EFFECT OF EARLY ENTERAL NUTRITION ON INTESTINAL PERMEABILITY, PROTEIN-LOSING ENTEROPATHY AND OUTCOME IN CANINE PARVOVIRAL ENTERITIS

by

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Résumé

Canine parvovirus (CPV) infection is characterized by a disruption of gut barrier function, which allows the systemic entry of bacteria and endotoxin, and the development of the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). Despite the lack of prospective data, conventional wisdom has dictated that "gut rest" with initial *nil per os* (NPO) remains the nutritional treatment of choice for CPV enteritis. However, early enteral nutrition (EEN) has been shown to be superior to starvation in human critical illnesses associated with gut barrier dysfunction. Documented benefits of EEN include improved intestinal permeability, reduced incidences of bacteremia, endotoxemia, SIRS and MODS, decreased catabolism, and improved clinical outcome.

A prospective, randomized, controlled clinical trial was conducted to evaluate the effect of EEN on intestinal permeability, protein-losing enteropathy, and clinical outcome in naturally occurring severe CPV enteritis in 30 puppies.

Parvoviral infection was confirmed by fecal electron microscopy, and dogs were hospitalized for 6 days. Dogs were randomly assigned to 2 groups. Fifteen dogs received *nil per os* until vomiting had ceased for 12 hours, after which a low fat diet was fed (initial NPO group; control). Fifteen dogs were fed immediately (Pedigree® Canine Concentration Instant Diet) by nasoesophageal tube (EEN group). All other treatments were identical. Disease severity was semi-quantified by a clinical scoring system. Intestinal permeability was assessed using urinary lactulose and rhamnose recoveries (%L and %R) and L/R ratios. Fecal α_1-proteinase inhibitor concentrations (α_1-PI) quantified protein-losing enteropathy.

Enteral tube feeding was not associated with any significant complications. The median time taken to normalization of habitus and appetite, and the resolution of vomiting and diarrhea, was consistently 1 day shorter for the EEN group for each parameter. Body weight remained stable in the NPO group, while EEN was associated

with significant weight gain (8.4% by day 6). This supports reduced catabolism with EEN.

Compared with reference values, urinary %Ls were elevated, %Rs reduced, and L/R ratios increased throughout the study for both groups. %L behaved significantly differently between groups (p=0.035), with a progressive decrease in the EEN group *vs.* a progressive increase in NPO. This may indicate earlier repair of intestinal epithelial necrosis, or improved tight junction structure and/or function due to EEN. Such an improvement in gut barrier function might potentially limit endotoxin and/or bacterial translocation. The decreased %R in both groups is consistent with villus atrophy. There were no significant differences in %R or L/R ratios between the two groups over time.

Fecal α_1-PI concentrations were increased throughout the study in both groups. There were no significant differences between the declines over time for fecal α_1-PI concentrations between groups.

Thirteen of 15 NPO dogs (87%) and all of the EEN dogs (100%) survived (non-significant; p = 0.48).

This study demonstrates that EEN may be effectively instituted in CPV enteritis, and supports the use of EEN in gut barrier dysfunction.

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CHAPTER I

1. LITERATURE REVIEW

1.1 CANINE PARVOVIRUS INFECTION

1.1.1 General overview of canine parvoviral disease:

Canine parvovirus (CPV) is a small (24.5 nm diameter), non-enveloped, single-stranded DNA virus with an icosahedral shape.[1, 2] Following the emergence and recognition of CPV as an important canine pathogen in 1977 to 1978,[3-5] the virus rapidly led to a panzootic infection of susceptible populations of both domesticated and wild canids.[2] The original virus (CPV type-2) soon underwent antigenic change, with the newer viral strains (CPV type-2a and type-2b) replacing the original in North America, Germany and other regions worldwide.[6] In southern Africa, the original CPV-2 was similarly completely replaced by these new antigenic types, with CPV-2b predominating (66% of isolates).[7] These new strains also gained the ability to replicate and produce clinical disease in cats.[6] In each case the CPV strains spread worldwide, facilitated by the virion's extreme stability and resistance to adverse environmental conditions.[8] Infective virus can persist on inanimate fomites for greater than 5 months, in feces for at least 6 months (at room temperature), and most disinfectants and detergents fail to inactivate CPV.[8, 9] The virus may, however, be inactivated by sodium hypochlorite (household bleach), formalin and sunlight.[10, 11]

More than 1 million dogs are currently affected by CPV annually in the United States.[12] The disease was first reported in South Africa in December 1979, although cases had been occurring since the beginning of the same year.[13] The incidence of CPV infection increased rapidly throughout South Africa, with enteric CPV disease reaching epidemic proportions towards the end of 1980.[14] During the following 2 decades, the disease led to considerable suffering and expenditure.[15, 16] During 1988-1993, approximately 300 dogs

(2.8% of all sick dogs presented to the Onderstepoort Veterinary Academic Hospital (OVAH) Outpatients clinic) were annually suspected of suffering from CPV enteritis, and consequently admitted to the infectious disease isolation ward for intensive treatment.[15] Seasonal variation in cases admitted to the OVAH isolation ward has clearly been demonstrated, with a peak incidence during the summer months (September to January) and a trough during winter (May to July).[15, 16] Meteorological factors that closely correlate with this seasonal distribution are wind speed and inverse humidity; harsh, dry and windy conditions have thus been speculated to enhance the susceptibility of mucous membranes to CPV exposure and subsequent invasion.[15] Seasonality in the occurrence of CPV clinical cases has similarly been reported in North America[17] and Canada.[18]

In susceptible canine populations, parvovirus infection often presents as a severe and potentially life-threatening enteric disease.[2] Although severe or fatal illness may be produced, serologic and experimental evidence suggests that subclinical infections are common and likely represent the most frequent outcome of exposure.[19-23] It has been suggested that the variation in the severity of clinical signs in CPV enteritis may stem primarily from variations in the individual host's response to infection, rather than from differing viral characteristics.[20] Dogs under six weeks of age are generally immune to infection, due to maternally derived humoral immunity.[2] As this immunity wanes, animals become increasingly susceptible to infection.[2] Animals under 1 year of age have the highest morbidity and mortality rates, with a peak incidence at 6 to 24 weeks of age.[2, 17] Most adult dogs have acquired protective immunity through either vaccination or natural infection.[2] Breed susceptibility to severe CPV disease has been suggested most consistently for the Rottweiler and Doberman pinscher.[18, 24, 25] Other breeds that may be at increased risk include the German shepherd dog and the American pit bull terrier,[18] while a clinical observation has been made that black Labrador retrievers may be more severely affected than yellow-colored Labrador retrievers.[24] Vaccination with modified live CPV-2 is an effective means of preventing infection; vaccination in high-risk areas is usually initiated at 6 to 8 weeks of age, with 3 to 4 boosters until 18 weeks of age.[26] Some authors currently recommend annual revaccination.[26] Pregnant dogs and puppies younger than 5 weeks may be vaccinated with the less efficacious killed vaccines.[26]

Since parvoviruses require host cell enzymes involved in DNA synthesis for their replication, they exhibit tropism for the rapidly replicating cells of the intestinal crypt epithelium, and of lymphoid and hematopoietic tissues.[20] The production of viral proteins by the host cell's nucleus results in unrestricted viral proliferation in these sites, with consequent cell death.[2, 27] Within a day of experimental oral inoculation, CPV localizes in the thymic cortex. Over the next 2-3 days the germinal centers of lymph nodes and the splenic white pulp are infected. This is followed by a predominantly non-cell associated viremia on days 3 and 4 post-infection, with eventual viral localization in the proliferative zones of the intestinal crypt epithelium between days 4-7.[9, 20, 28] Small intestinal crypt epithelial cells, a specific target population for viral replication in CPV infection, normally have a high mitotic index.[24] In rapidly growing puppies this mitotic index is even higher, facilitating greater CPV replication, and further predisposing this age group to severe disease.[24] Following experimental inoculation, intranuclear viral antigen may be demonstrated in thymocytes, lymph nodes and gut-associated lymphoid tissues (GALT), and in the intestinal crypt epithelium of the duodenum, jejunum and ileum.[28] Immunocytochemical studies have clearly shown that intestinal mucosal changes closely follow viral localization in the crypt epithelium.[28] Intestinal crypt necrosis, mucosal collapse and a severe diarrhea occur, approximately 5 days following experimental oral inoculation, which is coincidental with viral localization in the mitotically active zones of intestinal crypt epithelium.[20] An extremely short phase of intestinal viral replication is limited to the proliferative zone of the crypts, the extent of which determines the severity of the resultant crypt necrosis,[28] and also the severity of consequent clinical signs.[20] The majority of dogs fecally excrete infective virus during the period between 4 to 6 days following oral inoculation.[28] Fecal shedding of CPV continues for a maximum of 12 to 22 days following initial infection.[9] The development of a rapid humoral immune response, commencing 5 days post-infection, is likely responsible for the termination of viremia.[20, 28] The leakage of neutralizing antibody into the damaged intestinal tract may similarly eliminate virus from the intestinal mucosa, terminating fecal viral excretion.[28] During the period of clinical illness, virus may be readily detected in fecal samples by electron microscopy.[1, 24]

Histologic lesions of CPV infection are seen primarily in the proliferating cells of lymphoid tissues, the small intestine and bone marrow. Mesenteric and gut-associated lymph node germinal centers, splenic nodules and the thymic cortex show depletion of lymphoid cells.[20] Lymphoid follicles of Peyer's patches may show coagulative necrosis and hemorrhage.[20] The extent of lymphoid necrosis in Peyer's patches, lymph nodes, thymus and spleen is greater in more severely affected dogs.[20] Viral replication within the intestinal villus crypt cells leads to collapse of the epithelium with villus blunting and atrophy.[2, 19] Lesions may be focal or segmental, and are most severe in the ileum and jejunum; classic lesions include necrosis of the crypt epithelium accompanied by lamina propria collapse, resulting in dilated crypts filled with necrotic debris.[19, 20, 29] However, even in severe cases, intestinal epithelial regeneration usually occurs.[29] Remaining unaffected intestinal crypts become elongated and are lined by hyperplastic epithelium with a high mitotic index.[19] The small intestine has been suggested to lose its digestive and/or absorptive capacities during the period of villus atrophy, since destruction of the germinal intestinal epithelium precludes normal mature villus epithelial cell renewal, and it has been speculated that malabsorption and protein-losing enteropathy might persist until the intestinal villi have been repaired.[2, 29] However, although these abnormalities may intuitively be expected to occur, neither digestive nor absorptive abnormalities have, to my knowledge, been definitively documented nor quantified in CPV enteritis. Since intestinal epithelial cell turnover is rapid (1-3 days), any loss of absorptive capacity would be expected to be of short duration.[2]

Parvoviral infection therefore classically presents as severe necrotizing enteritis with pansystemic lymphoid tissue depletion.[19, 20, 29] Clinical signs of CPV infection are usually first observed 4 to 7 days following experimental oral exposure,[19, 20, 30, 31] although the incubation period may vary from 2 to 14 days.[2] Initial clinical signs may include depression, anorexia, lethargy, vomiting, diarrhea (which may be watery, mucoid, hemorrhagic and foul-smelling), pyrexia, and shock.[17, 29, 31] Intussusception can complicate severe disease.[17, 29] A second, distinct syndrome of CPV infection, namely acute viral myocarditis, was a significant clinical entity for only a few years following the

initial emergence of CPV infection.[32] This syndrome produced cardiac failure in puppies less than 3 months old.[13, 14, 33, 34] The myocardial form of the disease has presently virtually disappeared, since nearly all dams are now immune, either due to vaccination or subclinical infection.[19] Perinatal infection is prevented by maternal antibody until an age where myocardial cells no longer facilitate extensive CPV replication.[19, 24] A third clinical syndrome of CPV infection, fatal perinatal (3 to 9 days old) disease characterized by systemic necrotizing vasculitis, has additionally been described.[35]

Clinicopathologic and hematologic features of naturally occurring CPV enteritis have been reported.[17, 27, 36-40] Panleukopenia, especially neutropenia, is common in severe disease, while a transient lymphopenia is reportedly the most common leukocytic abnormality.[20, 27, 29, 37, 40] Up to 86% of cases may become leukopenic during the first 4 days of hospitalization.[27] Neutropenia has been suggested to be primarily the result of a net consumption of neutrophils at the injured intestinal mucosa, rather than a primary failure of granulopoiesis, since infection and destruction of granulocytic precursor cells is an inconsistent feature of CPV infection.[20, 28, 30, 40] Following experimental oral inoculation, CPV antigen is very inconsistently present in bone marrow (as detected by immunofluorescence and immunoperoxidase techniques), and very minor destructive changes are visible microscopically in the bone marrow.[28] However, 92% of 75 dogs with naturally occurring CPV enteritis showed myelophthisis (complete depopulation) on post mortem bone marrow light microscopy, supporting the role of reduced granulopoiesis in severe CPV disease.[41] The neutrophil nadir is usually observed at the time of the most severe clinical illness, and coincides with the peak in granulocyte colony-stimulating factor concentrations, which are significantly elevated.[40] Toxic changes in neutrophils have been suggested to be the result of secondary bacteremia and/or endotoxemia due to a loss of intestinal mucosal integrity.[24] Some investigators have found a significant correlation between the degree of leukopenia or lymphopenia at admission and a poor prognosis for survival,[17, 31] while others have found no such correlation.[27] Due to this discrepancy, it has been suggested that leukopenia may indicate the presence of more severe disease and the need for aggressive therapy, but should not be used as a sole prognostic indicator.[17] Viral replication in the lymphopoietic system results in

lymphopenia,[9, 42, 43] which is more marked in severely affected or symptomatic dogs than in dogs with subclinical infections.[24] Left-shift neutrophilia, monocytosis and lymphocytosis may occur during the recovery phase.[27, 30] Anemia with panhypoproteinemia results from intestinal blood loss followed by rehydration, and may be exacerbated by concomitant gastrointestinal parasitism.[27, 29] Erythropoeisis is not significantly affected in CPV infection.[28, 30] A decrease in packed cell volume (PCV), averaging 11 units, has been described in up to 86% of cases during a 6-day hospitalization period.[27] Significant hypoalbuminemia (92% of cases) and hypogammaglobulinemia (50%) are the result of protein-losing enteropathy (PLE).[37] Decreases in plasma proteins to subnormal values may occur in up to 94% of cases.[27] Hypoproteinemia parallels the decrease in PCV, and these variables reach their nadirs on the same day.[27] Significant hyperalpha-2-globulinemia (100% of cases in 1 study) may be related to profound tissue damage and inflammation, since circulating leukocyte endogenous mediators induce the production of hepatic acute-phase proteins.[37] Fibrinogen may be increased, possibly contributing to the maintenance of plasma oncotic pressure.[27, 44] Anorexia, vomiting and diarrhea may result in hypokalemia (approximately 25% of cases), which contributes to muscular weakness and depression.[27, 29, 36, 38] Pre-renal azotemia and hyperphosphatemia, associated with dehydration, are present in 30% of dogs.[27] Decreased total serum calcium concentrations, attributed to hypoalbuminemia, may occur in 15% of dogs.[27] Hypochloridemia and hyponatremia occur in approximately 30% of cases,[27, 36, 38] while hypochloridemic metabolic alkalosis is the most common acid-base abnormality reported.[36] Total and ionized blood magnesium concentrations are not significantly altered.[39] Hypoglycemia occurs in approximately 10% of cases at time of admission, while hyperglycemia is more common (37%), presumably due to a stress response.[27] Evidence for hepatocellular disease is present in approximately 25% of dogs, and includes elevations in alkaline phosphatase (not attributable to young age), alanine aminotransferase and mild hyperbilirubinemia.[27] It has been speculated that hepatic enzyme elevations might reflect a toxic hepatopathy induced by gut-origin toxins, following their absorption through the necrotic intestinal lining.[27] Hyperamylasemia may be present in a small percentage of dogs.[27] Evidence of hypercoagulability, without the presence of disseminated intravascular coagulopathy, has been demonstrated in 100%

(9/9) of dogs with naturally occurring CPV infection.[44] The hypercoagulable state was characterized by significantly decreased antithrombin III activity, elevated fibrinogen concentrations, moderate prolongation of the activated partial thromboplastin times, increased fibrin degradation product (FDP) concentrations, and normal D-dimer concentrations.[44] Four of the 9 dogs also had catheter-associated venous thrombosis or phlebitis.[44] It was postulated that the hypercoagulable state was the result of pro-inflammatory cytokine cascade activation following sepsis and endotoxemia.[44]

Immunosuppression in CPV infection may stem both from its adverse effects on the innate immune system (afforded by neutrophils, NK cells and macrophages), as well as suppression of the antigen-specific acquired immune system (dependent on T-lymphocyte function and B-cell mediated antibody production).[42] The common occurrence of panleukopenia, and especially neutropenia, has already been discussed. Although lymphopenia, lymph node necrosis, thymic and Peyer's patch atrophy and decreased serum gammaglobulin concentrations occur in CPV infection,[20, 30, 37] humoral immunity to the virus itself paradoxically appears to be relatively unaffected.[9, 20, 24] Lymphocyte blastogenesis response to phytomitogens is significantly depressed in naturally acquired CPV infection.[45] The immunosuppression in CPV-infected dogs (that are actively shedding fecal virus) might be the result of either a direct interaction between the virus and lymphocytes, changes in cell population dynamics, or alterations in mitogen receptors.[45] Lymphocyte blastogenesis was also decreased in some dogs (3/8) following modified-live CPV vaccination.[46] Although this was of short duration (approximately 4 days), it was consistently reproduced following repeated vaccine administrations.[46] In another study, 2 of 5 gnotobiotic puppies vaccinated with modified-live distemper vaccine, followed 3 days later by oral CPV infection, developed severe canine distemper encephalitis; dogs that received the distemper vaccine (without CPV infection) showed no clinical signs of distemper encephalitis.[43] In contrast, a preliminary study of oral CPV inoculation in 3 beagle pups showed only a mild, transient (1 or 2 days) suppression of lymphocyte blastogenesis.[47] The findings of the above studies, together with the fact that natural CPV infection may predispose dogs to other infectious diseases such as

hemobartonellosis and distemper, imply that at least some degree of suppression of antigen-specific immunity also occurs.[48, 49]

The mortality rate of CPV enteritis has been variously reported as 11%,[27] 21%,[39] 16% to 35%,[42] 36%,[25] and greater than 50%.[24] Aggressive therapy and supportive care have, however, achieved survival rates of 85% to 96% in individual institutions.[12, 29] Significantly improved survival has also been documented for dogs treated at a tertiary care hospital as compared with those treated at local veterinary clinics.[12] This higher survival rate was, however, associated with a significantly increased duration of hospitalization (median of 6 versus 3 days).[12] Death is usually attributed to dehydration, electrolyte imbalances, endotoxic shock, or overwhelming bacterial sepsis related to disruption of gut-barrier function and leukopenia.[26]

1.1.2 The influence of co-morbid disease on the severity of parvoviral enteritis:

The exact influence of co-morbid disease on CPV disease expression, and other factors that may predispose to severe disease, remain uncertain.[17, 19, 24] Early attempts to experimentally reproduce fatal, severe CPV infection were generally unsuccessful.[9, 21, 30, 43, 50] The severe disease observed in the field did not seem to be reliably reproducible experimentally, whether in gnotobiotic[43] (except for *Toxocara canis*)[9] dogs, dogs obtained from closed kennels,[30] or in dogs from the normal canine population.[21, 50] One experimental study compared CPV infection between specific pathogen-free (SPF) and conventionally raised dogs; more pronounced clinical signs were produced in the latter group, although both groups showed milder clinical signs than those observed in field cases.[9] These observations prompted the question whether additional factors may be involved in determining the severity of disease in naturally occurring CPV infection. However, serologic evidence suggests that subclinical infection is common and likely represents the most frequent outcome of exposure, and that the severe clinical disease with high mortality seen in the field is the exception rather than the rule for CPV infection.[21-23] Although this may account for the inability of experimental studies to reliably reproduce severe disease, these findings are disparate from experiences in some

animal shelters, pet stores and kennels, where morbidity in pups has exceeded 90% and mortality has been greater than 50%.[24] Additional factors apparently affect the pathophysiologic consequences of CPV infection.[24] It has been postulated that disease severity might be related to the rate of both lymphoid and intestinal cell turnover, with higher rates of turnover being correlated with greater viral replication and consequent cell destruction.[2, 20, 24, 31] It has also been suggested that the presence of intestinal parasites in puppies may enhance the severity of CPV infection, by stimulating increased mitotic activity of intestinal epithelial cells in response to parasite-induced cellular injury.[9, 24, 31] The disease may progress more rapidly and be more severe in animals in which the intestinal barrier is already compromised by concurrent enteric pathogens such as *Clostridium perfringens, Escherichia coli, Campylobacter, Salmonella*, coronavirus, or intestinal parasites (e.g. hookworm, *Giardia*, and coccidia).[2, 9, 21, 24, 42, 51] Supporting this hypothesis, clinical manifestations of CPV infection in 3 SPF pups pre-infected with *Giardia canis* were significantly more severe compared with *Giardia*-free CPV-inoculated SPF controls.[9] Furthermore, a study (first published in a review article) of experimental CPV infection produced only mild clinical signs with no deaths in 45% (14/31) of intestinal parasite-free dogs, while more severe disease with 35% mortality was produced in 88% (23/26) of intestinally parasitized dogs.[24] The types of intestinal parasites were unfortunately not stated in this latter study.[24] Other authors have claimed that there is little direct evidence supporting the importance of simultaneous infection, parasitism, and stress in CPV enteritis.[19] A study examining the effects of stress and immunosuppression in experimental CPV infection found no significant influence on clinical signs, the serologic response, or the duration of viremia in SPF pups immunosuppressed by anti-canine thymocyte serum or corticosteroids, compared with non-immunosuppressed controls.[9] One study of experimental CPV infection in 22 dogs noted no difference in the quantity or type of intestinal bacterial or parasitic flora, including *Giardia* and *Coccidia ohioensis*, between asymptomatic and symptomatic CPV infected dogs.[20] Similarly, no correlation has been found between either giardiasis or coccidiosis and large outbreaks of naturally occurring CPV enteritis.[17] Severe CPV enteritis outbreaks have also occurred in closed colonies of dogs that were judged entirely free of helminthiasis.[17] In addition to bacterial or viral infections and parasitism,

conditions that may also contribute to stress include inadequate nutrition, extreme environmental conditions (hot and humid or cold and damp), an unsanitary environment, and surgical procedures.[24] Supporting this hypothesis, it has been observed that the impact of stress factors on CPV disease expression in urban areas appears to be inversely related to socioeconomic conditions.[24] Experimental oral CPV infection in 7 non-SPF crossbreed puppies induced severe clinical signs of disease, similar to those observed in field outbreaks.[31] The authors postulated that severe disease developed in these dogs due to recent weaning (2 weeks prior to CPV inoculation), with resultant dietary stress, which may have caused increased intestinal and lymphoid cell turnover.[31] A light parasite burden (*Toxocara canis*, *Dipylidium caninum* and *Ancylostoma caninum*) in all the dogs may have contributed to increased intestinal epithelial turnover.[31] Another experimental study induced clinically apparent enteric disease in 22 of 24 conventional crossbreed puppies following oral CPV inoculation.[52] These puppies had been fasted for 24 hours prior to and 48 hours following challenge, and the investigators postulated that enhanced intestinal epithelial cell turnover, induced by starvation followed by refeeding, promoted CPV replication with the production of clinical enteritis.[52] In this study, 4 dogs that were not fasted at the time of viral exposure remained clinically healthy.[52]

A retrospective study comparing the disease status of 2248 hospitalized South African dogs originating from a poor peri-urban community with 1922 dogs originating from an affluent community, identified significant differences in epidemiology and disease prevalence between the 2 groups.[16] Those from the developing community were mainly young crossbreed dogs, and suffered primarily from infectious, parasitic and traumatic diseases.[16] Enteritis, suspected to be due to CPV, was common in this group (11% of all diagnoses), as were toxocariasis (4.6%) and ancylostomiasis (4.8%).[16] In contrast, dogs from the developed community were mainly adult or old purebred dogs, and suffered primarily from organ diseases, while infectious and parasitic diseases were less common.[16] Suspected CPV infection was uncommon in this group (1.9% of all diagnoses), as were toxocariasis (0.5%) and ancylostomiasis (1.8%).[16]

A prospective descriptive study of the health status of 220 dogs (older than 3 months of age) in a rural South African town of low socioeconomic status found that the mean condition score (CS) of all the dogs was 1.93, representing a body condition between poor and moderate, with very little subcutaneous fat.[53] Seven percent of dogs were emaciated (CS < 1).[53] Internal parasite quantification by the modified McMaster technique revealed ancylostomiasis in 40% of dogs (mean eggs per gram of feces, 296 EPG), *Toxocara leonina* in 5% (29 EPG) and *Toxocara canis* in 2% (13 EPG).[53] The prevalence of ancylostomiasis was high compared with 18% in the more affluent urban Pretoria area.[54] On average, the dogs also had hypochromic anemia, decreased serum iron, and hypoalbuminemia, probably due to verminosis (especially ancylostomiasis) and malnutrition.[53]

These studies[16, 53] have important ramifications for the epidemiology of CPV infection in southern Africa, considering the possible influence of co-morbid disease on the severity of CPV disease expression. Intestinal parasitism, nutritional stress and hypoalbuminemia potentially predispose dogs in poorer areas to more severe CPV disease. Coupled with an unsanitary environment and reduced emphasis on preventive vaccination in these communities,[16] CPV infection in socioeconomically deprived areas of southern Africa may be more severe than that observed in more affluent communities. Lending support to this suggestion are observations that the impact of stress factors on CPV disease expression in urban areas appears to be inversely related to socioeconomic conditions, and that CPV infection in resource-deprived communities is associated with high morbidity and mortality.[24]

1.1.3 Gut barrier dysfunction and systemic complications in parvoviral enteritis:

1.1.3.1 Introduction

The gastrointestinal tract (GIT) has endocrine, metabolic, immunologic and barrier functions, in addition to nutrient absorption.[55] The last fifteen years have seen increased interest in the role of intestinal barrier failure (the so-called “gut-hypothesis”) in the

development of systemic infection and the systemic inflammatory response syndrome (SIRS), as well as the multiple organ dysfunction syndrome (MODS) in the critically ill patient.[55-57] Normal GIT immunologic and mucosal barrier functions prevent the absorption of intraluminal microbes and their products, and therefore assist in the prevention and control of disease.[58-60] However, critical illness, whether primarily extra-intestinal or intestinal in origin, may have a tremendous impact on a healthy gut.[58] Depending on the severity of the insult, intestinal mucosal functions may be compromised; gut barrier function, nutrient digestion and absorption, and GIT metabolic activities may be impaired, which may negatively influence the outcome of disease.[58]

