necrotic debris and epithelial regeneration are also commonly observed.[36] The significant mucosal thinning of the duodenum and jejunum observed in this study is believed to correlate with these histopathological findings of villus sloughing, mucosal erosion and ulceration and crypt necrosis. Because cell turnover in the gastrointestinal tract is rapid (1 - 3 days), intestinal malabsorption is short-lived and recovery from the enteric form of the disease in the absence of secondary complications is rapid with appropriate therapy.[41] Complications are attributable to the disruption of the intestinal barrier function, which results in secondary bacteraemia and endotoxaemia, and the development of systemic inflammatory response and multiple organ dysfunction syndromes.[53] Follow-up ultrasonographic examinations were not performed on the patients in this study and hence the ultrasonographic progression of the disease over time was not documented.

Slight differences in gastrointestinal wall thickness can be observed between distended and contracted intestinal segments.[1] Previous studies in people have demonstrated a difference of 2 mm between the intestinal wall thickness measured in a distended or non-distended status.[3,7] Distended, fluid filled bowels can compound

the observed mucosal thinning but, in the author's opinion are an unlikely primary cause of the mucosal thinning in this study as the overall duodenal and jejunal wall thickness remained within normal limits when compared to the values obtained in the normal Beagle puppies. In a distended state, the intestinal wall becomes uniformly thinner. There was no thinning of any of the other wall layers in this study, in fact, a significant increase in the mean sum of thicknesses of the submucosa, muscularis and serosa was noted. This thickening of the remaining wall layers was such that there was no overall difference between the duodenal and jejunal wall layer thicknesses in parvoviral infected *vs*. normal puppies. The cause of this increased thickness of the submucosa, muscularis and serosa in parvoviral infected puppies is uncertain. A possible explanation could be the histological presence of widespread subserosal haemorrhage (which may extend into the muscularis and submucosa) in puppies with parvoviral enteritis.[54] Widespread transmural vascular congestion was also noted on the two histopathological evaluations conducted in our study. A second possible aetiology could be transmural oedema however this was not found histopathologically in our study or the literature.

Mucosal hyperechoic striations or speckles have been described in dogs with chronic enteropathies (although food responsive diarrhoea is rarely associated with mucosal echogenicity changes)[55] and may be seen in cases of inflammatory bowel disease,[13,54] lymphangiectasia and villous histiocytic sarcoma.[13] The mucosal striations are thought to result from reflected ultrasound waves from dilated lacteals with mucosal speckles presumably representing a partial section through a dilated lacteal. Other aetiologies, including focal accumulations of reflective substances such as mucous, cellular debris, protein, fibrous tissue or gas in the mucosal crypts, have been suggested.[55] The mucosal hyperechogenicity observed in some of the parvoviral infected puppies is likely a combination of mucous, cellular debris and protein accumulation - the end products of the extensive necrosis and inflammation which characterizes this disease histopathologically. This degree of extensive hyperechoic speckling within the duodenal or jejunal mucosa has not been described before.

An intussusception is a recognized complication of severe enteritis (including parvoviral enteritis)[47]. Intussusceptions can result in mechanical ileus and fluid accumulation oral to the intussusception.[18] Cases with intussusceptions were specifically excluded from this study and thus the generalised atony or decreased gastrointestinal motility observed in the puppies under investigation was attributed to functional ileus due to the inflammation associated with parvoviral enteritis.

### 5.2.2 Peritoneal fluid

In the majority of parvoviral infected puppies only a mild amount of anechoic free peritoneal fluid was observed and this is believed to be normal and not associated with the underlying disease process based on observations of a similar mild amount of anechoic free peritoneal fluid in the group of normal Beagle puppies. Total serum protein levels were within normal limits in the 15% of puppies in which a moderate amount of anechoic free peritoneal fluid was observed. Serum albumin levels were not measured and hence the exact cause of this effusion is unknown.

### 5.2.3 Jejunal lymph nodes

The jejunal lymph node size and appearance in parvoviral enteritis (mean thickness 7.3 ± SD 2.1 mm) is comparable to that found in the normal Beagle puppies (mean thickness 7.1 mm ± SD 2.2 mm) and thus parvoviral infection does not appear to be associated with ultrasonographically apparent gross changes or our hypothesized regional lymph node enlargement. Histopathologically, severe cortical depletion of lymphocytes (cortical atrophy) within jejunal lymph nodes has been described in canine parvoviral enteritis.[56] A possible explanation for the absence of ultrasonographic evidence of jejunal lymphadenomegally in parvoviral enteritis may be due to the opposing effect of this severe cortical atrophy.

### 5.2.4. Clinical correlation with ultrasonographic findings

The correlation between clinical score and ultrasonographic findings suggest a trend towards more severe ultrasonographic changes being observed in the more

moribund puppies. The absence of correlation between ultrasonographic findings and patient outcome is likely due to the variable length of hospitalisation prior to death and thus prolonged interval between ultrasonographic examination/clinical score (both conducted within 24 hours of admission) and death. Further studies documenting the daily ultrasonographic progression of changes in puppies suffering from parvoviral enteritis are needed before ultrasonographic prognosticators or further conclusions can be drawn from this study.

### 5.2.5 Correlation with histopathology

The prolonged time interval between ultrasonographic examination and post mortem histopathology made it impossible to draw direct conclusions between ultrasonographic findings and histopathology lesions. Further studies on in-vitro, water-bath specimens of the gastrointestinal tract in CPV cases are needed to directly correlate ultrasonographic with histopathological findings. However, albeit 4-6 days post ultrasound evaluation, the post mortem findings in the two puppies presented in this report did correspond to the general CPV ultrasonographic changes observed in this study. The histopathological findings of essentially normal stomach and colon walls correspond with the normal ultrasonographic assessment of the stomach and colon walls in puppies suffering from canine parvoviral enteritis. Furthermore, the widespread jejunal and duodenal villous sloughing and necrosis noted on histopathology correspond to the ultrasonographic findings of extensive mucosal thinning and necrosis (evident as diffuse hyperechoic mucosal speckling) seen with CPV.

### 5.2.6 Limitations of the study

Firstly, this study did not examine the ultrasonographic appearance of other gastrointestinal diseases and the changes observed in puppies with parvoviral enteritis cannot be considered pathognomonic for the disease. Duodenal corrugation has for example been described in other causes of enteritis such as lympho-plasmacytic enteritis and haemorrhagic duodenitis. It is a non-specific finding and may also be seen with peritonitis, neoplasia and most commonly, pancreatitis.[20] Other changes observed with parvoviral enteritis such as fluid filled atonic bowels can

be seen in functional and mechanical ileus of which there are many causes. Secondly, it was beyond the scope of this study to evaluate the ultrasonographic progression of parvoviral enteritis over time and hence follow-up ultrasonographic examinations were not conducted.

