Effect of Parvoviral Enteritis on Plasma Citrulline Concentration in Dogs

O. Dossin, S.I. Rupassara, H.-Y. Weng, D.A. Williams, P.J. Garlick, and J.P. Schoeman

Background: Plasma citrulline concentration is a reliable marker of global enterocyte mass in humans and is markedly decreased in diffuse small intestinal diseases. However, the relationship between acute intestinal damage and plasma citrulline concentration in dogs has never been documented.

Hypothesis: That dogs with parvoviral enteritis have a lower plasma citrulline concentration than healthy dogs and that plasma citrulline concentration is a predictor of death in puppies with parvoviral enteritis.

Animals: Sixty-one dogs with spontaneous parvoviral enteritis and 14 healthy age-matched control dogs.

Methods: Observational cohort study. Plasma citrulline concentration was measured by liquid chromatography and tandem mass spectrometry in blood samples collected at admission and each day until death or discharge from the hospital. Parvovirus enteritis was confirmed by electron microscopy on a fecal sample.

Results: Median (interquartile range) plasma citrulline concentrations at admission were 2.8 μmol/L (range: 0.3, 49.0; P < .001 versus controls) in survivors (n = 49), 2.1 μmol/L (range: 0.5, 6.4, P < .001 versus controls) in nonsurvivors (n = 12) and 38.6 μmol/L (range: 11.4, 96.1) in controls (n = 14), respectively. There was no significant difference in plasma citrulline concentration between survivors and nonsurvivors within the parvovirus-infected puppies, and plasma citrulline concentration was not significantly associated with outcome in parvoviral enteritis. There were no significant changes in plasma citrulline concentration over the 8-day follow-up period.

Conclusion and Clinical Importance: Parvovirus enteritis is associated with a severe decrease in plasma citrulline concentration that does not appear to have any significant prognostic value.

Keywords: Amino acid; Blood; Canine; Enteroctye; Necrosis.

Canine parvoviral enteritis is a worldwide infectious disease that preferentially affects young dogs. The virus induces severe and extensive damage to the enterocytes. In affected dogs, lesions are characterized by extensive necrosis of the epithelial cells, with nearly complete loss of intestinal glands and collapse of the overlying villi. The histologic lesions parallel the presence of the virus in the enterocytes. Therefore, parvoviral enteritis can be considered as a spontaneous model of acute and extensive necrosis of small intestinal enterocytes in dogs.

Citrulline is a nonessential amino acid that is not associated with a known genetic codon. Thus, its presence in a protein is always the result of a posttranslational modification. Citrulline is primarily synthesized in 2 organs, the liver and the gut. However, the citrulline produced by the liver is locally metabolized in the urea cycle, whereas the intestinal citrulline is absorbed in the blood stream and distributed in the body. In humans, rats, and pigs, plasma citrulline is essentially derived from the production in the enterocytes from alimentary and plasma glutamine. In dogs, the intestine remains an important source of citrulline, although the liver might also contribute to the plasma citrulline concentration. In humans with short bowel syndrome or villous atrophy, plasma citrulline concentration is correlated to the length of the remaining small intestine or the severity of the intestinal lesions, respectively.

The purpose of this study was to measure plasma citrulline concentrations in dogs with parvoviral enteritis compared with healthy dogs.

We hypothesized that dogs with parvoviral enteritis have reduced plasma citrulline concentrations compared with healthy dogs, and that plasma citrulline could be used as a prognostic indicator in this disease.