1.1.3.2 Normal gut barrier function

The intestinal mucosa in health maintains an effective barrier to the systemic entry of intraluminal bacteria, antigens and toxins.[61, 62] Integral to the normal functioning of this barrier are the epithelium and gut-associated lymphoid tissue (GALT).[61] The natural defense mechanisms of the GIT include mechanical as well as immunologic facets.[63] The maintenance of an intact intestinal epithelium prevents transepithelial migration of intraluminal bacteria and their toxins, whereas the preservation of epithelial tight junctions prevents their paracellular movement.[61, 62] Although both a normally functioning immune system and an intact intestinal epithelium are important components of the gut barrier, an intact mucosa has been demonstrated to maintain an effective barrier to bacterial translocation during suppression of cell-mediated immunity.[64, 65] This suggests that cell-mediated immunity, both systemically and within the GALT, serves only a supportive or secondary role to the epithelial barrier, as regards the prevention of bacterial translocation.[64, 65] Further GIT mechanical defense mechanisms include peristalsis, which serves a “housekeeper” function to keep the GIT relatively free of debris, digestive secretions (e.g. gastric acid, digestive enzymes and bile) that destroy bacteria and viruses, a protective mucus layer elaborated by goblet cells (trapping and preventing agents from entering the intestinal mucosa), and a normal bacterial flora that prevents colonization by pathogenic organisms.[63] Immunologic facets include all the processes, both antigen-specific and nonspecific, that protect against the deleterious

effects of the intestinal luminal contents.[63] The GIT contains factors that are antigenic or non-antigenic, injurious or nutritious, and persistent or transient.[63] This necessitates a versatile immune system that will eliminate injurious agents yet be tolerant of persistent harmless antigens.[63] Gut-associated lymphoid tissue consists of both aggregated lymphoid tissue, including Peyer's patches, lymphoid nodules, and mesenteric lymph nodes, and non-aggregated tissue, including lamina propria and intraepithelial lymphocytes.[66] The aggregated lymphoid components of GALT serve as the afferent arm of the GIT immune response, whereas the non-aggregated tissues comprise the efferent arm.[66] The GALT comprises 50% of the body's total lymphoid tissue,[66] and accounts for approximately 80% of immunoglobulins secreted across the intestinal mucosa.[67] Potential responses of GALT to intraluminal antigens (e.g. bacteria or nutritive elements) include exclusion, tolerance, or inflammation; these responses may not be mutually exclusive.[66] Small intestinal Peyer's patches and dendritic cells sample luminal antigen, sensitise resident T and B-lymphocytes, which leads to the local development of appropriate cell-mediated and humoral immune responses.[61, 63] The latter is characterized by the local production of immunoglobulin A (IgA) by B cells located in the intestinal lamina propria. This IgA is transported across the mucosa by secretory component into the mucin layer, where luminal antigens (e.g. bacteria, viruses, enterotoxins and parasites) are bound or inactivated.[61, 63, 67, 68] This prevents antigen attachment to and uptake by the intestinal mucosa, the key event in invasive infection.[67]

1.1.3.3 Intestinal bacterial flora alterations in parvoviral enteritis

Significant alterations in the normal intestinal flora have been extensively documented in CPV enteritis, and it has been established that concomitant or secondary intestinal bacterial infection plays an integral role in the development of clinical signs of disease.[21, 51, 69-72] *Clostridium perfringens* was isolated from jejunal specimens in 69% (74/108) of dogs that succumbed to naturally occurring CPV enteritis.[69] Small intestinal histologic abnormalities were more severe in the dogs with concurrent *C. perfringens* infection than in those without,[69] and these more severe lesions were associated histologically with Gram-positive bacilli.[69] This study indicated that *C. perfringens* frequently proliferates in

the intestinal tract in CPV enteritis, leading to mucosal infection and potentially complicating the course of the disease.[69] Virulence factors of enteric *E. coli* isolates were studied in 44 dogs with fatal diarrhea, in which the history (diarrhea with or without bloody feces), signalment (mean age, 56 days) and gross small intestinal pathologic lesions were suggestive of CPV enteritis.[51] Canine parvovirus was identified as an intercurrent pathogen in 43% (19/44) of these dogs.[51]. The *eaeA* gene (encoding for proteins that produce attaching and effacing lesions) was identified in bacteria from all 44 dogs; control dogs were unfortunately not examined.[51] This study indicated that attaching and effacing *E. coli* strains might be primary or secondary pathogens in dogs with diarrhea, and also that these enteric strains commonly proliferate in CPV enteritis.[51] Another study isolated *E. coli* from 61% (17/28) of fecal samples cultured in naturally occurring CPV enteritis in puppies, confirming that enteric coliforms commonly proliferate in CPV enteritis.[71] *Escherichia coli* proliferation in the small intestine may have caused more severe disease and death in 1 dog experimentally infected with CPV.[21] *Campylobacter* species have also been isolated from the feces of 47% (23/49) of naturally occurring CPV enteritis cases, compared with 14% (4/28) of controls; a statistically significant difference.[70] Whether this bacterial colonization predisposed the gut to CPV infection, or whether it was secondary to CPV disease, is unknown.[70]

1.1.3.4 Bacterial & endotoxin translocation, SIRS and MODS in parvoviral enteritis

Endotoxin (lipopolysaccharide) is present in the outer cell wall of all Gram-negative bacteria, and both Gram-negative bacteria and endotoxin are normally present in the GIT.[73] Bacterial translocation is defined as the passage of viable indigenous bacteria from the GIT through the intestinal epithelial mucosa to the mesenteric lymph nodes and other organs.[74, 75] Indigenous Gram-negative enteric bacilli (e.g. *E. coli*, *Proteus* and *Enterobacter* spp.) translocate more efficiently than Gram-positives, while obligate anaerobes translocate only in small numbers.[75] Intestinal endotoxin transmigration is not necessarily associated with the passage of intact bacteria, since endotoxins transmigrate more readily than intact Gram-negative bacteria.[60] One or more of 3 pathophysiologic conditions, commonly observed in critically ill patients, is required for bacterial

translocation to occur.[55, 62, 74, 76] These are (1) functional or physical loss of the gut mucosal barrier with hyperpermeability, (2) impaired host immunity, and (3) intestinal bacterial overgrowth.[55, 62, 74, 76]

Bacterial translocation *per se* may not be clinically significant in the presence of a fully functional immune system.[77] Bacterial translocation to mesenteric lymph nodes occurred in 52% of 50 healthy dogs undergoing elective ovariohysterectomy, while no bacteria were isolated from portal blood samples in any of the dogs.[78] The destruction of translocated bacteria in mesenteric lymphnodes is dependent on the host's immunological competence and bacterial virulence factors.[76, 77] In severely immunocompromised patients, viable translocated bacteria may disseminate to extra-intestinal sites and establish septic foci.[77]

Since 1980, bacteremia and endotoxemia have been postulated to occur in CPV enteritis, as a consequence of a breakdown in the gut barrier.[34] Bacteremia and systemic bacterial infections have been extensively documented in naturally occurring CPV enteritis, as a consequence of enteric bacteria entering the systemic circulation.[17, 72, 79-81] Bacteremia caused by *E. coli*,[72, 79] *Klebsiella* and *Enterobacter* spp.,[80] and *Bacteroides* spp.[72] have been described, as have bacterial pneumonia[17] and bacterial myocarditis.[81] *Candida* organisms have similarly led to multisystemic infection following intestinal invasion in CPV enteritis.[82]

Escherichia coli bacteremia and endotoxemia was described in 3 dogs with naturally occurring severe CPV enteritis (2 of these dogs died).[72] Concomitant intestinal *E. coli* overgrowth was demonstrated, with serologically similar strains being isolated from the blood.[72] *Bacteroides* spp. bacteremia was also present in all 3 dogs.[72] The intestinal flora was thus shown to be instrumental in the development of the diarrhea, endotoxemia and shock seen in CPV enteritis.[72] In a further study of 98 dogs that succumbed to CPV enteritis in which liver or lung were aerobically cultured, septicemic colibacillosis was diagnosed in 90% of cases.[79] Disruption of gut-barrier function (due to small intestinal mucosal and lymphoid tissue necrosis) was thought to have predisposed to systemic

bacterial invasion.[79] Histologic examination of the lungs of these cases demonstrated pulmonary edema or alveolitis compatible with the adult respiratory distress syndrome (ARDS) in 69% of dogs.[79] Due to the retrospective nature of this postmortem study, the percentages of septicemia and ARDS may not reflect the true incidence of these phenomena in CPV enteritis, although the findings do indicate that these sequelae commonly occur in fatal CPV disease.[79] ARDS in these cases might have been induced by endotoxemia and/or tumor necrosis factor (TNF).[79] These findings support the likelihood that CPV intestinal pathology increases the risk of bacterial translocation, resultant *E. coli* bacteremia, as well as SIRS, MODS (e.g. ARDS) and death.[79, 83] Endotoxemia was also documented following experimental severe CPV enteritis in 10 mongrel puppies.[72] Plasma endotoxin levels gradually increased during the study, with high levels being demonstrable for prolonged periods (10 to 30 days) following initial CPV inoculation.[72] It is noteworthy that clinical signs of CPV disease were only present from 3 to 10 days after CPV inoculation,[72] indicating that endotoxemia persists even during the recovery phase. The maximum endotoxin levels in these dogs were 73.6 ± 11.2 pg/ml (mean ± SE), compared with 2.3 ± 0.6 pg/ml in healthy controls.[72]

Fourteen of 17 dogs with naturally occurring parvoviral enteritis had endotoxemia, and 7 of the 17 dogs also had measurable TNF levels, while neither TNF nor endotoxin could be detected in normal controls.[83] An increase in TNF activity was predictive of mortality, while there was a trend for increasing endotoxin activity over time to be predictive of death.[83] There was no correlation between endotoxin and TNF activity.[83] Three of the 4 dogs in this study that died had increases in TNF activity, endotoxin, or both.[83] The authors deduced from their results and those of previous studies on sepsis that the activation of the systemic cytokine cascade, which limits the ability to treat affected patients successfully, is integral to the pathophysiology of parvoviral enteritis.[83] It was speculated that measures to limit endotoxemia and SIRS might improve survival in CPV enteritis.[83] A further study detected significantly higher plasma endotoxin concentrations in 40 dogs with naturally occurring CPV than in normal dogs or dogs recovered from CPV.[12] This study detected no relationship between increasing endotoxin concentration and outcome.[12]

Dogs experimentally treated with endotoxin may develop vomiting, bloody mucoid diarrhea, gastrointestinal mucosal desquamation with hemorrhagic enteritis, hepatosplanchnic pooling of blood and marked systemic arterial hypotension.[73, 84-86] In addition, puppies during the first 6 months of life are approximately 4 times more sensitive to the lethal effects of endotoxin than adult dogs.[84] Endotoxemia may thus be important in further compromising an already dysfunctional gut barrier in CPV enteritis.

A clinically significant relationship may exist between the state of intestinal barrier dysfunction, Kupffer cell function, the hypermetabolic response to injury or sepsis, and distant organ injuries.[55] Gut-derived endotoxin may suppress Kupffer cell activity.[55] This impaired activity may potentiate the systemic effects of gut-barrier failure by allowing gut-derived bacteria or endotoxin to reach the systemic circulation, rather than being cleared from the portal circulation.[55] In addition, endotoxin absorbed from the canine gut may bypass the portal circulation and hepatic clearance, by entering the systemic circulation *via* lymphatic drainage.[60] Translocated intestinal bacteria or endotoxin need not, however, reach the portal or systemic circulation to induce a systemic inflammatory state.[56] Instead, loss of gut-barrier function with intestinal bacteria or endotoxin reaching the lamina propria, especially if superimposed on an existing gut injury, could induce a local intestinal inflammatory response and/or potentiate the subsequent production and/or release of cytokines from the GALT and resident gut macrophages.[56] The gut may therefore serve as a source of proinflammatory cytokines and vasoactive substances, as well as a priming bed for neutrophils, which may lead to SIRS or MODS.[56]

In summary, bacterial and endotoxin translocation, septicemia and endotoxemia, and the development of SIRS and MODS occurs in CPV enteritis. It is likely that the loss of gut barrier function in CPV enteritis underlies these phenomena.

1.2 THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS) AND THE MULTIPLE ORGAN DYSFUNCTION SYNDROME (MODS)

1.2.1 Introduction:

In patients with life-threatening critical illness, a major threat to survival is often not the primary underlying illness, or even a single complication thereof, but, rather, progressive secondary dysfunction or failure of several organ systems.[87] Risk factors for MODS are diverse and include infection (Gram-negative or Gram-positive bacteria, protozoa, viruses or fungi),[55] non-infectious inflammatory conditions, immune system activation, traumatic or burn injury, ischemia, and toxins.[88] Many processes that induce an excessive or prolonged inflammatory response are capable of initiating a systemic inflammatory cascade culminating in MODS.[55] Considering the host's relatively limited repertoire of responses to insults, it is not surprising that similar mediator systems and multiple organ dysfunctions are observed even though the initiating events may differ.

In MODS, organ injury does not develop in direct response to the original insult itself, but instead is largely a consequence of the host's endogenously produced mediators.[55, 87] Current evidence suggests that a massive inflammatory reaction, resulting from systemic cytokine release in response to the initiating event, is the common pathway underlying both SIRS and multiple organ dysfunctions.[55, 89, 90] The pro-inflammatory reaction is mediated by substances including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-12, interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor, thromboxane, prostaglandins (PG), oxygen free radicals, and platelet activating factor.[89, 90] This pro-inflammatory reaction is countered by a compensatory anti-inflammatory reaction, dubbed the compensatory anti-inflammatory response syndrome (CARS).[89] The function of this anti-inflammatory reaction is to down-regulate synthesis of proinflammatory mediators and to modulate their effects, thereby attempting to restore homeostasis.[89] Mediators of CARS include, amongst others, IL-4, IL-10, IL-11, IL-13, soluble TNF-α receptors, IL-1 receptor antagonists, and transforming growth factor-β1.[89, 90] Predominance of either of these opposing arms of the immuno-

inflammatory response in an individual patient may result in SIRS, CARS, or the mixed antagonists response syndrome (MARS), in which features of SIRS are present in a patient with CARS.[89] Should CARS predominate, immunosuppression may result, with increased susceptibility to infection.[89]

1.2.2 The "gut hypothesis" of MODS:

The basic element of the physiologic continuum of MODS is an excessive or persistent immuno-inflammatory response.[55] In the gut hypothesis of MODS, an initiating clinical event that alters multiple homeostatic mechanisms, such as shock or tissue hypoperfusion, leads to impaired oxygen delivery to the gut, with resulting intestinal injury, increased intestinal permeability, and gut-barrier dysfunction.[55, 56, 91-93] A direct gastrointestinal insult, such as GIT inflammation, chemotherapy or radiation may have a similar effect.[62] A similar scenario may also exist during systemic inflammatory or infectious states, where activation of endogenous inflammatory mediators leads to decreased intestinal oxygen delivery, as well as impairment of intestinal barrier function.[55] This manifests as disruption of the intercellular tight junctions and the development of patchy mucosal erosions, necrosis, and submucosal edema.[91, 93] Ischemia-reperfusion injury may further aggravate the extent of tissue injury.[55, 56] Intestinal bacterial or endotoxin translocation is not a prerequisite for the development of SIRS or MODS.[59, 83] However, in the gut hypothesis of MODS, intestinally-derived portal or systemic endotoxemia or bacteremia (Gram-negative or Gram-positive) may serve as the triggers that initiate, perpetuate, or exacerbate the hypermetabolic and immuno-inflammatory responses of the septic state, and thereby promote the development of MODS.[55, 56, 91] Endotoxin and bacteria induce cytokine release by resident gut tissue macrophages, recruit neutrophils, T and B lymphocytes and platelets to the site of injury, stimulate neutrophil oxidant and protease production, promote pro-inflammatory endothelial cell phenotypes, and activate the coagulation and complement systems.[55, 89] Once initiated, this cycle of systemic inflammation may become self-perpetuating.[55, 58, 94] The products of activated macrophages (TNF-α, IL-1, IL-6, PGE_2), neutrophils and endothelial cells, as well as the activated coagulation and complement systems, may

impair GIT oxygen delivery by their microcirculatory effects, and by thus leading to further increases in intestinal permeability, create a feedback loop which potentiates additional intestinal endotoxin or bacterial translocation.[55, 58, 94] Endotoxin administration increases intestinal mucosal permeability of healthy humans, as measured by urinary lactulose and rhamnose excretion ratios.[58] Intestinal hyperpermeability therefore plays an important role in the development and perpetuation of gut-barrier failure.[58] The above gut hypothesis fits well with the "two-hit hypothesis" of MODS, where an initial insult, with its resultant loss of gut mucosal barrier function, leads to activation of the systemic inflammatory cascade.[91] The initial insult thus primes the patient so that, should a second insult occur, the pro-inflammatory response will be greatly amplified.[55, 91]

The "gut origin of MODS" hypothesis was supported by the findings of a prospective controlled study of 279 human surgical (predominantly laparotomy) patients.[77] Diagnoses in these patients were diverse and consisted predominantly of GIT neoplasia, inflammatory GIT disorders, pancreatic and biliary disease.[77] Bacterial translocation to mesenteric lymph nodes occurred in 21% of patients; amongst these patients, enteric coliforms were responsible for sepsis in 65%, with *E. coli* being the most common organism isolated from mesenteric lymph nodes (48%) and septic foci (53%).[77] The organisms responsible for post-operative infections in these patients were all gut-derived bacteria that had previously been shown to translocate.[77] In a different study, more than 30% of 50 human patients with non-inflammatory bowel disease undergoing laparotomy were shown to have portal bacteremia, also confirming the occurrence of bacterial translocation in non-inflammatory gastrointestinal disease.[95]

In addition to occurring in CPV enteritis,[17, 72, 79-81] bacterial translocation with resultant bacteremia has been documented in dogs with experimental shock,[96] bowel ischemia and reperfusion,[97] hypoxia[98] and acute pancreatitis.[99]

1.2.3 Gut barrier dysfunction and intestinal hyperpermeability in critical illness:

Increased intestinal permeability has been proposed as one of the major mechanisms by which bacterial translocation and the systemic absorption of intestinal toxins are promoted.[58, 77] A wide range of primary intestinal as well as non-intestinal critical illnesses have been associated with intestinal epithelial hyperpermeability, including sepsis, endotoxemia, hemorrhagic shock, trauma, acute pancreatitis, inflammatory bowel disease, intestinal surgery, and severe thermal injury.[58, 100, 101] Endotoxemia has been demonstrated in a large variety of human critical illnesses, further suggesting that gastrointestinal dysfunction occurs in sepsis.[58] Gut permeability is increased as early as 24h after burn injury, with the extent of the injury being correlated to the degree of intestinal hyperpermeability.[58] Hypoalbuminemia and capillary leak syndromes that commonly occur in critically ill patients may additionally result in intestinal edema, impaired GIT peristalsis, small intestinal bacterial overgrowth (SIBO) and increased intestinal permeability.[55]

The mechanisms responsible for the above-mentioned increases in intestinal epithelial permeability are incompletely understood.[58, 100] Hyperpermeability may be induced by oxidant stress, adenosine triphosphate (ATP) depletion, acidosis, inadequate tissue perfusion, endothelial damage, and pro-inflammatory cytokine release.[102] Hypovolemia and ischemia-reperfusion injury may lead to cellular hypoxia and decreased intestinal mucosal pH, which may further promote intestinal epithelial hyperpermeability.[58] Oxidant injury or increased intracellular calcium concentrations have been suggested to be the mechanisms responsible for the loosening of intestinal epithelial tight junctions, which lead to increased intercellular permeability.[58] Depletion of ATP and inflammatory cytokine release has similarly produced hyperpermeability under experimental conditions.[58] The actions of certain cytokines, notably IFN-γ and IL-4, may be especially important.[100] Another factor contributing to increased intestinal epithelial permeability in certain inflammatory conditions may be the excessive release of the pluripotent signaling and effector molecule nitric oxide (NO).[58, 100] Expression of inducible NO synthase (iNOS) may be induced in multiple cell types following exposure to proinflammatory

cytokines and/or lipopolysaccharide.[100] It has been documented that IFN-γ acts synergistically with IL-1β and TNF-α to induce functional iNOS expression, leading to increased permeability of cultured enterocyte monolayers.[100] An essential second messenger role for NO in producing pro-inflammatory cytokine-induced intestinal epithelial hyperpermeability, and therefore decreased gut barrier function, has been demonstrated.[100] It is likely that endothelial damage and hyperpermeability result from the combined effects of the above-mentioned, and more diverse, mediators.[58]

1.3 CURRENT TREATMENT RECOMMENDATIONS IN PARVOVIRAL ENTERITIS

The fundamental treatment of CPV is based on first principles rather than on clinical studies. The few clinical trials that have been performed have investigated novel adjunctive therapies.

The treatment of CPV enteritis is mainly supportive, as it is in other causes of severe enteritis.[29, 103] Standard treatment includes intravenous crystalloid fluid therapy (lactated Ringer's with 2.5% or 5% dextrose and potassium chloride); colloidal products if indicated (plasma, whole blood, or synthetic colloids); intravenous, broad-spectrum, bactericidal antibiotics; antemetic and prokinetic drugs; and the eradication of intestinal parasites.[29, 42] In addition to providing albumin, immunoglobulins and antithrombin III, plasma and whole blood also contain plasma proteinase inhibitors, which may be beneficial in the modulation of the systemic inflammatory response in CPV enteritis.[29, 44]

Novel adjunctive therapies that have been investigated include recombinant human granulocyte colony-stimulating factor,[104] recombinant feline interferon-omega,[105] hyperimmune serum transfusion,[106] anti-endotoxin antibody,[39, 71] and recombinant bactericidal/permeability-increasing protein (an endotoxin-neutralizing agent).[12] Anti-endotoxin therapy has shown marked variation in efficacy.[12, 39, 71] Following an initial report of significantly increased survival with such therapy,[71] further studies have

demonstrated either no significant effects on outcome, duration of hospitalization or plasma endotoxin concentrations,[12] or even significantly reduced survival rates.[39] Granulocyte colony-stimulating factor has not been beneficial.[104] Although interferon treatment has been claimed to be beneficial,[105] the experimental design and execution were poorly described, and the mortality in non-interferon-treated dogs was unacceptably high (62%), as to cast some doubt on the conclusions of this study.

Conventional wisdom dictates that "gut rest" with initial *nil per os* (NPO) remains the nutritional treatment of choice for CPV enteritis.[8, 26, 29, 42] It has been recommended that food should be withheld for a period ranging from 24[26, 29] to 48[8, 26, 29] or 72 hours[42] after vomiting has ceased. It is advised that water should be offered during the 24-hour period following the cessation of vomiting, and only once vomiting of water does not occur, should enteral nutrition be initiated with small bland feedings.[8, 26] An easily digestible, high-carbohydrate, low-fat diet has been recommended for this purpose.[29] All of these recommendations stem from the notion that the diseased gut in CPV infection should be "rested" in order to decrease the severity of vomiting and diarrhea.

This currently advised treatment strategy of initial NPO has never been subjected to objective scientific scrutiny. A few relatively recent publications have speculated on the potential benefits of nutritional support in CPV enteritis.[26, 29, 107] It has been postulated that dogs with severe CPV enteritis and prolonged hospitalization might require nutritional support in order to prevent catabolism and immune dysfunction associated with a negative nitrogen balance.[29] The feeding of liquefied diets via nasogastric tubes has similarly been advocated to reverse catabolic processes in CPV infection, although this was only recommended for less severe cases, or once vomiting has ceased.[107] It has also been stated that early enteral nutrition in CPV enteritis may be important to promote intestinal regeneration.[29, 107] The addition of glutamine (0.5 g/kg/day) to drinking water has been recommended to promote GIT healing in the recovery phase of CPV enteritis[107] and to decrease bacterial translocation, rather than avoiding the oral route completely.[26] Partial parenteral nutrition (PPN), although not supporting all the patient's nutritional needs, has additionally been recommended to provide short-term support for dogs that are

expected to recover soon.[29, 107] Although all of the above recommendations are currently unsubstantiated by CPV-specific research, they do reflect a collective consciousness of the benefits of enteral nutritional support in critical illness.

The mortality rate of human patients with established MODS has not improved appreciably in the 20 years following its initial description, despite the use of improved antibiotics and increasingly sophisticated critical care.[55, 87, 90] A similar situation presently exists in the treatment of CPV enteritis. It has been speculated that measures to limit endotoxemia and SIRS might improve survival in CPV enteritis.[83] Treatments that decrease the severity of disease, reduce the duration of hospitalization and reduce mortality are needed to reduce the emotional and financial costs incurred with CPV infection.[12]

1.4 NUTRITION IN CRITICAL ILLNESS

1.4.1 The hypermetabolism and catabolism of critical illness and SIRS:

Acute illness or sepsis is accompanied by an increased metabolic rate, hypermetabolism (protein and fat catabolism), a negative nitrogen balance, peripheral resistance to insulin, augmented hepatic acute phase protein and glucose synthesis, and a loss of lean body mass.[108-111] Accelerated generalized protein catabolism of visceral and skeletal muscles in hypermetabolic states leads to a negative nitrogen balance.[108, 110, 111] Endogenous amino acids in sepsis are utilized both as gluconeogenic precursors, and for the synthesis of hepatic acute-phase proteins.[108, 110, 111] Protein and energy intake are concomitantly also reduced in sepsis.[108]

The hypermetabolism and catabolism of critical illness and sepsis is the net result of a complex interdependent cascade of neuroendocrine counterregulatory hormones, and a multitude of inflammatory mediators (most notably TNF-α and IL-1).[58, 90, 110-112]

- The neuroendocrine component consists of an increase in sympathetic activity, a decrease in parasympathetic activity, and hypothalamic-pituitary-adrenal axis activation leading to plasma elevations of the counterregulatory hormones (catecholamines, glucocorticoids, glucagon, and growth hormone), with resultant peripheral resistance to insulin.[58, 110-112] These counterregulatory hormones cause an increase in metabolic rate that is related to the severity of the initiating stimulus.[113]
- The acute-phase response and SIRS may also contribute significantly to hypermetabolism and catabolism, through the actions of the pro-inflammatory mediators IL-1, IL-6, IL-8, TNF-α, IFN-γ and NO, amongst others.[90, 112] Furthermore, endotoxemia, as well as inducing TNF-α and IL-1 release leading to hypothalamic stimulation, may also directly stimulate adrenocortical and adrenomedullary responses.[110] Since the acute-phase response and SIRS carry great metabolic costs, therapies that reduce cytokine generation may reduce the nutrient-losing effects of systemic inflammatory states.[112]

Considering the above, dogs suffering from severe CPV enteritis are almost certainly in a hypermetabolic and catabolic state. Vomiting and protein-losing enteropathy will lead to additional nutrient losses. The currently advised treatment strategy of therapeutic nutrient exclusion (by recommending NPO) does not address these metabolic derangements in CPV enteritis, and probably exacerbates them.

1.4.2 Effects of enteral nutrients on gut-barrier function and SIRS in critical illness:

1.4.2.1 Introduction

The most important stimulus for intestinal mucosal growth, cell mass, integrity and function is the presence of nutrients within the gut lumen.[58, 67, 114] The presence of nutrients provides local substrate for cellular oxidation, creates a mechanical stimulus for increased enterocyte proliferation, promotes splanchnic microvascular blood flow, stimulates motility, secretion of secretory IgA and mucus, and activates the parasympathetic nervous system.[62, 114-116] Enteral nutrients also have indirect trophic

effects mediated through the increased production of trophic GIT hormones, which act via autocrine, paracrine, or endocrine pathways.[62]

Changes in small bowel structure and function occur when enteral nutrients are excluded, such as during malnutrition, starvation or during total parenteral nutrition administration.[58] When appropriate enteral nutrients are not provided, the small intestine may be more susceptible to impairment of its digestive, absorptive, and barrier functions.[67] The absence of luminal nutrients leads to reduced small bowel villus height and suppressed crypt cell proliferation.[109] For instance, intestinal mucosal atrophy has been shown to occur in rats after 72h of bowel rest.[117] When a hypermetabolic critically ill patient does not receive nutritional support, the gastrointestinal tract atrophies and its walls become more permeable to intraluminal bacteria and toxins.[118] Prolonged anorexia may therefore contribute to sepsis.[118] A delay of more than 2 days before implementing enteral feeding in the critically ill patient may also severely affect immune function and wound healing.[119]

Enteral nutritional support initiated early in the course of disease in critical illness may limit or prevent immunosuppression, hypoalbuminemia and muscle weakness, and by maintaining adequate gut-barrier function by supporting structural and functional intestinal integrity, limit or prevent bacterial and endotoxin translocation, the development of endotoxemia and/or bacteremia, and subsequent SIRS and MODS.[62, 119] Enteral nutritional intervention may modulate local GIT inflammatory mediator production and the ensuing systemic response, and attenuate the response to “second hits”.[90] Several studies have shown reductions in septic morbidity in humans following early enteral feeding in inflammatory conditions associated with trauma, thermal injury, and major surgery.[67, 120]

It therefore stands to reason that the provision of enteral nutrients in CPV enteritis may promote enterocyte proliferation and intestinal mucosal repair, and thus gut-barrier function.