## 5.3 Future studies

This study did not examine the ultrasonographic appearance of other paediatric gastrointestinal diseases such as severe verminosis, giardiasis, coccidiosis or distemper viral infection. Although these diseases may be associated with some of the changes observed in canine parvoviral enteritis (such as fluid filled, atonic intestines), based on their pathophysiologies, they are unlikely to cause the mucosal thinning observed in this study. Studies on the ultrasonographic appearance of these diseases are needed before further conclusions can be drawn from this study.

Daily ultrasonographic examinations of puppies suffering from canine parvoviral enteritis are needed to further understand the progression of this disease over time as well as the possible ultrasonographic indicators of clinical improvement or deterioration. It is the author's opinion that as early as 1 week post recovery from CPV, the gastrointestinal tract is ultrasonographically essentially normal – this can be attributed to the immense regenerative capacity of the intestinal epithelium. This hypothesis however remains to be proven.

# CHAPTER 6 CONCLUSION

This study provides reference values for gastrointestinal wall, duodenal and jejunal mucosal thicknesses for Beagle puppies up to 12 weeks of age. Prominent jejunal lymph nodes and a mild amount of anechoic free peritoneal fluid are considered normal findings in healthy puppies. The author believes that this data will facilitate improved future assessment of gastrointestinal disease in puppies.

Ultrasonography is by no means a method for diagnosing parvoviral enteritis. Ultrasonographic changes which may be considered indicative of canine parvoviral enteritis include: fluid filled, atonic small and large intestines; duodenal and jejunal mucosal layer thinning with or without indistinct wall layers and irregular luminal-mucosal surfaces; duodenal and/or jejunal extensive hyperechoic mucosal speckling and duodenal and/or jejunal corrugations. Each of the above described changes cannot be considered pathognomonic for canine parvoviral enteritis but if observed during abdominal ultrasound of the paediatric patient, the clinician should be alerted of the likelihood of underlying parvoviral infection.

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# APPENDICES

## APPENDIX A
## CLIENT CONSENT FORM

**Research project: Gastrointestinal ultrasonographic changes of canine parvovirus infection**

The following consent form is to be completed by the patient's owner or an authorised person:

**Please encircle yes or no where necessary below:**

1) Have you read the information sheet on the research project?
   Yes / No
2) Have you had the opportunity to ask questions about this research project?
   Yes / No
3) Are you happy with the answers you have received?
   Yes / No
4) Do you grant consent for your puppy to participate in our research project for the duration of its hospitalisation?
   Yes / No
5) Do you give permission for us to shave the hair on his/her belly?
   Yes / No
6) I hereby also give permission that a full post mortem examination can be performed on my dog in the event of death or euthenasia.
   Yes / No

Name of owner:

_____

Name of patient: Breed:

_____

Signature of owner / authorised representative:

_____

Date: Time:

_____

# APPENDIX A
## VORM VIR EIENAAR TOESTEMMING

**Navorsingsprojek: Maagdermkanaal ultraklank veranderinge in honde met parvovirusinfeksie**

Die vorm moet deur die pasiënt se eienaar / gemagtigde persoon ingevul word:

**Omkring asseblief Ja of Nee, waar toepaslik:**

1) Het u die inligtingsblad oor die navorsingsprojek gelees?
   Ja / Nee
2) Het u die geleentheid gehad om oor die navorsingsprojek uit te vra?
   Ja / Nee
3) Is u vrae bevredigend beantwoord?
   Ja / Nee
4) Verleen u toestemming dat u hondjie deelneem in ons navorsingsprojek gedurende sy hospitalisasie?
   Ja / Nee
5) Verleen u toestemming vir ons om die hare op u hondjie se buik te skeer?
   Ja / Nee
6) Hiermee gee ek ook toestemming dat ‘n volledige nadoodse ondersoek op my hond uitgevoer mag word in die geval van dood / genadedood.
   Ja / Nee

Naam van eienaar:

_____

Naam van hondjie: Ras:

_____

Handtekening van eienaar / gemagtigde verteenwoordiger :

_____

Datum: Tyd:

_____

# APPENDIX B
## CLIENT INFORMATION SHEET

**Research project: Gastrointestinal ultrasonographic appearance of canine parvovirus infection**

Due to your puppy's vomiting and diarrhoea, we suspect that he/she may be suffering from canine parvo viral enteritis (so called "catflu"). It has been advised that your puppy be admitted to the Onderstepoort Veterinary Academic Hospital's Isolation ward for intensive treatment. Your puppy will be placed on intravenous fluids, and will receive various drugs such as antibiotics, deworming and drugs to help stop the vomiting.

We are conducting a study to determine what a puppy's intestines look like ultrasonographically if infected with canine parvovirus. We would like to perform an abdominal ultrasound examination on your puppy. In order to do this we will need to clip some of your puppy's hair on his/her abdomen. An ultrasound examination is non-painful and non-invasive and your puppy will be lying comfortably on a blanket during this time. The ultrasound exam may detect pathology of which the clinician was not aware. This may help in the earlier diagnosis and treatment of complications.

This study will also involve an additional faecal test in order to confirm whether your puppy has parvovirus infection or not. The cost of these tests or procedures will be covered by the research project's funds. No additional costs will be incurred for the ultrasound examination.

Your decision whether or not to allow your puppy to participate in this study will in no way influence the treatment of this case.

Thank-you for allowing your puppy to be included in this study. If you have any questions regarding the research project, please do not hesitate to discuss them with the duty clinician or myself.

Dr N Stander
Department of Companion Animal Clinical Studies,
University of Pretoria.
Room 4-64
Tel: 012 529 8289 (within clinic hours)

# APPENDIX B
## KLIËNT INLIGTINGSBLAD

**Navorsingsprojek: Maagdermkanaal ultraklank veranderinge in honde met parvovirusinfeksie**

As gevolg van u hondjie se erge vomisie en diarree, vermoed ons dat hy/sy dalk hond parvovirus enteritis (die sogenaamde „katgriep“) onderlede kan hê. Dit word aanbeveel dat u hondjie opgeneem word na die Isolasie-eenheid van die Onderstepoort Veterinêre Akademiese Hospitaal vir intensiewe behandeling. U hondjie sal binne-aarse vloeistof ontvang en sal ook verskeie ander medikasie ontvang soos antibiotika, ontwurming en middels wat naarheid en vomisie onderdruk.