Material and Methods

Animals

The dogs used in this study were enrolled at the Onderstepoort Veterinary Academic Hospital of the University of Pretoria for an unrelated study reviewed by the institutional animal use and care committee. Three groups of dogs were studied: survivors and nonsurvivors of parvovirus enteritis and healthy control dogs. Parvovirus-infected dogs were included in the study based on the following criteria: clinical presentation of acute severe diarrhea necessitating admission to the intensive care unit and diagnosis of parvoviral enteritis confirmed by the presence of viral particles in the feces on transmission electron microscopy. Concurrent Babesia spp. parasites and Ehrlichia spp. were excluded by blood smear evaluation. Dogs were excluded if they presented clinical signs of canine distemper (eg, conjunctival or nasal discharge or neurological signs) and if distemper viral particles were detected in the feces. A routine fecal panel to screen for ova, coccidia, and giardia was performed on each dog. Routine blood work, including a CBC and...
a chemistry panel, was performed on the day of admission in all the
dogs. The parvovirus-infected dogs were treated with a combination of
IV fluid therapy and IV administration of ampicillin\(^b\) and gent-
amicin.\(^b\) Buprenorphine\(^c\) was used to control abdominal pain, and
metoclopramide\(^d\) or ondansetron\(^*\) (in case of failure with meto-
clopramide) was administered to control vomiting. Dogs were
started on enteral feeding with a commercial diet\(^f\) soon after rehy-
dration was completed. Except for the amount of fluids and pain
medication and the antiemetic drugs, all the dogs received the same
administration. The dogs were considered as survivors on the day of dis-
charge if they were free of clinical signs. Dogs that died during their
hospital stay were classified as nonsurvivors. A 3rd group of age-
matched healthy puppies presented for routine vaccination were
used as controls. The dogs were considered as healthy based on his-

tory, physical examination, and routine blood work.

**Blood Sampling**

Blood samples were collected from the jugular vein into EDTA
tubes at admission before medical treatment. All the dogs were
fasted for at least 8 hours before sampling the blood at admission.
In parvovirus-infected dogs, blood samples were also collected ev-
every day until discharge or death. These dogs were fed through an
indwelling naso-esophageal tube after the day of admission. There-
fore, they were not fasted for the follow-up time points. The blood
was immediately centrifuged at +4°C and the plasma harvested and
stored at —80°C until analyzed.

**Citrulline Assays**

Citrulline was measured in the EDTA plasma by liquid chroma-
tography and tandem mass spectrometry (LC-MS/MS). The plasma
samples were deproteinized with acetonitrile (ACN, used plasma/
ACN of 1/3 v/v) and vacuum dried.\(^a\) The samples were added with
100µL of 200µM norvaline as the internal standard and vacuum
dried again. The vacuum dried samples were derivatized with propyl
chlororormate (20 µL) as described by Uutela et al.\(^{13}\) The derivati-
ized samples were vacuum dried and redissolved in 50µL of 0.1%
aqueous formic acid/ACN (10/1 v/v), and analyzed by LC-MS/MS\(^b\)
with electro-spray ionization. The column\(^c\) was eluted with mobile
phase consisting of 10 mM ammonium formate in water (A) and
10mM ammonium formate in methanol (B) according to Leco Cor-
poration recommendations (Rapid Sample Preparation and LC-
leco.com/resources/application_note_sub/pdf/separation_science-296.pdf).\(^d\) The samples were run in positive ion mode and the
relative signal intensities of the compounds were used for quantifi-
cation. Concentrations of citrulline were estimated based on a
reference curve prepared by commercial standards.\(^e\)

The between day coefficients of variation (CV) in a series of 5
repeated measurements of the citrulline concentrations were 13.6,
11.7, and 8.7%, respectively, for low (1 µM), medium (20µM), and
high (50µM) ranges of the method. The within day CV on 5 re-
peated measurements were 12.5, 8.4, and 6.3%, respectively, for
low, medium, and high concentrations over the range of linearity.

**Statistics**

Sex and breed distributions were compared among the 3 study
groups by a \(\chi^2\)-test. Age and weight were compared between the
groups with Kruskal-Wallis and posthoc Dunn's tests. Clinicopat-
ologic data at admission were compared between the 2 groups of
parvovirus-infected dogs with a Mann and Whitney test. Plasma
citrulline concentrations were evaluated for normality of the distri-
bution by the Shapiro-Wilk test. The data were normally distributed
after log-transformation. Therefore, results are reported as median
and range.

A general linear model including age and weight was used to
compare the citrulline concentration among the 3 groups at admis-
sion. Posthoc pairwise comparisons were performed with a
Bonferroni adjustment. Cox proportional hazard model was used to
evaluate whether citrulline concentration on the day of admission
was associated with the hazard rate of dying. The dogs that were
discharged were considered as survivors at the day of discharge, and
the day of death was reported for the nonsurvivors. Age and weight
were also included as covariates in the Cox model. Only parvovirus-
infected dogs were included in the survival analysis. The duration of
the follow-up period for survivors and nonsurvivors were the inter-
val between hospital admission and discharge or death. At the day of
admission, correlations between plasma citrulline concentrations
and serum creatinine or albumin concentrations were analyzed by
Spearman's test in the parvovirus affected dogs group.