The following section scrutinizes the above-mentioned beneficial effects of enteral nutrition more closely, by comparing the provision of enteral nutrients to conditions where such nutrition is excluded, namely total parenteral nutrition and malnutrition states.

1.4.2.2 Enteral nutrition *vs.* total parenteral nutrition

Enteral nutrition (EN) appears to have significant benefits over total parenteral nutrition (TPN) in preventing intestinal mucosal alterations. Compared with EN, TPN in animal models results in mucosal atrophy, increased intestinal permeability, and increased bacterial translocation.[67] These changes are rapidly reversible with appropriate EN.[58] Total parenteral nutrition in rats is associated with significant small intestinal mucosal atrophy, as compared with EN.[121, 122]

Gut-associated lymphoid tissue is exquisitely sensitive to the type and route of nutrition.[67, 123] Enteral nutrition supports the gut-barrier and GALT, and is superior to TPN in this regard.[67] In mice, normal numbers of small intestinal lymphocytes and GALT cell profiles are maintained with EN.[123] In contrast, TPN is associated with significant reductions in GALT cell mass (both T and B cells) within Peyer's patches, lamina propria, and intraepithelial spaces of the small intestine.[123] Significant reductions in mucosal and lamina propria CD4/CD8 lymphocyte ratios[121, 123] and intestinal IgA production accompany these changes during TPN.[123] Following TPN, mucosal immunity in mice recovers within 5 days of reinstituting an enteral diet.[123]

Diverse critical illnesses in human ICU patients were associated with intestinal epithelial hyperpermeability, as assessed by urinary lactulose/rhamnose (L/R) recovery ratios in 24 patients.[114] The early institution of EN in this scenario was associated with a significant progressive decrease in GIT permeability, whereas TPN resulted in a continued increase in permeability.[114] However, a statistically significant difference in urinary L/R ratios between these 2 groups of patients was only observed 9 days after the institution of nutrition, while no significant difference was noted by day 6 (measurements were taken on days 3, 6 and 9).[114] Such a reversal of intestinal hyper-permeability in the patients

receiving EN might parallel a reduction in bacterial translocation, and the development of sepsis, SIRS, and MODS.[114] A substantially higher (55% *vs.* 15%; but statistically non-significant) mortality was observed in the TPN patients.[114]

Damage to the tight junctions between enterocytes is presumably responsible for the increase in intestinal permeability (as assessed by differential saccharide absorption and excretion tests; e.g. urinary L/R recovery ratios) observed when enteral nutrients are excluded, such as during TPN.[58] Bacteria or endotoxin are physically too large to traverse these tight junctions; the maximal aperture size of these tight junctions is however large enough to allow the passage of bacteria-derived peptides and small dietary antigens that are usually limited to the gastrointestinal lumen.[58] These absorbed molecules can activate intra-epithelial lymphocytes to secrete IFN-γ, which in turn increases the intestinal mucosal permeability and activates macrophages and neutrophils.[58] This cascade of events may in part contribute to the alterations in gut structure and function observed during TPN.[58]

Enteral feeding seems to modulate the acute phase response and preserves visceral protein metabolism, which suggests downregulation of the splanchnic cytokine response.[109, 120] Parenteral nutrition, by contrast, predisposes to an exaggerated cytokine mediator production and acute-phase response.[109] TPN in humans also enhances splanchnic and systemic TNF-α and C-reactive protein production, whereas EN has not shown such effects.[120] Furthermore, TPN causes a significant rise in free radical production, both in stable and critically ill human infants.[124]

Early EN has generally been advised over TPN in humans,[58, 125, 126] to prevent further or limit existing gut atrophy or injury.[58] In disease states where the GIT is the primary site of disease or dysfunction, the administration of standard intravenous TPN formulations may not deliver adequate nutrients to the GIT for mucosal metabolism and repair.[127]

Critically ill humans who are fed enterally may have significant reductions in the severity of septic complications and length of hospital stay, as compared with patients receiving

TPN.[58] However, some studies have not observed any significant differences in the incidence of septic morbidity between EN and TPN.[128]

1.4.2.3 Malnutrition

Malnutrition in animal models is associated with small intestinal mucosal and villus atrophy,[94] loss of intestinal weight, RNA and protein content,[129] increased transepithelial absorption of macromolecules (bovine serum albumin),[130] significantly decreased goblet cell-derived mucin levels,[131] significantly reduced intestinal secretory IgA concentrations,[132] marked impairment of mucosal immune responses,[133] and Gram-negative GIT bacterial overgrowth.[94] Such impaired mucosal immunity[133] and reduced s-IgA secretion[132] are rapidly reversible with refeeding.

The systemic and gut mucosal immunosuppressive effects of mild to moderately severe protein-energy malnutrition (PEM) in humans and laboratory animals are well established.[110, 134, 135] The effects of PEM on lymphoid function include prominent atrophy of lymphoid tissues, reduced cell-mediated immunity due to a reduction in differentiated T lymphocytes and reduced lymphocyte DNA synthesis, and significantly decreased CD4+/CD8+ ratios, while serum antibody responses generally remain near normal.[134, 135] The concentrations and activity of most complement components are also decreased in PEM, with reduced intracellular destruction of phagocytosed bacteria.[135]

Mucosal immunity is specifically affected in PEM.[134, 135] Protein-energy malnutrition in children increases the morbidity and mortality from infectious diarrheal disease, and also profoundly increases the average duration of each diarrheal episode.[135] Mucosal immunity is adversely affected by PEM in humans and animals, as a result of decreased intra-epithelial lymphocytes and plasma cells, reduced salivary s-IgA secretion, and decreased “homing” of mesenteric lymphoblasts to the GIT.[134] Increased bacterial adherence to mucosal epithelial cells has also been described.[135, 136] The above changes in immune responses occur early during the course of PEM.[135] As a result of such

immunosuppression, GIT bacterial overgrowth may occur. This, coupled to decreased gut immune function, may permit bacterial translocation.[134]

Malnourished animals challenged with pro-inflammatory stimuli show enhanced bacterial translocation and mortality from sepsis, as compared with normally nourished controls.[94, 137]

- Protein-malnourished mice exposed to endotoxin have a significantly higher incidence of bacterial translocation to systemic organs, and are significantly more susceptible to the lethal effects of endotoxin than normally nourished mice.[94] These effects are directly correlated to the duration of protein malnutrition.[94] The synergistic effect of protein malnutrition plus endotoxin in promoting bacterial translocation and death may be related to endotoxin initiating bacterial translocation by increasing intestinal permeability, and the combined immunosuppressive effects of malnutrition and endotoxin consequently impair the ability to clear the translocating bacteria.[94]
- Similar results were obtained in a study investigating the effects of the substance zymosan on intestinal structure and barrier function in normally nourished and protein malnourished mice.[137] Zymosan is a non-bacterial, non-endotoxin inflammatory agent capable of inducing a systemic inflammatory response, through the activation of complement and the stimulation of neutrophils and macrophages.[137] In this study, bacterial translocation to systemic organs was significantly greater in the malnourished mice.[137] Translocation in malnourished mice was related to intestinal mucosal injury (villus and crypt atrophy and epithelial disruption) and intestinal Gram-negative bacterial overgrowth.[137] This study confirmed that protein malnutrition predisposes to the development of inflammatory-induced, gut-origin septic states.[137]

An elegant study, conducted in 53 critically ill humans, classified nutritional status as well nourished, mildly to moderately malnourished, or severely malnourished.[57] This study was particularly significant in that it was conducted in a relevant population of hospitalized patients, rather than at the extremes of malnutrition observed in many experimental studies.[57] Both malnourished groups showed a significant increase in

urinary lactulose/mannitol (L/M) excretion ratios compared with the well-nourished controls, due to a relative increase in the amount of lactulose absorbed.[57] There was a significant inverse correlation between L/M ratios and the degree of malnutrition.[57] The significantly increased intestinal permeability was closely associated with abnormal mucosal immunology.[57] Duodenal immunohistochemistry demonstrated a significantly increased number of lamina propria macrophages and mucosal CD3+ T cells in malnourished patients, consistent with local immuno-inflammation.[57] Additionally, enhanced expression of the major histocompatibility complex class II antigen on macrophages, T cells and duodenal enterocytes, suggested cellular activation in the malnourished groups.[57] Coupled with a significantly decreased duodenal mucosal expression of the anti-inflammatory cytokine IL-10 in malnourished patients, these findings suggest a relative shift from anti-inflammatory and humoral immune mechanisms to cellular immunity and pro-inflammatory gut cytokine profiles in malnutrition.[57] Significantly increased serum IL-6 and a heightened acute phase response (measured by serum C-reactive protein levels) in these malnourished patients correlated directly with the severity of malnutrition.[57] A significant positive correlation between serum IL-6 and L/M ratios might indicate that these changes were the consequence of gut barrier failure in malnutrition.[57] Serum concentration of anti-endotoxin-core antibody (IgG), an indirect measure of chronic exposure to endotoxin, was similar between the study groups.[57] This finding might have been a genuine reflection of endotoxin exposure, although it was speculated that impaired systemic humoral immunity in malnutrition might have precluded antibody generation.[57]

A study of 101 humans undergoing elective major GIT resection found that relatively protein depleted patients (39% mean protein loss) had a significantly greater incidence of major complications, pneumonia, and length of hospital stay, as compared with non-protein depleted patients.[138]

1.4.2.4 Clinical efficacy of enteral nutrition in critical illness

This section reviews the effects of EN on humans with severe acute pancreatitis, bowel resection, intractable diarrhea of infancy, thermal injury, multisystem trauma and those undergoing high-risk surgery. The nutritional treatment regimens for all these conditions traditionally included a period of initial starvation.

1.4.2.4.1 *Acute pancreatitis*

Early EN in severe acute pancreatitis (SAP) in humans has recently been the focus of much investigation.[92, 120, 139-142] Poor outcome in SAP is associated with a high incidence of SIRS and sepsis.[120, 140] It has been stated that bowel rest during SAP deprives the gut of nutrients and negatively affects its structure and function.[92] Enteral nutrition usually is initiated late in the course of the disease, and therefore cannot prevent intestinal barrier failure and possible bacterial translocation, or offset the hypermetabolic state.[92] Notwithstanding, gut rest has traditionally been the treatment of choice for SAP.[120]

In a recent study comparing TPN with nasojejunal total EN in 34 people with SAP, there was a reduction in the requirement for ICU care in the EN group, incidence of intra-abdominal sepsis, multiple organ failure, need for operative intervention, and mortality as compared with the TPN group.[120] Enteral feeding resulted in reduced disease severity, improved clinical outcome (after 7 days of nutrition), preservation of visceral protein metabolism, suggesting downregulation of the splanchnic cytokine response, and significant attenuation of the acute-phase response, reducing systemic exposure to endotoxin and reducing oxidant stress.[120] This study suggested that contrary to conventional wisdom, enteral feeding is practical, feasible and desirable in the management of human patients with SAP.[120] Similar results were found in a randomized, prospective trial in 38 patients with SAP, which indicated that early nasojejunal EN was associated with significantly reduced septic complications as compared with TPN.[139] The authors speculated that the observed difference might be related to an improvement of gut immunity and structure, and a restoration of normal gut microflora by EN.[139] Reduced

mortality, improved nutritional and immunological status, improved intestinal motility and reduced septic complications were also found in a recent retrospective study of continuous nasojejunal EN in human SAP.[140] Early nasogastric EN in 22 humans with SAP has recently also been demonstrated to be safe and well tolerated.[141]

In contrast, however, a study of 27 humans with SAP found no amelioration of the inflammatory response (as measured by serum concentrations of IL-6, soluble TNF receptor I, or C-reactive protein) associated with EEN, as compared with an initial *nil per os* treatment.[142] The authors of this study also reported no beneficial effect of EN on intestinal permeability, as measured by urinary lactulose and rhamnose (L/R) excretion ratios.[142]

1.4.2.4.2 *Bowel resection*

Conventional treatment following bowel resection in humans has entailed starvation until the passage of flatus.[101] A randomized trial of immediate postoperative enteral tube feeding versus conventional postoperative intravenous fluids was conducted in 30 humans following elective partial bowel resection.[101] Gut mucosal permeability was measured by urinary lactulose/mannitol (L/M) excretion ratios.[101] Urinary recoveries of L/M on postoperative day 5 were significantly elevated compared with preoperative values in the intravenous fluids group, while mucosal permeability in the enteral feeding group remained stable.[101] Nitrogen balance on the 1st postoperative day was negative in patients receiving conventional postoperative intravenous fluids, but positive in all enterally fed patients, thus demonstrating a significant preservation of a positive nitrogen balance by EN.[101] Immediate EN was also found to be safe and well tolerated in this scenario.[101]

1.4.2.4.3 *Intractable diarrhea of infancy*

This diarrheal syndrome is associated with villus atrophy, significant malabsorption, malnutrition, immunocompromise, and GIT infection (including rotavirus and

salmonellosis).[143] Human infants (younger than 3 months of age) with severe intractable diarrhea responded more favorably to EN rather than to TPN, in a prospective, randomized clinical trial.**[143]** Continuous nasogastric EN in this setting was associated with significantly faster resolution of malabsorption and diarrhea, fewer complications, significantly less expensive hospitalization and a significant reduction in hospitalization time.[143]

1.4.2.4.4 *Thermal injury*

Moderate to major burn injury is associated with bacterial translocation,[74] and aggressive EN in human burn patients has dramatically improved sepsis-induced mortality.[115] Mechanisms whereby thermal injury might promote bacterial translocation include impairment of intestinal epithelial cell function, disruption of intestinal mucous production, interference with secretory immunity and the disruption of intestinal microflora.[74]

Immediate postburn EN in guinea-pigs, as compared with nutrition initiated 3 days postburn, was associated with significantly greater jejunal mucosal weight and thickness, significantly reduced catabolic hormone responses (plasma cortisol and glucagon), and inhibition of the expected rise in resting metabolic expenditure.[144] Post-burn hypermetabolism and hypercatabolism may thus be significantly reduced by early EN.[144] In another study, immediate postburn EN in guinea pigs was associated with significantly reduced bacterial translocation to systemic organs, and enhanced bacterial killing ability (after 24 to 48 hours of EN), as compared with acute postburn starvation.[145] Such early EN also significantly decreased the postburn hypermetabolic response (as measured by lower resting energy expenditure, plasma cortisol and urinary catecholamine metabolites), and effected higher intestinal mucosal and body weight, as compared with acute postburn starvation.[146] Reduced bacterial translocation was correlated with the reduced hypermetabolic response, suggesting that translocation may be an important initiator of the hypermetabolism in burn injury.[146] Enteral nutrition administered 12 hours following 50% burn injury in guinea-pigs preserved gut-barrier function against

translocation of enteric *Candida albicans*, among the most serious postburn infections.[115] Significantly reduced *C. albicans* colonization in the ileum was similarly effected by this treatment, as compared with starved animals.[115]

Prior to these studies, EN was generally not initiated until the 4th postburn day, since the GIT was considered to be functioning poorly in the resuscitation period.[144] Early EN has however been shown to be tolerated well immediately following injury.

1.4.2.4.5 *Multisystem trauma*

By maintaining the gut-barrier and preserving normal immunologic function, early EN significantly reduces the incidence of septic complications and improves clinical outcome in critically injured humans (trauma and high-risk surgical procedures), as compared with either starvation or TPN.[67]

A significantly lower incidence of septic morbidity (pneumonia, intra-abdominal abscessation, intravenous line sepsis, and infections per patient) was demonstrated in patients fed enterally within 24 hours (versus TPN) in a study of blunt and penetrating abdominal trauma in 98 human ICU patients.[147] As explanation for these differences, the authors speculated that EN improves gut immunity, and restores gut architecture and microflora.[147] Immediate EN following multisystem trauma in humans also improved nitrogen balance and significantly reduced septic morbidity, as compared with TPN following an initial 5 day period of fasting.[109] Enteral nutrition in this latter study was also associated with significantly higher albumin 10 days post trauma, and with significantly reduced acute-phase protein responses.[109] Immediate EN following major abdominal trauma requiring celiotomy in humans similarly caused significant improvement in nitrogen balance and significantly reduced septic morbidity, as compared with fasting for 5 days.[148] This study did not demonstrate significant differences in serum albumin.[148] A prospective, randomized study in 63 humans with severe multiple trauma illustrated a significantly more rapid normalization of serum C-reactive protein, IL-6 and IL-8 levels in early EN (within 24 hours of injury), as compared with either TPN or

starvation.[149] Lower mortality and reduced infectious complications were observed in the early EN group.[149]

Conventional practice in humans with major abdominal trauma traditionally consisted of delaying EN until 3 to 5 days postinjury.[148] The above studies indicate that immediate postoperative nutrition in these patients is simple, safe, feasible, cost effective and preferable to either TPN or starvation.

1.4.2.4.6 *High-risk surgical patients*

A meta-analysis from 8 prospective, randomized human trials comparing early EN within 48 hours post-surgery (118 patients) with TPN (112 patients) in high-risk surgical patients, found significantly reduced septic complications in the EN group.[150] Eighty-five percent of patients tolerated EN well.[150] Early post-operative EN maintained immunocompetence, improved wound healing, and decreased septic morbidity.[150] End-of-study nutritional markers (albumin and transferrin) were higher in the EN group, although this was not statistically significant.[150]

Previous to studies indicating the benefits of early EN, nutritional support had conventionally been delayed by 5 to 7 days after surgery.[150]

1.4.3 Concluding remarks on enteral nutrition in human critical illness:

Following a review of 39 research articles[151] pertaining to the effect of EN in critically ill humans, it was recommended that:

- EN be used in preference to TPN whenever possible;
- EN be instituted as early as possible in the course of illness (within 24 hours), initially at low rates and subsequently increased as tolerated; and that
- Polymeric formulas are used in preference to elemental formulas.[151]

A consensus statement on nutrition in critically ill people has similarly recommended the use of EN, initiated as soon as possible, as the preferred method of substrate delivery.[125]

1.4.4 Veterinary nutrition recommendations in critical illness:

The importance of nutritional support for hospitalized and critically ill small animal patients has been extensively addressed in the literature.[113, 116, 119, 152-163] Enteral nutrition[113, 116, 152-154, 156-162, 164] and nasoesophageal or nasogastric tube feeding[113, 119, 153, 156, 157, 160, 162, 163] have received particular attention.

It has been suggested that the incidence of protein-energy malnutrition in hospitalized veterinary patients may be similar to, if not greater than, the reported 30% to 65% in humans.[110, 118, 153] Veterinarians have an ethical responsibility to ensure that hospitalized patients obtain adequate nutrition.[118] Similar to recommendations in humans, nutritional support in seriously ill veterinary patients should be enteral, rather than parenteral, and initiated during the early, catabolic phase of disease.[118, 119, 152, 153, 157-159, 162] Such early EN for critically ill animals may maintain intestinal function, preventing bacterial and endotoxin translocation.[110, 118]

Enteral nutrition therapy should be started gradually, to deliver the fully calculated amount by the third day of treatment.[119] In insufficiently resuscitated patients with GIT hypoperfusion, intestinal epithelial oxygen demand may be increased beyond delivery capacity by the introduction of nutrients into the GIT.[165] It has therefore been advised that EN be initiated only after the patient has been hemodynamically stabilized.[116]

General parameters suggested to identify dogs at risk of protein-energy malnutrition, and thus candidates for nutritional support, have included

- recent weight loss equal to or greater than 5-10% of usual body weight,
- partial or total anorexia for 3 to 5 days, and
- serum albumin < 2.1 g/dl.[113, 152, 155, 156]

Inadequate nutrition should specifically not be allowed to persist in animals with resting energy expenditures increased above 20% to 25%, or in young animals.[113] Vomiting, diarrhea, blood loss, sepsis and pyrexia may increase the risk of malnutrition in veterinary patients.[118] Animals with severe protein-losing enteropathy may especially benefit from nutritional intervention.[118]

1.4.5 Enteral tube feeding:

Nasogastric and nasoesophageal tube feeding are well described in the veterinary literature.[113, 119, 153, 156, 157, 160, 162, 163] Tube feeding in animals is easy and practical, and considerably less costly than TPN.[113, 118, 157, 162] Tube feeding is an appropriate means of providing enteral nutritional support to critically ill animals, and has been associated with few complications.[113] Nasoesophageal tubes are preferred in most cases, since traversing the cardiac sphincter may result in gastric reflux with subsequent caudal esophagitis.[119]

A stated prerequisite for the successful application of enteral tube feeding has been the presence of a functional gastrointestinal tract.[164] However, in human and animal models with significant viral and bacterial diarrhea, nasogastric feeding and rehydration have been successfully accomplished.[166, 167] Nasogastric tubes have also been used successfully in enteral rehydration and feeding of a limited number of dogs suffering from parvoviral enteritis.[163] Gastrointestinal dysfunctions such as vomiting, diarrhea and abdominal distention are not absolute contraindications to enteral tube feeding in humans.[168] Vomiting is usually associated with bolus feeding, and is not a contraindication to oral fluid administration.[55, 169]

A controlled, randomized study assessing the efficacy of oral versus intravenous (IV) rehydration therapy in 470 children with severe gastroenteritis of various etiologies found that nearly 99% of the children with severe forms of dehydration, diarrhea, and vomiting were treated adequately with oral rehydration therapy alone.[170] Nearly all the patients tolerated rapid administration (40 ml/kg per hour) of oral fluid well.[170] Severe vomiting was not a limiting factor in any of the patients, 50% of whom had a history of severe vomiting.[170] Severe diarrhea (present in 72%) similarly did not prevent successful oral treatment.[170] The frequency of vomiting (during the first 6 hours) and duration of diarrhea were significantly lower in the orally treated children; their electrolyte and acid-base status improved more rapidly than in the IV group; and their weight gain at time of discharge was significantly greater than for the IV group.[170] Oral rehydration therapy in acute gastroenteritis in small animals has similarly been reported.[169, 171] The authors argued that the recommendation that the oral route of rehydration and nutrition should be avoided in acute gastroenteritis is a misconception, since there is little evidence to support the notion that "gut rest" is of benefit.[171] Withholding oral nutrition is neither essential nor helpful to recovery from most acute diarrheal illnesses.[171]

1.4.6 Complications of naso-enteral tube feeding:

Enteral tube feeding in human ICUs often results in grossly inadequate nutritional support, both as a result of physicians prescribing inadequate amounts to be fed and the inappropriate cessation of feedings by nursing staff.[168, 172] Prescribed amounts may vary from 66%[168] to 78%[173] of the patient's actual caloric requirements. Of this prescribed amount, only 76%[168, 174] to 87%[173] may actually be delivered to the patient. The amounts that are actually being fed may thus be as little as 52% of the patients' caloric requirements.[168] The incidence of such inadequate nutritional intake is significantly higher for EN than for TPN.[128] Human ICUs with well-defined feeding protocols deliver significantly greater enteral volumes than ICUs without such protocols.[174]

Side effects of enteral tube feeding include diarrhea,[168, 172, 174] high gastric residual volumes (i.e. gas and fluid remaining in the stomach from the previous feeding),[172, 174]

abdominal distention,[172, 174] vomiting,[172, 174] tube displacement,[168] regurgitation[172] and aspiration due to tube misplacement. One large prospective multicenter study documented the following frequency of complications in humans: high gastric residuals (39% of patients), diarrhea (14.7%), abdominal distention (13.2%), vomiting (12.2%), and regurgitation (5.5%).[172] Clinically significant aspiration pneumonia causes minimal morbidity (1 to 4% of patients) and minimal mortality.[168] Enteral feeding has to be abandoned due to uncontrollable complications in 11% to 15% of human patients.[150, 172, 174] Complications attributable to enteral tube feeding were reported in 8 (32%) of 25 dogs, consisting of the removal of NG tubes (6 dogs), vomiting (1), and diarrhea (2).[162]

Gastric emptying is significantly delayed in humans with a large variety of non-GIT critical illnesses, which may cause intolerance to EN and gastric bacterial overgrowth.[175] Opioid analgesia in particular is significantly associated with delayed gastric emptying.[175] In critically ill humans who have large volumes of gastric aspirates (residuals) and are therefore intolerant of nasogastric feeding, a single low dose of intravenous erythromycin significantly improves gastric emptying, allowing the continuation of enteral feeding.[176] Gastric decompression may also be required in these patients.[125] Gastric decompression (by aspirating gastric gas through the nasogastric tube) can also be used in animals prior to EN administration, especially in patients with ileus or persistent vomiting.[177]

Diarrhea associated with tube feeding can be virtually abolished in humans by addition of psyllium (a hydrophilic hemicellulose mucilage) to the enteral formulation.[178, 179] This effect has been attributed to psyllium's water-binding capacity.[178, 179]

1.4.7 Determination of nutrient requirements in critical illness:

An estimate of the animal's nutrient requirements is needed to calculate the minimum amount of food necessary to sustain critical physiologic processes, provide substrates for protein synthesis and gluconeogenesis, and provide energy needed to meet the additional demands of wound repair, immunity, and cell division and growth.[157]

The resting energy requirement (RER) is a patient's energy requirement at rest, in a thermoneutral environment, in a postabsorptive state.[160] The following linear formula is used for animals weighing more than 2 kg: RER (kcal/day) = (30 x Wt_{kg}) + 70.[160] The maintenance energy requirement (MER) is the amount of energy needed to maintain stable body weight, with allowance for limited activity and thermoregulation, in a moderately active adult animal.[157] The MER for dogs is 1.5 to 2 times RER.[157] The illness energy requirement (IER) is the energy required during illness or injury.[160] Accurate, direct measurements of energy expenditure in sick dogs are not available.[157] Suggested IER in dogs are 1.2 x RER in medical patients,[158] 1.5 x RER in critical illness,[160] 1.5-2.0 x RER in acute disease processes complicated by sepsis,[154] and 2.0 x RER in burn patients.[158] However, the use of the above illness factors to determine a patient's final caloric needs has been discouraged, due to the extreme difficulty of discriminating between patients with differing illness factors.[157] Instead, it has been suggested that since IER varies between RER and MER, individual patients' caloric intake be increased or decreased toward the MER or RER, respectively, on the basis of physical examination findings.[157]

It is often impractical or impossible to provide all nutrition to a patient enterally, especially in the presence of frequent vomiting or severe diarrhea.[55, 121, 126, 177] However, benefits from EN in humans may be obtained when approximately 25%-40% of nutrient requirement is delivered by this route;[180] and even a small amount of EN may have important GIT protective effects in critically ill animals.[118, 119, 181] In animals that do not tolerate total EN, supplemental partial (peripheral) parenteral nutrition may be used.[181]

The enteral feeding of 25% of required nutrients (minimum luminal nutrition) in rats has not shown significant benefits in preventing the small intestinal mucosal atrophy and abnormal mucosal lymphocyte subsets associated with TPN.[121] In another study, minimum luminal nutrition in rats significantly reduced the gut mucosal atrophy associated with TPN, but without decreasing bacterial translocation.[122] At least 50% of goal calories are required to establish gut integrity and effectively stop bacterial

translocation, in rats.[168] Small volumes of enteral feedings in human infants significantly increase host bactericidal activity, as compared with TPN.[182]

