Effect of Early Enteral Nutrition on Intestinal Permeability, Intestinal Protein Loss, and Outcome in Dogs with Severe Parvoviral Enteritis

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A randomized, controlled clinical trial investigated the effect of early enteral nutrition (EN) on intestinal permeability, intestinal protein loss, and outcome in parvoviral enteritis. Dogs were randomized into 2 groups: 15 dogs received no food until vomiting had ceased for 12 hours (mean 50 hours after admission; NPO group), and 15 dogs received early EN by nasoesophageal tube from 12 hours after admission (EEN group). All other treatments were identical. Intestinal permeability was assessed by 6-hour urinary lactulose (L) and rhamnose (R) recoveries (%L, %R) and L/R recovery ratios. Intestinal protein loss was quantified by fecal α_1-proteinase inhibitor concentrations (α_1-PI). Median time to normalization of demeanor, appetite, vomiting, and diarrhea was 1 day shorter for the EEN group for each variable. Body weight increased insignificantly from admission in the NPO group (day 3: 2.5 ± 2.8%; day 6: 4.3 ± 2.3%; mean ± SE), whereas the EEN group exhibited significant weight gain (day 3: 8.1 ± 2.7%; day 6: 9.7 ± 2.1%). Mean urinary %L was increased, %R reduced, and L/R recovery ratios increased compared to reference values throughout the study for both groups. Percent lactulose recovery decreased in the EEN group (admission: 22.6 ± 8.0%; day 6: 17.9 ± 2.3%) and increased in the NPO group (admission: 11.0 ± 2.6%; day 6: 22.5 ± 4.6%, *P* = .035). Fecal α_1-PI was above reference values in both groups and declined progressively. No significant differences occurred for %R, L/R ratios, or α_1-PI between groups. Thirteen NPO dogs and all EEN dogs survived (*P* = .48). The EEN group showed earlier clinical improvement and significant weight gain. The significantly decreased %L in the EEN versus NPO group might reflect improved gut barrier function, which could limit bacterial or endotoxin translocation.

Key words: Alpha$_1$-proteinase inhibitor; Canine; Gut barrier function; Lactulose; Rhamnose.

Disease caused by canine parvovirus (CPV) affects more than a million dogs per year in the United States.[1] Parvoviral infection is characterized by severe enteritis, anorexia, vomiting, hemorrhagic diarrhea, and shock.[2] The published fatality rate is 16–35%,[3] although intensive therapy has achieved survival rates of up to 85–96%.[1,2] Treatment is primarily supportive and symptomatic. Novel adjunctive drugs have been investigated, but results have been disappointing or variable.[1,4–6] There is a distinct need for therapies that decrease disease severity and hospitalization time, improve survival, and reduce treatment cost.[1,7]

Despite the lack of controlled clinical studies, conventional wisdom has dictated that "gut rest," achieved by allowing no ingestion of food, remains the nutritional therapy of choice for CPV enteritis. The recommended duration of starvation ranges from 24 to 72 hours after vomiting has ceased.[2,3,8]

Canine parvovirus exhibits tropism for rapidly replicating cell populations of the intestinal crypt epithelium and lymphoid and hematopoietic tissues.[9] Small intestinal viral proliferation causes extensive epithelial necrosis with villus blunting and atrophy.[2,9] Lymphoid necrosis and atrophy occurs in gut-associated and systemic lymphoid tissues.[9] Bacteremia,[10,11] endotoxemia,[1,7,10] high serum tumor necrosis factor concentrations,[7] and multiple organ dysfunctions[11] occur frequently. The disruption of gut barrier function in CPV enteritis likely underlies bacterial and endotoxin translocation, resultant bacteremia and endotoxemia, and development of the systemic inflammatory response and multiple organ dysfunction syndromes.