Ons is besig met ‘n studie oor die utrasonografiese voorkoms van die derms in hondjies met parvovirus. Ons sal graag ‘n ultraklank ondersoek op u hondjie se buik wil uitvoer. Dit sal nodig wees om die hare op u hondjie se buik te skeer. Hierdie ultraklank prosedure is nie pynlik nie en u hondjie sal die heeltyd gemaklik op ‘n kombers lê. Die ultraklank ondersoek mag dalk patologie ontdek waarvan die klinikus nie bewus is nie en sodoende bydra tot die vroeë diagnosering en behandeling van komplikasies.

‘n Addisionele fekale toets sal op u hondjie uitgevoer word om te bevestig of u hondjie wel parvovirus infeksie het. Die kostes vir hierdie toets sowel as enige ander kostes wat verband hou met die projek (uitgesluit kostes vir die standaard behandeling van parvovirus) sal deur die projek se fondse gedra word.

U besluit om u hondjie te laat deelneem aan die studie of nie, sal op geen manier die behandeling van u hondjie beïnvloed nie.

Dankie dat u toelaat dat u hondjie ingesluit kan word in die studie. Indien u enige navra oor die navorsingsprojek het, is u welkom om dit met my of met die klinikus aan diens te bespreek.

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# APPENDIX C

## GENERAL TREATMENT GUIDELINES FOR CANINE PARVOVIRAL ENTERITIS

(Adapted from current OVAH parvoviral treatment protocol)

General treatment for all dogs will consist of the following:

- All dogs will have an intravenous catheter placed at time of admission.

- Initial intravenous fluid therapy will be aimed at correcting dehydration within 6 hours. Degree of dehydration (%) will be determined according to accepted guidelines. The fluid used for this purpose will be 1L Ringer's Lactate®, with 20ml of 50% dextrose solution, and 1 vial (20mEq) potassium chloride (Sabax Potassium Chloride) added. The volume of fluid needed to effect rehydration will be determined using the following formula: (Body mass x 10 x % dehydration = volume in ml). Following this initial period of fluid replacement, serum potassium and glucose concentration will be determined, and potassium chloride and glucose added to the intravenous fluid according to deficits.

- Once the rehydration phase has been completed (approximately 6 hours post-admission), the patients will be started on enteral feeding either via syringe feeding, or via a naso-oesophageal tube. Enteral feeding will consist of any of the following: Hill's a/d; Hill's can i/d; Eukanuba feline intestinal diet; Eukanuba intestinal for puppies; skinless chicken.

- Once the rehydration phase had been completed, the maintenance phase of intravenous fluid therapy will be initialized: The fluid used for this purpose will be Ringer's Lactate®, spiked with potassium chloride and 50% dextrose. Daily serum potassium and glucose concentration will be determined for this purpose, and potassium chloride and dextrose added to the intravenous fluid according to deficits. The rate of administration of this fluid will be tailored to the individual patient's needs. The total amount of daily fluid requirement (enteral and parenteral) will be estimated as follows: maintenance fluid requirements + ongoing losses. The ongoing loss due to the diarrhoea is estimated to be 10-20 ml/kg/24 hrs initially, and will be adjusted during the course of treatment.

- Antibiotic administration:
  - Amoxycillin
    (a) Initially Amoxil® intravenous (i/v), 15mg/kg, tid. Once rehydration had been effected and peripheral perfusion judged adequate (absence of hypothermia, capillary refill time > 2 seconds, normalized skin tugor), the formulation will be changed to amoxycillin per os (capsules or suspension)

    (b) Clamoxyl RTU® injectable suspension subcutaneous (s/c), 20 mg/kg, bid, and once no vomition had occurred for 24 hours, the formulation will be changed to

(c) Clamoxyl tablets® per os, 20g/kg, bid, or Amoxycillin suspension per os, 20mg/kg, bid.

The amoxycillin treatment will be combined with gentamicin treatment, to effect greater gram-negative spectrum to the antibiotic regime, as follows:

- Gentamicin (Genta® 20 PHENIX)
  (a) Due to the risk of acute renal failure development, gentamicin will only be administered after the rehydration phase of therapy had been completed (see above).

  (b) Dose: 2.2 mg/kg, i/v, tid.

- Antemetic therapy:
  - Metoclopramide (Clopamon®) at 0.2-0.4 mg/kg every 6-8 hours OR 2mg/kg/24hr via continuous rate i/v infusion as standard treatment,
  - or prochlorperazine (Stemetil®) at 0.1 mg/kg, i/v. every 4-6 hours if metoclopramide is ineffective in controlling vomition.

- Sucralfate (Ulsanic® suspension) at 1 ml per 3 kg, p/o, every 6 hours, to persistently vomiting dogs to prevent reflux oesophagitis.

- Fenbendazole (Panacur® BS) 50 mg/kg, oid, p/o for 5 days, irrespective of whether helminth eggs are identified on faecal flotation.

- Heated cages if hypothermia is present.

- Daily intravenous potassium supplementation if hypokalaemia is present

- Plasma transfusion (20 ml/kg) if albumin < 15 g/dl or total serum protein < 35 g/dl.

- Whole blood transfusion if haematocrit < 15%

# APPENDIX D
## ADDITIONAL INFORMATION SHEET

Patient: **STICKER**

E.M. Positive for parvovirus? YES / NO
E.M. Positive for coronavirus? YES / NO
E.M. Positive for distempervirus? YES / NO

**TREATMENT / CLINICAL PARAMETERS**
(To be recorded on same date and time as the ultrasound examination)

| **TREATMENT** | **DETAILS** |
|---|---|
| Antemetics | |
| Fluids | |
| Gastric protectants | |
| Anthelmintics | |
| Antibiotics | |
| Plasma/Blood | |
| **LABORATORY PARAMETER** | |
| Potassium | |
| Haematocrit | |
| Total Protein | |
| Glucose | |

**PATIENT OUTCOME**

Died / Recovered

Date died / recovered: …….

Time died / recovered: …….

Days to recovery (resolution of clinical signs) / death? ……..

Complications developed? …….

If so, describe …….