The term "microenteral nutrition" describes the feeding of small amounts of elemental diets (initially less than 0.25 ml/kg/hour) to anorexic patients.[177] The goal is not to meet systemic caloric or protein needs, but rather to improve GIT blood flow, prevent GIT mucosal atrophy and mechanical dysfunction, prevent down-regulation of mucosal enzymes and to help preserve gut immune function.[177] Microenteral nutrition may specifically benefit patients with severe vomiting that cannot yet tolerate complete EN, in the transition phase to full EN.[177] Microenteral nutrition should be initiated within 2-12 hours of hospital admission.[177] Microenteral nutrition is well tolerated in small animals, and has not been associated with significant stimulation of vomiting.[177] However, there have been no controlled human or veterinary studies to determine whether microenteral nutrition has any impact on patient outcome.[177]

1.4.8 Elemental *vs.* complex diets:

Several studies support the preferential feeding of complex protein diets, rather than elemental diets, in critical illness:

- Rats receiving oral amino acid diets consistently show decreased gut growth and distal intestinal mass compared with those receiving isocaloric and isonitrogenous complex protein or peptide diets.[183] Complex fiber-based intact protein diets produce the greatest gut and total body growth effects.[183] In rats, dietary fiber (cellulose) significantly improves gut barrier function as measured by a reduction in the incidence and magnitude of bacterial translocation, compared with diets lacking fiber.[184]
- The feeding of liquid low-residue defined formula (pure amino acids or hydrolyzed proteins) diets in rats leads to a significantly decreased proximal (approximately the proximal half) small intestinal mucosal weight, protein and DNA content, as compared with a normal residue control diet containing intact proteins.[185]

- Enteral feeding with intact protein or peptides stimulates greater secretion of gut trophic hormones and tissue growth factors (e.g. insulin-like growth factor I) as compared with amino acids.[183] The feeding of elemental diets in rats has also been shown to lead to significantly decreased gastric (antral) and serum gastrin concentrations, as compared with rats fed on normal rat food.[186] This may have important clinical significance, since gastrin is an important regulator of GIT growth, secretion and function.[186]
- The intestinal absorption of most essential and non-essential amino acids in pigs is greater, more rapid and more homogeneous when small peptides are fed as compared with when free amino acids are fed.[187] This difference may be related to the fact that small peptides, which are transported by enterocyte cytosol or membrane hydrolysis, do not compete for intestinal transport sites as in the case of free amino acids.[187]
- The feeding of a complex diet (containing intact proteins) in chemotherapy (methotrexate)-induced enteritis in cats was associated with reduced morbidity (vomiting and diarrhea), significantly reduced proximal small intestinal damage and reduced bacterial translocation to mesenteric lymph nodes, as compared with the feeding of a purified diet (elemental; containing free amino acids).[188]

However, the following theoretical disadvantage to feeding a complex diet in CPV enteritis, rather than an elemental diet, deserves comment:

- Following acute gastroenteritis with disruption of the intestinal mucosa, increased quantities of intact ingested dietary proteins are absorbed.[189] Allergy-prone human infants exposed to allergens during the first 3 months of life are more prone to developing allergic disease than are infants exposed after 3 months of age.[189] Following initial sensitization, re-exposure to luminal GIT antigens later in life may lead to a local intestinal cellular immune-mediated hypersensitivity reaction, resulting in inflammatory bowel disease.[189] [While hypersensitivities to intact proteins or peptides may develop by this mechanism, similar allergic responses to individual amino acids have not been reported, to the best of my knowledge. Possibly amino acids are too small to serve as efficient allergens?] In an experimental study, opening of the intercellular tight junctions (*zonulae occludentes*) with intercellular

macromolecular penetration has also been demonstrated to occur 2 hours following surgical trauma to guinea pig intestines.[190]

- Loss of intestinal barrier function to macromolecules may also occur as a response to malnutrition.[130, 191] Severe protein deficiency of 2 months' duration in rats has been associated with 10-fold increases in intestinal transmucosal movement of macromolecules (bovine serum albumin; molecular weight 60,000).[130] The presence of macromolecular tracer molecules within apical tight junctions was associated with damage to and separation of these intercellular tight junctions.[130] The observed increased intestinal permeability to bovine serum albumin in malnutrition was thus postulated to occur *via* intercellular routes.[130] It was speculated that antibodies produced to such circulating foreign proteins of dietary origin may result in sensitization of the host with resultant antigen-antibody complex deposition in various organs.[130] Increased intestinal permeability to macromolecules has similarly been demonstrated in malnourished children, although it was suggested that macromolecular absorption occurred transcellularly, rather than paracellularly.[191]
- Feeding an elemental diet during CPV enteritis may therefore potentially minimize macromolecular antigen exposure, while still providing adequate nutrition, although the hypertonicity of such diets may conceivably worsen diarrhea.

1.4.9 Immuno-nutrition: "immune-enhancing" enteral nutrients:

Substances with purported immuno-modulating properties (including glutamine, arginine, omega-3 polyunsaturated fatty acids, nucleotides, branched chain amino acids and peptides) have been reviewed in the human[125, 192] and veterinary[116] medical literature.

Arginine, an essential amino acid in the dog, enhances cell-mediated immunity and protein and collagen synthesis during wound healing, and may be required in supra-physiological amounts during stress.[193] Omega-3 fatty acids (obtained from fish oils) have immune-modulating and anti-inflammatory effects.[125] Enteral diets supplemented with eicosapentaenoic acid from fish oil rapidly promote a shift toward the formation of less inflammatory eicosanoids by stimulated macrophages, without impairing

macrophage bactericidal function.[194] Nucleotides may play a role in the proliferation of intestinal crypt epithelium and lymphocytes, and in cellular DNA and RNA synthesis.[125] Branch-chain amino acids may augment protein synthesis, while peptides may enhance enteral protein absorption.[125]

Of all the "immuno-nutrients", the amino acid glutamine has probably received the widest scientific attention. Intestinal mucosal weight, DNA content and villus heights decrease significantly in the absence of glutamine.[58] Glutamine also serves as an important substrate for immuno-competent cells of the GALT, which use glutamine as a precursor of purine and pyrimidine synthesis.[55] Glutamine may thus limit or speed the repair of intestinal mucosal injuries[58] and potentially prevent gut-barrier dysfunction.[55] Glutamine is a preferred energy substrate for enterocytes and other rapidly dividing cells, plays a role in immune function preservation, and is considered a conditionally essential amino acid during stress.[55] An additional nitrogen-sparing effect has been shown when glutamine is supplemented during hypermetabolic states.[55] In addition, glutamine is an essential part of glutathione, an important free radical scavenger.[55] A multitude of studies have examined the effects of dietary glutamine supplementation on intestinal structure and function, and the systemic consequences of such effects, in a wide variety of pathologic states. For the purpose of this review, suffice to say that widely contradictory results have been obtained regarding the effects and usefulness of glutamine supplementation in critical illness.[195-201] Beneficial effects of glutamine supplementation have included the following: significantly increased intestinal mucosal cellularity in 5-fluorouracil chemotherapeutic toxicity in rats[201]; a significant reduction in bacterial translocation and septic morbidity, coupled with reduced plasma soluble TNF receptors, in humans with severe multiple trauma[200]; reduced gut permeability with significantly decreased bacterial translocation and infectious complications in acute pancreatitis in rats[199]; and significantly reduced total hospital costs in critically ill humans (by 30%).[197] In contrast, other studies have documented the following effects of glutamine supplementation: no preservation of intestinal function as measured by small intestinal cellular proliferation, villus tip length or villus surface area, and intestinal permeability (urinary lactulose/mannitol ratios) in methotrexate-induced enterotoxicosis in cats[198]; no

beneficial effects on GIT mucosal healing in rota- or coronaviral diarrhea in calves[195]; no significant effects on intestinal morphology or bacterial translocation following endotoxin challenge in rats[196]; and no effect on mortality in critically ill humans.[197]

A meta-analysis of 12 randomized controlled trials (1482 patients) compared standard EN with those receiving immune-enhancing enteral diets (containing arginine, omega-3 fatty acids and nucleotides, with or without glutamine or branch-chain amino acids) in critically ill humans with sepsis, trauma or major surgery.[202] Immunonutrition showed no effect on mortality, but was associated with significant reductions in infectious morbidity, ventilator days, and length of hospital stay.[202] There was no evidence of any detrimental effect of immunonutrition, and the authors concluded that any critically ill patient suitable for enteral feeding might potentially benefit from immune-enhancing enteral feeds.[202] A later prospective, randomized trial compared early enteral feeding with an immune-enhancing formula (supplemented with arginine, nucleotides and omega-3 fatty acids from fish oil), with an enteral feed without these nutrients in 176 septic human ICU patients.[203] The immune-enhancing diet in this study was associated with significant reductions in mortality, incidence of bacteremia and infection rate.[203] Additionally, a prospective, double-blinded randomized study in 29 human ICU trauma patients found significant reductions in the occurrence of SIRS, significantly reduced MODS scores, and lower acute-phase parameters (C-reactive protein and fibrinogen) with the feeding of an immune-enhancing diet (supplemented with arginine, nucleotides and omega-3 fatty acids from fish oil), as compared with a control enteral diet.[204]

1.4.10 Monitoring the efficacy of nutritional support:

Monitoring the effectiveness of nutritional support is difficult, particularly in short-term studies.[154] Biologic indicators of nutritional status, such as the concentrations of total serum proteins, serum albumin, acute-phase proteins, and total leukocyte counts are influenced by multiple non-nutritional factors.[162]

Hypoalbuminemia in critical illness or sepsis may be the result of decreased hepatic synthesis (related to decreased amino acid intake or hepatopathy), increased losses through protein-losing enteropathy or nephropathy, exudation into the perivascular space, or the dilutional effects of fluid resuscitation and water retention.[108, 112, 125, 205] The long serum half-life of albumin, namely 8.2 days in dogs,[206] together with the increased energy expenditure and protein catabolism in critical illness and sepsis, makes this protein a relatively insensitive measurement of acute changes in nutrient intake.[108] Thus a period in excess of the 1 to 7 days of nutritional support provided in most ICU dogs would be required for appreciable albumin changes to occur as a consequence of increased synthesis.[162]

A definitive test of nutritional status is not available.[205] General clinical assessment in hospitalized humans (including physical examination, weight loss, anorexia, vomiting and diarrhea) has been found to be a valid and reproducible technique for evaluating nutritional status (protein-energy malnutrition).[205] Classification of patients as either normal, mildly or severely malnourished by these criteria showed significant correlation with more objective measurements of nutritional status, including the percentages of ideal body weight, ideal lean body weight and body fat, the creatinine-height index, serum albumin and transferrin concentrations, delayed cutaneous hypersensitivity reactions, and total-body nitrogen and potassium measurements.[205] Such clinical assessment also correlated with clinical morbidity in these patients, as assessed by the incidence of infection and total hospitalization time.[205] Clinical assessment of nutritional status in hospitalized humans, specifically weight loss greater than 10%, is as reliable an indicator of malnutrition as more complex tests of nutritional status.[125]

Considering the catabolic effects of pro-inflammatory cytokines and neuro-endocrine mediators in sepsis and critical illness, nutritional support in such patients may at most be expected to maintain lean body mass or attenuate its rate of decline, rather than effecting actual weight gain.[108] The primary objective of nutritional support in most critically ill patients should thus be weight maintenance, rather than weight gain.[160] The daily measurement of body weight has been suggested to be the most practical and readily

available method of assessing the therapeutic effect of halting the loss of body weight.[113, 162]

1.4.11 Experimental diet chosen for CPV trial:

Pedigree® Canine Concentration Instant Diet[a] (Appendix 1) is a complex diet formulated specifically for debilitated and stressed dogs.[193] Its main indications are anorexia, malnutrition, metabolic stress, and inability to eat. The diet is designed specifically for enteral tube feeding, being formulated as a powder which when reconstituted forms a homogenous liquid suspension.[193] The diet contains 41% intact proteins, 18% fat, and 3% crude fiber on a dry matter basis.[193] It contains a particularly rich source of glutamine, namely milk proteins.[193] It is also a rich source of arginine, containing levels more than adequate to support growth in the dog.[193]

1.5 INTESTINAL FUNCTION TESTS

1.5.1 Intestinal permeability tests:

1.5.1.1 Introduction

Intestinal permeability and epithelial functional integrity may be non-invasively assessed by dual-sugar (disaccharide/monosaccharide) differential intestinal permeability tests. The underlying principle involves the passive, non-carrier-mediated transmucosal diffusion of orally administered sugars of differing sizes, with their subsequent excretion and quantification in urine.[207-211] Intestinal permeability to a sugar is an inverse function of its cross-sectional diameter.[212] The permeation of the monosaccharide rhamnose (molecular diameter 8.3 angstrom; molecular weight 164 Daltons) in healthy dogs is 7 to 13-fold greater than that of the disaccharide lactulose (9.5 Å; 342 D).[213, 214] Since the

[a] Pedigree® Canine Concentration Instant Diet, Waltham, Melton Mowbray, UK

sugars traverse the epithelium by different pathways and in differing amounts, their urinary recoveries provide information of intestinal structure and function.

Diverse intestinal pathologic states are accompanied by increased disaccharide permeation, decreased monosaccharide permeation, and resultant elevation of the urinary disaccharide/monosaccharide excretion ratios. Relevant examples include:

- Acute viral gastroenteritis[215-218] and severe necrotizing enterocolitis in children.[219]
- Combination cancer chemotherapy in humans.[198, 220, 221]
- Human critical illnesses associated with gut barrier dysfunction, bacteremia, endotoxemia, SIRS and MODS: these include multiple trauma and shock,[222-225] severe burn injury,[226, 227] and bacterial sepsis.[228]
- Small intestinal bacterial overgrowth (anaerobic or aerobic) in dogs.[229, 230]

Altered intestinal permeability test results are thus clearly *not specific* for the type of intestinal pathology present.[209]

The precise transepithelial permeation pathways of probes utilized in intestinal permeability tests have not been definitively established.[208, 209, 231, 232] According to the *classical* and most widely cited *hypothesis*, smaller monosaccharides (e.g. rhamnose) permeate transcellularly via small aqueous pores of high incidence in enterocyte cell-membranes.[207, 209, 211] In contrast, the larger disaccharides (e.g. lactulose) are hypothesized to permeate paracellularly via larger aqueous channels of low incidence, located in intercellular tight junctions.[207, 209, 211] This hypothesis correlates well with intestinal mucosal microstructure: intercellular tight junctions constitute only a small proportion (<5%) of the total intestinal epithelial surface area, which is compatible with the theory of a small population of large pores,[209] while cell membranes constitute approximately 95% of the intestinal surface area, compatible with the large population of small pores.[209] Lactulose may also permeate paracellularly through areas of epithelial disruption (i.e. necrosis, ulceration, erosion, or the extrusion zones of exfoliated cells).[207, 209, 215, 219, 233, 234] Increased lactulose permeation (and urinary excretion) may thus reflect intestinal epithelial tight junction microstructural and/or functional impairment, or

mucosal necrosis. In contrast, decreased rhamnose permeation would be consistent with villus atrophy, with reduced surface area available for rhamnose permeation.[207, 209, 233]

An *alternative hypothesis* for the transmucosal permeation pathways of permeability probes proposes that both the small and large probes traverse the epithelium paracellularly through tight junctions.[208, 231, 232] In this hypothesis, the permeation of larger probes (e.g. lactulose) is confined to the more permeable intestinal crypt tight junctions, whereas smaller probes (e.g. rhamnose) may permeate via tight junctions throughout the crypt-villus axis.[208, 231, 232] The structure of tight junctions on the villi differs from those in the intestinal crypts: ultrastructural tight junction strand counts are higher for villus tight junctions (mean 6.03) than for those in the crypts (4.45), with good correlation between such tight junction structure and function (paracellular resistance).[235] It has further been hypothesized that lactulose, due to its large diameter, may be physically restricted in its ability to move between villi to reach the crypt tight junctions; in contrast, the smaller rhamnose may more freely gain access to the spaces between villi, allowing its permeation throughout the crypt-villus axis.[208, 231, 232] Villus atrophy would thus allow greater access of the larger lactulose to the crypts, with increased lactulose permeation; concomitantly decreased rhamnose permeation would be the result of reduced surface area available for rhamnose permeation.

Another factor that may affect rhamnose permeation is "*solvent drag*". The opposite directions of blood flow in villus arterioles and venules maintain villus tip hyperosmolality, as a result of countercurrent multiplication.[236] Villus tip hyperosmolality enhances water absorption, which may induce coupled "solvent drag" of monosaccharide-sized hydrophilic sugars, while larger molecules such as lactulose remain unaffected.[232, 237] Decreased rhamnose permeation might thus additionally reflect impaired efficiency of countercurrent multiplication, as a result of either villus atrophy (with shortening of villus vasculature), intestinal ischemia, or passive congestion.[232]

By expressing the sugars' urinary recoveries as a *ratio* (*disaccharide/monosaccharide*), factors unrelated to mucosal permeability (e.g. vomiting, gastric emptying, intestinal

transit time, dilution of test solution by intestinal contents, and glomerular filtration rate) are excluded, since both markers would be expected to be equally affected.[207-209] There is general agreement that lactulose permeates through tight junctions[207, 209, 211] or areas of epithelial disruption,[207, 209, 215, 219, 233, 234] and may thus be an appropriate measure of the functional and/or physical gut barrier. In contrast, the permeation of rhamnose may be affected by mucosal factors not directly related to intestinal integrity (e.g. villus atrophy,[207, 209, 233] "solvent drag" and intestinal blood flow aberrations,[232, 237] and potentially also altered enterocyte membrane phospholipid composition[209]). Although expressing urinary sugar recoveries as a ratio excludes the influence of non-mucosal factors, individual sugar recoveries may thus provide more specific information of epithelial structure and function. For some intestinal diseases, expressing permeability test results as a ratio may increase their sensitivity in detecting intestinal abnormalities,[238] since it accentuates the contrasting effects of the increased permeation of a disaccharide, and the decreased permeation of a monosaccharide.[213] However, diverse intestinal pathologic states (predominantly those accompanied by epithelial disruption) may in fact lead to increased monosaccharide recoveries,[198, 233, 234] and expressing recovery results as a ratio may in these circumstances decrease their sensitivity in documenting intestinal abnormalities. Some of the *limitations* in the interpretation of test results by the *use of a ratio alone* have been previously noted.[232, 234, 239] Notwithstanding, the majority of human and animal studies report only urinary excretion ratios, without consideration of the individual sugars' recoveries.

It is uncertain whether increased permeability to lactulose-sized molecules (342 Daltons) may be extrapolated to imply similarly increased intestinal permeability to bacteria or endotoxin (typically $>10^6$ Daltons aggregates).[228, 240] Some studies have documented significant associations between increased intestinal permeability and the development of bacteremia, SIRS or MODS,[222, 224, 226, 227] while others have not.[198, 223, 225] One of the factors that may contribute to these differing results may be that intestinal permeability tests evaluate only one component of gut barrier function, namely the epithelium itself, while GALT function and mucosal immunity are not assessed.[231]

1.5.1.2 Urinary lactulose/rhamnose kinetics and recoveries in healthy dogs

Urinary lactulose/rhamnose excretion ratios in puppies are variable in the peri-weaning period, but remain stable from 8 weeks of age.[241] Apparent breed differences in dogs have been suggested but not conclusively proven, other than for the Irish setter, which has increased intestinal permeability.[242]

Following *intravenous* administration, the 24-hour urinary excretion of lactulose is 96%, indicating that it is minimally metabolized, whereas that of rhamnose is 72%, indicating that approximately 28% is metabolized.[243] The mean urinary recoveries of lactulose and rhamnose at 6 hours after *orogastric* administration in dogs exceed 85% of the total urinary recoveries of the sugars at 12 hours,[244] rendering 6-hour urine collection periods suitable for clinical studies.

Normal 6-hour urinary recovery of lactulose (as a percentage of *orally* administered dose) in healthy dogs has been reported as 3.7 ± 2.6% (mean ± SD)[214] and 1.3% (mean).[213] Normal 6-hour urinary rhamnose recoveries (as a percentage of *orally* administered dose) have been reported as 27.9 ± 4.4% (mean ± SD)[214] and 17% (mean).[213] Normal 6-hour urinary lactulose/rhamnose excretion ratios have been variably reported as: 0.08 ± 0.03 (mean ± SD);[245] 0.10 ± 0.01 (mean ± SEM);[246] 0.19 ± 0.07 (mean ± SD);[242] and 0.24 ± 0.02 (mean ± SEM).[241]

1.5.2 Quantification of protein-losing enteropathy:

After albumin and the immunoglobulins, proteinase inhibitors form the group of plasma proteins with third-highest concentration in plasma.[247] They have been grouped in a super family of glycoproteins known as serine proteinase inhibitors.[247] α1-Proteinase inhibitor (α_1-PI) is the prototype of this group of inhibitors,[247] with a molecular weight similar to that of albumin.[248]

The fecal concentration of α_1-PI provides a sensitive and specific quantitative measure of protein-losing enteropathy, in diseases with transmucosal loss of plasma, lymph, or intercellular fluid.[249] This stems from the fact that, unlike albumin, α_1-PI is excreted in the faeces essentially undegraded, due to its inhibitory activity on gastrointestinal luminal proteases.[249] Since the mechanism for protein-losing enteropathy in CPV enteritis is related to intestinal inflammation and erosion,[29] fecal α_1-PI concentrations would be expected to be increased in CPV infection.

There is significant correlation between random fecal samples and 24-hour pooled fecal sample collections for fecal α_1-PI concentrations in dogs.[249] Due to the species-specificity of α_1-PI immunoassays, a canine α_1-PI enzyme-linked immunosorbent assay (ELISA) has recently been developed for the protein's quantification in fecal extracts.[249] This ELISA has high precision, accuracy, and reproducibility.[249]

1.6 STUDY MOTIVATION: SUMMARY

Canine parvovirus (CPV) has a worldwide distribution,[2] and reportedly affects more than a million dogs per annum in the United States alone.[12] The published mortality rate remains high, generally ranging from 11-36%,[25, 27, 39, 42] although intensive therapy has achieved survival rates of up to 85-96% in individual institutions.[12, 29] Treatment is supportive, consisting of intravenous crystalloids and colloids, broad-spectrum bactericidal antibiotics, antemetic and prokinetic agents, analgesics, eradication of intestinal parasites, and nursing care.[29, 42] Novel adjunctive therapies have been investigated, but results have been disappointing or variable.[12, 39, 71, 104] There is a distinct need for therapies that decrease disease severity and hospitalization time, improve survival, and reduce treatment cost.[12, 83]

Despite the lack of prospective data, conventional wisdom has dictated that "gut rest" with initial *nil per os* remains the nutritional treatment of choice for CPV enteritis.[8, 26, 29,

[42] The recommended duration of such a period of starvation has ranged from 24 to 48[8, 26, 29] or 72 hours[42] after vomiting has ceased.

Small intestinal viral proliferation causes extensive epithelial necrosis with lamina propria collapse, villus blunting and atrophy.[2, 19, 20, 29] Lymphoid necrosis and atrophy occurs in gut-associated and systemic lymphoid tissues.[20] Bacteremia,[72, 79] endotoxemia[12, 72, 83] and elevated serum tumor necrosis factor concentrations[83] are frequent events. Multiple organ dysfunction syndrome[55] may occur in severe disease, as evidenced by the development of the acute respiratory distress syndrome in 69% of fatal cases.[79] The body of evidence suggests that disruption of gut barrier function in CPV enteritis underlies bacterial and endotoxin translocation, resultant bacteremia and endotoxemia, and the development of SIRS and MODS.

The last fifteen years have witnessed extensive interest in the role of intestinal barrier dysfunction on the development of septicemia, endotoxemia, septic foci, SIRS and MODS in critical illness.[55-57] Critical illnesses associated with gut barrier dysfunction in humans and animals include severe acute pancreatitis,[120, 139, 140] inflammatory and non-inflammatory bowel disease,[77, 95, 101] severe burn injury,[74, 115, 145, 146] multisystem trauma,[109, 147-149] and high-risk surgery.[150]

The nutritional management of these disorders had traditionally consisted of an initial period of starvation, ranging from 3-7 days.[101, 120, 144, 148, 150] However, the most important stimulus for intestinal mucosal growth, repair, integrity and function is the presence of nutrients within the gut lumen.[58, 62, 67, 114-116] Alterations in small bowel structure and function occur when enteral nutrients are excluded, such as during starvation or total parenteral nutrition (TPN).[58] The absence of luminal nutrients in animals and humans leads to significant small intestinal mucosal atrophy and suppressed crypt cell proliferation;[67, 109, 117, 121, 122] increased intestinal permeability to intraluminal bacteria and toxins;[67, 118] significant reductions in gut-associated lymphoid tissue cell mass, CD4/CD8 lymphocyte ratios and IgA production;[121, 123] and enhanced pro-inflammatory cytokine generation and acute-phase responses.[109]

Acute critical illness, sepsis and SIRS are accompanied by an increased metabolic rate and energy expenditure, protein and fat catabolism, a negative nitrogen balance, and loss of lean body mass.[109-111] Protein-losing enteropathy, anorexia and vomiting in CPV enteritis would further contribute to malnutrition. Malnourished animals challenged with pro-inflammatory stimuli show significantly enhanced bacterial translocation and mortality from sepsis, as compared with normally nourished controls.[94, 137] Malnutrition in human critical illness is additionally associated with significantly increased intestinal permeability and inflammation, intestinal pro-inflammatory cytokine generation, increased systemic inflammatory mediator concentrations, and a heightened acute phase response.[57]

Early enteral nutrition (EN) has been shown to be superior to either starvation or TPN in critical illnesses associated with gut barrier dysfunction. Documented benefits of early EN include improved intestinal mucosal permeability,[101, 114] weight[146] and motility;[140] reduced incidence of bacteremia,[145, 146] endotoxemia[120] and septic morbidity;[67, 109, 120, 139, 140, 147-150] attenuation of the acute-phase response[120, 149] and reduced multiple organ failures;[120, 222] improved immunological status;[140, 150] reduced hypermetabolism and catabolism;[101, 109, 144, 146, 148] improved clinical outcome,[67, 120] and decreased mortality.[115, 120, 140, 149] Significantly higher survival was also recently documented in dogs and cats receiving EN supplemental to partial parenteral nutrition, as compared to partial parenteral nutrition alone.[250] Evidence underscoring the benefits of early EN (versus either starvation or TPN) in human critical illness has led to the following recommendations: (1) EN should be instituted as early as possible during the course of illness, and (2) EN should be used in preference to TPN whenever achievable.[125, 151]

The efficacy of the currently advised strategy of initial starvation in CPV enteritis has never been scientifically investigated. A prospective, randomized, controlled clinical trial was conducted to evaluate the effect of early EN on intestinal permeability, protein-losing enteropathy, and clinical outcome in naturally occurring severe CPV enteritis.

CHAPTER II

2. OBJECTIVES

To determine whether early enteral nutrition (as compared to initial starvation) in severe parvoviral enteritis in puppies positively influences:

1. Intestinal permeability (as assessed by urinary lactulose and rhamnose recoveries),

2. Protein-losing enteropathy (fecal α_1-proteinase inhibitor concentrations), and

3. Disease outcome, as measured by clinical scoring (habitus, appetite, vomiting and diarrhea), and mortality rate.

CHAPTER III

3. RESEARCH QUESTIONS

The following questions were addressed:

1. Does early enteral nutrition restore (i.e. reverse the abnormal) intestinal permeability and protein-losing enteropathy in canine parvoviral enteritis more rapidly than initial starvation?

2. Does early enteral nutrition in parvoviral enteritis improve recovery and outcome, as compared to initial starvation?

CHAPTER IV

4. BENEFITS

1. The effect of enteral nutrition on intestinal permeability, protein-losing enteropathy and outcome in parvoviral enteritis was studied, and the study's results may alter the disease's current treatment regimen.