Critical illnesses associated with gut barrier dysfunction, bacteremia, endotoxemia, systemic inflammatory response syndrome, and multiple organ dysfunction syndrome include severe acute pancreatitis,[12] inflammatory and noninflammatory bowel disease,[13] severe burn injury,[14] multisystem trauma,[15,16] and high-risk surgery.[17] The nutritional management of all these disorders has traditionally consisted of an initial period of starvation, ranging from 3 to 7 days.[12,13,15,17] However, the most important stimulus for intestinal mucosal growth, repair, and integrity is the presence of nutrients within the gut lumen.[18,19] The absence of luminal nutrients leads to marked small intestinal mucosal atrophy and suppressed crypt cell proliferation,[16,20,21] marked reductions in gut-associated lymphoid tissue cell mass and function,[20] increased intestinal permeability to bacteria and toxins,[22] and enhanced pro-inflammatory cytokine generation and acute-phase responses.[16]

Early enteral nutrition (EN) is superior to either starvation or total parenteral nutrition (TPN) in critical illnesses associated with gut barrier dysfunction. Documented benefits of early EN include reduced intestinal mucosal permeability[13,18,23]; increased weight[14] and motility; reduced incidence of bacteremia,[14] endotoxemia,[12] and septic morbidity[12,15–17]; attenuation of the acute-phase response[12] and reduced incidence of multiple organ failure[12,23]; improved immunological status[17]; reduced catabolism and preservation of a positive nitrogen balance[13–16]; and improved clin-

ical outcome.[12,23] Significantly higher survival also was recently documented in dogs and cats receiving EN in addition to partial parenteral nutrition, as compared to parenteral nutrition alone.[24] Evidence underscoring the benefits of early EN 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 or starvation whenever possible.[25]

Intestinal permeability and epithelial integrity can be noninvasively assessed by differential sugar intestinal permeability tests. The underlying principle involves the passive, non–carrier-mediated transmucosal diffusion of sugars of different sizes administered PO with their subsequent excretion and quantification in urine.[26–29] Intestinal permeability to a sugar is an inverse function of its cross-sectional diameter.[30] The permeation of rhamnose (molecular diameter 8.3 angstroms, molecular weight 164 daltons) in healthy dogs is 7- to 13-fold greater than that of lactulose (9.5 Å, 342 daltons).[31,32] Because the sugars traverse the epithelium by different pathways and in differing amounts, their urinary recoveries provide information about intestinal structure and integrity. Lactulose and rhamnose are widely accepted markers of intestinal permeability in dogs and humans. Many intestinal diseases are accompanied by an increased permeation of lactulose and a decreased permeation of rhamnose. Alpha$_1$-proteinase inhibitor (α_1-PI) is a plasma protein, of which concentration in feces provides a sensitive and specific quantitative measure of gastrointestinal protein loss in diseases with transmucosal loss of plasma, lymph, or intercellular fluid.[33]

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, intestinal protein loss, and clinical outcome in naturally occurring severe CPV enteritis.

Materials and Methods

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 sex, 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. The attending veterinarian was blinded as to which treatment group the dog would be assigned. The diagnosis of CPV infection was confirmed by electron microscopy of feces.

Dogs were required to have no evidence of concurrent coronavirus infection on fecal electron microscopy, of coccidial oocysts on fecal hyperosmolar sugar flotation, and of 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 sulfate flotation tests on the first 2 days of hospitalization.

The Research and Ethics Committees of the University of Pretoria approved the study, and written consent was obtained from all dogs' owners.

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. After admission (day 1), all dogs were rehydrated over 6 hours with lactated Ringer's solution[a] with added dextrose[b] (final concentration 2.5%) and potassium chloride[c] (20 mEq/L). Further maintenance fluid requirements were met with crystalloid fluids (Electrolyte no. 2 with 5% glucose)[d] and potassium chloride added according to deficits.[2] Volumes of fluids administered IV were individualized for each patient on the basis of clinical assessment.

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

Plasma transfusions (20 mL/kg) were administered if serum albumin decreased below 1.5 g/dL and the dog deteriorated clinically. Hydroxyethyl starch[j] boluses (5–20 mL/kg) were administered IV if adequate crystalloid resuscitation failed to correct shock.

Nutritional Groups

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

NPO Group. Fifteen dogs were starved (nothing PO; NPO group) until vomiting had ceased for 12 hours, after which small amounts of a low-fat diet[k] were offered 6 times per day. Dogs that refused to eat this diet voluntarily after a period of 12 hours were then force-fed small amounts 6 times per day. Water was provided ad libitum throughout.

