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Calcium and magnesium abnormalities in puppies with parvoviral enteritis

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OBJECTIVE
To determine the association between ionized calcium (iCa) and/or total magnesium (tMg) and the development of sepsis and to investigate whether iCa or tMg is associated with mortality in puppies with canine parvoviral enteritis (CPE).

METHODS
64 client-owned puppies with CPE were enrolled in this prospective cohort study. Serum iCa and tMg were measured daily from admission until death or discharge. Fifteen healthy client-owned puppies were used as controls.

RESULTS
Mean iCa concentrations of the CPE group on admission were significantly lower compared to the control group (1.35 mmol/L vs 1.52 mmol/L). Ionized calcium concentrations of nonsurvivors were significantly higher compared to survivors on day 2 but not on any other days. Puppies that were hypercalcemic on day 2 were also significantly more likely to die than normocalcemic puppies (OR, 10.7; 95% CI, 1.7 to 71). Ionized calcium was not associated with the development of sepsis on any day. In contrast, mean admission tMg concentrations of the CPE group were significantly higher compared to the control group (0.72 mmol/L vs 0.63 mmol/L). However, tMg concentrations were not significantly different between survivors and nonsurvivors nor were they associated with the development of sepsis on any day.

CONCLUSIONS
On admission, puppies with CPE had lower iCa and higher tMg compared to healthy puppies, and higher iCa a day after initiation of treatment was associated with increased odds of mortality.

CLINICAL RELEVANCE
The results of this study provide insight into calcium homeostasis in critically ill young dogs with CPE.

Keywords: parvovirus, magnesium, systemic inflammatory response syndrome (SIRS), calcium, sepsis

Canine parvoviral enteritis (CPE) is a common cause of acute, life-threatening enteritis in young puppies.[1] Despite the extensive availability of vaccination, infection with canine parvovirus remains associated with high morbidity rates.[2] While gastrointestinal disturbances and immunosuppression are the most recognized sequalae of CPE, a less apparent systemic inflammatory response syndrome (SIRS) also develops in many puppies.[2] The term SIRS was first introduced in 1991,[3] and it has since been adapted for use in animals.[4] Sepsis has been defined as the development of SIRS secondary to the presence of an identifiable infection.[5,6] It has been found that puppies with CPE that meet at least 3 SIRS criteria at admission have greater odds of nonsurvival.[7]

The importance of reliable biomarkers in puppies with CPE lies in its potential to help determine disease severity, duration of hospitalization, and prognosis of patients while also guiding decision making on treatment options and euthanasia. Multiple studies[8,9,10,7,11] have investigated potential prognostic

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indicators in puppies affected by CPE, including endocrine, hematological, and biochemical parameters. Rapidly available and easily accessible biomarkers include a low Hct and decreased blood glucose concentration on admission. Both have been associated with decreased survival rates.[11] Additionally, a normal total WBC count and lymphocyte count 24 hours after admission has a positive predictive value of 100% for survival.[10] Lymphopenia and hypoalbuminemia on admission are both associated with a longer hospitalization period.[7]

Circulating calcium is found in 3 forms: ionized calcium (iCa), protein bound, and complexed (eg, with lactate, citrate, or bicarbonate). Notably, iCa is the most metabolically active component.[12] The fractions of calcium that exist as either ionized, bound, or complexed can be influenced by albumin and other protein levels, acid-base status, and the availability of potential chelators.[12] Moreover, serum calcium levels are regulated by parathyroid hormone (PTH), 1,25-dihydroxyvitamin D, and calcitonin.[12,13] Like calcium, magnesium (Mg) is also found in ionized, protein-bound, and complexed forms within serum, and it is a necessary element for PTH synthesis and secretion.[12]

Ionized hypocalcemia (iHypoCa) is an important electrolyte disturbance that is well documented among septic human patients, and it is increasingly recognized in critically ill animals.[13] The incidence of iHypoCa ranges from 16% in critically ill dogs to 24% in dogs that meet 2 or more SIRS criteria.[6,14] Ionized hypocalcemia has also been associated with an increased duration in hospital stay in puppies with CPE[15] as well as mortality in dogs evaluated in an emergency room or intensive care setting.[16]