2. Canine parvoviral infection is an economically important disease in Southern Africa and globally. More effective therapy may have an economic impact by reducing hospitalization time.

3. The results of this study may be of value in other animal and human diseases where gut barrier dysfunction with its sequelae (bacteremia, endotoxemia, SIRS and MODS) occurs.

4. The usefulness of gastrointestinal function tests in canine critical illness was assessed.

5. The research conducted served as partial fulfillment of the investigator's M.Med.Vet.(Med.) degree.

CHAPTER V

5. MATERIALS AND METHODS

5.1 STUDY DESIGN

Client-owned dogs presented to the Onderstepoort Veterinary Academic Hospital (OVAH) with clinical signs indicative of CPV enteritis were considered for inclusion. Dogs between 8 and 24 weeks of age, of any breed or gender, and weighing between 3 and 20 kg were eligible for inclusion. Only dogs with clinical signs of sufficient severity to warrant hospitalization and intensive therapy, as assessed by the admitting veterinarian, were included. This veterinarian was blinded as to which treatment group the dog would be assigned to. The diagnosis of CPV infection was confirmed by fecal electron microscopy.

Dogs were required to be negative for concurrent coronavirus infection on fecal electron microscopy, for coccidial oocysts on fecal hyperosmolar sugar flotation, and for hematogenous parasites (*Babesia, Ehrlichia* or *Hepatozoon* spp.) on peripheral stained blood smear. Giardiasis was excluded in all dogs by the absence of trophozoites on a fecal "wet mounted" slide at admission, and 2 consecutive negative zinc sulphate flotation tests on the first 2 days of hospitalization.

The Research and Ethics Committees of the University of Pretoria approved the study (OVARU project number 36.5.398), and written consent was obtained from all dogs' owners.

5.2 STANDARD TREATMENTS

All dogs were hospitalized for a minimum of 6 days, and were housed separately in heated cages in the OVAH infectious diseases isolation unit. Following admission (day

1), all dogs were rehydrated over 6 hours, using Ringer-Lactate[b] with added dextrose[c] (final concentration of 2.5%) and potassium chloride[d] (20 mEq/l). Further maintenance fluid requirements were met with Electrolyte no.2 with 5% glucose[e], and potassium chloride added according to deficits.[29] Volumes of fluids administered were individualized for each patient, based on clinical assessment.[251]

Antimicrobial therapy consisted of amoxycillin (15 mg/kg, q8h, IV[f] until vomiting had ceased for 24h; followed by 20 mg/kg, q12h, PO[g], for 10 days), and gentamicin[h] (6.6 mg/kg, q24h, IV, for 5 days) initiated once euhydration had been achieved. Metoclopramide[i] (2 mg/kg/24hr, continuous rate infusion, IV) was administered as antemetic until vomiting had ceased for 24 hours. All dogs received anthelmintic therapy with fenbendazole[j] (50mg/kg, q24h, PO, for 5 days).

Plasma transfusions (20 ml/kg) were administered if serum albumin decreased below 15 g/dL and the dog deteriorated clinically. Hydroxyethyl starch[k] boluses (5 to 20 ml/kg, IV) were administered if adequate crystalloid resuscitation failed to correct shock.

5.3 NUTRITIONAL GROUPS

Dogs were randomly assigned by way of sealed envelopes to either of 2 nutritional groups:

[b] Intramed Ringer-Lactate Solution, Fresenius Kabi, Port Elizabeth, SA
[c] Intramed Dextrose 50%, Fresenius Kabi, Port Elizabeth, SA
[d] Sabax Potassium Chloride, Adcock Ingram Critical Care, Johannesburg, South Africa
[e] Intramed Electrolyte No. 2, Fresenius Kabi, Port Elizabeth, SA
[f] Amoxil® injectable, SmithKline Beecham, Bergvlei, SA
[g] Clamoxyl® palatable tablets, Pfizer Animal Health, Sandton, SA
[h] Genta® 20 PHENIX Aqueous injectable solution, Logos Agvet, Halfway House, SA
[i] Clopamon®, Intramed, Randburg, SA
[j] Panacur® BS, Hoechst Roussel Vet Specialties, Halfway House, SA
[k] Haes-Steril® 10%, Fresenius Kabi, Midrand, SA

5.3.1 Initial NPO group:

Fifteen dogs were starved (*nil per os*; initial NPO group) until vomiting had ceased for 12 hours, after which small amounts of a low fat diet[l] were offered 6 times per day. Dogs that refused to eat this diet voluntarily after a period of 12 hours were then force-fed 6 times per day. Water was provided *ad lib* throughout.

5.3.2 EEN group:

Fifteen dogs received early enteral nutrition (EEN group), commencing 12 hours following admission. A nasoesophageal feeding tube was placed in the distal third of the esophagus,[113] and a lateral cervico-thoracic survey radiograph confirmed correct tube placement. Tube feeding was performed by continuous rate infusion through an open gravity-drained system, with food being reconstituted every 12 hours. A commercial canine complex diet[m] (Appendix 1) was fed, formulated as a suspension for tube feeding in critical illness.[193] The diet contains 41% intact proteins, 18% fat, and 3% crude fiber on a dry matter basis.[193] The quantity of food to be administered was calculated by multiplying the manufacturer's recommended quantity[193] (Appendix 2) by an illness factor of 1.5.[154, 160] One third of this amount was fed on day 1, two-thirds on day 2, and the full volume from day 3 onwards.[119] The suspension was diluted to approximate isosmolality (by reconstituting 47g of the powder with 200ml of water, instead of the recommended 100ml). Once vomiting had ceased for 24 hours, the feeding tube was removed. Small amounts of the same low fat diet fed to the NPO group were then offered 6 times per day. Dogs that refused to eat this diet voluntarily after 12 hours were force-fed 6 times per day. Water was provided *ad lib* throughout.

Enteral feeding was interrupted for a period of 2 hours preceding, and 6 hours during intestinal permeability testing on days 2, 4 and 6 (see *Intestinal Permeability Testing*, below).

[l] Pedigree® Canine Low Fat Diet, Waltham, Melton Mowbray, UK
[m] Pedigree® Canine Concentration Instant Diet, Waltham, Melton Mowbray, UK

5.4 CLINICAL SCORING AND OBSERVATIONS

A daily scoring system was applied whereby clinical variables (i.e. habitus and appetite, and the severity of vomiting and diarrhea) were awarded numerical values, to semi-quantify clinical response to therapy:

Habitus score was defined as: 3 (collapsed or moribund), 2 (severely depressed), 1 (mildly to moderately depressed), or 0 (normal).

Appetite score: 2 (no interest in food), 1 (voluntarily eats small amounts), or 0 (normal). Appetite scores in both nutritional groups were based on the dogs' interest in eating a small amount of the low fat diet offered (note: also on NPO days in the NPO group).

Vomiting score: 3 (severe; ≥ 6 times per 12h), 2 (moderate; 2-5 times per 12h), 1 (mild; once per 12h), or 0 (vomiting absent).

Fecal score: 3 (watery, bloody diarrhea), 2 (watery diarrhea, not bloody), 1 (soft or pasty feces), or 0 (well-formed).

An overall *clinical score* was calculated as the sum of the above 4 scores. The primary investigator (AJM) awarded all scores.

Blood glucose[n] and serum potassium[o] concentrations were determined at admission, and thereafter as clinically indicated. Micro-hematocrit and body weight were determined daily, and serum albumin[p] concentrations on days 1, 2, 4 and 6.

5.5 INTESTINAL PERMEABILITY TESTING

Intestinal permeability was assessed using a differential saccharide (lactulose and L-rhamnose) intestinal permeation and 6-hour urinary excretion test. The first test dose was administered as soon as the dogs were rehydrated (approximately 6 hours after admission), while dosing on days 2, 4 and 6 was performed in the mornings. The test solution was formulated immediately prior to each dosing, by dissolving lactulose[q] and

[n] Reflolux® S Haemo-Glukotest®, Boehringer, Mannheim, Germany
[o] Reflotron® Reflovet®, Boehringer, Mannheim, Germany
[p] Technicon RA®-1000™, Miles Inc., Tarrytown, NY, USA
[q] Lactulose, Sigma, Atlasville, SA

rhamnose[r] in tap water to produce a solution with concentrations of 33.3 mg/ml rhamnose and 33.3 mg/ml lactulose, and an osmolality of approximately 305 mOsm/l (isosmolar). This solution was dosed at a rate of 3 ml/kg body weight. All food was withheld for 2 hours preceding and 6 hours during permeability testing. Immediately prior to test solution dosing, the urinary bladder was emptied by manual expression, followed by catheterization or cystocentesis. The test solution was then dosed by syringe into the caudal oropharynx. Urine was intermittently (q1.5h) collected by manual bladder expression, and the bladder was finally entirely emptied by manual expression, followed by catheterization or cystocentesis, 6 hours following oral dosing. Any vomiting or urinating during the 6 hours was recorded. All urine produced over the 6 hours was pooled, and the total volume recorded. A 10 ml aliquot was stored at -80º C, following the addition of sodium azide[s] (10µl of a 10% solution) as preservative. Sugars remain stable in stored urine for several months.[207, 209] Urine samples were batched, and transported on dry ice to the laboratory where separation and quantification of lactulose and rhamnose was performed by high-pressure liquid chromatography, as described.[239] The laboratory was blinded as to which treatment group the samples originated from. The urinary recoveries of lactulose (%L) and rhamnose (%R) were expressed as a percentage of orally administered dose, and the urinary lactulose-to-rhamnose recovery ratio (L/R_{rec}) calculated from the %L and %R values. For dogs that vomited or urinated during the urine collection period, %L and %R, and therefore also L/R_{rec}, could not be calculated. For these samples, only isolated urinary lactulose-to-rhamnose ratios (L/R_{iso}) were expressed.

5.6 FECAL PROTEIN LOSS QUANTIFICATION

Fecal samples (approximately 2 grams) were collected on days 1, 2, 4 and 6, weighed, and frozen at -20º C. There is significant correlation between such random fecal samples and 24-hour pooled fecal sample collections for fecal α_1-PI concentrations in dogs.[249] In order to more accurately compare fecal α1-PI concentrations between samples of

[r] L-Rhamnose, Sigma, Atlasville, SA
[s] Sodium azide, Sigma, Atlasville, SA

differing water contents, α1-PI concentrations were expressed on a dry weight basis. For this purpose specimens were lyophilized[t] (freeze-dried) prior to transportation on dry ice to the laboratory. Fecal lyophilization does not affect α_1-PI concentrations.[252] The laboratory was blinded as to which treatment group the samples originated from. Dry fecal samples were homogenized (using a mortar), and a representative amount reconstituted with water. Quantification of α1-PI by ELISA was performed, according to described methodology.[249] Fecal α1-PI concentrations were finally expressed on a dry matter basis.

Fecal α1-PI concentrations in healthy puppies have not been published. Fecal samples from 10 healthy puppies presented for vaccination at the OVAH were thus analyzed in the same manner as described above for fecal α1-PI concentrations (dry matter basis). These puppies were healthy (as determined by clinical examination), of the same age and body weight ranges as the CPV-infected puppies, and their fecal analyses were negative for coccidial oocysts (on hyperosmolar sugar flotation) and giardiasis (absence of trophozoites on a fecal "wet mounted" slide, and a negative zinc sulphate flotation test).

5.7 STATISTICAL ANALYSES

Data were analyzed with the assistance of a biostatistician[u] using standard statistical software[v], and graphs were plotted using a statistical software package[w]. Categorical variables (habitus, appetite, vomiting and fecal scores) were compared between the 2 treatment groups (NPO *vs*. EEN) within days using Fisher's exact test. Comparability between the 2 groups at admission for all continuous variables was tested with the Student's t-test for normally distributed data or the Mann-Whitney rank sum test for non-normally distributed data. Analysis of variance (ANOVA) was used to describe changes from admission values at each time point within each of the 2 groups for the following continuous variables: clinical score, body weight, hematocrit, serum albumin, %L, %R,

[t] Modulyo lyophilizer, BOC Ltd, Crawley, UK
[u] Dr PJ Becker, Medical Research Council, Pretoria, SA
[v] Stata® Release 6, Stata Corporation, College Station, TX, USA
[w] SigmaPlot for Windows v. 4.00, SPSS Inc., Chicago, IL, USA

fecal α1-PI, as well as for the natural log (log_e; ln) values of L/R_{iso} and L/R_{rec}. The L/R_{iso} and L/R_{rec} data were log_e-transformed due to non-normally distributed datasets. Responses over time for the continuous variables were compared between the 2 treatment groups using generalized estimation equations (GEE). GEE modeling describes repeated measures (i.e. time series data), and is specifically appropriate for unbalanced designs and incomplete data sets (i.e. missing values in the time series).[253, 254] Such incomplete data sets were present for %L, %R, and L/R_{rec}, due to vomiting (common) or urinating (rare) during intestinal permeability testing. Mortality between groups was compared using Fisher's exact test. Significance for all analyses was defined as $p < 0.05$.

CHAPTER VI

6. RESULTS

6.1 ANIMALS

Thirty dogs were included in the study. Both the NPO and EEN groups consisted of 15 dogs each. There were no significant differences between groups for age (NPO: range 8-24 weeks, median 17 weeks; EEN: range 9-24 weeks, median 16 weeks) or gender (NPO: 7M, 8F; EEN: 9M, 6F) (Appendix 3).

6.2 CLINICAL VARIABLES

At admission, there were no significant differences between groups for habitus (Figure 1), appetite (Figure 2), vomiting (Figure 3), fecal scores (Figure 4), or the composite clinical scores (Figure 5). Admission scores were: vomiting (NPO and EEN: range 0-3, median 2), appetite (NPO and EEN: all score 2), feces (NPO and EEN: range 2-3, median 3), habitus (NPO: range 1-2, median 1; EEN: range 1-3, median 2), and clinical score (NPO: range 5-10, median 7; EEN: range 6-11, median 9) (Appendix 4 & 5).

Appetite differed significantly between the groups on day 2 ($p = 0.02$), and improved faster in the EEN group as compared to the NPO group. No significant differences were detected between groups in the other categorical variables. However, the median time taken to the normalization of habitus and appetite, and the resolution of vomiting and diarrhea, was consistently 1 day shorter for the EEN group. This was reflected in a significant improvement in the composite clinical score from admission values by day 2 in EEN ($p < 0.0001$), versus day 3 in NPO ($p = 0.0005$).

There was no significant difference in body weight between groups at admission. Body weight was significantly increased ($p < 0.003$) from admission on all days in the EEN

group, with a mean weight increase of 8.4% on day 6, while no significant changes in body weight occurred in NPO (3.7% mean weight increase on day 6) (Figure 6; Appendix 6). Groups did not behave significantly differently over time (GEE) for clinical score or body weight.

6.3 ADDITIONAL TREATMENTS

Two NPO dogs and 2 EEN dogs received hydroxyethyl starch treatment, and 1 EEN dog received a plasma transfusion. In the EEN group, syringe feeding was performed in 2 dogs; in 1 (a dachshund) the tube could not be passed through the nasal cavity, and another removed its tube on day 3.

6.4 SERUM ALBUMIN

There were no significant differences between groups at admission in serum albumin. Serum albumin concentrations decreased significantly from admission in both groups on days 2 (NPO: $p = 0.001$; EEN: $p = 0.002$) and 4 (NPO: $p = 0.01$; EEN: $p = 0.009$), followed by significant increases on day 6 from day 4 values (NPO: $p = 0.0002$; EEN: $p = 0.01$) (Figure 7; Appendix 7). The 1 EEN dog that received a plasma transfusion on day 3 was excluded from albumin analyses thereafter. Groups did not behave significantly differently over time (GEE) for serum albumin concentration.

6.5 INTESTINAL PERMEABILITY

Several dogs vomited or urinated during the 6-hour intestinal permeability-testing period. For such dogs' urine samples, the urinary recoveries of lactulose (%L) and rhamnose (%R) as a percentage of orally administered dose, and therefore also the L/R_{rec} ratio, could not be calculated. Only isolated urinary lactulose-to-rhamnose ratios (L/R_{iso}) were expressed for these samples (Appendices 8-10). During a further 6 permeability tests, no urine whatsoever was collected, due to persistent urinating; %L, %R, L/R_{rec} and L/R_{iso}

thus remain unknown for these tests (Appendices 8-10). Fecal contamination of 2 urine samples rendered these unsuitable for analysis (Appendices 8-10).

There were no significant differences between groups at admission for %L, %R, $\log_e(L/R_{iso})$ or $\log_e(L/R_{rec})$.

Urinary recovery of lactulose as a percentage of orally administered dose (%L) was elevated above the laboratory reference range (1.5-5.8%) throughout the study period for both groups (Figure 8; Appendix 8). Lactulose recovery behaved significantly differently between treatment groups ($p = 0.035$; GEE modeling), characterized by a progressive decrease in %L in the EEN group *vs.* a progressive increase in the NPO group over time. Changes from admission values for %L were not significant for either group, although %L was significantly increased on day 6 from day 2 values in NPO ($p = 0.04$).

Urinary recovery of rhamnose as a percentage of orally administered dose (%R) was reduced below the reference range (17.3-42.6%) throughout the study period in both groups (Figure 9; Appendix 8). Rhamnose recovery decreased progressively over time in both groups, although changes from admission values were not significant for either group. Rhamnose recoveries were significantly decreased from day 2 values on days 4 ($p = 0.01$) and 6 ($p = 0.005$) in EEN, and on day 6 ($p = 0.05$) in NPO.

Note: The L/R_{iso} and L/R_{rec} data (Appendix 9) were $\log_e$-transformed due to non-normally distributed datasets.

$Log_e(L/R_{rec})$ was significantly increased from admission values in both groups on days 4 (NPO: $p = 0.004$; EEN: $p = 0.04$) and 6 (NPO: $p < 0.001$; EEN: $p = 0.005$) (Figure 10; Appendix 10). $Log_e(L/R_{iso})$ was similarly significantly increased from admission in both groups on days 4 (NPO: $p = 0.003$; EEN: $p = 0.0001$) and 6 (NPO: $p = 0.0001$; EEN: $p = 0.0001$) (Figure 11; Appendix 10).

Groups did not behave significantly differently over time (GEE) for %R, $\log_e(L/R_{iso})$ or $\log_e(L/R_{rec})$.

6.6 FECAL PROTEIN LOSS

The normal puppies' fecal α1-PI concentrations (dry matter basis) were 44.7 ± 25.1 μg/g (mean ± SD), with a normal range of 22.8-80.2 μg/g (95% confidence interval) (Appendix 11).

For CPV puppies, there were no significant differences in fecal α1-PI concentrations between groups at admission. Fecal α1-PI concentrations were elevated above the normal range for the majority of the study in both groups (Figure 12; Appendix 12). Significant decreases in fecal α1-PI concentrations from admission were observed on days 2 ($p = 0.03$), 4 ($p = 0.006$) and 6 ($p = 0.0004$) in NPO, and on day 6 ($p = 0.006$) in EEN. Groups did not behave significantly differently over time (GEE) for fecal α1-PI concentrations.

6.7 OUTCOME

Thirteen of 15 dogs (87%) in the NPO group and all 15 of the EEN dogs survived; this difference was not statistically significant ($p = 0.48$). Both fatalities in the NPO group occurred on day 2.

6.8 FIGURES

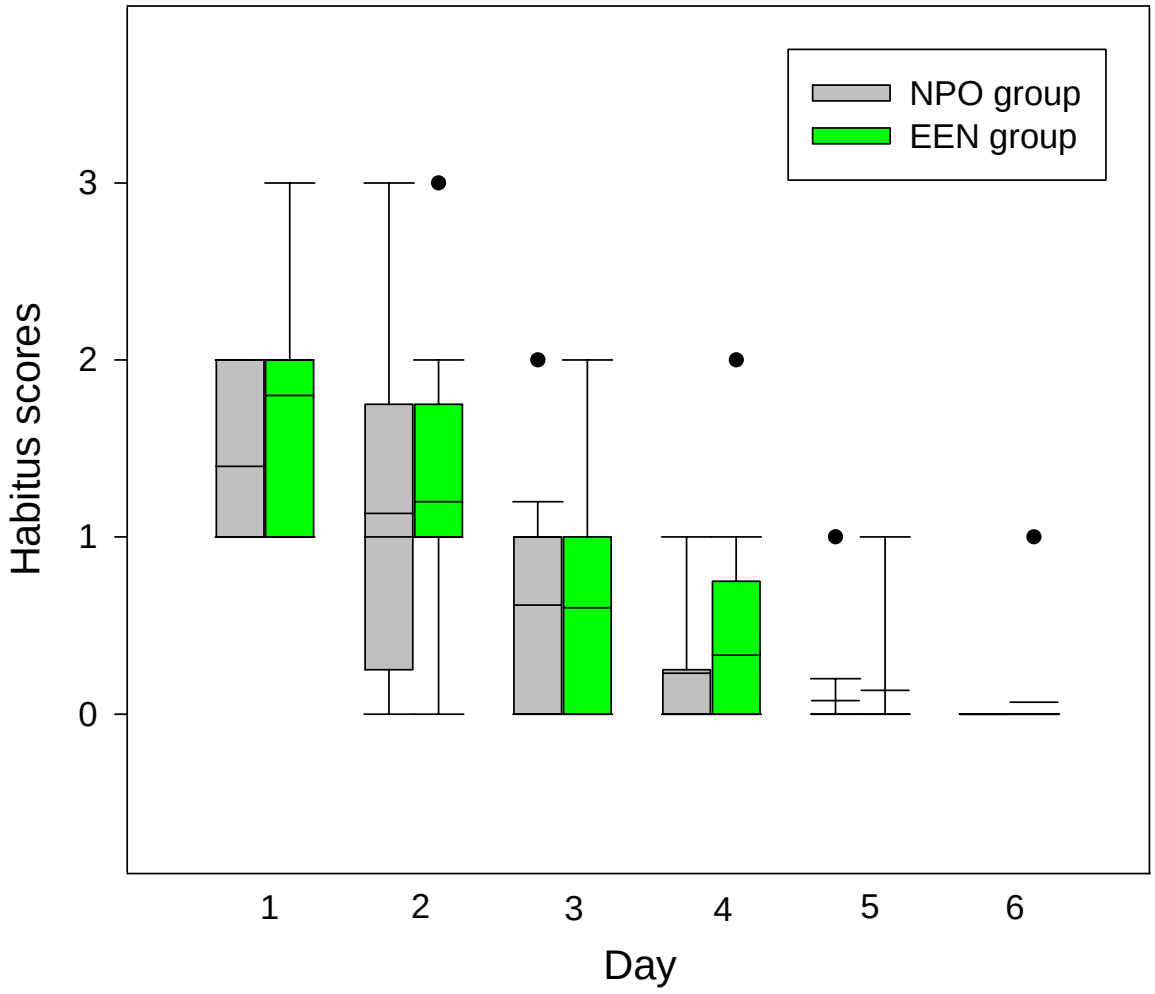

Figure 1: Habitus scores in 30 dogs with parvoviral enteritis. Data are shown as mean (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). Black dots represent outliers. NPO = Nil per os; EEN = Early enteral nutrition.
Score 0 = normal
Score 1 = mildly to moderately depressed
Score 2 = severely depressed
Score 3 = collapsed or moribund.

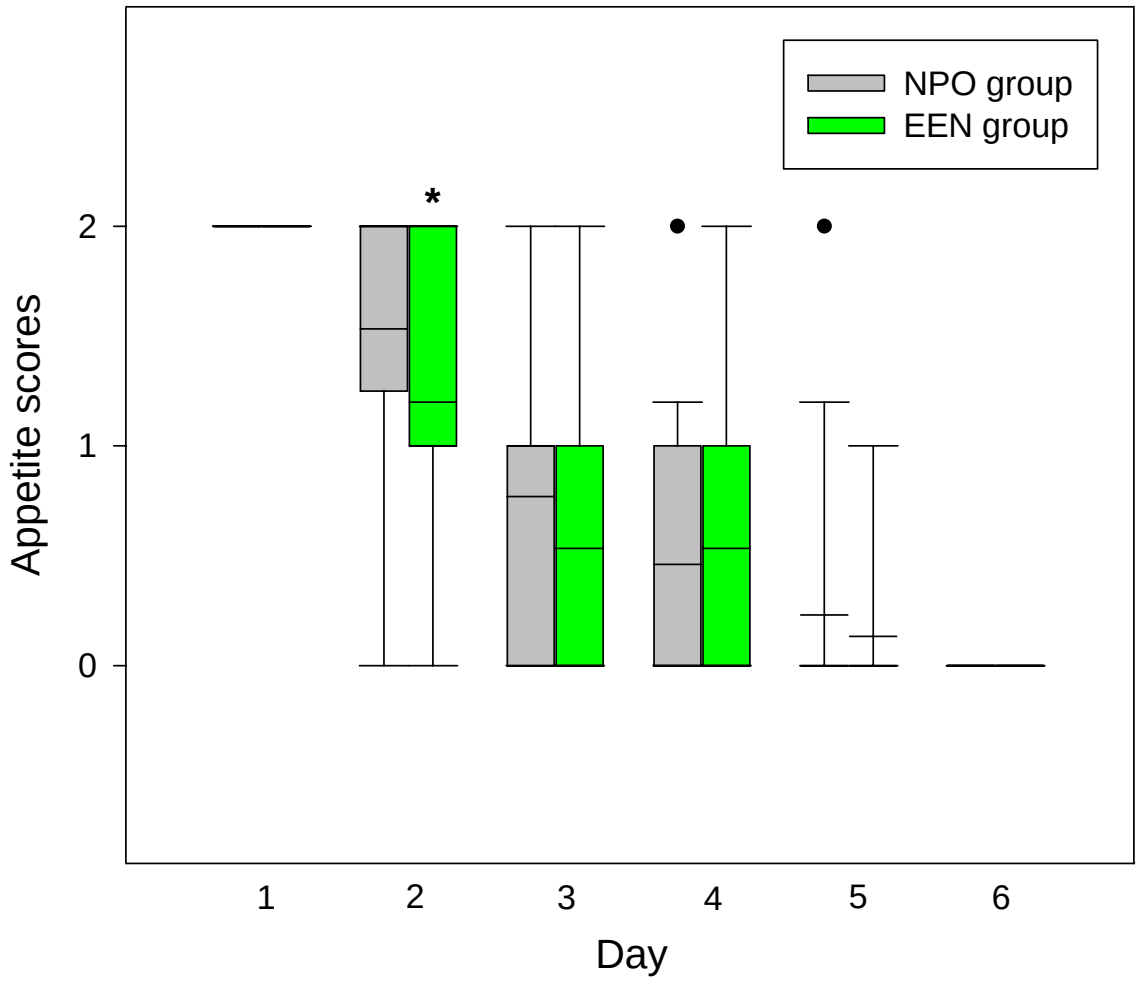

Figure 2: Appetite scores in 30 dogs with parvoviral enteritis. Data are shown as mean (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). Black dots represent outliers. The asterisk indicates a statistically significant difference between the groups. NPO = Nil per os; EEN = Early enteral nutrition.
Score 0 = normal
Score 1 = voluntarily eats small amounts
Score 2 = no interest in food.