EEN Group. Fifteen dogs received early enteral nutrition (EEN group) beginning 12 hours after admission. A nasoesophageal feeding tube was placed in the distal 3rd of the esophagus, and a lateral cervicothoracic 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[l] was fed, formulated as a suspension for tube feeding during critical illness. The diet contained 41% intact proteins, 18% fat, and 3% crude fiber on a dry matter basis. The quantity of food to be administered was calculated by multiplying the manufacturer's recommended quantity by an illness factor of 1.5. One third of this amount was fed on day 1, two thirds on day 2, and the full volume from day 3 onward. The suspension was diluted to approximate isosmolality (by reconstituting 47 g of the powder with 200 mL of water, instead of the recommended 100 mL). The feeding tube was removed when the dog had not vomited for 24 hours. 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 libitum 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).

Clinical Scoring

A daily scoring system was applied whereby clinical variables (ie, general attitude and appetite and the severity of vomiting and diarrhea) were awarded numerical values to semiquantify clinical disease severity (Table 1). A daily composite clinical score was calculated as the sum of the above 4 scores. The primary investigator (AJM) awarded all scores.

Body weight was determined daily, and serum albumin concentrations were measured on days 1, 2, 4, and 6.

Table 1. Daily clinical scoring system used to semiquantify clinical disease severity in dogs with parvoviral enteritis.

Scoring Categories	Numerical Score			
	0	1	2	3
General attitude	Normal	Mild to moderate depression	Severe depression	Collapsed or moribund
Appetite[a]	Normal	Voluntarily eats small amounts	No interest in food	N/A
Vomiting	Absent	Mild; once per 12 hours	Moderate; 2–5 times per 12 hours	Severe; ≥6 times per 12 hours
Feces	Well-formed	Soft or pasty feces	Watery diarrhea, non bloody	Watery, bloody diarrhea
Composite clinical score	Sum of the above 4 scores			

N/A, not applicable.

[a] Appetite scores in both the no food and early enteral nutrition groups were based on the dogs' interest in eating a small amount of the low-fat diet offered.

Intestinal Permeability Testing

Intestinal permeability was assessed by differential sugar recovery of lactulose and L-rhamnose. The 1st test dose was administered as soon as the dogs were rehydrated (approximately 6 hours after admission), whereas dosing on days 2, 4, and 6 was performed in the mornings. The test solution was formulated immediately before each dosing by dissolving lactulose[m] and rhamnose[n] 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 3 mL/kg body weight. All food was withheld as previously described. Immediately before 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, rather than through the feeding tube in the EEN dogs, to ensure consistency between groups. 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 after dosing PO. Any vomiting or urination during the 6-hour period was recorded. All urine collected over the 6 hours was pooled, and the total volume was recorded. A 10-mL aliquot was stored at −80°C after the addition of sodium azide[o] (10 μL of a 10% solution) as preservative. Urine samples were batched and transported on dry ice to the Gastrointestinal (GI) Laboratory at Texas A&M University for analysis by high-pressure liquid chromatography[p,q,r] with pulsed amperometric detection,[s] as described.[34] The laboratory was blinded as to which treatment group the samples originated from. Urinary recoveries of lactulose (%L) and rhamnose (%R) were expressed as a percentage of dose administered PO, and the urinary lactulose to rhamnose recovery ratio (L/R) was calculated. Dogs that vomited or urinated during the urine collection period were censored from %L, %R, and L/R calculations.

Fecal α₁-Proteinase Inhibitor Concentration

Fecal samples (~2 g) were collected on days 1, 2, 4, and 6; weighed; and frozen at −20°C. In order to more accurately compare fecal α_1-PI concentrations between samples of differing water content, α_1-PI concentrations were expressed on a dry matter basis. For this purpose, specimens were lyophilized[t] before transportation on dry ice to the GI Laboratory at Texas A&M University for analysis. The laboratory was blinded as to which treatment group the samples originated from. Dry fecal samples were homogenized (with a mortar), and a representative amount was reconstituted with water. Quantification of α_1-PI was performed by enzyme-linked immunosorbent assay according to described methodology.[33] Fecal α_1-PI concentrations were finally expressed on a dry matter basis.