# APPENDIX E

## CLINICAL SCORING ASSESSMENT

Patient name and number: **STICKER**

Date:

Day number: 0 (admission),1,2,3,4,5,6,7 or 8. (Encircle choice)

Temperature: Pulse: Respiration rate:

**Encircle the applicable choice under 1 – 8 below:**

| | | |
|---|---|---|
| **1) Habitus** | 1 | Collapsed / moribund |
| | 2 | Severe depression |
| | 3 | Mild-to-moderate depression |
| | 4 | Normal |
| **2) Appetite** | 1 | No interest in food |
| | 2 | Voluntarily eats small amounts of food offered |
| | 3 | Voluntarily eats moderate amounts of food offered (but not normal) |
| | 4 | Normal |
| **3) Vomition** | 1 | Severe (≥ 6 times per 12h) |
| | 2 | Moderate (3-5 times per 12h) |
| | 3 | Mild (1-2 times per 12h) |
| | 4 | Absent |
| **4) Faecal consistency** | 1 | Watery diarrhoea, bloody |
| | 2 | Watery diarrhoea, not bloody |
| | 3 | Soft |
| | 4 | Well-formed |
| **5) Mucous membranes** | 1 | Congested |
| | 2 | Pale |
| | 3 | Normal |

## CLINICAL SCORING ASSESSMENT

| | | |
|---|---|---|
| **6) Capillary refill time** | 1 | > 2 seconds |
| | 2 | < 1 second |
| | 3 | 1-2 seconds |
| **7) Abdominal palpation** | 1 | Tense and / or painful |
| | 2 | Gassy and fluid filled intestines |
| | 3 | Thickened or doughy |
| | 4 | Normal |
| **8) Borborygmi** | 0 | Absent |
| | 1 | Present, but auscultate with difficulty |
| | 2 | Present, auscultate easily |
| | 3 | Audible without the use of a stethoscope |

**Score for a normal clinical assessment:** 26-29

# APPENDIX F
## ULTRASOUND EXAMINATION FORM

| | | | | |
|---|---|---|---|---|
| **Owner name** | | Patient sticker | | |
| **Owner number** | | | | |
| **Patient name** | | | | |
| **Patient number** | | | | |
| **Microchip number** | | | | |
| **Video recording no.** | | | | |
| **Sex** | Male Female | | | |
| **Age in weeks** | | **Group** | 1 | OTAU beagle |
| **Weight in kg** | | | 2 | EM parvovirus positive |
| **Date of admission** | | **Time of admission** | | |
| **Date of U/S exam** | | **Time of U/S Exam** | | |
| **Hours starved** | | **Hours post admit** | | |

### EXTRA-INTESTINAL ASSESSMENT

| | | | |
|---|---|---|---|
| **General abdominal scan** | | | |
| **Abdominal fluid** | | | |
| Amount and location | None | | |
| | Mild | Single or multiple small fluid collections of up to 5 mm wide | |
| | Moderate | Larger fluid accumulations sufficient to separate abdominal structures | |
| | Severe | Intestines freely floating in the fluid | |
| Echogenicity | Homogenous | Echogenic | |
| Additional comments | | | |
| **Jejunal lnn** | | | |
| Maximum thickness | | | |
| Echopattern | Homogenous | Heterogenous | |
| Echogenicity | Anechoic | Hypoechoic | Hyperechoic |
| Indication of number | One | few (3-5) | plenty (>5) |
| Ease of locating lnn | Not found | Difficult to find | Easily seen |
| Other lnn seen | Yes | No | Which? |
| Additional comments | | | |
| **Comments / Incidental findings** | | | |
| **Transducer frequency** | | | |

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# GASTROINTESTINAL ASSESSMENT

| | Duodenum | | Jejunum RHS | | Jejunum LHS | | Stomach | | Colon | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Peristaltic contractions** | | | | | | | | | | |
| Number observed / 90sec | | | | | | | | | | |
| Nature of contractions (weak / normal) | | | | | | | | | | |
| **Measurements** (longitudinally) | Collapsed | Distended | Collapsed | Distended | Collapsed | Distended | Collapsed | Distended | Collapsed | Distended |
| Total GIT segment diam | | | | | | | | | | |
| Luminal width | | | | | | | | | | |
| Wall width | | | | | | | | | | |
| Mucosal width | | | | | | | | | | |
| Muscularis width | | | | | | | | | | |
| | | | | | | | | | | |
| **GIT wall layering** | | | | | | | | | | |
| Intact layering | | | | | | | | | | |
| Altered layering | | | | | | | | | | |
| Lost layering | | | | | | | | | | |
| Echogen of muc vs musc | equal hyper hypo | | equal hyper hypo | | equal hyper hypo | | equal hyper hypo | | equal hyper hypo | |
| Echogencity of mucosa | anech hyper hypo | | anech hyper hypo | | anech hyper hypo | | anech hyper hypo | | anech hyper hypo | |
| Specify changes in other layers | | | | | | | | | | |
| **Corrugations?** | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Absent |
| **GIT luminal content** | | | | | | | | | | |
| F = Fluid G = Gas M = Mucosal A = Alime | F G M A | | F G M A | | F G M A | | F G M A | | F G M A | |
| **Lesion distribution** | | | | | | | | | | |
| Segmental / Multisegmental / diffuse | | | | | | | | | | |
| **Additional comments** | | | | | | | | | | |
| **Transducer frequency** | | | | | | | | | | |

P

# APPENDIX G
## Results datasheet for normal puppies
Age, weight, sex and clinical parameters

| BEAGLE PUPPY | AGE (weeks) | WEIGHT (kg) | SEX | TEMPERATURE | PULSE (bpm) | RESPIRATION (bpm) | CLINICAL SCORE |
|---|---|---|---|---|---|---|---|
| 1 | 8 | 3.0 | Female | 38.8 | 150 | 40 | 26 |
| 2 | 8 | 3.1 | Male | 38.2 | 180 | panting | 26 |
| 3 | 8 | 3.2 | Male | 38.7 | 200 | 50 | 26 |
| 4 | 8 | 2.8 | Male | 38.0 | 180 | 60 | 26 |
| 5 | 7 | 2.6 | Female | 38.3 | 150 | panting | 26 |
| 6 | 7 | 2.7 | Male | 37.2 | 180 | panting | 26 |
| 7 | 7 | 2.4 | Female | 38.8 | 180 | panting | 26 |
| 8 | 7.5 | 2.6 | Male | 38.2 | 150 | panting | 26 |
| 9 | 7.5 | 2.3 | Male | 38.2 | 150 | panting | 26 |
| 10 | 7.5 | 2.5 | Female | 38.1 | 180 | panting | 26 |
| 11 | 7.5 | 2.7 | male | 38.5 | 180 | panting | 26 |
| 12 | 7.5 | 2.4 | Female | 38.2 | 150 | panting | 26 |
| 13 | 7.5 | 2.7 | Male | 38.1 | 150 | panting | 26 |
| 14 | 11 | 3.6 | Female | 38.5 | 162 | 42 | 26 |
| 15 | 12 | 3.5 | Male | 38.9 | 180 | 36 | 26 |
| 16 | 12 | 3.6 | Male | 38.8 | 150 | 40 | 26 |
| 17 | 9 | 2.9 | Male | 37.9 | 150 | panting | 26 |
| 18 | 9 | 3.1 | Male | 37.3 | 140 | panting | 26 |
| 19 | 9 | 2.8 | Female | 38.2 | 160 | panting | 26 |
| 20 | 9 | 2.8 | Female | 38.5 | 150 | panting | 26 |
| 21 | 9 | 2.9 | Male | 38.0 | 140 | panting | 26 |
| 22 | 12 | 4.9 | Male | 38.0 | 150 | 32 | 26 |
| 23 | 12 | 5.0 | Male | 38.4 | 140 | 40 | 26 |