Moreover, critical illness has been identified as the most common cause of iHypoCa in dogs.[17] Some proposed pathomechanisms for the development of hypocalcemia in critical illness include vitamin D deficiency or resistance, acquired or relative hypoparathyroidism, and hypomagnesemia.[13]

Hypomagnesemia is a common occurrence in critically ill people and animals,[18] but it has not been associated with outcome in puppies infected with CPE.[19,20] Magnesium depletion has been associated with reduced PTH secretion and impaired skeletal responsiveness to PTH.[18] A significant correlation has also been found between total serum calcium and total Mg (tMg) concentrations in dogs with hypomagnesemia.[21]

Hypocalcemia has been documented in puppies with CPE infection[15]; however, its association with the development of sepsis has not been reported. The relationship between hypocalcemia, hypomagnesemia, and the development of sepsis in CPE has also not been investigated. A better understanding of calcium and Mg regulation may help guide diagnostic and treatment strategies.

Therefore, the aims of this study were (1) to determine the association between serum iCa and/or tMg and the development of sepsis and (2) to investigate whether serum iCa and/or tMg concentrations are associated with mortality in puppies with CPE.

Methods

Study population

This prospective cohort study was performed in 64 client-owned puppies that were naturally infected with CPE and admitted to the isolation ward of the Onderstepoort Veterinary Academic Hospital for severe CPE in 2006. Fifteen age-matched, healthy, client-owned puppies that presented for routine vaccination at the same facility were used as controls. Although a formal power analysis was not done for the purpose of this study, the sample size would achieve 80% power to detect an OR of 6 for the association between an electrolyte abnormality and mortality, assuming 25% prevalence of the abnormality and 15% mortality in the normal concentration group. Written consent from all owners was required prior to enrollment in the study. The study was reviewed and approved by the university's animal use and care committee (REC092-22). Endocrine and citrulline data on the same cohort of puppies have previously been published.[9,22]

A diagnosis of CPE was suspected based on presenting clinical signs and confirmed by the detection of canine parvovirus particles in feces using electron microscopy. In addition, patients were considered eligible for the study if they (1) had a body weight of > 3 kg, (2) were admitted to the isolation ward due to the severity of clinical signs, (3) had no blood-borne parasites detected on capillary blood smear evaluation, and (4) tested negative for canine distemper virus on electron microscopy. At admission, a peripheral blood smear was made of blood collected from the ear and examined under a light microscope. A fecal sample was also collected. A fecal flotation, fecal wet preparation, and a fecal smear were performed and examined under a light microscope. Lastly, puppies that received treatment for CPE prior to admission were excluded.

All the CPE puppies were treated according to the standards set out by the first author's institution and as determined by the attending clinician. This included IV Ringer lactate fluid therapy; electrolyte replacement; antibiotic, antiemetic, antiulcer, and prokinetic treatment; deworming; enteral feeding; and blood or plasma transfusions if required. Puppies were fed a commercial diet (A/D; Hill's). If a puppy did not eat by itself within 1 day of admission, early enteral nutrition was implemented by placing a nasogastric tube.

Puppies presenting for routine vaccinations were included in the control group if they were deemed clinically healthy based on their clinical parameters (temperature, pulse, respiration rate, abdominal palpation, capillary refill time, and mucous membrane color) and routine laboratory testing (CBC determination and serum biochemical analysis).

Blood sampling, storage, and analysis

At admission and prior to any treatment, a clinical examination was performed on each puppy. Blood samples were collected at admission on day 1 and daily thereafter between 8:00 AM and 11:00 AM

until either death or discharge. Blood samples from the control puppies were collected in the consulting room. Blood was collected via jugular venipuncture and placed into a serum tube. The blood samples were allowed to clot at room temperature, and the tubes were centrifuged. The serum was collected and stored at −80 °C to ensure the accuracy of iCa measurements until analyzed.[23] Samples were stored for the duration of the study period and analyzed in a single batch at the end of the study period. Ionized calcium was adjusted to pH 7.40.