Statistical Analysis

Data were analyzed with the assistance of a biostatistician and standard statistical software,[u] and graphs were plotted with a statistical software package.[v] Categorical variables (general attitude, appetite, vomiting, and fecal scores) were compared between the 2 treatment groups (NPO versus EEN) within days by 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 nonnormally 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, serum albumin concentration, %L, %R, fecal α_1-PI, and $\log_e$ (ln) values of L/R. The L/Rs were ln-transformed because of nonnormally distributed data sets. Data sets for %L, %R, and L/R were incomplete because of vomiting or urinating during permeability testing, so responses over time for the continuous variables were compared between the 2 treatment groups with generalized estimation equations (GEE). GEE modeling describes repeated measures (ie, time series data) and is specifically appropriate for unbalanced designs and incomplete data sets (ie, missing values in the time series).[35] Mortality between groups was compared by Fisher's exact test. Significance for all analyses was defined as $P < .05$.

Results

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 sex (NPO: 7 males, 8 females; EEN: 9 males, 6 females).

At admission, there were no significant differences between groups for general attitude, appetite, vomiting, fecal scores, or the composite clinical scores. Admission scores ranged from 0 to 3 for vomiting (median 2 for both groups), was 2 for all dogs for appetite, ranged from 2 to 3 for feces (median 3 for both groups), ranged from 1 to 2 for the NPO group and 1 to 3 for the EEN group for general attitude (median 1 for NPO and 2 for EEN), and ranged from 5 to 10 for the NPO group and 6 to 11 for the EEN group for clinical score (median 7 for NPO and 9 for EEN).

Appetite improved significantly faster in the EEN group compared to the NPO group on day 2 ($P = .02$). No significant differences were detected between groups in the other categorical variables. However, the median time taken to the normalization of general attitude and appetite and the resolution of vomiting and diarrhea were consistently 1 day shorter for the EEN group. This was reflected in a significant improvement in the composite clinical score from ad-
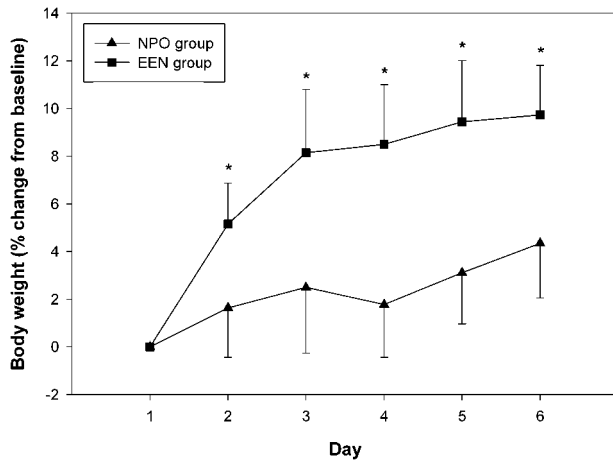
Fig 1. Body weight changes presented as percent change from base-line values for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Asterisks indicate significant differences from baseline. NPO, nothing PO; EEN, early enteral nutrition.

Fig 2. Serum albumin concentrations in 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Asterisks indicate significant differences from baseline values. NPO, nothing PO; EEN, early enteral nutrition.

mission values by day 2 in EEN ($P <$ .0001) versus day 3 in NPO ($P =$ .0005).

There was no significant difference in body weight between groups at admission. Body weight was significantly increased ($P <$ .003) from admission on all days in the EEN group, whereas no significant changes in body weight occurred in the NPO group (Fig 1). Groups did not behave significantly differently over time (GEE) for clinical score or body weight.

Two NPO dogs and 2 EEN dogs received hydroxyethyl starch treatment, and 1 EEN dog received a plasma transfusion (day 3) because of severe hypoalbuminemia (1.2 g/dL) and continued bloody diarrhea and vomiting. In the EEN group, syringe force-feeding was performed in 2 dogs; in 1 dog (a Dachshund), the tube could not pass through the nasal cavity, and another dog removed its tube on day 3.

Serum albumin concentration was not significantly different between groups at admission. Albumin decreased significantly from admission in both groups on days 2 (NPO: $P =$ .001; EEN: $P =$ .002) and 4 (NPO: $P =$ .01; EEN: $P =$ .009), followed by significant increases on day 6 (compared to values on day 4; NPO: $P =$ .0002; EEN: $P =$ .01; Fig 2). The 1 EEN dog that received a plasma transfusion on day 3 was excluded from albumin analyses thereafter. Serum albumin concentrations of groups were not significantly different over time.