The follow-up plasma citrulline concentrations until discharge or
death were compared between the survivors and nonsurvivors using
a mixed model including time, outcome, and interaction between
time and outcome.

Statistical analyses were performed with commercial statistical
softwares\(^\text{17,18}\) and \(P < .05\) was considered significant.

**Results**

**Dogs**

The study sample was composed of 75 dogs, including
49 survivors, 12 nonsurvivors, and 14 healthy dogs. The
median ages were 4 months (range: 1, 9 months), 2.5
months (range: 2, 5 months), and 3 months (range: 2, 6
months) in the survivor, nonsurvivor, and healthy dogs,
respectively. There was no statistically significant differ-
ce in age among the 3 groups (\(P = .149\)). There were 29
females and 32 males in the study. The nonsurvivor
group was composed of 5 females and 7 males, whereas
24 females and 25 males were in the survivor group. The
control group was composed of 5 females and 9 males.
There was no significant difference in sex distribution
among the 3 groups (\(P = .653\)). The breed distribution
was not different among the groups. The survivor group
was composed of 6 Boerboels, 8 Dachshunds, 5 Jack
Russell Terriers, 18 others breeds, and 12 mixed-breed
dogs. The nonsurvivors were 1 Boerboel, 1 Dachshund, 2
Jack Russell Terriers, 5 other breeds, and 2 mixed-breed
dogs. In the control group, there were 4 Boerboels, 1
Dachshund, 2 Jack Russell Terriers, 2 other breeds, and 5
mixed-breed dogs. The median weight was 5.7 kg (range:
1.8, 44.0 kg) in the survivor group, 4.3 kg (range: 2.3,
6.3 kg) in the nonsurvivors group, and 4.3 kg (range: 2.0,
10.7 kg) in the healthy group (\(P = .045\), Kruskal-Wallis
test). The nonsurvivors had a significantly lower body
weight than did the survivors (\(P < .05\)). As shown in
Table 1, the nonsurvivors had lower total leucocytes,
bands, lymphocytes, and eosinophils counts when com-
pared with survivors on the CBC at the day of admission.
However, serum albumin, globulin, ALT, and creatinine
were not different between the 2 groups.

**Plasma Citrulline Concentrations**

There was a significant difference (\(P = .001\)) in plasma
citrulline concentration between infected and healthy
groups [survivors: 2.8 µmol/L (range: 0.3, 49.0), non-
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Table 1. Laboratory data in survivors and nonsurvivors.

<table>
<thead>
<tr>
<th></th>
<th>Survivors (n = 49)</th>
<th>Nonsurvivors (n = 12)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes (10⁹/L)</td>
<td>5.7 (0.4-23.6)</td>
<td>1.4* (0.3-16.5)</td>
<td>6.0-15.0</td>
</tr>
<tr>
<td>Neutrophils (10⁹/L)</td>
<td>2.9 (0.0-19.1)</td>
<td>0.4 (0.0-13.9)</td>
<td>3.0-11.5</td>
</tr>
<tr>
<td>Bands (10⁹/L)</td>
<td>0.4 (0.0-2.8)</td>
<td>0.1* (0.0-0.5)</td>
<td>0.0-0.5</td>
</tr>
<tr>
<td>Lymphocytes (10⁹/L)</td>
<td>0.8 (0.1-4.3)</td>
<td>0.5* (0.1-1.4)</td>
<td>1.0-4.8</td>
</tr>
<tr>
<td>Monocytes (10⁹/L)</td>
<td>0.6 (0.0-2.4)</td>
<td>0.4 (0.1-1.3)</td>
<td>0.15-1.35</td>
</tr>
<tr>
<td>Eosinophils (10⁹/L)</td>
<td>0.1 (0.0-1.4)</td>
<td>0.0* (0.0-0.1)</td>
<td>0.1-1.25</td>
</tr>
<tr>
<td>Basophils (10⁹/L)</td>
<td>0.0 (0.0-0.2)</td>
<td>0.0 (0.0-0.1)</td>
<td>0.0-0.1</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.38 (0.08-0.61)</td>
<td>0.36 (0.22-0.60)</td>
<td>0.27-0.56</td>
</tr>
<tr>
<td>Platelets (10⁹/L)</td>
<td>394 (134-898)</td>
<td>348 (108-527)</td>
<td>200-500</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>22.1 (12.0-33.4)</td>
<td>19.0 (13.4-27.1)</td>
<td>27.0-35.0</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>30.4 (13.3-46.5)</td>
<td>28.3 (18.1-45.9)</td>
<td>20.0-37.0</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>54.0 (32.0-279.0)</td>
<td>43.0 (32.0-99.0)</td>
<td>40.0-133.0</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>27 (7-312)</td>
<td>32 (12-88)</td>
<td>9-73</td>
</tr>
</tbody>
</table>

n, number of dogs.
Data are presented as median and range.