bpm = beats or breaths per minute

# APPENDIX H
## Results datasheet for normal puppies
Ultrasonographic measurements in millimetres

| BEAGLE PUPPY | Jejunal lymph thickness | Duodenal wall thickness | Duodenal mucosa thickness | Duodenal wall-mucosal thickness | Jejunal wall thickness | Jejunal mucosa thickness | Jejunal wall-mucosal thickness | Stomach wall thickness | Colon wall thickness |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.5 | 3.4 | 2.1 | 1.3 | 1.2 | 0.6 | 0.6 | 3.2 | 1.0 |
| 2 | 9.2 | 3.5 | 2.5 | 1.0 | 2.6 | 1.8 | 0.9 | 3.2 | - |
| 3 | 6.4 | 4.3 | 3.0 | 1.3 | 2.2 | 1.4 | 0.9 | 3.0 | 1.5 |
| 4 | 6.8 | 4.8 | 3.8 | 1.0 | 2.7 | 1.7 | 1.1 | 2.5 | - |
| 5 | 7.9 | 3.5 | 2.6 | 0.9 | 2.8 | 1.6 | 1.2 | 2.5 | 1.3 |
| 6 | 7.9 | 3.3 | 2.3 | 1.0 | 2.7 | 1.7 | 1.0 | 2.4 | 1.4 |
| 7 | 12.5 | 4.2 | 3.0 | 1.2 | 2.4 | 1.4 | 1.0 | 2.7 | 1.3 |
| 8 | 9.7 | 4.3 | 3.2 | 1.1 | 3.4 | 2.5 | 0.9 | 2.9 | 2.0 |
| 9 | 5.3 | 4.1 | 2.6 | 1.5 | 2.7 | 1.7 | 1.0 | 2.3 | 1.3 |
| 10 | 9.2 | 3.8 | 3.0 | 0.8 | 1.5 | 1.0 | 0.5 | - | 1.3 |
| 11 | 4.4 | 4.4 | 3.2 | 1.2 | 2.8 | 1.7 | 1.1 | 2.2 | 1.3 |
| 12 | 6.7 | 3.2 | 2.4 | 0.8 | 2.6 | 1.6 | 1.0 | - | 1.2 |
| 13 | 5.4 | 3.8 | 2.6 | 1.2 | 2.5 | 1.4 | 1.1 | 2.2 | 1.3 |
| 14 | 7.2 | 4.5 | 3.2 | 1.3 | 2.4 | 1.4 | 1.0 | 2.6 | 1.3 |
| 15 | 5.4 | 4.8 | 3.2 | 1.6 | 2.9 | 2.0 | 0.9 | 3.7 | 1.4 |
| 16 | 8.0 | 3.4 | 2.5 | 0.9 | 2.6 | 1.5 | 1.2 | 3.2 | 1.6 |
| 17 | 7.7 | 3.2 | 2.3 | 0.9 | 2.4 | 1.5 | 0.9 | 2.7 | 0.9 |
| 18 | 7.7 | 3.2 | 2.0 | 1.2 | 2.9 | 1.9 | 1.0 | 2.5 | 1.2 |
| 19 | 7.0 | 3.4 | 2.2 | 1.2 | 1.3 | 0.6 | 0.7 | - | - |
| 20 | 7.5 | 3.6 | 2.4 | 1.2 | 2.4 | 1.5 | 0.9 | 3.0 | 0.7 |
| 21 | 9.4 | 3.2 | 2.2 | 1.0 | 2.9 | 1.7 | 1.2 | - | - |
| 22 | 4.8 | 4.3 | 3.3 | 1.0 | 2.8 | 1.8 | 1.1 | 2.7 | 1.0 |
| 23 | 6.2 | 3.7 | 2.5 | 1.2 | 3.2 | 2.0 | 1.3 | 2.7 | 1.0 |

# APPENDIX I
## Results datasheet for puppies with parvoviral enteritis
Age, weight, sex and breed

| PUPPY | AGE (weeks) | WEIGHT (kg) | SEX | BREED |
|---|---|---|---|---|
| **1** | 12 | 7.6 | Male | pitbull terrier |
| **2** | 24 | 5.7 | Male | crossbreed |
| **3** | 18 | 12.0 | Female | Boerboel |
| **4** | 20 | 2.8 | Male | Irish terrier |
| **5** | 8 | 7.1 | Male | Boerboel |
| **6** | 8 | 7.4 | Male | Boerboel |
| **7** | 8 | 3.6 | Female | German Shepherd Dog |
| **8** | 8 | 3.7 | Male | German Shepherd Dog |
| **9** | 12 | 7.5 | Male | Boerboel X |
| **10** | 12 | 1.4 | Male | Maltese X |
| **11** | 8 | 3.9 | Male | Boerboel |
| **12** | 8 | 7.8 | Male | rottweiler |
| **13** | 8 | 4.3 | Male | bull-terrier |
| **14** | 12 | 2.7 | Male | pitbull terrier X |
| **15** | 6 | 1.4 | Male | boxer |
| **16** | 20 | 1.9 | Male | Jack Russel terrier |
| **17** | 20 | 1.8 | Female | Jack Russel terrier |
| **18** | 12 | 2.5 | Female | dachshund |
| **19** | 20 | 5.0 | Male | Jack Russel terrier |
| **20** | 16 | 12.4 | Male | dalmation |
| **21** | 20 | 9.3 | Male | crossbreed |
| **22** | 8 | 4.7 | Male | German Shepherd Dog |
| **23** | 8 | 5.4 | Male | German Shepherd Dog |
| **24** | 8 | 5.0 | Female | German Shepherd Dog |
| **25** | 8 | 5.2 | Male | German Shepherd Dog |
| **26** | 8 | 5.4 | Female | German Shepherd Dog |
| **27** | 8 | 5.0 | Male | German Shepherd Dog |
| **28** | 8 | 4.7 | Female | German Shepherd Dog |
| **29** | 16 | 12.5 | Female | bull terrier |
| **30** | 12 | 1.8 | Male | Jack Russel terrier |
| **31** | 15 | 2.5 | Male | dachshund |
| **32** | 15 | 2.6 | Male | crossbreed |
| **33** | 10 | 2.7 | Male | crossbreed |
| **34** | 8 | 3.6 | Female | Rhodesian ridgeback |
| **35** | 8 | 7.6 | Male | German Shepherd X |
| **36** | 13 | 8.2 | Male | rottweiler X |
| **37** | 16 | 3.4 | Male | Jack Russel terrier |
| **38** | 12 | 2.6 | Male | Jack Russel terrier |
| **39** | 12 | 4.6 | Male | Jack Russel terrier |
| **40** | 9 | 5.8 | Male | pitbull terrier |