There were no significant differences between groups at admission for %L, %R, or $\log_e$(L/R). Urinary recovery of lactulose as a percentage of dose administered PO (%L) was above the laboratory reference range (1.5–5.8%) throughout the study period for both groups (Fig 3). Lactulose recovery behaved significantly differently between treatment groups ($P =$ .035; GEE modeling), characterized by a progressive decrease in %L in the EEN group versus 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 higher on day 6 from day 2 values in the NPO group ($P =$ .04).

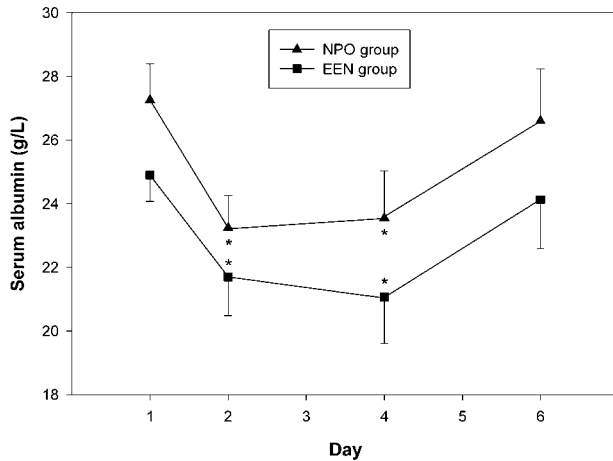Urinary recovery of rhamnose as a percentage of dose

administered PO (%R) was reduced below the reference range (17.3–42.6%) throughout the study period in both groups (Fig 4). Rhamnose recovery decreased progressively over time in both groups, although changes from admission values were not significant for either group. Rhamnose recoveries decreased significantly from day 2 values on days 4 ($P =$ .01) and 6 ($P =$ .005) in EEN dogs and on day 6 ($P =$ .05) in NPO dogs.

$\log_e$(L/R) increased significantly from admission in both groups on days 4 (NPO: $P =$ .004; EEN: $P =$ .04) and 6 (NPO: $P <$ .001; EEN: $P =$ .005; Fig 5). Groups did not behave significantly differently over time (GEE) for %R, or L/R.

There were no significant differences in fecal α_1-PI concentrations between groups at admission. Fecal α_1-PI concentrations were above the in-house reference range (determined in 10 clinically healthy puppies) for the majority
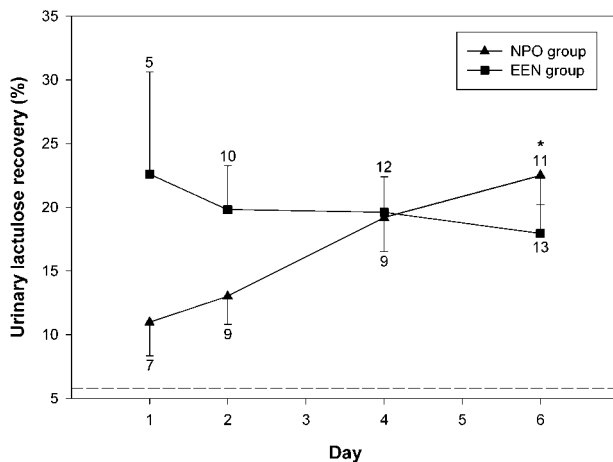