A CBC was performed every day (Cell Dyn 3700; Abbott Laboratories). Blood chemistry performed on admission included total serum protein, albumin, globulins, ALT, tMg, bilirubin, urea creatinine (NExCT/VetEX Alfa Wassermann; Bayer), sodium, potassium, and iCa (865 pH; Blood Gas Analyzer; Chiron Diagnostics Ltd). Blood chemistry on subsequent days included total serum protein, sodium, potassium, iCa, and tMg.

Ionized calcium and tMg measurements were classified as hypo-, normo-, and hypercalcemia and hypo-, normo-, and hypermagnesemia. The reference interval (RI) for iCa was set at 1.30 to 1.55 mmol/L to account for age-specific variation.[24] The RI for tMg was set at 0.6 to 1.2 mmol/L based on institutional RI (Onderstepoort Veterinary Clinical Pathology Laboratory). The CPE puppies were then classified based on outcome (fulfilment of ≥ 3 SIRS criteria and death vs survival). To fulfill the SIRS criteria, at least 3 of the following criteria had to be met: heart rate > 150 beats per minute, respiratory rate > 30 breaths per minute, temperature > 39.4 °C or < 37.2 °C, and/or a WBC count > 19,000 cells/µL or < 5,000 cells/µL.[25] Puppies were assigned a SIRS score out of 4 depending on how many SIRS criteria were met upon admission. The puppies were then reevaluated daily before blood sample collection to assign a new SIRS score. Sepsis was defined as the development of SIRS secondary to the presence of an identifiable infection. As a result, all puppies diagnosed with parvovirus infection that met the SIRS criteria were classified as having sepsis by definition.

Statistical analysis

Data were analyzed using commercially available statistical software (Stata, version 16; StataCorp). The Shapiro-Wilk test and histogram evaluation were used to assess continuous data for normality. Mean concentrations of iCa and tMg were compared between CPE and control groups on day 1 using a Student *t* test. Linear mixed models with Bonferroni adjustment for multiple comparisons were used to compare iCa and tMg concentrations between outcomes on each day as well as within outcomes for each day compared to day 1. The associations of hypo- and hypercalcemia (vs normocalcemia) on each day with mortality and of hypo- and hypermagnesemia (vs normomagnesemia) were assessed using ORs and a Fisher exact test. The associations of hypo- and hypercalcemia and hypo- and hypermagnesemia on day 1 with the development of sepsis on any day were assessed using Fisher exact tests. The associations of hypo- and hypercalcemia and hypo- and hypermagnesemia with the presence of sepsis using all daily data were assessed using mixed-effects multiple logistic regression. For all tests, significance was set at $P < .05$.

Results

Study population

Sixty-four puppies with CPE and 15 healthy control puppies were included in the study **(Table 1)**. The puppies with CPE consisted of 33 (52%) intact males and 31 (48%) intact females. There were 52 (81%) survivors and 12 (19%) nonsurvivors. Of the 12 nonsurvivors, only 1 was euthanized when it became agonal. Nonsurvivors (median, 4.4 kg; IQR, 3.0, 5.3) weighed significantly less on admission than survivors (median, 5.8 kg; IQR, 3.8, 9.2) (P = .017). Healthy control puppies consisted of 10 (66%) intact male and 5 (33%) intact female puppies. On day 1, 3 (20%) of the control puppies, 13 (26%) of the survivors, and 6 (50%) of the nonsurvivors met 3 or more SIRS criteria **(Table 2)**. One survivor (2%) and 2 nonsurvivors (17%) received whole-blood transfusions as part of their treatment protocol. The survivor received the transfusion on day 1 and the nonsurvivors on days 1 and 4, respectively. Eight survivors (15%) received fresh frozen plasma transfusions—2 on day 3, 4 on day 4, 1 on day 5, and 1 on day 6. Two nonsurvivors (17%) received fresh frozen plasma on day 2 and day 3, respectively. No puppies received any IV or oral calcium supplementation nor were they administered any medication that could have impacted calcium and Mg absorption.

Table 1—Summary of the signalment and clinical data (day 1) in the control group and the survivor and nonsurvivor groups of puppies with canine parvoviral enteritis (CPE).