*P < .05 versus survivors, Mann and Whitney test.

survivors: 2.1 µmol/L (range: 0.5, 6.4) and healthy dogs: 38.6 µmol/L (range: 11.4, 96.1)] (Fig 1). Posthoc analysis showed that citrulline concentrations in the survivors and nonsurvivors were both significantly lower than in the healthy group (P < .001). However, there was no significant difference in plasma citrulline concentration between survivors and nonsurvivors (P = .168). The plasma citrulline concentration in all the parvovirus-infected dogs was 2.7 µmol/L (range: 0.3, 49.0).

Median serum creatinine and albumin concentrations of parvovirus-infected dogs at admission were 53 µmol/L (range: 32, 279 µmol/L) and 22.1 g/L (range: 12.0, 33.4 g/L), respectively. Plasma citrulline concentrations were not correlated with either serum albumin concentrations (r = 0.082, P = .54) or serum creatinine concentrations (r = 0.162, P = .22) on the day of admission in parvovirus-infected dogs. Fifty-two dogs had a serum albumin concentration below the reference range (median 20.9 g/L, range: 12.0, 26.4 g/L). Three parvovirus-infected dogs had serum creatinine concentrations above the reference range, with values of 142, 155, and 279 µmol/L. Their citrulline concentrations were 3.8, 7.0, and 2.8 µmol/L, respectively. Three of the parvovirus-infected dogs had an increased serum ALT activity (ALT >73 UI/L) with values of 74, 88, and 312 UI/L. Their respective plasma citrulline concentrations were 5.3, 6.4, and 1.7 µmol/L. The dog with the highest ALT activity was a nonsurvivor, the other 2 were survivors.

There was no association between the hazard rate of dying and plasma citrulline concentration (hazard ratio = 0.76, 95% confidence interval [CI]: 0.52-1.11, P = .15) or age (hazard ratio = 0.82, 95% CI: 0.55-1.23, P = .34). However, weight was negatively and significantly associated with the hazard rate of dying of parvoviral enteritis (hazard ratio = 0.73, 95% CI: 0.54-0.99, P = .045). This result indicates that each kilogram increase in weight
Table 2. Follow-up citrulline plasma concentration of hospitalized puppies until discharge or death.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors</td>
<td>2.4 (0.3-49.0)</td>
<td>2.1 (0.4-33.2)</td>
<td>2.5 (0.2-20.5)</td>
<td>2.3 (0.4-17.9)</td>
<td>2.5 (0.7-9.9)</td>
<td>2.3 (1.3-4.9)</td>
<td>1.8 (1.5-3.0)</td>
<td>1.6;4.6</td>
</tr>
<tr>
<td></td>
<td>n = 49</td>
<td>n = 43</td>
<td>n = 33</td>
<td>n = 21</td>
<td>n = 13</td>
<td>n = 7</td>
<td>n = 4</td>
<td>n = 2</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>2.1 (0.5-6.4)</td>
<td>2.0 (0.6-9.6)</td>
<td>4.3 (0.7-8.8)</td>
<td>2.2 (1.3-11.6)</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 12</td>
<td>n = 8</td>
<td>n = 5</td>
<td>n = 4</td>
<td>n = 4</td>
<td></td>
<td>n = 1</td>
<td></td>
</tr>
</tbody>
</table>

n, number of dogs in each group at each time point. Results are median and range. There were no statistically significant differences between the 2 groups and over time.

would reduce the mortality rate by 0.73 after adjusting for age and plasma citrulline concentration.

The results of the follow-up plasma citrulline concentrations are reported in Table 2. There were no significant changes in plasma citrulline concentration over time (P = .872), and no significant difference between the 2 groups (P = .597 survivors versus nonsurvivors) (Table 2).