Fig 3. Urinary lactulose recoveries (%L) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. The asterisk indicates a significant difference from day 2 value (NPO group). Dashed line indicates upper limit of normal laboratory range (5.8%). NPO, nothing PO; EEN, early enteral nutrition.
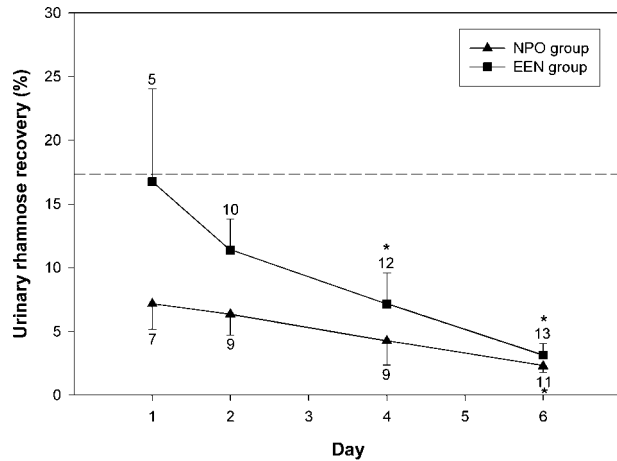
Fig 4. Urinary rhamnose recoveries (%R) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from day 2 values. Dashed line indicates lower limit of normal laboratory range (17.3%). NPO, nothing PO; EEN, early enteral nutrition.
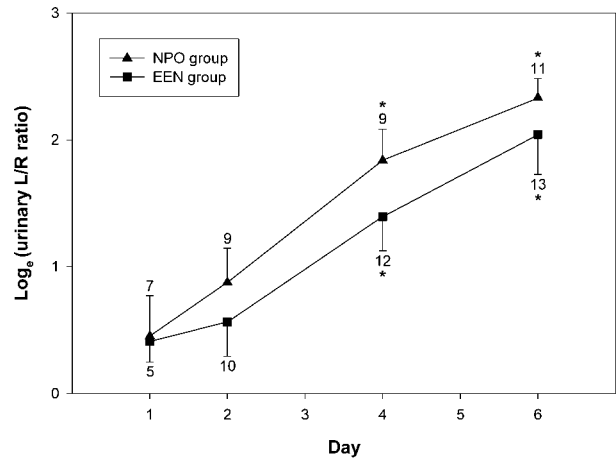

Fig 5. Log_e (urinary lactulose/rhamnose ratios) for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from baseline values. NPO, nothing PO; EEN, early enteral nutrition.

of the study period in both groups (Fig 6). Significant decreases in fecal α_1-PI concentrations from admission were observed on days 2 ($P = .03$), 4 ($P = .006$), and 6 ($P = .0004$) in the NPO group and on day 6 ($P = .006$) in the EEN group. The 1 EEN dog that received a plasma transfusion was not excluded from fecal α_1-PI analyses, in order to prevent bias in favor of EEN. Groups did not behave significantly differently over time (GEE) for fecal α_1-PI concentrations.

Thirteen of 15 dogs (87%) in the NPO group and all 15 dogs in the EEN group survived. This difference was not statistically significant.

Discussion

Early EN of dogs with severe CPV enteritis was associated with more rapid clinical improvement compared to withholding food during the early stages of the disease, as evidenced by the faster normalization of general attitude and appetite and the resolution of vomiting and diarrhea. The more rapid clinical improvement in dogs with early EN has the potential to reduce hospitalization time, expense, or both. Investigator bias cannot be excluded in the awarding of clinical scores for general attitude and appetite, but vomiting and fecal scores were more objective in character. The clinical scoring system proved to be practical and useful for assessing clinical disease severity in CPV enteritis.

The marked increase in body weight with early EN supports reduced catabolism. The prevention of protein-energy malnutrition in CPV infection could have important ramifications because malnutrition is associated with markedly increased intestinal inflammation and pro-inflammatory cytokine generation.[36] Although fluid therapy might have contributed to increased body weight in both groups, intravenous fluid therapy was based on the clinical assessment of hydration status, so the greater enteral fluid administration in the EEN group should not have led to greater weight

gains compared to the NPO group. Serum albumin concentration is an insensitive measure of nutritional status, and intestinal protein loss further limits its utility in this regard.

The precise transepithelial permeation pathways of probes used for intestinal permeability tests have not been established definitively.[27,28,37,38] According to the classical hypothesis, monosaccharides (eg, rhamnose) permeate transcellularly via small aqueous pores of high incidence in enterocyte cell membranes.[26,28,29] In contrast, disaccharides (eg, lactulose) are hypothesized to permeate paracellularly via larger aqueous channels of low incidence, located in the region of intercellular tight junctions.[26,28,29] The progressive decrease in %L in the EEN group, as compared to a continued increase in the NPO group, might thus reflect improved integrity of epithelial tight junctions attributable to
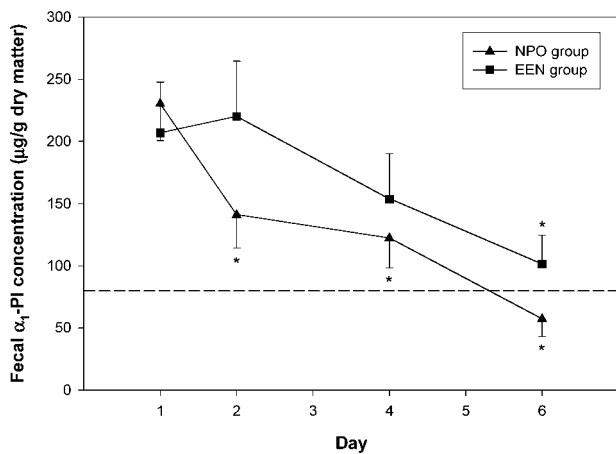