Parameter	Control group (n = 15)	Survivors (n = 52)	Nonsurvivors (n = 12)
Age (mo)	3.73 ± 1.71	4.12 ± 1.77	3.13 ± 1.25
Sex (male/female)	10/5	25/27	8/4
Weight (kg)	7.37 ± 5.34	8.02 ± 6.99	4.23 ± 1.33[a]
Length of hospitalization		4.62 ± 2.77	2.58 ± 1.44[b]
Hct (L/L)	0.34 ± 0.07	0.38 ± 0.10	0.37 ± 0.10
Albumin (g/L)	23.34 ± 3.17	22.19 ± 4.17	20.77 ± 4.99

Values are reported as mean ± SD.
[a]Significantly different (P = .017). [b]Significantly different (P = .003).

Table 2—Summary of puppies meeting 3 or more systemic inflammatory response syndrome criteria in the control group and the survivor and nonsurvivor groups of puppies with CPE.

	Admission (day 1) CPE (n = 62) Control (n = 15)	Day 2 CPE (n = 54)	Day 3 CPE (n = 41)	Day 4 CPE (n = 27)
Control	3/15 (20%)			
Survivors	13/50 (26%)	3/46 (7%)	3/36 (8%)	2/24 (8%)
Nonsurvivors	6/12 (50%)	4/8 (50%)	2/5 (40%)	1/3 (33%)

Table 3—Ionized calcium data in the control group and the survivor and nonsurvivor groups of puppies with CPE.

Analyte categories	Admission (day 1) CPE (n = 56) Control (n = 15)	Day 2 CPE (n = 42)	Day 3 CPE (n = 31)	Day 4 CPE (n = 20)
iCa: ($\bar{x}$ ± SD) (mmol/L)				
Control group	1.52 ± 0.12			
Survivors	1.37 ± 0.11	1.45 ± 0.13	1.45 ± 0.11	1.42 ± 0.10
Nonsurvivors	1.31 ± 0.17	1.60 ± 0.15[a]	1.49 ± 0.14	1.24 ± 0.11
iCa: n (%) < 1.30 mmol/L				
Control group	1/15 (7%)			
Survivors	7/44 (16%)	1/36 (3%)	1/27 (4%)	2/18 (11%)
Nonsurvivors	3/12 (25%)	0/6 (0%)	0/4 (0%)	1/2 (50%)
iCa: n (%) > 1.55 mmol/L				
Control group	8/15 (53%)			
Survivors	2/44 (5%)	3/36 (8%)	5/27 (19%)	2/18 (11%)
Nonsurvivors	0/12 (0%)	3/6 (50%)	1/4 (25%)	0/2 (0%)

iCa = Ionized calcium.
[a]Significantly higher compared to survivor group at $P < .05$.

Serum iCa

Ionized calcium values were available for 56 puppies on admission (day 1). Mean iCa concentrations of the CPE group on admission were significantly lower compared to the control group ($P < .001$). On day 2, iCa concentrations of nonsurvivors were higher compared to survivors ($P = .038$). Moreover, day 2 hypercalcemic puppies were more likely to die than normocalcemic puppies (OR, 10.7; 95% CI, 1.7 to 71; $P = .031$; **Table 3**). In contrast, iCa was not significantly different between survivors and nonsurvivors on any other days **(Figure 1)**. Calcium concentrations were also not associated with the development of sepsis on any day.

Total Mg

Mean admission serum tMg concentrations of the CPE group were significantly higher compared to the control group ($P = .040$), yet the distribution between hypo-, normo-, and hypermagnesemia was similar between the CPE and control groups **(Table 4)**. Total Mg concentrations were not significantly different between survivors and nonsurvivors on any day **(Figure 2)** nor were they associated with mortality or the development of sepsis on any day.