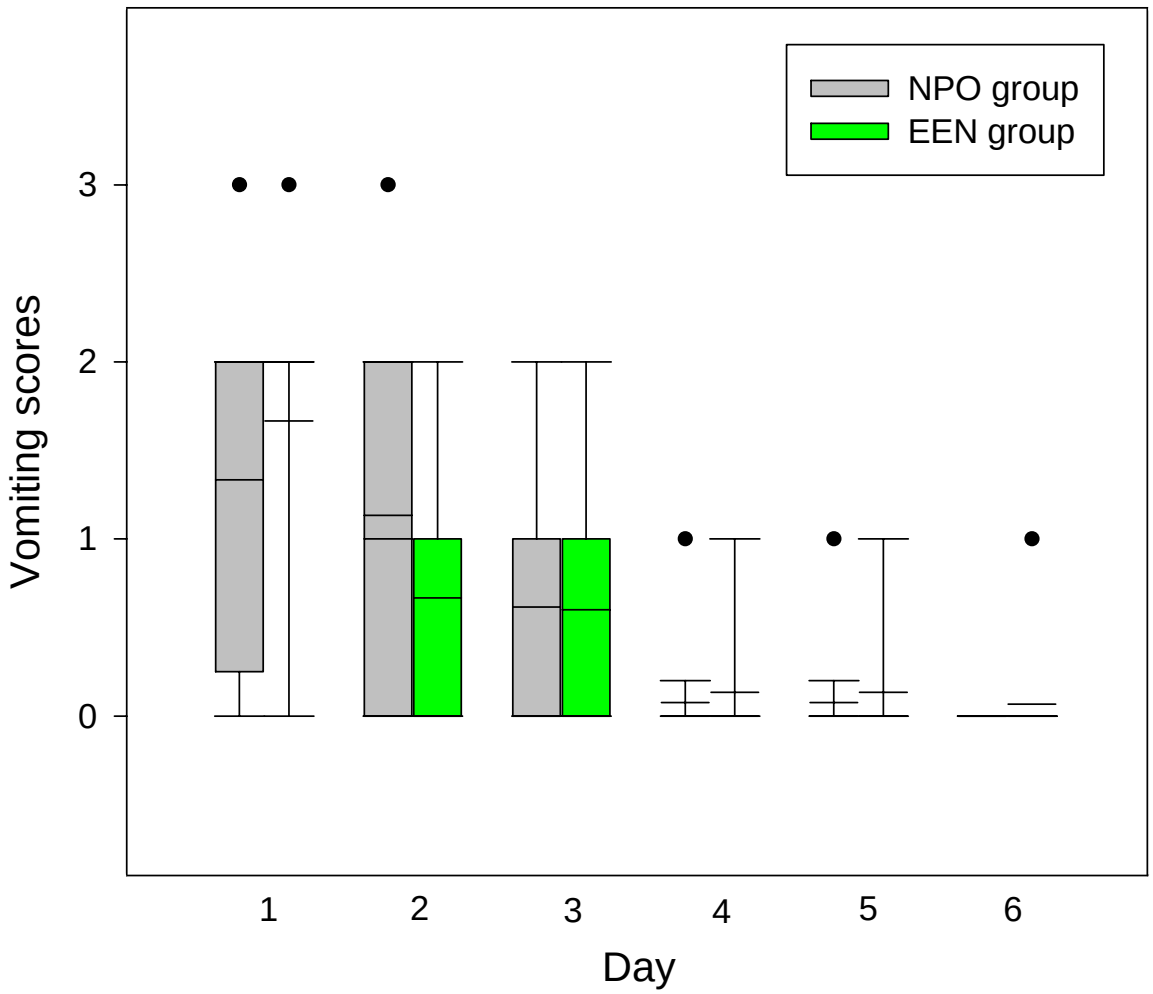

Figure 3: Vomiting scores in 30 dogs with parvoviral enteritis. Data are shown as mean (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). Black dots represent outliers. NPO = Nil per os; EEN = Early enteral nutrition.
Score 0 = vomiting absent
Score 1 = mild vomiting; once per 12h
Score 2 = moderate vomiting; 2-5 times per 12h
Score 3 = severe vomiting; ≥ 6 times per 12h.

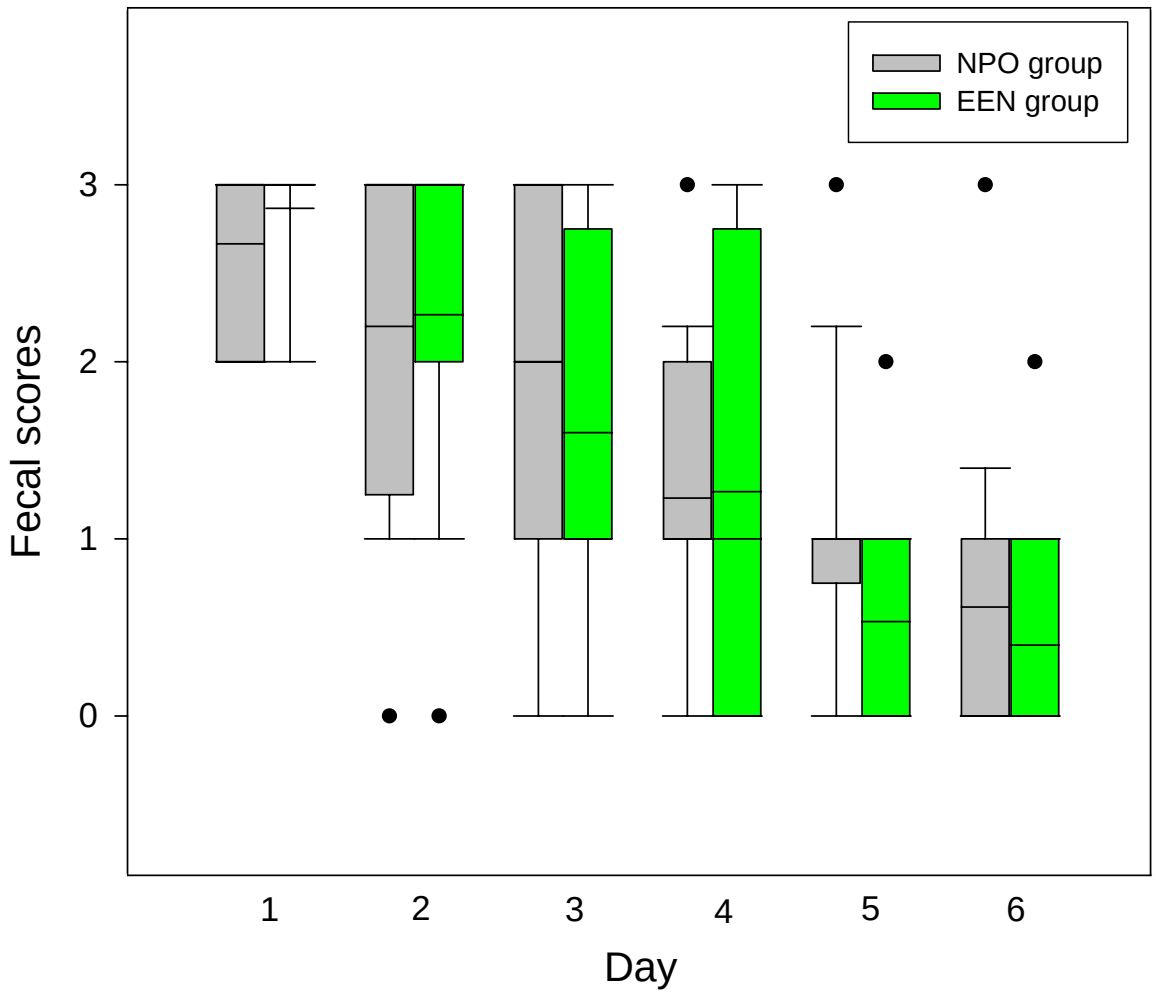

Figure 4: Fecal scores in 30 dogs with parvoviral enteritis. Data are shown as mean (horizontal line within box), 25th and 75th percentiles (horizontal ends of boxes), and 10th and 90th percentiles (T-bars). Black dots represent outliers. NPO = Nil per os; EEN = Early enteral nutrition.
Score 0 = well-formed feces
Score 1 = soft or pasty feces
Score 2 = watery diarrhea, not bloody
Score 3 = watery, bloody diarrhea.

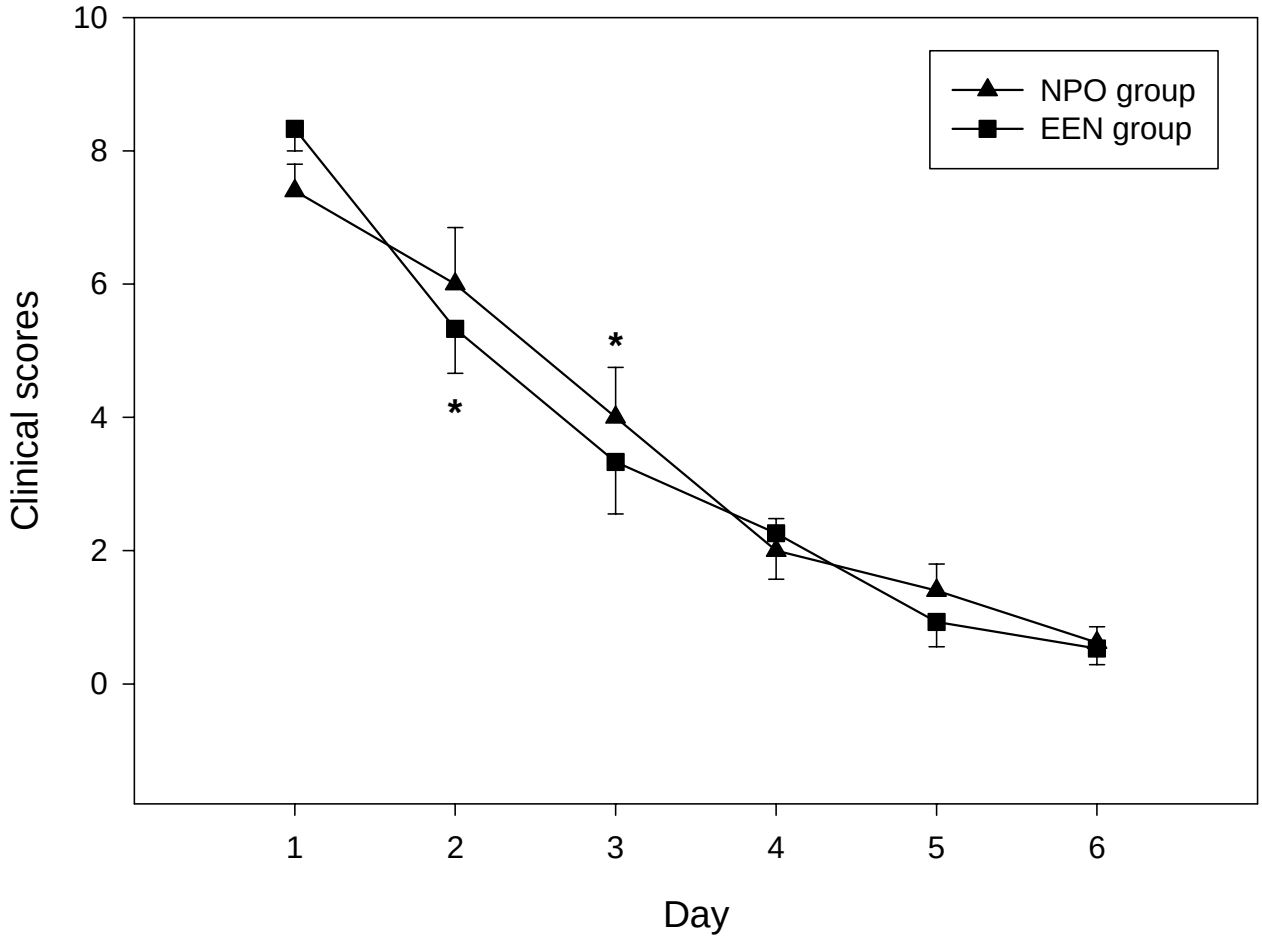

Figure 5: Overall clinical scores in 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Asterisks indicate the first day where a significant difference from the admission score was found. NPO = Nil per os; EEN = Early enteral nutrition.
Clinical score = habitus + appetite + vomiting + fecal score.

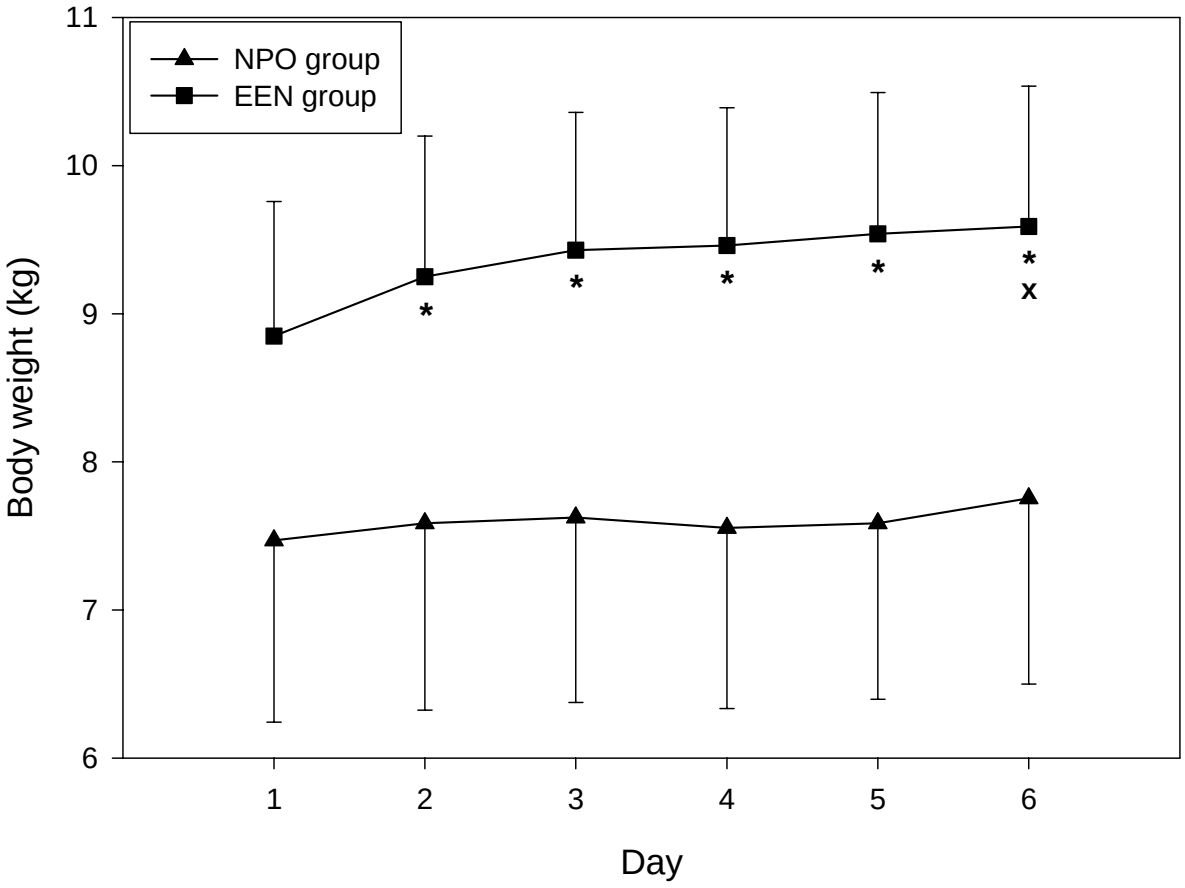

Figure 6: Body weight changes for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Asterisks indicate significant differences from admission weights. NPO = Nil per os; EEN = Early enteral nutrition.
x = 8.4% (mean) increase from baseline value.

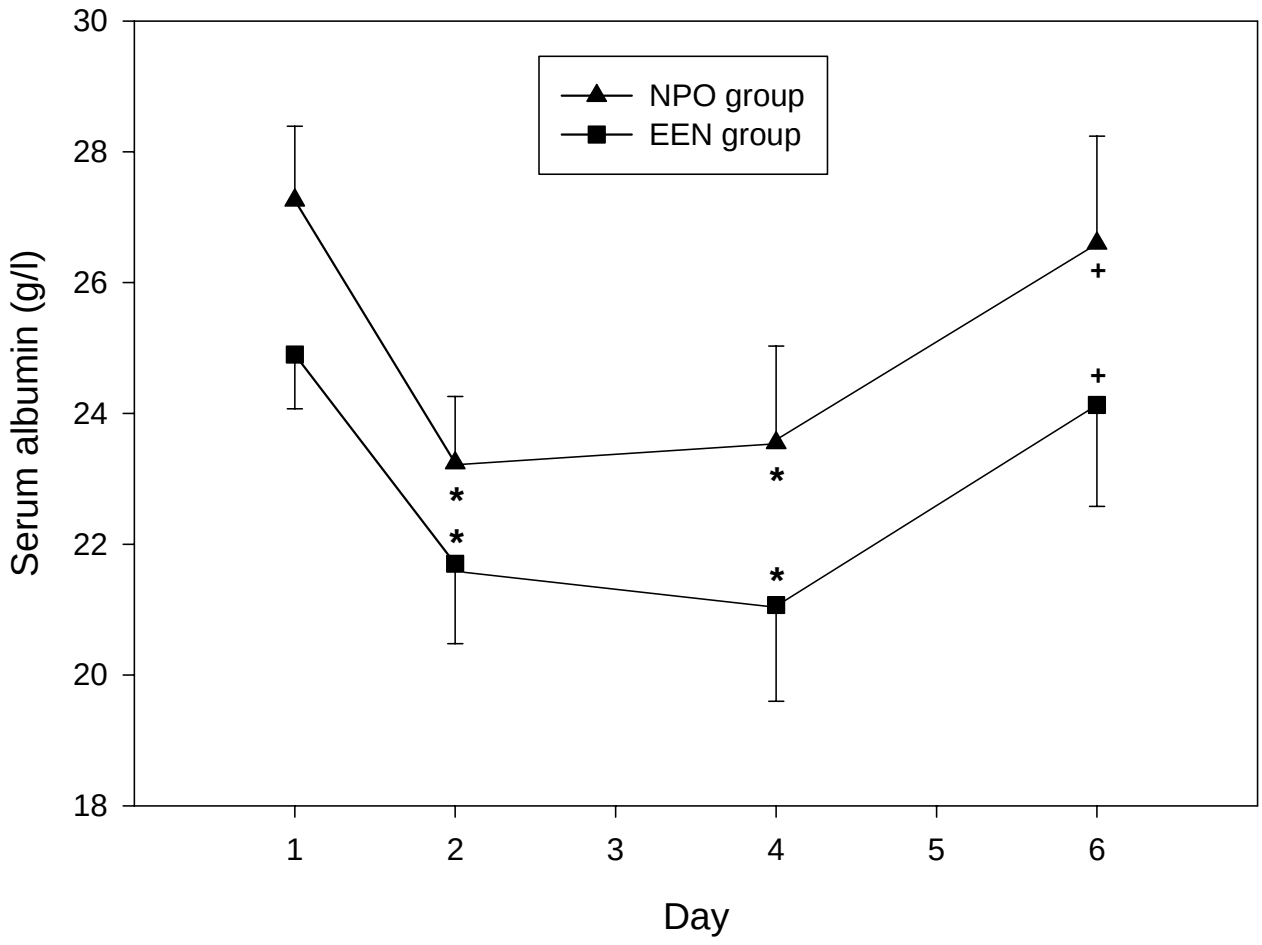

Figure 7: Serum albumin changes for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Asterisks indicate significant differences from admission values. NPO = Nil per os; EEN = Early enteral nutrition.
Crosses (+) indicate significant differences from day 4 values.

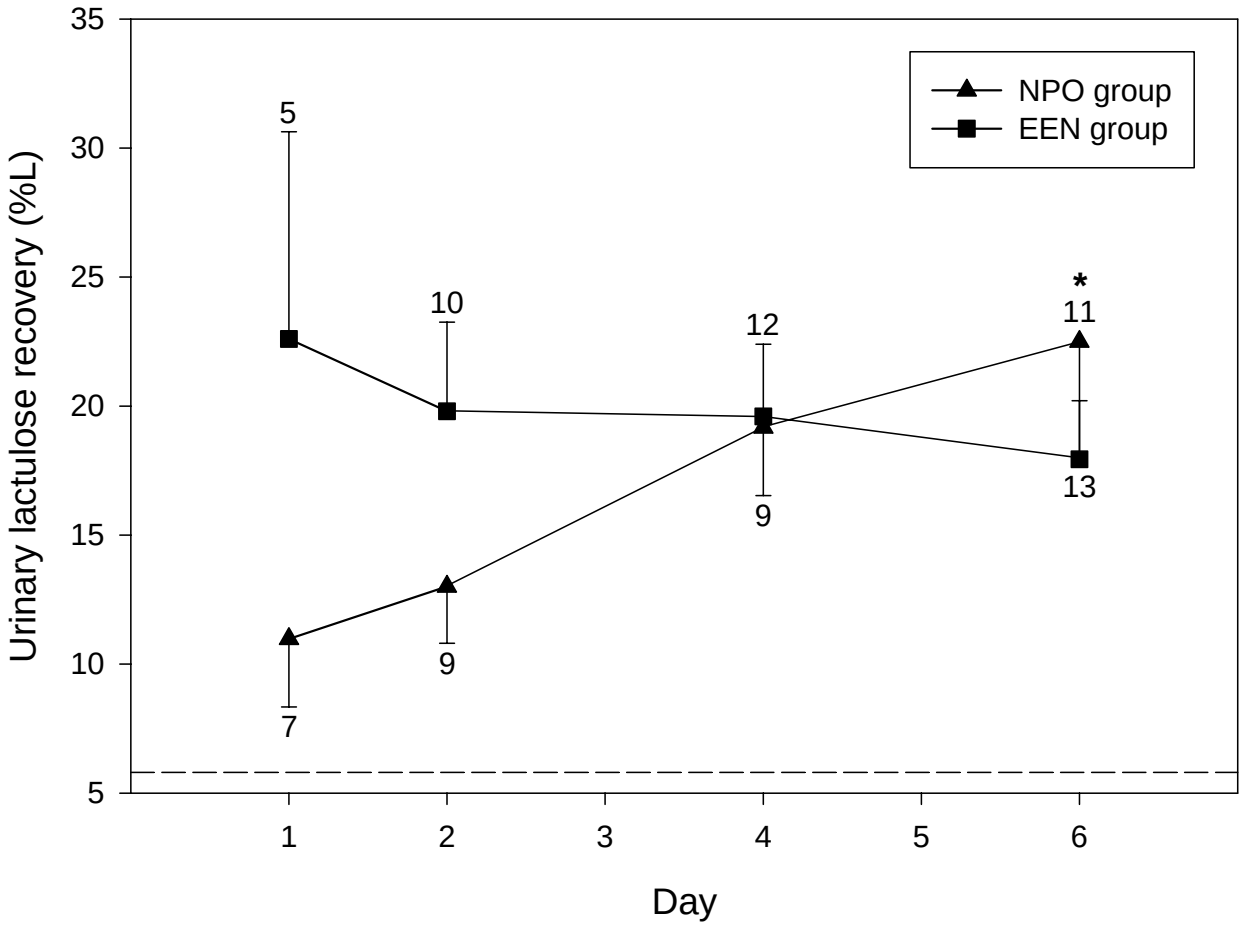

Figure 8: Urinary lactulose recoveries (%L) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. The asterisk indicates a significant difference from the day 2 value (NPO). Dashed line indicates upper limit of normal laboratory range (5.8%). NPO = Nil per os; EEN = Early enteral nutrition.

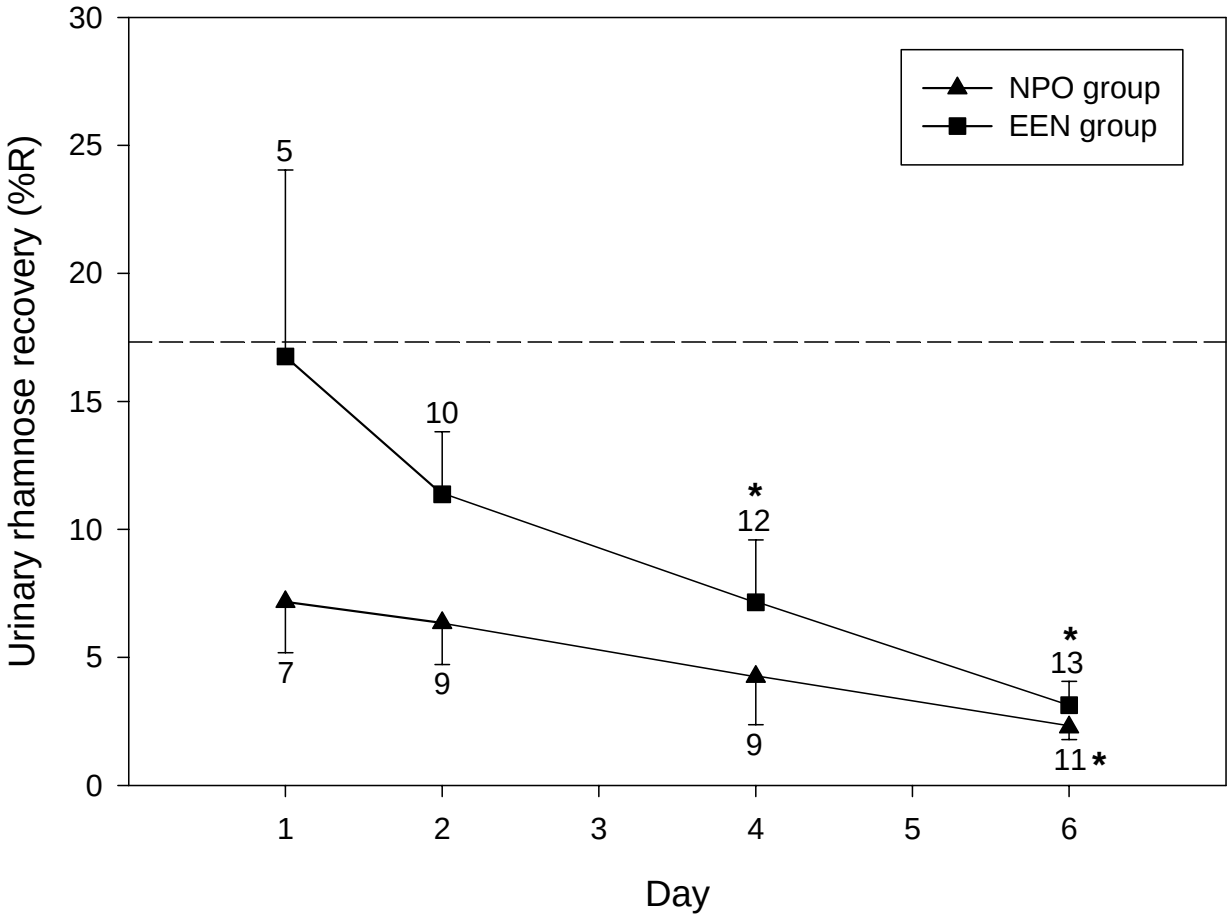

Figure 9: Urinary rhamnose recoveries (%R) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from day 2 values. Dashed line indicates lower limit of normal laboratory range (17.3%). NPO = Nil per os; EEN = Early enteral nutrition.