Fig 6. Fecal α_1-proteinase inhibitor (α_1-PI) concentrations for 30 dogs with parvoviral enteritis. Data are presented as mean with standard error. Numerical values indicate numbers of observations per group per time point. Asterisks indicate significant differences from baseline values. Dashed line indicates upper limit of the in-house reference range (80.2 μg/g). NPO, nothing PO; EEN, early enteral nutrition.

early EN. In part, this may be a result of decreased intestinal inflammation because pro-inflammatory cytokines (tumor necrosis factor-α and interferon-γ) impair tight junction structure and function.[39] Lactulose might also permeate paracellularly through a disrupted epithelium (ie, necrosis, ulceration, or erosion),[26,28,40,41] and the decreased %L in dogs with early EN could thus indicate earlier repair of intestinal epithelial necrosis. Earlier repair of epithelial necrosis or improved tight junction structure or function with early EN potentially might have improved gut barrier function with decreased transmucosal passage of luminal compounds. Translocation of bacteria, endotoxin, or luminal antigens could locally intensify intestinal inflammation[19,42] or systemically initiate the systemic inflammatory response syndrome and the multiple organ dysfunction syndrome.[42] Because intestinal inflammation,[19,42] endotoxemia,[43] and the systemic inflammatory response syndrome[19] might further increase intestinal permeability, a reduction in any of these events could limit further gut barrier compromise. The decreased %L with early EN could also indicate earlier intestinal flora normalization because small intestinal bacterial overgrowth, known to occur in CPV enteritis,[44] increases intestinal permeability.[45]

The decreased %R in both treatment groups is consistent with villus atrophy, with reduced surface area for rhamnose permeation.[26,28,40] Further %R decreases over time in both groups might reflect progressive villus atrophy, intestinal ischemia or congestion,[38,46] or altered immature enterocyte membrane phospholipid composition and transcellular membrane pores.[28]

Failure to demonstrate a difference between groups in L/R ratio changes over time, even though lactulose permeations differed significantly, could be a result of small patient numbers and the large interindividual variability of this parameter within data points. By expressing the urinary sugar recoveries as a ratio, factors unrelated to mucosal permeability (eg, vomiting, gastrointestinal motility, glomerular filtration rate, and others) are excluded because both markers should be equally affected.[26–28] Differences in gastrointestinal motility between groups might thus potentially have contributed to the differing lactulose permeations. It is of interest that the highest L/R ratios in both groups occurred at the time of discharge from hospital, when intestinal morphological integrity would be expected to be most normal. This brings to question whether L/R ratios accurately reflect the severity of intestinal epithelial disruption in CPV enteritis. There is general agreement that lactulose permeation is an appropriate measure of the functional or physical gut barrier,[26,28,29,40,41] whereas rhamnose permeation can be affected by mucosal factors not directly related to intestinal integrity.[26,28,38,40,46] The individual sugar recoveries could thus provide more specific information relating to epithelial integrity and structure.

There were no significant differences between the declines over time for fecal α1-PI concentrations for the NPO and EEN groups. Although α1-PI decreased more slowly in the EEN group, this decline was not accompanied by a concomitantly greater decrease in serum albumin concentrations, as compared to NPO. The ability of enteral nutrients to stimulate increased intestinal blood flow,[18] could in part account for the slower decline of intestinal protein loss

in the EEN group. The increase in %L over time in the NPO group, in combination with the decreased fecal α1-PI loss over time, might indicate that the continued increase in lactulose permeation in the NPO group occurred primarily through altered tight junctions, rather than through a disrupted epithelium because the latter would be expected to be associated with greater intestinal protein loss.