Concurrent calcium and Mg abnormalities

Two puppies in the control group had concurrent ionized hypercalcemia and hypomagnesemia. Six other puppies with ionized hypercalcemia all had low normal Mg values. In contrast, the remaining 4 control puppies with hypomagnesemia were normocalcemic. In the CPE survivor group, 2 puppies had concurrent ionized hypercalcemia and hypomagnesemia upon admission. The puppies were siblings admitted on the same day. In the nonsurvivor group, 1 puppy had concurrent iHypoCa and hypermagnesemia upon admission. On day 2, there

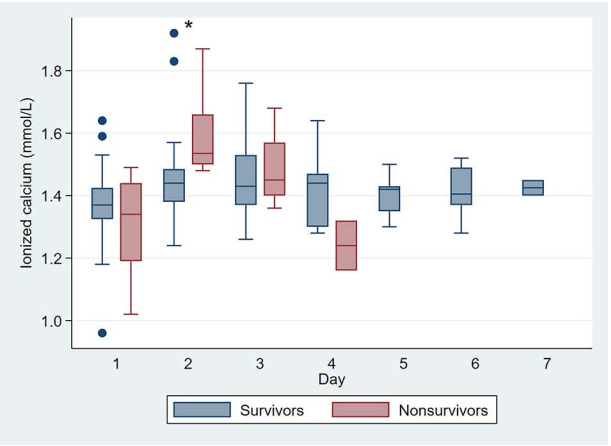

Figure 1—Serial daily serum ionized calcium measurements in 64 puppies with canine parvoviral enteritis. Horizontal lines indicate the median, box extends from 25th (Q1) to 75th (Q3) quartile, whiskers indicate fence values (lowest value ≥ Q1 – [1.5 X IQR] and highest value ≤ Q3 + [1.5 X IQR]), and dots show outliers. *Ionized calcium concentrations of nonsurvivors were significantly higher compared to survivors ($P = .038$).

Table 4—Total magnesium data in the control group and the survivor and nonsurvivor groups of puppies with CPE.

Analyte categories	Admission (day 1) CPE (n = 62) Control (n = 15)	Day 2 CPE (n = 54)	Day 3 CPE (n = 41)	Day 4 CPE (n = 27)
tMg ($\bar{x}$ ± SD) (mmol/L)				
Control group	0.63 ± 0.10			
Survivors	0.70 ± 0.13	0.77 ± 0.15	0.74 ± 0.11	0.73 ± 0.10
Nonsurvivors	0.77 ± 0.23	0.77 ± 0.15	0.75 ± 0.02	0.85 ± 0.13
tMg n (%) < 0.6 mmol/L				
Control group	6/15 (40%)			
Survivors	10/50 (20%)	8/46 (17%)	4/36 (11%)	3/24 (13%)
Nonsurvivors	3/12 (25%)	1/8 (13%)	0/5 (0%)	0/3 (0%)
tMg n (%) > 1.2 mmol/L				
Control group	0/15 (0%)			
Survivors	0/50 (0%)	0/46 (0%)	0/36 (0%)	0/24 (0%)
Nonsurvivors	1/12 (8%)	0/8 (0%)	0/5 (0%)	0/3 (0%)

tMg = Total magnesium.

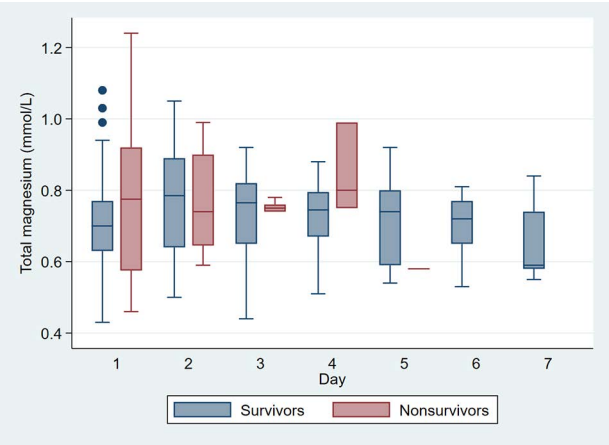

Figure 2—Serial daily serum total magnesium measurements in puppies with canine parvoviral enteritis. Horizontal lines indicate the median, box extends from Q1 to Q3, whiskers indicate fence values (lowest value ≥ Q1 – [1.5 X IQR] and highest value ≤ Q3 + [1.5 X IQR]), and dots show outliers.

was 1 puppy in the survivor group with iHypoCa and hypomagnesemia. On days 3 and 4, there were no concurrent iCa or tMg abnormalities in any puppies.