# APPENDIX J
## Results datasheet for puppies with parvoviral enteritis
Clinical score, assessment of biochemical parameters and patient outcome

| PUPPY | TEMPERATURE (˚C) | PULSE (bpm) | RESPIRATION (bpm) | CLINICAL SCORE | POTASSIUM (mmol/l) | GLUCOSE (mmol/l) | TOTAL SERUM PROTEIN (g/l) | Ht (%) | DAYS HOSPITALISED | OUTCOME |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38.7 | 116 | 28 | 14 | 3.14 | 4.98 | 50 | 36 | 9 | Alive |
| 2 | 38.7 | 100 | 60 | 13 | 3.41 | 4.7 | 66 | 47 | 4 | Alive |
| 3 | 38.5 | 152 | 28 | 13 | 3.5 | 3.3 | 58 | 49 | 6 | Alive |
| 4 | 38.7 | 90 | 40 | 16 | 3.76 | 3.9 | 54 | 42 | 4 | Alive |
| 5 | 37.7 | 100 | 24 | 14 | 4.4 | 7.1 | 44 | 38 | 4 | Dead |
| 6 | 37.0 | 110 | 32 | 17 | 3.35 | 5.6 | 52 | 33 | 6 | Dead |
| 7 | 37.0 | 180 | 40 | 11 | 2.94 | LO | 38 | 39 | 2 | Dead |
| 8 | 38.2 | 88 | 44 | 15 | 3.35 | 4.5 | 44 | 25 | 4 | Dead |
| 9 | 39.0 | 140 | 48 | 12 | 2.9 | 5.1 | 65 | 37 | 3 | Dead |
| 10 | 37.9 | 240 | 72 | 13 | 2.56 | 4 | 50 | 32 | 1 | Dead |
| 11 | 38.8 | 180 | 24 | 12 | 3.68 | 6.3 | 50 | 34 | 9 | Alive |
| 12 | 40.0 | 220 | 36 | 12 | 3.48 | - | 52 | 28 | 1 | Dead |
| 13 | 38.9 | 104 | 30 | 15 | 3.6 | - | 50 | 28 | 4 | Dead |
| 14 | 38.5 | 84 | 32 | 19 | 3.8 | 4.4 | 46 | 19 | 3 | Dead |
| 15 | 38.3 | 154 | 32 | 13 | 4.26 | 6.8 | 44 | 29 | 6 | Dead |
| 16 | 38.3 | 120 | 28 | 18 | 3.23 | 4.5 | 48 | 36 | 5 | Alive |
| 17 | 37.9 | 180 | 20 | 13 | 3.25 | 2.8 | 44 | 45 | 8 | Alive |
| 18 | 38.2 | 180 | 48 | 15 | 4.3 | 3 | 52 | 30 | 4 | Dead |
| 19 | 39.2 | 200 | 56 | 14 | 3.28 | 4.6 | 76 | 54 | 4 | Alive |
| 20 | 37.5 | 160 | 16 | 14 | 5.01 | 4.8 | 65 | 37 | 5 | Alive |

bpm = beats or breaths per minute
Ht = haematocrit

# APPENDIX J
## Results datasheet for puppies with parvoviral enteritis
Clinical score, biochemical parameters and patient outcome

| PUPPY | TEMPERATURE (˚C) | PULSE (bpm) | RESPIRATION (bpm) | CLINICAL SCORE | POTASSIUM (mmol/l) | GLUCOSE (mmol/l) | TOTAL SERUM PROTEIN (g/l) | HT (%) | DAYS HOSPITALISED | OUTCOME |
|---|---|---|---|---|---|---|---|---|---|---|
| 21 | 39.6 | 200 | 40 | 11 | 3.42 | 2.8 | 60 | 50 | 7 | Alive |
| 22 | 36.9 | 96 | 24 | 16 | 4.99 | - | 56 | 45 | 6 | Alive |
| 23 | 38.4 | 102 | 38 | 21 | 5.02 | - | 50 | 28 | 3 | Alive |
| 24 | 37.9 | 100 | 36 | 14 | 4.5 | - | 45 | 36 | 5 | Alive |
| 25 | 38.3 | 100 | 48 | 21 | 5.09 | - | 48 | 32 | 3 | Alive |
| 26 | 38.2 | 110 | 30 | 15 | 5.7 | - | 58 | 37 | 6 | Alive |
| 27 | 37.8 | 88 | 20 | 14 | 4.46 | - | 50 | 39 | 3 | Alive |
| 28 | 38.2 | 120 | 32 | 13 | 4.3 | - | 40 | 47 | 8 | Alive |
| 29 | 39.2 | 96 | 36 | 14 | 3.2 | 3.2 | 44 | 46 | 5 | Alive |
| 30 | 38.6 | 120 | 32 | 14 | 4.13 | 7.8 | 50 | 28 | 10 | Alive |
| 31 | 37.8 | 160 | 24 | 16 | 3.59 | 6.5 | 60 | 42 | 3 | Alive |
| 32 | 37.2 | 180 | 20 | 12 | 4.32 | 4.7 | 71 | 52 | 2 | Alive |
| 33 | 37.9 | 120 | 20 | 16 | <2 | 6 | 42 | 34 | 4 | Dead |
| 34 | 38.1 | 120 | 20 | 13 | 4.3 | 4.5 | 50 | 30 | 11 | Alive |
| 35 | 40.3 | 200 | 44 | 11 | 2.88 | 3.1 | 58 | 30 | 8 | Alive |
| 36 | 38.3 | 128 | 68 | 19 | 3.68 | 5.6 | 42 | 26 | 7 | Alive |
| 37 | 38.7 | 120 | 30 | 18 | 2.6 | 3.9 | 52 | 41 | 4 | Alive |
| 38 | 38.1 | 150 | 24 | 12 | - | - | - | - | 6 | Alive |
| 39 | 38.5 | 80 | 30 | 14 | - | - | - | - | 4 | Alive |
| 40 | 38.6 | 190 | 20 | 16 | 5.46 | 5.3 | 53 | 41 | 5 | Alive |