Discussion

This study showed that the median plasma citrulline concentration is drastically decreased (by 93%) in dogs with parvoviral enteritis compared with healthy dogs.

The importance of plasma citrulline concentration has been mostly studied in humans, rodents, and pigs. In these species, citrulline is synthesized almost exclusively in the enterocytes, and the liver has no effect on plasma citrulline concentrations. Because of this, the measurement of plasma citrulline has been proposed as a marker of global enterocyte mass and metabolic activity in these species. Several experimental studies have shown that intestinal resection was associated with a severe reduction in plasma citrulline concentration in rodents and pigs, whereas a similar reduction has been observed in people with short bowel syndrome. There is a strong and consistent correlation between the remaining intestinal length and plasma citrulline concentration in adults and children. To the best of our knowledge, the effect of intestinal resection on plasma citrulline concentration has never been documented in dogs.

Parvoviral enteritis in dogs induces a severe necrosis of small intestinal epithelial cells, especially in the crypts, but also atrophy of the villi with complete collapse of mucosal architecture. This pathology is similar to an acute form of the human villus atrophy-associated diseases, the conditions which induce the most severe citrulline depletion in people. It is because of these similarities that we chose parvoviral enteritis to document plasma citrulline variations in dogs with acute intestinal damage.

In healthy research dogs, the enterocytes produce citrulline as in other species, but the liver has been reported to contribute to plasma citrulline concentration. In dogs with chronic liver failure, plasma citrulline concentration can be decreased by 46%, whereas the concentration is not changed in humans with liver failure. However, in canine acute liver disease, plasma citrulline concentration remains unchanged. Parvoviral enteritis is usually not associated with liver failure, significant liver enzyme activities increases, or hepatic lesions. Only one of the infected dogs had a significantly increased ALT activity (302 U/L), confirming that acute liver damage is unlikely in our cohort. Therefore, it is unlikely that liver failure is a significant factor of reduced plasma citrulline concentration in the current study. Recent studies reported that among several other amino acids, plasma citrulline concentrations were decreased in critically ill dogs as well as in humans with sepsis. In septic human patients, the hypo-citrullinemia has been hypothesized to be secondary to decreased synthesis in the enterocytes because of decreased availability of glutamine. Moreover, intestinal damage is a major concern in critical care cases mostly because of splanchnic hypoperfusion. Parvoviral enteritis in dogs is a critical condition, which is frequently associated with sepsis. Therefore, it is possible that the decrease in plasma citrulline that we observed was also related to these complications (sepsis, critical illness), and not just to the primary intestinal damage. However, the magnitude of the citrulline reduction in the present study was much more marked than those reported in septic human patients or critically ill dogs and more in line with levels reported in humans with very severe intestinal conditions such as short bowel syndrome, severe villous atrophy, or chemotherapy/radiation induced intestinal damage. In humans, the 2 conditions inducing the most severe decrease in plasma citrulline concentration are severe and diffuse villous atrophy and viral enteritis. Our findings are consistent with these previous reports. Lower plasma citrulline concentrations have been reported to be associated with the translocation of intestinal bacteria in human patients undergoing myeloablation treatment, logically implying that the most severe enterocyte damage is associated with an increased likelihood of bacterial translocation. It would be interesting to document whether a decrease in plasma citrulline concentration is associated with digestive bacterial translocation in dogs.

In the present study there was a trend for plasma citrulline concentration to be lower in the nonsurvivor group when compared with survivors. It is likely that the relatively low number of nonsurvivors explains the lack of significance because of low power. The same observation can also be reported about the survival analysis. However, the difference in the median citrulline
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Plasma citrulline concentration can be increased in humans with moderate to severe renal impairment. This increase is most likely a combination of decreased conversion of citrulline to arginine and reduced renal elimination of citrulline. Citrulline was not correlated with creatinine concentration. However, only 3 dogs (5%) had a creatinine concentration above the upper limit of the reference range. This is a very small number to definitely exclude any correlation between plasma citrulline and creatinine concentrations, but all 3 of these dogs had decreased citrulline concentrations. We did not find a significant correlation between plasma citrulline and albumin concentrations despite the fact that 52 dogs had mild to severe hypoalbuminemia. In humans, citrullinemia has been positively correlated with albuminemia. It is possible that initial dehydration at admission might have masked the severity of hypoalbuminemia in certain dogs, and therefore induced a bias in the severity of hypoalbuminemia. It would have been valuable to control albuminemia after fluid therapy.