The higher survival in the EEN versus the NPO group was not statistically significant. However, we are unaware of any previous studies documenting 100% survival with any therapeutic regimen in severe, naturally occurring CPV enteritis. Dogs in the initial NPO group were force-fed from an earlier time than is traditionally advised, and enteral nutrient administration in the EEN group was interrupted for 8 hours on the days of intestinal permeability testing. Had this not been the case, observed differences between groups might potentially have been more pronounced. Two of the NPO dogs were Rottweilers, a breed with reported susceptibility to more severe CPV disease, whereas there were no Rottweilers in the EEN group. One of the NPO group mortalities was a Rottweiler, which might have been related to more severe disease, rather than being attributable to the NPO treatment. The remaining Rottweiler had less severe CPV disease.

Enteral tube feeding was not associated with notable complications. High volumes of gastric residual gas and food secondary to ileus, a frequent complication of tube feeding in humans, can be relieved by aspirating gastric contents through the nasogastric tube before EN administration.[47] We detected moderate gastric tympany in 2 EEN dogs, and nasogastric tubes might thus be preferable in CPV enteritis. Because of vomiting, the amount of EN that effectively reached the intestine in the EEN group is unknown. Some studies suggest that at least 25% of total daily caloric requirements should be delivered enterally to prevent intestinal mucosal atrophy.[20,21]

This study demonstrates that early EN can be instituted successfully in CPV enteritis, even with severe vomiting and diarrhea. The significant weight gain in the EEN group indicates at least partially efficient nutrient digestion and absorption. It has been noted that CPV provides a suitable animal model for human sepsis research.[1] This study supports the use of early EN in gut barrier dysfunction.

Further studies are required to determine whether early EN in CPV enteritis reduces the incidence of endotoxemia, bacteremia, or the systemic inflammatory response syndrome. Additional trials should investigate the optimal dietary composition for CPV enteritis. Enteral diets containing intact proteins or peptides (ie, the test diet in this study) might stimulate intestinal mucosal growth to a greater degree than do free amino acids.[48] However, because undigested proteins can be absorbed in increased quantities during acute gastroenteritis,[49] feeding intact proteins might produce a breach in oral tolerance, with resultant intestinal inflammation or hypersensitivity responses. The addition of dietary nonfermentable insoluble fiber (eg, cellulose) could further decrease bacterial translocation.[50,51] 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 and

length of hospitalization, as compared to standard EN diets in human critical illness and sepsis.[52] Such formulations might be beneficial in CPV enteritis. Furthermore, combined EN and parenteral nutrition[24] could yield optimal results.

Footnotes

a Intramed Ringer-Lactate Solution, Fresenius Kabi, Port Elizabeth, South Africa

b Intramed Dextrose 50%, Fresenius Kabi, Port Elizabeth, South Africa

c Sabax Potassium Chloride, Adcock Ingram Critical Care, Johannesburg, South Africa

d Intramed Electrolyte No. 2, Fresenius Kabi, Port Elizabeth, South Africa

e Amoxil® injectable, SmithKline Beecham, Bergvlei, South Africa

f Clamoxyl® palatable tablets, Pfizer Animal Health, Sandton, South Africa

g Genta® 20 PHENIX Aqueous injectable solution, Logos Agvet, Halfway House, South Africa

h Clopamon®, Intramed, Randburg, South Africa

i Panacur® BS, Hoechst Roussel Vet Specialties, Halfway House, South Africa

j Haes-Steril® 10%, Fresenius Kabi, Midrand, South Africa

k Pedigree® Canine Low Fat Diet, Waltham, Melton Mowbray, UK

l Pedigree® Canine Concentration Instant Diet, Waltham, Melton Mowbray, UK

m Lactulose, Sigma, Atlasville, South Africa

n L-Rhamnose, Sigma, Atlasville, South Africa

o Sodium azide, Sigma, Atlasville, South Africa

p 717+ Autosampler, 625 pump, Waters Corp, Milford, MA

q Carbopac™ PA10 analytic and guard columns, DIONEX Corp, Sunnyvale, CA

r Duros CC-30-S-PK, ELDEX Laboratories, Napa, CA

s Electrochemical detector 464, Waters Corp, Milford, MA

t Modulyo lyophilizer, BOC Ltd, Crawley, UK

u Stata® Release 6, Stata Corporation, College Station, TX

v SigmaPlot for Windows v. 4.00, SPSS Inc., Chicago, IL

Acknowledgments

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