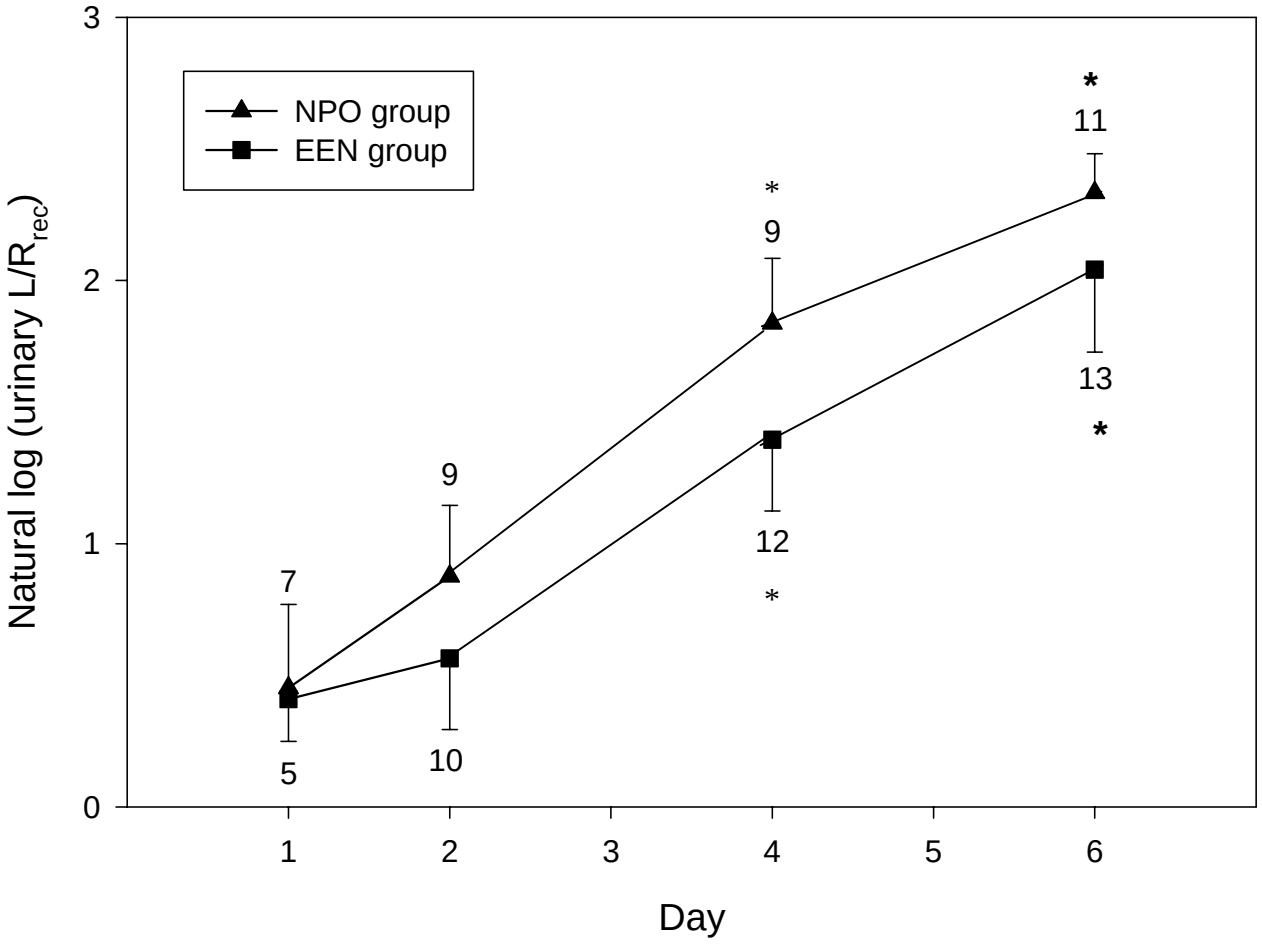

Figure 10: Log$_e$ (urinary L/R$_{rec}$) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from baseline values. NPO = Nil per os; EEN = Early enteral nutrition.

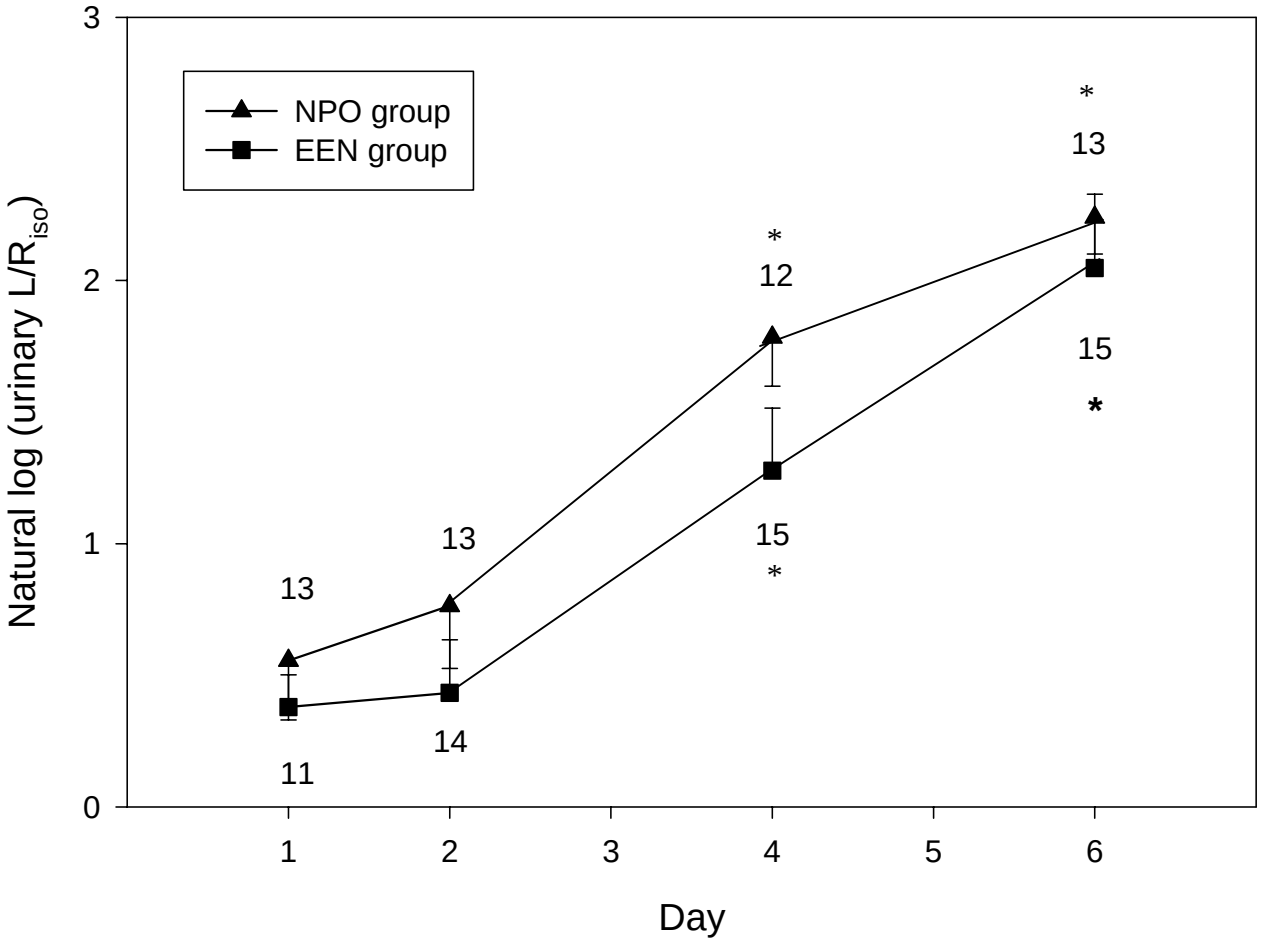

Figure 11: Log$_e$ (urinary L/R$_{iso}$) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from baseline values. NPO = Nil per os; EEN = Early enteral nutrition.

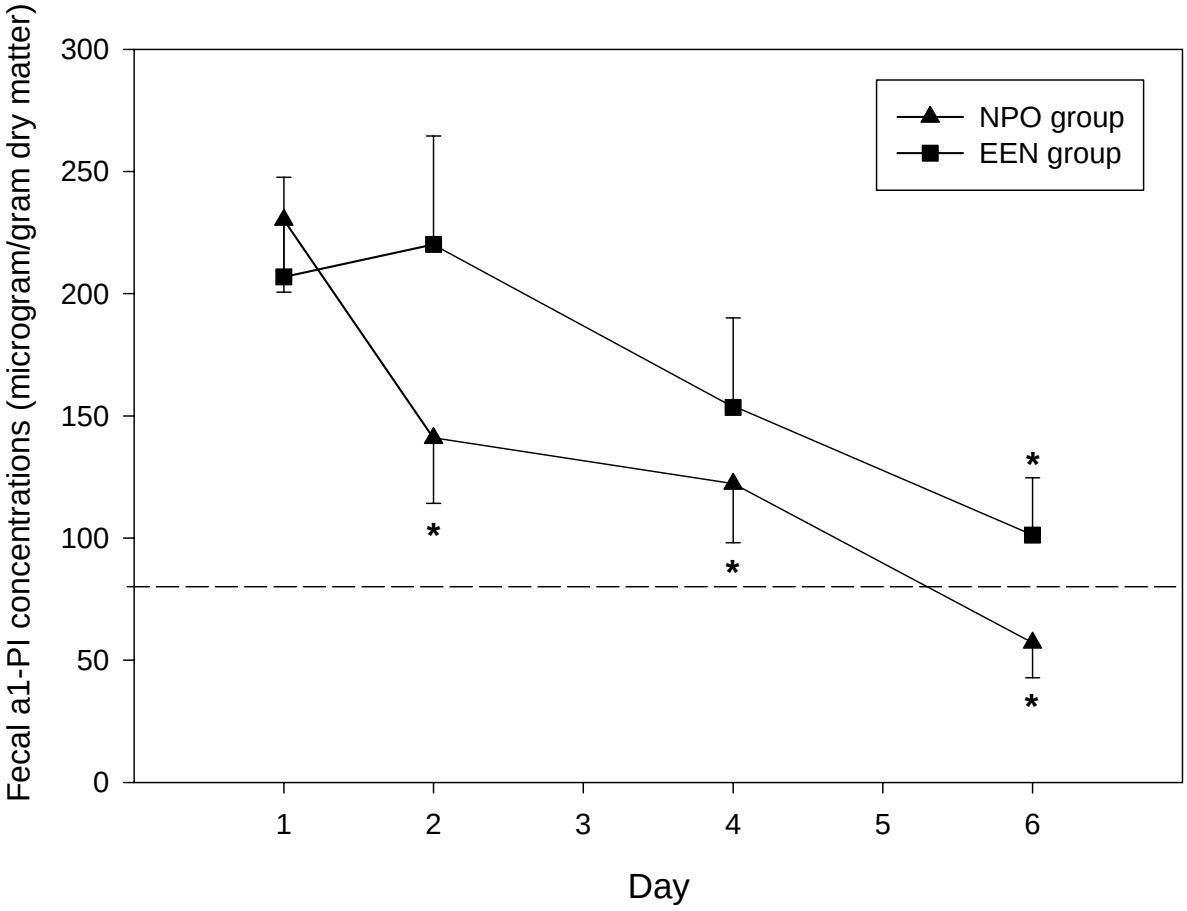

Figure 12: Fecal α_1–proteinase inhibitor concentrations for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from baseline values. Dashed line indicates upper limit of local Onderstepoort normal range (80.2 μg/g). NPO = Nil per os; EEN = Early enteral nutrition.

CHAPTER VII

7. DISCUSSION

Early EN in severe CPV enteritis was associated with more rapid clinical improvement than initial NPO, as evidenced by faster normalization of habitus and appetite, and the resolution of vomiting and diarrhea. Investigator bias cannot be excluded in the awarding of habitus and appetite scores; however, vomiting and fecal scores were more objective in character. Significantly faster resolution of vomiting, diarrhea and disease severity has also been documented in human EEN, compared with initial starvation or TPN.[120, 143, 222] Hospitalization time was not assessed in this trial, since all dogs were hospitalized for 6 days for intestinal permeability testing. The more rapid clinical improvement in EEN has the potential to reduce hospitalization time and/or expense.

The significantly increased body weight in EEN supports reduced catabolism and hypermetabolism. The measurement of body weight in hospitalized humans is a valid and reproducible technique for the evaluation of nutritional status, with significant correlation to more objective tests.[125, 205] Reduced catabolic responses and/or preservation of a positive nitrogen balance in EEN, as compared to initial starvation or TPN, have previously been demonstrated.[101, 109, 120, 144, 146, 148] The prevention of protein-energy malnutrition in CPV infection may have significant ramifications, since malnutrition impairs mucosal and systemic immunity,[131-135] increases the duration of diarrhea and mortality of infectious enteritis in humans,[135] and significantly increases infectious morbidity and hospitalization time following intestinal surgery.[138] It is possible that fluid therapy contributed to increased body weight in both groups, since weights on day 1 were measured prior to rehydration. Dogs in the EEN group received fluids enterally, in addition to IV fluids; however, IV fluid therapy was individualized for each patient based on the clinical assessment of hydration status, so that the greater enteral fluid administration in EEN should not have led to greater body weight gains as compared to NPO. Serum albumin concentration is an insensitive measure of nutritional status, due to

its long serum half-life (8.2 days in dogs).[206] Diverse non-nutritional factors also contribute to hypoalbuminemia in critical illness, including decreased hepatic synthesis, protein-losing enteropathy or nephropathy, perivascular exudation, and hemodilution by fluid resuscitation or water retention.[108, 112, 125, 205]

Parvoviral enteritis was accompanied by severely increased intestinal permeability. The urinary recovery of lactulose (%L) was elevated above the laboratory reference range throughout the study period for both groups. At admission (day 1), %L was increased (mean value) above the top-normal reference range by a factor of 1.9 in the NPO group, and by a factor of 3.9 in the EEN group. The urinary recovery of rhamnose (%R) was reduced below the reference range throughout the study period for both groups. At admission, %R was decreased (mean) below the bottom-normal reference range by a factor of 0.4 in the NPO group, and by a factor of 0.97 in the EEN group. Urinary L/R_{iso} ratios were elevated above the laboratory reference range[246] throughout the study period for both groups. At admission, L/R_{iso} was increased (mean) above the normal reference range (mean) by a factor of 24.9 in the NPO group, and by a factor of 15.8 in the EEN group.

The precise transepithelial permeation pathways of probes utilized in intestinal permeability tests have not been definitively established.[208, 209, 231, 232] According to the classical and most widely cited hypothesis, smaller monosaccharides (e.g. rhamnose) permeate transcellularly via small aqueous pores of high incidence in enterocyte cell-membranes.[207, 209, 211] In contrast, the larger disaccharides (e.g. lactulose) are hypothesized to permeate paracellularly via larger aqueous channels of low incidence, located in intercellular tight junctions.[207, 209, 211] The progressive decrease in %L over time in the EEN group, as compared to a continued increase in the NPO group, may thus reflect improved microstructure and/or function of epithelial tight junctions attributable to EEN. This may be due in part to decreased intestinal inflammation, as lactulose permeation in human Crohn's disease correlates to the severity of intestinal inflammation,[231] and pro-inflammatory cytokines (tumor necrosis factor-α and interferon-γ) impair tight junction structure and function.[255] Lactulose may also permeate

paracellularly through areas of epithelial disruption (i.e. necrosis, ulceration, erosion, or the extrusion zones of exfoliated cells),[207, 209, 215, 219, 233, 234] and the decreased %L in EEN may thus also indicate earlier repair of intestinal epithelial necrosis. The decreased %L in EEN may additionally indicate earlier normalization of intestinal flora, since small intestinal bacterial overgrowth increases intestinal permeability.[229, 230] The decreased %R in both the EEN and NPO groups is consistent with villus atrophy, with reduced surface area available for rhamnose permeation.[207, 209, 233] Further %R decreases over time in both groups may potentially be related to alterations in immature enterocytes' membrane phospholipid composition and fluidity, and thus transcellular membrane pores,[209] since re-epithelialization following crypt necrosis in CPV enteritis is associated with epithelial cells of aberrant morphology.[256]

Similar to the findings in our study, early EN in acute viral gastroenteritis in children decreases intestinal permeability, while starvation significantly increases lactulose permeation.[218] Early EN in human critical illness has also been demonstrated to progressively decrease intestinal permeability, as compared to initial starvation or TPN.[114, 222] Since short-term (36 hours) starvation *per se* does not alter intestinal permeability in healthy humans,[257] the differences in intestinal permeability between EN and starvation are attributable to the beneficial effects of EN on intestinal epithelial structure and function. Since the decreased lactulose permeation in our EEN *vs.* NPO group may reflect earlier repair of intestinal epithelial necrosis or improved tight junction structure or function, EEN might potentially have been associated with decreased transmucosal passage of luminal compounds. Translocation of bacteria, endotoxin or luminal antigens may locally propagate intestinal inflammation,[56, 58] or systemically initiate SIRS and MODS.[55, 56] Since intestinal inflammation,[56, 58] endotoxemia,[84-86] and SIRS[55, 58] may lead to further increases in intestinal permeability, a reduction in any of these phenomena could limit or prevent further gut barrier compromise. Early EN, as compared to starvation or TPN, has previously been associated with decreased incidences of bacteremia,[145, 146] endotoxemia,[120] SIRS[120, 149] and MODS[120, 222] in diseases associated with gut barrier dysfunction.

An alternative hypothesis for the transmucosal permeation pathways of permeability probes proposes that both the small and large probes traverse the epithelium paracellularly through tight junctions.[208, 231, 232] In this hypothesis, the permeation of larger probes (e.g. lactulose) is confined to the more permeable intestinal crypt tight junctions, whereas smaller probes (e.g. rhamnose) may permeate via tight junctions throughout the crypt-villus axis.[208, 231, 232] The decreased %R in both our study groups might thus reflect villus atrophy, with decreased surface area for rhamnose permeation. It has further been hypothesized that lactulose, due to its large diameter, may be physically restricted in its ability to move between villi to reach the crypt tight junctions; in contrast, the smaller rhamnose may more freely gain access to the spaces between villi, allowing its permeation throughout the crypt-villus axis.[208, 231, 232] Villus atrophy would thus allow greater access of the larger lactulose to the crypts, with increased lactulose permeation. Decreased %L over time in EEN *vs.* NPO may therefore reflect more rapid villus regeneration in EEN, although the lack of a concomitant increase in %R in EEN may not support this conclusion.

The opposite directions of blood flow in villus arterioles and venules maintains villus tip hyperosmolality, as a result of countercurrent multiplication.[236] Villus tip hyperosmolality enhances water absorption, which may induce coupled "solvent drag" of monosaccharide-sized hydrophilic sugars, while larger molecules such as lactulose are unaffected.[232, 237] The decreased %R in both our study groups may thus also reflect impaired efficiency of countercurrent multiplication, as a result of either villus atrophy (with shortening of villus vasculature), intestinal ischemia, or passive congestion.[232]

Urinary L/R ratios in puppies are variable in the peri-weaning period, but remain stable from 8 weeks of age.[241] Changes over time in urinary L/R ratios were similar between treatment groups. Failure to demonstrate a difference in L/R ratios, even though lactulose permeations differed significantly, may be due to small patient numbers and large standard deviations within data points. By expressing the sugars' urinary recoveries as a ratio (disaccharide/monosaccharide), factors unrelated to mucosal permeability (e.g. vomiting, gastric emptying, intestinal transit time, dilution of test solution by intestinal

contents, and glomerular filtration rate) are excluded, since both markers would be expected to be equally affected.[207-209] Differences in gastro-intestinal motility between groups may thus have contributed to the differing lactulose permeations, although experimentally induced increased or decreased gastro-intestinal motility does not significantly affect lactulose permeation in humans.[258] Dilution of test solution by intestinal contents is unlikely, since dogs were fasted for the 2 hours preceding and 6 hours during permeability testing.

It is of interest that the highest L/R ratios in both treatment groups occurred at the time of discharge from hospital, when clinical signs of intestinal disease had resolved and fecal protein loss was at its lowest. Since intestinal morphological integrity would intuitively be expected to be most normal at this time, this brings to question whether L/R ratios accurately reflect the severity of the physical and/or functional disruption to the intestinal epithelium in CPV enteritis. There is general agreement that lactulose permeates through tight junctions[207, 209, 211] or areas of epithelial disruption,[207, 209, 215, 219, 233, 234] and may thus be an appropriate measure of the functional and/or physical gut barrier. In contrast, the permeation of rhamnose may be affected by mucosal factors not directly related to intestinal integrity (e.g. villus atrophy,[207, 209, 233] "solvent drag" and intestinal blood flow aberrations,[232, 237] and potentially also altered enterocyte membrane phospholipid composition[209]). Although expressing urinary sugar recoveries as a ratio excludes the influence of non-mucosal factors, individual sugar recoveries may provide more specific information of epithelial structure and function. Some of the limitations in the interpretation of test results by the use of a ratio alone have been previously noted.[232, 234, 239] We recommend that studies utilizing intestinal permeability tests report the recoveries of individual sugars, in addition to their ratios.

Fecal α1-PI concentrations (dry matter basis) were elevated above the local Onderstepoort reference range (44.7 ± 25.1 μg/g; mean ± SD) for the majority of the study period for both groups. At admission, fecal α1-PI was increased (mean) above the reference range (mean) by a factor of 5.1 in the NPO group, and by a factor of 4.6 in the EEN group. There were no significant differences between the declines over time for

fecal α_1-PI concentrations between NPO and EEN; however, α_1-PI concentrations decreased more slowly in EEN. Since enteral nutrients may stimulate increased intestinal blood flow,[114, 116] this may in part account for the slower decline in the EEN group. This slower decline of fecal α_1-PI concentrations in EEN was not accompanied by a concomitantly greater decrease in serum albumin concentrations as compared to NPO.

The higher survival rate in EEN *vs.* NPO was not statistically significant, but the sample size was small. However, we are unaware of any previous studies with 100% survival in severe, naturally occurring CPV enteritis. Early EN in CPV enteritis may thus be associated with reduced mortality. Since we were concerned about the effects of prolonged starvation, dogs in the initial NPO group were force-fed from an earlier time than that traditionally advised. Enteral nutrient administration in the EEN group was additionally interrupted for 8 hours on the days of intestinal permeability testing. Had the NPO dogs been starved for longer, and EN administration been uninterrupted in the EEN group, observed differences between groups might potentially have been more outspoken. Two of the NPO dogs were Rottweilers, a breed with reported susceptibility to more severe CPV disease, while there were no Rottwielers in the EEN group. One of the NPO dogs that died was a Rottweiler, and this dog's death may potentially have been the result of more severe disease, rather than being attributable to the NPO treatment.

Enteral tube feeding was not associated with significant complications. We detected moderate gastric tympany in 2 EEN dogs, although this did not measurably aggravate vomiting. The most frequently documented complication of tube feeding in humans (39% of patients), which may necessitate the temporary discontinuation of EEN, is high volumes of gastric residual gas and food, secondary to ileus.[172] Gastric decompression may be performed in these cases prior to EN administration, by aspirating gas through the nasogastric tube.[168] Nasogastric tubes may thus be preferable in CPV enteritis, at least in selected patients, particularly since concurrent opioid analgesia may further delay gastric emptying. Due to the presence of vomiting, the amount of EN that effectively reached the intestine is unknown. Some experimental studies suggest that at least 25% of calculated nutrients should be delivered enterally to prevent intestinal mucosal atrophy,[121, 122] while

others propose that even smaller volumes may improve gut structure-function and systemic immunity.[177, 181, 182]

General consensus maintains that early EN should be the preferred route of substrate delivery in human critical illness and sepsis,[125, 126, 151] which has similarly been emphasized in the veterinary literature.[110, 118, 157] Although it had previously been speculated that at least some form of enteral nutrient delivery might be beneficial in CPV enteritis,[26, 29, 107] the treatment of this disease has remained an exception to this recommendation. This likely stems from the severity of intestinal necrosis, vomiting and diarrhea, and the assumption that nutrient digestion and absorption will be inefficient. However, both EN[143, 163, 259] and oral rehydration[163, 169-171] have been successfully applied in humans and animals with severe acute gastroenteritis. Enteral nutrition, as compared to TPN, has also been associated with significantly faster resolution of malabsorption in human infantile diarrhea.[143] The present study demonstrates that EEN may be successfully instituted in CPV enteritis, even in the presence of severe vomiting and diarrhea. The significant weight gain in EEN might also indicate that at least partially efficient nutrient digestion and absorption occurs. This study supports the use of EEN in gut barrier dysfunction.

Further studies are required to determine whether early EN in CPV enteritis reduces the incidence of endotoxemia, bacteremia, or SIRS. Additional trials should investigate the optimal dietary composition for CPV enteritis. Enteral diets containing intact proteins or peptides (i.e. our test diet) stimulate intestinal mucosal growth to a greater degree than do free amino acids.[183, 186, 198] However, a potential disadvantage to feeding intact proteins exists; intact, undigested dietary proteins are absorbed in increased quantities in acute gastroenteritis.[189, 260] Following initial sensitization, later re-exposure to luminal antigens may theoretically lead to inflammatory bowel disease.[189] The addition of dietary fiber may further improve gut barrier function and decrease bacterial translocation.[184, 198] Enteral formulations containing immune-enhancing nutrients (arginine, omega-3 polyunsaturated fatty acids and nucleotides, with or without glutamine or branch-chain amino acids), have produced significant reductions in infectious morbidity,[202] length of

hospitalization,[202] and incidence of SIRS and MODS[204] in human critical illness and sepsis, as compared to standard EN diets. Such immunonutrition formulations may potentially be of benefit in CPV enteritis. Furthermore, a strategy of combined EN and partial parenteral nutrition[250] may yield optimal results in CPV enteritis.

CHAPTER VIII

8. CONCLUSIONS

8.1 The study's results have addressed the research questions, as follows:

i. ***Does early enteral nutrition restore (i.e. reverse the abnormal) intestinal permeability and protein-losing enteropathy in canine parvoviral enteritis more rapidly than initial starvation?***

The significant difference in the behavior of urinary lactulose recovery (%L) between groups might indicate earlier repair of intestinal epithelial necrosis, or improved tight junction structure and/or function due to EEN. Such an improvement in gut barrier function with EEN might potentially limit endotoxin and/or bacterial translocation, with a decreased magnitude of the resultant systemic inflammatory response. This may lead to decreased development of MODS with reduced mortality due to EEN.

Early EN did not have a beneficial effect on the magnitude or rate of decline of intestinal protein loss.

ii. ***Does early enteral nutrition in parvoviral enteritis improve recovery and outcome, as compared to initial starvation?***

Dogs receiving EEN recovered faster clinically, which may reduce hospitalization time and expense. The significant weight gain in EEN might reflect reduced catabolism. Early EN in CPV enteritis may be associated with reduced mortality.

Early enteral tube feeding was a feasible and safe alternative to NPO treatment in CPV enteritis. The study's results support the use of EEN in CPV enteritis.

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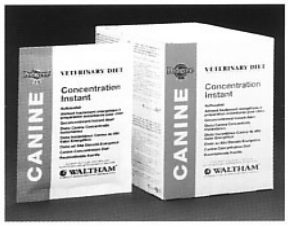
APPENDICES

Appendix 1: Analysis of the enteral formulation fed in naturally occurring canine parvoviral enteritis (EEN group)[193]



PEDIGREE CANINE CONCENTRATION INSTANT DIET

Product description

PEDIGREE Canine Concentration Instant Diet is a highly palatable and easily digestible, complete liquid suspension diet for dogs. When made into a liquid suspension, the diet is suitable for bowl feeding, syringe feeding or enteral feeding through large and small gauge tubes. The Diet provides a concentrated and balanced source of nutrition.

Presentation

A spray-dried powder in 47g sachets.