bpm = beats or breaths per minute
Ht = haematocrit

# APPENDIX K
## Results datasheet for puppies with parvoviral enteritis
Ultrasonographic measurements in millimetres

| PUPPY | Jejunal lymph thickness | Duodenal wall thickness | Duodenal mucosa thickness | Duodenal wall-mucosal thickness | Jejunal wall thickness | Jejunal mucosa thickness | Jejunal wall-mucosal thickness | Stomach wall thickness | Colon wall thickness |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.3 | 3.5 | 1.7 | 1.8 | 2.9 | 0.7 | 2.2 | 3.6 | 2.0 |
| 2 | 9.2 | 3.8 | 1.8 | 2.0 | 3.6 | 2.1 | 1.5 | 3.3 | 1.2 |
| 3 | 8.3 | 4.2 | 2.2 | 2.0 | 3.7 | 1.2 | 2.6 | 4.4 | 1.2 |
| 4 | 7.1 | 4.3 | 1.7 | 2.6 | 3.1 | 1.6 | 1.5 | 2.7 | - |
| 5 | 10..4 | 3.1 | 2.8 | 0.3 | 3.3 | 1.9 | 1.4 | 4.0 | 2.0 |
| 6 | 7.5 | 3.6 | 1.6 | 2.0 | 3.4 | 1.2 | 2.2 | 2.9 | 1.7 |
| 7 | 8.6 | 3.6 | 2.6 | 1.0 | 2.6 | 2.0 | 0.7 | 2.7 | 1.2 |
| 8 | 10.6 | 3.6 | 1.6 | 2.0 | 3.2 | 1.5 | 1.7 | 3.8 | 1.3 |
| 9 | 9.6 | 3.1 | 0.1 | 3.0 | 3.0 | 1.5 | 1.5 | 3.5 | 1.4 |
| 10 | 5.6 | 3.6 | 1.7 | 1.9 | 2.4 | 1.3 | 1.1 | 3.0 | 1.5 |
| 11 | 10.5 | 3.5 | 0.7 | 2.8 | 3.1 | 1.5 | 1.6 | 2.5 | 1.3 |
| 12 | 10.4 | 4.3 | 2.2 | 2.1 | 3.7 | 1.3 | 2.4 | 3.3 | 1.3 |
| 13 | 11.1 | 4.0 | 2.2 | 1.8 | 3.5 | 1.2 | 2.3 | 3.2 | 1.6 |
| 14 | 11.2 | 5.3 | 3.4 | 1.9 | 3.1 | 1.9 | 1.2 | 2.8 | 1.4 |
| 15 | 5.2 | 3.5 | 2.3 | 1.2 | 2.4 | 1.0 | 1.4 | 1.6 | 1.7 |
| 16 | 5.0 | 4.0 | 2.4 | 1.6 | 2.7 | 1.4 | 1.3 | 3.1 | 1.3 |
| 17 | 6.5 | 3.8 | 2.0 | 1.8 | 2.3 | 1.2 | 1.1 | 2.5 | - |
| 18 | 8.9 | 2.9 | 1.3 | 1.6 | 1.7 | 0.8 | 0.9 | 2.6 | - |
| 19 | 8.1 | 4.9 | 2.1 | 2.8 | 2.2 | 1.1 | 1.1 | 3.0 | 1.1 |
| 20 | 11.8 | 4.3 | 2.2 | 2.1 | 2.9 | 1.4 | 1.5 | 3.9 | - |

# APPENDIX K
## Results datasheet for puppies with parvoviral enteritis
Ultrasonographic measurements in millimetres

| PUPPY | Jejunal lymph thickness | Duodenal wall thickness | Duodenal mucosa thickness | Duodenal wall-mucosal thickness | Jejunal wall thickness | Jejunal mucosa thickness | Jejunal wall-mucosal thickness | Stomach wall thickness | Colon wall thickness |
|---|---|---|---|---|---|---|---|---|---|
| 21 | 12.2 | 4.2 | 2.0 | 2.2 | 3.4 | 1.4 | 2.1 | 2.4 | 1.5 |
| 22 | 6.9 | 4.1 | 2.5 | 1.6 | 2.9 | 1.6 | 1.3 | 2.6 | 1.4 |
| 23 | 10.4 | 2.5 | 1.1 | 1.4 | 2.8 | 1.3 | 1.6 | 2.6 | 1.2 |
| 24 | 6.6 | 4.5 | 2.5 | 2.0 | 2.1 | 1.1 | 1.0 | 1.9 | 1.3 |
| 25 | 4.6 | 2.9 | 1.9 | 1.0 | 3.0 | 1.6 | 1.4 | 2.8 | 1.3 |
| 26 | 7.9 | 3.4 | 1.7 | 1.7 | 3.6 | 1.8 | 1.8 | 3.3 | - |
| 27 | 5.8 | 4.1 | 1.8 | 2.3 | 2.4 | 1.0 | 1.4 | 3 | 0.8 |
| 28 | - | 3.8 | 1.9 | 1.9 | 3.4 | 1.7 | 1.8 | 2.5 | 1.1 |
| 29 | 9.0 | 3.0 | 2.0 | 1.0 | 3.3 | 1.3 | 2.0 | 5.3 | 1.1 |
| 30 | 6.0 | 3.1 | 1.0 | 2.1 | 2.4 | 0.8 | 1.6 | 2.4 | 1.3 |
| 31 | 5.4 | 3.1 | 1.7 | 1.4 | 2.5 | 1.1 | 1.5 | 2.5 | 1.2 |
| 32 | 9.2 | 3.1 | 1.9 | 1.2 | 2.3 | 1.3 | 1.1 | 2.3 | 0.9 |
| 33 | 6.9 | 2.8 | 1.2 | 1.6 | 2.7 | 0.8 | 1.9 | 4.4 | 1.7 |
| 34 | 5.9 | 3.9 | 2.0 | 1.9 | 2.6 | 1.0 | 1.7 | 3.9 | 1.8 |
| 35 | 6.4 | 2.9 | 1.1 | 1.8 | 3.0 | 0.9 | 2.1 | 3.9 | 0.8 |
| 36 | 8.1 | 3.2 | 1.9 | 1.3 | 2.6 | 1.4 | 1.2 | 3.6 | 1.4 |
| 37 | 7.1 | 3.1 | 1.9 | 1.2 | 2.2 | 1.1 | 1.2 | 2.1 | 0.8 |
| 38 | 6.9 | 3.1 | 1.7 | 1.4 | 2.3 | 0.8 | 1.5 | 2.7 | 1.1 |
| 39 | 6.0 | 3.8 | 1.8 | 2.0 | 2.6 | 1.2 | 1.4 | 2.2 | 0.9 |
| 40 | 9.0 | 3.7 | 1.5 | 2.2 | 3.0 | 1.2 | 1.9 | 2.5 | 0.9 |