Finally, this study suggests that citrulline supplementation might be considered in parvovirus-infected dogs. It has been shown in humans and rats that oral citrulline supplementation is a better way to restore arginine pools than direct arginine supplementation, mostly because the vast majority of orally supplemented arginine is metabolized by arginase in the liver during its 1st pass. Because dogs, like humans, convert most of the enterocyte-derived citrulline to arginine in their kidneys, it is possible that citrulline supplementation might help to restore arginine pools in situations of depletion, such as critical illness, especially because intestinal absorption of citrulline after oral administration is effective in healthy dogs. Citrulline supplementation might be even more important in parvoviral enteritis, because plasma citrulline concentrations remained low over the 1 week of follow-up period. However, to the best of our knowledge, there is no data documenting intestinal absorption of citrulline in severe intestinal damage in dogs.

A large reduction in plasma citrulline concentration has been associated with hepatic encephalopathy in humans with inherited hypocitrullinemia, probably because of impairment of the urea cycle because of a lack of citrulline availability. The findings of our study should encourage consideration of possible plasma hyperammonemia in dogs with parvoviral enteritis, especially in cases of severely reduced citrulline concentration. To the best of our knowledge this has never been reported in parvovirus-infected puppies.

This study has several limitations. First, it is a prospective study as far as enrollment is concerned, but the citrulline measurements have been performed retrospectively on stored plasma. Citrulline has been reported to be stable in human plasma with or without deproteinization over 12 weeks at −20 and −70°C, in plasma stored at −18°C for 6 months, and in nondeproteinized plasma stored at −80°C for more than a year. The concentrations of other amino acids such as glutamine, glutamate, arginine, and ornithine are affected by storage. In this study, the plasma was stored for 36 months at −80°C before analysis. This prolonged storage could have affected citrulline stability. However, the control samples are contemporary with the samples from the parvovirus-infected dogs and were stored and handled under the same conditions. Moreover, we compared the control population with a group of 8 research dogs sampled just before citrulline measurements, and did not find any statistically significant difference between the 2 groups (unpublished data). Also, the plasma citrulline concentrations of our control population were comparable to others reported in healthy dogs.

Secondly, we cannot definitely conclude that the decrease in the plasma citrulline reported here is exclusively the consequence of the intestinal damage, especially because sepsis and critical illness have also been associated with hypocitrullinemia in humans and dogs, respectively.

Finally, the small number of nonsurvivors as well as the low number of dogs after day 5 of follow-up is also a limitation. It could have been valuable to measure citrulline follow-up concentrations for a longer time period to document when plasma citrulline concentration is back to the normal range in parvovirus-infected dogs.

We conclude that plasma citrulline concentration may be a valuable marker of enterocyte damage based on this preliminary study. However, additional prospective studies including cases with chronic gastrointestinal diseases, massive intestinal resection, chemotherapy-associated intestinal damage, and other nonintestinal conditions (eg, renal and liver diseases) need to be investigated to confirm these preliminary findings. These results also suggest that the effect of oral citrulline supplementation should be documented in prospective studies enrolling parvovirus-infected dogs.
Footnotes

1. Ampicillin: Ampicillin-Fresenius, Fresenius Kabi, Midrand, South Africa
2. Gentamicin: Gentax 50, Virbac Animal Health, Halfway House, South Africa
3. Buprenorphine: Torbugesic, Fort Dodge, South Africa
4. Metoclopramide: Clopamion injection, Aspen Pharmaceutic, Woodmead, South Africa
5. Ondansetron: Zofran 4 mg injection, Pharmaplan, Midrand, South Africa
6. A/D, Hill's, Hout Bay, South Africa
7. Savant AS 110 Speed Vac, Savant, Thermo Fisher Scientific, Waltham, MA
8. LC-MSD-Trap-XCT plus, Agilent Technologies, Santa Clara, CA
9. Zorbax Eclipse XDB C18 (2.1 x 50 mm, 3.51µm), Agilent Technologies
10. LECO Corporation, Saint Joseph, MI
11. Sigma Aldrich, St Louis, MO
12. SPSS version 17.0, SPSS Inc, Chicago, IL
13. SAS/STAT, SAS Inc, Cary, NC

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References