Ingredients

Milk protein-hydrolysate, milk protein, maltodextrin, deydrated egg yolk, vegetable oil, minerals, vitamins, prebiotic (fructo-oligosaccharide), L–carnitine

Typical Analysis

Calculated metabolisable energy (ME) per 100g of powder: 1828 kJ/437 kcal
per 100ml as fed: 636 kJ/152 kcal

Nutrient	Unit	per 100 g "as is"	per 100 ml as fed	per 400 kcal
Moisture	g	4.0		
Protein	g	41.0	14.3	37.5
Fat	g	18.0	6.3	16.5
NFE	g	26.5	9.2	24.3
Ash	g	7.5	2.6	6.9
Crude fibre	g	3.0	1.0	2.7
EFA	g	1.8	0.6	1.6
Calcium	g	1.4	0.49	1.3
Phosphorus	g	1.3	0.45	1.2
Sodium	g	0.4	0.14	0.4
Potassium	g	0.7	0.24	0.6
Magnesium	mg	70	24	64.1
Iron	mg	15.0	5.22	13.7
Copper	mg	1.5	0.52	1.4
Manganese	mg	0.6	0.21	0.5
Zinc	mg	12.0	4.17	11.0
Iodine	µg	200	70	183.1
Selenium	µg	20	7	18.3
Vitamin A	IU	1500	522	1373
Vitamin D	IU	100	35	91.5
Vitamin E	mg	25	9	22.9
Thiamin (B_1)	mg	1.1	0.38	1.0
Riboflavin (B_2)	mg	1.0	0.35	0.9
Niacin	mg	10.0	3.48	9.2
Pyridoxine (B_6)	mg	1.0	0.35	0.9
Pantothenic acid	mg	5.0	1.74	4.6
Folic acid	µg	150	52	137.3
Cobalamin (B_{12})	µg	10	3.5	9.2
Biotin	µg	150	52	137.3
Choline	mg	590	205	540
Ca/P Ratio		1.08	1.08	1.08

Appendix 2: Manufacturer's feeding guidelines for the diet fed in canine parvoviral enteritis (EEN group)[193]

Feeding guide for puppies – Concentration Instant

Expected Adult Bodyweight (kg)	Age of puppy (months)								
	1	2	3	4	5	6	9	12	18
	Volume of Diet per day (ml)†								
1	90	120	140	140	120	90	*	*	*
5	140	240	280	330	340	350	300	*	*
10	180	320	430	520	540	570	530	*	*
15	250	450	680	770	820	830	730	*	*
20	320	570	890	1010	1040	1070	940	850	*
25	390	700	1050	1210	1320	1340	1230	1040	*
30	410	750	1110	1360	1500	1630	1430	1220	*
40	430	790	1300	1600	1790	1930	1890	1570	*

* Commence feeding with a standard dog food, unless the puppy requires continued special feeding. In this instance, refer to the feeding guide for adult dogs.
† To nearest 10ml of solution
47g sachet/100 ml water

Appendix 3: Signalment of 30 dogs with parvoviral enteritis

Study Dog Number	Breed	Age (months, weeks)	Sex
NPO group			
1	Fox terrier	5m4w	F
3	Crossbreed	5m4w	F
5	Boerboel	5m4w	M
6	Crossbreed	5m	M
9	Crossbreed	4m4w	M
12	Rottweiler	2m	F
14	Labrador retriever	4m	M
15	Bouvier des Flandres	5m4w	F
17	Crossbreed	3m3w	M
18	German shepherd dog	1m4w	F
19	Rottweiler	3m1w	M
21	German shepherd dog	4m1w	F
22	Crossbreed	3m2w	F
23	Fox terrier	2m3w	M
25	Maltese poodle	5m4w	F
EEN group			
2	Crossbreed	5m4w	F
4	Crossbreed	2m1w	M
7	Great Dane	4m	M
8	Great Dane	4m	M
10	Crossbreed	3m4w	M
11	German shepherd dog	4m4w	M
13	Boerboel	3m4w	M
16	Dalmatian	2m4w	F
20	Rhodesian ridgeback	5m	M
24	Dachshund	3m4w	F
26	Crossbreed	2m4w	F
27	Crossbreed	2m4w	M
28	Crossbreed	4m1w	F
29	Dachshund	3m4w	M
30	Boerboel	4m2w	F

m = Months; w = Weeks; M = Male; F = Female.

Appendix 4: Habitus, appetite and vomiting scores for 30 dogs with parvoviral enteritis

	Habitus score						Appetite score						Vomiting score					
Day	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
NPO																		
Dog #																		
1	1	1	1	0	0	0	2	2	1	1	0	0	2	2	1	1	0	0
3	2	1	1	1	0	0	2	2	2	1	0	0	2	2	2	0	0	0
5	1	2	1	0	0	0	2	2	1	0	0	0	1	2	0	0	0	0
6	2	1	0	0	0	0	2	2	0	0	0	0	1	0	0	0	0	0
9	2	0	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
12	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
14	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
15	1	2	2	1	1	0	2	2	2	2	1	0	2	2	2	0	0	0
17	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0
18	1	1	1	0	0	0	2	2	1	0	2	0	2	1	1	0	1	0
19	2	3	-	-	-	-	2	2	-	-	-	-	0	2	-	-	-	-
21	1	1	1	0	0	0	2	2	1	1	0	0	1	1	0	0	0	0
22	2	3	-	-	-	-	2	2	-	-	-	-	2	3	-	-	-	-
23	2	1	0	0	0	0	2	2	1	0	0	0	3	1	0	0	0	0
25	1	1	1	1	0	0	2	2	1	1	0	0	2	1	1	0	0	0
Median	**1**	**1**	**1**	**0**	**0**	**0**	**2**	**2**	**1**	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**0**	**0**
EEN																		
Dog #																		
2	2	1	2	1	1	0	2	2	2	2	1	0	2	2	2	0	1	1
4	2	1	1	0	0	0	2	1	1	1	0	0	2	2	1	0	0	0
7	2	2	2	2	1	1	2	2	2	2	1	0	2	1	1	1	0	0
8	2	1	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
10	2	2	1	1	0	0	2	2	1	1	0	0	2	1	2	1	0	0
11	1	2	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
13	3	1	1	0	0	0	2	2	0	0	0	0	0	1	1	0	0	0
16	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
20	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
24	3	3	1	1	0	0	2	2	1	1	0	0	3	2	1	0	1	0
26	1	1	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
27	1	1	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
28	2	1	0	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
29	1	1	1	0	0	0	2	1	0	0	0	0	2	0	0	0	0	0
30	2	1	0	0	0	0	2	1	1	1	0	0	2	1	1	0	0	0
Median	**2**	**1**	**0**	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**

Note: Missing values (-) recorded following death.

Appendix 5: Fecal and overall clinical scores for 30 dogs with parvoviral enteritis

	Fecal score						Clinical score					
Day	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
NPO												
Dog #												
1	3	3	3	2	1	0	8	8	6	4	1	0
3	3	3	3	1	1	0	9	8	8	3	1	0
5	3	3	3	1	1	1	7	9	5	1	1	1
6	3	2	2	1	1	1	8	5	2	1	1	1
9	3	3	3	1	0	0	9	4	3	1	0	0
12	2	1	0	0	0	0	5	1	0	0	0	0
14	2	1	1	1	1	0	5	1	1	1	1	0
15	2	2	2	2	1	0	7	8	8	5	3	0
17	2	0	0	0	0	0	5	0	1	0	0	0
18	3	3	3	2	2	1	8	7	6	2	5	1
19	3	2	-	-	-	-	7	9	-	-	-	-
21	3	3	2	1	1	1	7	7	4	2	1	1
22	3	3	-	-	-	-	9	11	-	-	-	-
23	3	1	1	1	1	1	10	5	2	1	1	1
25	2	3	3	3	3	3	7	7	6	5	3	3
Median	**3**	**3**	**2**	**1**	**1**	**0**	**7**	**7**	**4**	**1**	**1**	**0**
Mean (±SE)	N/A	N/A	N/A	N/A	N/A	N/A	**7.4 (0.4)**	**6.0 (0.9)**	**4.0 (0.8)**	**2.0 (0.5)**	**1.4 (0.4)**	**0.6 (0.2)**
EEN												
Dog #												
2	3	3	3	3	2	1	9	8	9	6	5	2
4	3	3	2	1	0	0	9	7	5	2	0	0
7	3	3	3	3	1	2	9	8	8	8	3	3
8	3	0	1	1	0	1	9	2	1	1	0	1
10	3	3	3	3	1	1	9	8	7	6	1	1
11	2	2	1	1	0	0	7	5	1	1	0	0
13	3	3	2	1	1	0	8	7	4	1	1	0
16	3	3	1	0	0	0	6	3	1	0	0	0
20	2	1	0	0	0	0	6	1	0	0	0	0
24	3	3	3	3	1	0	11	10	6	5	2	0
26	3	2	1	0	0	0	8	4	1	0	0	0
27	3	2	1	0	0	0	8	4	1	0	0	0
28	3	1	0	0	0	0	9	3	0	0	0	0
29	3	2	1	1	1	1	8	4	2	1	1	1
30	3	3	2	2	1	0	9	6	4	3	1	0
Median	**3**	**3**	**1**	**1**	**0**	**0**	**9**	**5**	**2**	**1**	**0**	**0**
Mean (±SE)	N/A	N/A	N/A	N/A	N/A	N/A	**8.3 (0.3)**	**5.3 (0.7)**	**3.3 (0.8)**	**2.3 (0.7)**	**0.9 (0.4)**	**0.5 (0.2)**

Note: Missing values (-) recorded following death.

Appendix 6: Body weight changes for 30 dogs with parvoviral enteritis

	Body weight (kg)					
Day	**1**	**2**	**3**	**4**	**5**	**6**
NPO						
Dog #						
1	4.9	4.9	4.9	4.9	5.4	5.4
3	4.6	5.1	5.0	5.0	4.8	4.9
5	12.6	12.6	13.9	13.5	13.0	13.0
6	3.2	3.5	4.0	3.5	3.5	3.0
9	13.4	14.5	14.0	14.0	14.0	15.0
12	5.1	5.0	5.0	6.0	6.0	5.8
14	11.5	11.0	11.5	11.0	10.8	12.0
15	15.0	15.5	14.5	14.4	14.4	14.5
17	3.7	4.3	4.2	4.0	4.2	4.5
18	6.0	5.8	6.0	5.7	6.0	5.8
19	8.7	8.5	-	-	-	-
21	10.6	10.4	10.3	10.2	10.3	10.4
22	10.6	10.6	-	-	-	-
23	3.1	2.9	2.8	2.9	2.9	3.0
25	3.4	3.1	3.0	3.1	3.3	3.5
Mean (±SE)	**7.47 (1.23)**	**7.59 (1.26)**	**7.62 (1.25)**	**7.55 (1.22)**	**7.59 (1.19)**	**7.75 (1.26)**
EEN						
Dog #						
2	9.1	9.5	9.7	10.0	9.6	9.2
4	7.8	8.1	8.4	8.6	8.3	8.6
7	11.1	11.1	10.5	10.6	11.1	11.5
8	9.5	10.3	11.0	10.5	10.4	10.6
10	3.6	4.5	5.0	5.0	5.0	4.8
11	16.0	17.5	17.5	17.5	18.4	17.5
13	13.7	14.0	14.0	14.0	13.5	14.5
16	8.8	9.5	10.0	9.0	10.2	10.0
20	10.2	10.0	10.0	10.5	10.0	10.0
24	3.2	3.1	3.2	3.3	3.4	3.4
26	9.1	9.3	9.4	9.5	9.7	10.0
27	6.7	7.0	7.2	7.2	7.3	7.3
28	10.0	10.2	10.3	10.5	10.5	10.6
29	4.0	4.3	4.5	4.5	4.6	4.6
30	9.9	10.4	10.8	11.2	11.1	11.2
Mean (±SE)	**8.85 (0.91)**	**9.25 (0.95)**	**9.43 (0.93)**	**9.46 (0.93)**	**9.54 (0.95)**	**9.59 (0.95)**

Note: Missing values (-) recorded following death.

Appendix 7: Selected clinical pathology of 30 dogs with parvoviral enteritis

	Potassium (mmol/l)	Glucose (mmol/l)	Albumin (g/l)				Hematocrit (%)			
Normal range	*3.6-5.1*	*3.3-5.5*	*27-35*				*37-55*			
Day	**1**	**1**	**1**	**2**	**4**	**6**	**1**	**2**	**4**	**6**
NPO										
Dog #										
1	3.90	7.2	31.4	24.6	20.1	23.7	45	46	35	35
3	4.55	3.6	29.8	21.0	23.9	28.7	38	31	33	37
5	3.12	4.1	23.8	23.8	21.5	25.3	42	33	33	33
6	3.84	4.7	23.8	19.0	21.7	22.7	35	32	32	29
9	3.64	4.5	27.7	24.7	25.8	29.4	41	32	33	36
12	4.61	4.1	27.0	21.7	24.1	24.5	35	33	35	34
14	3.81	4.4	26.6	26.6	29.9	30.3	35	40	41	40
15	4.57	3.1	27.6	27.6	18.6	21.8	35	42	36	38
17	3.25	7.7	29.6	27.7	32.7	35.4	31	31	33	34
18	4.62	6.1	18.4	14.6	12.5	14.8	36	23	25	24
19	4.25	3.8	29.6	23.3	-	-	40	33	-	-
21	4.78	3.3	20.3	19.5	20.0	22.5	45	40	38	39
22	3.49	5.8	27.0	-	-	-	46	36	-	-
23	3.62	4.5	35.7	23.3	27.2	30.4	35	26	27	29
25	4.20	4.5	30.6	27.9	28.2	36.3	50	44	38	42
Mean (±SE)	**4.02 (0.14)**	**4.8 (0.4)**	**27.3 (1.1)**	**23.2 (1.0)**	**23.6 (1.5)**	**26.6 (1.6)**	**39.3 (1.4)**	**34.8 (1.7)**	**33.8 (1.2)**	**34.6 (1.4)**
EEN										
Dog #										
2	4.88	3.5	31.3	21.5	17.3	18.1	44	37	37	31
4	4.03	5.1	26.1	22.4	18.1	22.3	46	49	45	43
7	3.37	5.3	25.1	21.2	17.4	15.2	25	35	33	29
8	3.73	3.7	27.6	25.8	28.6	28.9	45	33	32	34
10	4.48	3.9	19.4	17.5	12.0	27.1	32	32	31	30
11	3.99	4.0	23.9	19.7	23.7	26.6	37	33	32	33
13	3.30	2.0	22.5	21.9	20.4	23.6	42	39	37	34
16	4.00	4.4	28.3	28.1	32.0	33.9	37	35	37	39
20	3.50	3.3	28.8	29.2	28.9	33.0	32	30	32	35
24	3.23	2.0	20.7	9.3	17.8	20.3	39	50	37	38
26	3.82	6.2	25.4	25.1	26.1	29.2	34	34	33	37
27	3.70	3.6	23.6	21.5	15.7	16.3	37	37	30	38
28	3.00	3.2	22.4	19.6	22.5	26.5	30	24	23	29
29	4.70	9.8	26.4	23.0	18.4	25.0	31	38	33	32
30	4.12	5.0	22.4	19.6	17.2	15.9	33	34	33	34
Mean (±SE)	**3.86 (0.14)**	**4.3 (0.5)**	**24.9 (0.8)**	**21.7 (1.2)**	**21.1 (1.5)**	**24.1 (1.6)**	**36.3 (1.6)**	**36.0 (1.7)**	**33.7 (1.2)**	**34.4 (1.0)**

Note: Missing values (-) recorded following death.

Appendix 8: Urinary lactulose and rhamnose recoveries for 30 dogs with parvoviral enteritis.

Missing values are due to: ***V*** *= Vomiting;* ***U*** *= Urinating;* ***F*** *= Fecal contamination of the urine sample;* ***NS*** *= No sample collected (due to persistent urinating); or* ***DIED*** *= death.*

	Urinary Lactulose Recovery (Percentage of orally administered dose; %L)				**Urinary Rhamnose Recovery (Percentage of orally administered dose; %R)**			
Reference range	*1.5-5.8%*				*17.3-42.6%*			
Day	**1**	**2**	**4**	**6**	**1**	**2**	**4**	**6**
NPO: Dog #								
1	16.04	14.94	17.39	2.49	13.27	9.13	2.07	0.17
3	V	2.80	U	13.09	V	1.74	U	1.07
5	V	V	10.34	22.06	V	V	2.33	1.66
6	10.14	9.80	11.79	16.02	6.69	4.48	1.83	1.34
9	V	11.17	U	24.01	V	5.23	U	3.51
12	U	U	U	U	U	U	U	U
14	F	8.68	20.30	17.00	F	2.15	2.54	1.62
15	V	V	NS	14.85	V	V	NS	3.28
17	16.33	19.42	36.75	45.82	1.81	1.15	2.73	1.56
18	4.94	V	21.87	6.68	2.94	V	19.15	0.95
19	5.38	DIED	DIED	DIED	5.10	DIED	DIED	DIED
21	21.16	21.16	12.81	U	15.67	15.67	1.04	U
22	F	DIED	DIED	DIED	F	DIED	DIED	DIED
23	V	7.70	21.78	48.10	V	6.16	2.51	5.20
25	2.84	21.43	19.52	37.09	4.73	11.48	4.05	4.68
Mean (±SE)	**10.98 (2.64)**	**13.01 (2.20)**	**19.17 (2.64)**	**22.50 (4.56)**	**7.17 (1.99)**	**6.35 (1.63)**	**4.25 (1.88)**	**2.28 (0.49)**
EEN: Dog #								
2	V	7.80	U	1.52	V	5.38	U	0.72
4	44.49	V	11.34	21.72	39.16	V	4.33	2.19
7	NS	V	33.09	24.26	NS	V	30.19	12.05
8	V	26.26	11.39	U	V	3.66	0.88	U
10	NS	V	V	32.22	NS	V	V	6.96
11	U	6.94	24.06	22.35	U	3.04	2.85	1.12
13	U	V	8.21	17.16	U	V	5.13	1.91
16	17.55	29.10	41.63	26.16	6.47	6.76	5.66	1.86
20	7.47	3.14	15.94	U	4.56	11.82	1.21	U
24	NS	NS	14.60	7.87	NS	NS	16.44	4.41
26	5.10	12.91	22.35	20.44	4.40	5.93	3.00	1.23
27	V	27.85	U	13.12	V	16.29	U	1.64
28	NS	25.73	17.87	18.12	NS	16.90	2.98	0.51
29	38.43	25.01	12.92	17.80	29.15	17.29	2.89	0.40
30	U	33.32	21.78	10.44	U	26.59	10.25	5.73
Mean (±SE)	**22.60 (8.03)**	**19.80 (3.45)**	**19.60 (2.80)**	**17.94 (2.27)**	**16.75 (7.29)**	**11.37 (2.44)**	**7.15 (2.44)**	**3.13 (0.94)**

Appendix 9: Urinary lactulose: rhamnose ratios for 30 dogs with parvoviral enteritis

Missing values are due to: ***V*** *= Vomiting;* ***U*** *= Urinating;* ***F*** *= Fecal contamination of the urine sample;* ***NS*** *= No sample collected (due to persistent urinating); or* ***DIED*** *= death.*

	Urinary L/R_{rec} ratio				**Urinary L/R_{iso} ratio**			
Reference range	*0.10 ± 0.01 (mean ± SE)*				*0.10 ± 0.01 (mean ± SE)*			
Day	**1**	**2**	**4**	**6**	**1**	**2**	**4**	**6**
NPO: Dog #								
1	1.21	1.64	8.4	14.83	1.21	1.64	8.4	14.83
3	V	1.61	U	12.2	3	1.61	4.5	12.2
5	V	V	4.44	13.29	1.14	0.94	4.44	13.29
6	1.52	2.19	6.44	11.92	1.52	2.19	6.44	11.92
9	V	2.14	U	6.84	0.88	2.14	6.27	6.84
12	U	U	U	U	6.93	7.83	4.63	5.05
14	F	4.04	8	10.51	F	4.04	8	10.51
15	V	V	NS	4.52	3.13	1.32	NS	4.52
17	9	16.89	13.45	29.42	9	16.89	13.45	29.42
18	1.68	V	1.14	7.02	1.68	0.8	1.14	7.02
19	1.05	DIED	DIED	DIED	1.05	DIED	DIED	DIED
21	1.35	1.35	12.33	U	1.35	1.35	12.33	6.25
22	F	DIED	DIED	DIED	F	DIED	DIED	DIED
23	V	1.25	8.69	9.25	0.89	1.25	8.69	9.25
25	0.6	1.87	4.81	7.92	0.6	1.87	4.81	7.92
Mean (±SE)	**2.34 (1.12)**	**3.66 (1.68)**	**7.52 (1.29)**	**11.61 (2.01)**	**2.49 (0.72)**	**3.38 (1.24)**	**6.93 (1.01)**	**10.70 (1.80)**
EEN: Dog #								
2	V	1.45	U	2.11	0.82	1.45	1.28	2.11
4	1.14	V	2.62	9.92	1.14	1.14	2.62	9.92
7	NS	V	1.1	2.01	NS	0.99	1.1	2.01
8	V	7.18	13	U	3.15	7.18	13	3.71
10	NS	V	V	4.63	NS	1.48	1.5	4.63
11	U	2.28	8.45	20	1.88	2.28	8.45	20
13	U	V	1.6	9	1.33	0.91	1.6	9
16	2.71	4.31	7.36	14.07	2.71	4.31	7.36	14.07
20	1.64	0.27	13.16	U	1.64	0.27	13.16	17.39
24	NS	NS	0.89	1.78	NS	NS	0.89	1.78
26	1.16	2.18	7.45	16.63	1.16	2.18	7.45	16.63
27	V	1.71	U	8	1.17	1.71	5.88	8
28	NS	1.52	6	35.5	NS	1.52	6	35.5
29	1.32	1.45	4.47	44.5	1.32	1.45	4.47	44.5
30	U	1.25	2.13	1.82	1.11	1.25	2.13	1.82
Mean (±SE)	**1.59 (0.29)**	**2.36 (0.63)**	**5.69 (1.25)**	**13.07 (3.73)**	**1.58 (0.22)**	**2.01 (0.47)**	**5.13 (1.07)**	**12.74 (3.29)**

Appendix 10: Log_e-transformations of urinary lactulose: rhamnose ratios for 30 dogs with parvoviral enteritis

Missing values are due to: ***V*** *= Vomiting;* ***U*** *= Urinating;* ***F*** *= Fecal contamination of the urine sample;* ***NS*** *= No sample collected (due to persistent urinating); or* ***DIED*** *= death.*

	Log_e (urinary L/R_{rec})				Log_e (urinary L/R_{iso})			
Day	**1**	**2**	**4**	**6**	**1**	**2**	**4**	**6**
NPO: Dog #								
1	0.19	0.49	2.13	2.70	0.19	0.49	2.13	2.70
3	V	0.48	U	2.50	1.10	0.48	1.50	2.50
5	V	V	1.49	2.59	0.13	-0.06	1.49	2.59
6	0.42	0.78	1.86	2.48	0.42	0.78	1.86	2.48
9	V	0.76	U	1.92	-0.13	0.76	1.84	1.92
12	U	U	U	U	1.94	2.06	1.53	1.62
14	F	1.40	2.08	2.35	F	1.40	2.08	2.35
15	V	V	NS	1.51	1.14	0.28	NS	1.51
17	2.20	2.83	2.60	3.38	2.20	2.83	2.60	3.38
18	0.52	V	0.13	1.95	0.52	-0.22	0.13	1.95
19	0.05	DIED	DIED	DIED	0.05	DIED	DIED	DIED
21	0.30	0.30	2.51	U	0.30	0.30	2.51	1.83
22	F	DIED	DIED	DIED	F	DIED	DIED	DIED
23	V	0.22	2.16	2.22	-0.12	0.22	2.16	2.22
25	-0.51	0.63	1.57	2.07	-0.51	0.63	1.57	2.07
Mean (±SE)	**0.45 (0.32)**	**0.88 (0.27)**	**1.84 (0.25)**	**2.33 (0.15)**	**0.56 (0.23)**	**0.76 (0.24)**	**1.78 (0.19)**	**2.24 (0.14)**
EEN: Dog #								
2	V	0.37	U	0.75	-0.20	0.37	0.25	0.75
4	0.13	V	0.96	2.29	0.13	0.13	0.96	2.29
7	NS	V	0.10	0.70	NS	-0.01	0.10	0.70
8	V	1.97	2.56	U	1.15	1.97	2.56	1.31
10	NS	V	V	1.53	NS	0.39	0.41	1.53
11	U	0.82	2.13	3.00	0.63	0.82	2.13	3.00
13	U	V	0.47	2.20	0.29	-0.09	0.47	2.20
16	1.00	1.46	2.00	2.64	1.00	1.46	2.00	2.64
20	0.49	-1.31	2.58	U	0.49	-1.31	2.58	2.86
24	NS	NS	-0.12	0.58	NS	NS	-0.12	0.58
26	0.15	0.78	2.01	2.81	0.15	0.78	2.01	2.81
27	V	0.54	U	2.08	0.16	0.54	1.77	2.08
28	NS	0.42	1.79	3.57	NS	0.42	1.79	3.57
29	0.28	0.37	1.50	3.80	0.28	0.37	1.50	3.80
30	U	0.22	0.76	0.60	0.10	0.22	0.76	0.60
Mean (±SE)	**0.41 (0.16)**	**0.56 (0.27)**	**1.39 (0.27)**	**2.04 (0.31)**	**0.38 (0.12)**	**0.43 (0.20)**	**1.28 (0.24)**	**2.05 (0.28)**

Appendix 11: Fecal α_1-proteinase inhibitor concentrations in 10 healthy dogs originating from the Onderstepoort area

	Age (weeks)	Breed	Sex	Fecal α_1-proteinase inhibitor concentration (µg/g dry matter)
Normal puppies				
N1	12	Boerboel	M	91.8
N2	24	Rottweiler	M	42.6
N3	14	Bull Mastiff	F	37.4
N4	12	Staffordshire terrier	M	31.3
N5	12	Boxer	M	34.3
N6	16	Dachshund	F	30.3
N7	21	Crossbreed	F	21.8
N8	16	Boxer	M	26.2
N9	16	Boxer	M	41.3
N10	12	Border collie	M	89.6
Mean (±SD)	-	-	-	**44.7 (25.1)**
2.5th percentile	-	-	-	**22.8**
97.5th percentile	-	-	-	**80.2**

M = Male; F = Female.

Appendix 12: Fecal α_1-proteinase inhibitor concentrations in 30 dogs with parvoviral enteritis

	Fecal α_1-proteinase inhibitor concentration (µg/g dry matter)			
Day	**1**	**2**	**4**	**6**
NPO				
Dog #				
1	294.7	342.0	334.7	58.0
3	174.7	47.3	74.3	7.8
5	187.3	263.7	210.7	15.0
6	268.7	23.7	66.3	8.0
9	255.0	220.3	175.3	129.7
12	173.0	31.0	74.7	94.0
14	57.7	18.7	4.7	2.0
15	256.7	154.0	73.7	95.0
17	82.7	61.7	135.7	45.3
18	444.0	140.7	123.7	27.3
19	161.3	161.7	-	-
21	292.2	109.7	174.7	49.9
22	450.7	-	-	-
23	241.3	241.7	106.3	39.9
25	113.3	156.6	33.7	171.0
Mean (±SE)	**230.2 (29.6)**	**140.9 (26.7)**	**122.2 (24.1)**	**57.2 (14.3)**
EEN				
Dog #				
2	435.0	512.3	446.7	83.0
4	338.3	515.0	347.7	63.0
7	240.0	329.3	220.7	253.0
8	130.7	149.3	68.0	133.0
10	174.3	133.7	122.7	137.0
11	44.3	45.0	35.0	14.7
13	100.3	413.3	253.7	73.0
16	81.0	77.0	19.6	20.0
20	39.0	32.7	12.2	19.0
24	224.7	403.7	381.7	246.0
26	48.0	34.0	61.3	24.1
27	211.7	203.3	114.1	82.7
28	225.7	103.7	54.3	34.3
29	198.3	115.7	83.7	52.7
30	612.3	235.3	80.6	282.7
Mean (±SE)	**206.9 (40.8)**	**220.2 (44.4)**	**153.5 (36.6)**	**101.2 (23.5)**

Note: Missing values (-) recorded following death.