# APPENDIX L
## Results datasheet for puppies with parvoviral enteritis
Ultrasonographic descriptive parameters

| PUPPY | Duodenal corrugation | Jejunal corrugation | Hyperechoic duodenal mucosa | Hyperechoic jejunal mucosa | Irregular duodenal mucosa | Irregular jejunal mucosa | Indistinct duodenal wall layers | Indistinct jejunal wall layers |
|---|---|---|---|---|---|---|---|---|
| 1 | No | No | No | No | No | No | No | No |
| 2 | No | No | No | Yes | No | No | No | No |
| 3 | No | Yes | Yes | No | No | No | No | No |
| 4 | No | No | No | No | No | No | Yes | Yes |
| 5 | No | No | No | No | No | No | Yes | Yes |
| 6 | No | No | No | No | No | No | No | No |
| 7 | Yes | No | Yes | Yes | Yes | Yes | Yes | Yes |
| 8 | No | No | No | No | No | No | No | No |
| 9 | No | No | Yes | Yes | Yes | Yes | No | No |
| 10 | No | No | Yes | Yes | No | No | No | No |
| 11 | No | No | No | Yes | Yes | Yes | No | No |
| 12 | Yes | No | No | Yes | No | Yes | No | No |
| 13 | No | No | No | Yes | Yes | No | No | No |
| 14 | No | No | No | No | No | No | No | No |
| 15 | No | No | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | No | No | No | No | No | No | No | No |
| 17 | Yes | No | No | No | No | No | Yes | Yes |
| 18 | Yes | No | No | No | No | No | No | No |
| 19 | Yes | No | No | No | No | No | No | No |
| 20 | No | No | No | Yes | No | Yes | No | Yes |

# APPENDIX L
## Results datasheet for puppies with parvoviral enteritis
Ultrasonographic descriptive parameters

| PUPPY | Duodenal corrugation | Jejunal corrugation | Hyperechoic duodenal mucosa | Hyperechoic jejunal mucosa | Irregular duodenal mucosa | Irregular jejunal mucosa | Indistinct duodenal wall layers | Indistinct jejunal wall layers |
|---|---|---|---|---|---|---|---|---|
| 21 | No | No | No | Yes | Yes | Yes | No | No |
| 22 | No | No | No | No | No | No | No | No |
| 23 | No | No | No | Yes | No | Yes | No | Yes |
| 24 | No | No | No | No | No | No | Yes | Yes |
| 25 | No | No | No | No | No | No | No | Yes |
| 26 | No | No | No | Yes | No | No | Yes | Yes |
| 27 | Yes | Yes | No | Yes | No | Yes | Yes | Yes |
| 28 | No | No | No | No | No | No | No | No |
| 29 | No | No | No | No | No | No | No | No |
| 30 | Yes | No | No | No | Yes | Yes | Yes | Yes |
| 31 | Yes | No | No | No | No | No | Yes | Yes |
| 32 | Yes | No | No | Yes | No | Yes | No | Yes |
| 33 | No | No | No | Yes | No | Yes | Yes | Yes |
| 34 | Yes | Yes | No | Yes | No | No | Yes | Yes |
| 35 | Yes | No | No | No | No | Yes | Yes | Yes |
| 36 | No | No | No | Yes | No | No | Yes | Yes |
| 37 | Yes | No | No | No | No | Yes | Yes | Yes |
| 38 | Yes | No | No | Yes | Yes | Yes | Yes | Yes |
| 39 | Yes | No | No | Yes | No | Yes | Yes | Yes |
| 40 | No | No | Yes | Yes | Yes | Yes | Yes | Yes |

# APPENDIX M
## Results datasheet for puppies with parvoviral enteritis
Correlation between clinical score, intestinal corrugations and mucosal changes

| CLNICAL SCORE | Duodenal corrugation | Jejunal corrugation | Hyperechoic duodenal mucosa | Hyperechoic jejunal mucosa | Irregular duodenal mucosa | Irregular jejunal mucosa |
|---|---|---|---|---|---|---|
| 11 | X | | X | X | X | X |
| 11 | X | | | | | X |
| 11 | | | | X | X | X |
| 12 | | | X | X | X | X |
| 12 | | | | X | X | X |
| 12 | X | | | X | | X |
| 12 | X | | | X | | X |
| 12 | X | | | X | X | X |
| 13 | | | | X | | |
| 13 | | X | X | | | |
| 13 | | | X | X | | |
| 13 | | | X | X | X | X |
| 13 | X | | | | | |
| 13 | | | | | | |
| 13 | X | X | | X | | |
| 14 | | | | | | |
| 14 | | | | | | |
| 14 | X | | | | | |
| 14 | | | | X | | X |
| 14 | | | | | | |
| 14 | X | X | | X | | X |
| 14 | | | | | | |
| 14 | X | | | | X | X |
| 14 | X | | | X | | X |
| 15 | | | | X | | |
| 15 | X | | | | | |
| 15 | | | | X | X | |
| 15 | | | | | | |
| 16 | X | | | | | |
| 16 | | | X | X | X | X |
| 16 | | | | X | | X |
| 16 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | X | | | | | X |
| 18 | | | | | | |
| 19 | | | | X | | |
| 19 | | | | | | |
| 21 | | | | X | | X |
| 21 | | | | | | |