Discussion

In this study, iCa concentrations of nonsurvivors on day 2 were significantly higher compared to survivors, and puppies that were hypercalcemic on day 2 were more likely to die than normocalcemic puppies. A previous study[16] in dogs demonstrated that iCa and outcome have a nonlinear U-shaped relationship as progressively abnormal concentrations, be it higher or lower, have been associated with increased fatality rates. For example, cats with septic peritonitis that failed to normalize their iCa during hospitalization had decreased survival rates.[26] Additionally, it has been noted that the best time to prognosticate puppies with parvoviral enteritis may be 24 hours after admission.[27] Puppies are likely to have a poor prognosis if they have a persistent leuko- and lymphopenia, elevated cortisol concentrations, hypothyroxinemia, hypocholesterolemia, and elevated C-reactive protein and/or tumor necrosis factor concentrations 24 hours after the initiation of treatment.[27]

Only 5% of the puppies with CPE were hypercalcemic on day 1; however, that increased to 14% on day 2 and 19% on day 3. Pathological causes of ionized hypercalcemia include malignancy, primary hyperparathyroidism, hypoadrenocorticism, renal injury, osteolysis, hypervitaminosis D, and granulomatous disease.[17] Organ dysfunction, including acute kidney injury, is known to develop secondary to sepsis[28] and may have played a role in the calcium abnormalities observed in the puppies with CPE. Evidence of acute kidney injury has been reported in puppies with CPE; however, serum creatinine and urea are insensitive indicators of early kidney injury, and therefore acute kidney injury often remains undetected.[29] Additionally, serum creatinine is not considered a sensitive or specific marker for the diagnosis of early kidney injury,[30] puppies have lower creatinine than adults,[31] creatinine production is decreased in sepsis,[32] and puppies with CPE receive aggressive fluid therapy as part of their treatment, potentially diluting serum creatinine. Moreover, hypercalcemia has been described in humans with SIRS, acute kidney injury, and liver injury.[33] Extrarenal activation of the enzyme responsible for converting 25(OH)D3 to 1,25-dihydroxyvitamin D, 25-hydroxyvitamin D 1α-hydroxylase, in the liver may be responsible for the increase in calcium levels.[33]

The prevalence of iHypoCa in puppies with CPE on presentation in this study was 18%. This is similar to ranges reported from 16% in critically ill dogs to 24% in dogs that meet 2 or more SIRS criteria[6,14] but lower compared to a previous study[7] that reported the prevalence of hypocalcemia as 34% in puppies with CPE. However, this could be explained by their use of total calcium rather than iCa to determine calcium status in the abovementioned study. For

instance, total calcium has been shown to overestimate hypocalcemia and underestimate normocalcemia in critically ill hypoalbuminemic patients.[34] The accuracy of tCa to determine iCa status in dogs by adjusting for albumin or total protein have been evaluated with varying degrees of success, but it has ultimately been concluded that iCa remains the gold standard to confirm true calcium status and to avoid misdiagnosing iHypoCa.[35,36]

Although tMg was not associated with outcome in this study, a recent study[11] found a significant association between tMg on presentation and survival in puppies with CPE; for every 0.1 mmol/L increase in tMg concentration on admission, puppies had 2.50 times lower odds of survival. This discrepancy can potentially be ascribed to the small number of nonsurvivors in our study. Hypomagnesemia has been the main focus related to Mg regulation in critically ill people and animals[18]; however, hypermagnesemia has also been associated with a negative outcome.[37] As for hypermagnesemia, the most common etiologies reported are renal failure and iatrogenic causes,[18] and increased Mg concentrations have been associated with nonsurvival in dogs with acute kidney injury.[38] Although hypomagnesemia was more common in critically ill dogs, hypermagnesemic dogs were 2.6 times more likely to die compared to dogs that had normal Mg levels.[37] In the same study, hypomagnesemia was associated with a higher incidence of concurrent hypokalemia and hyponatremia.[37] It has been postulated that the additive effect of dysregulation of ion concentrations can affect clinical outcome due to their impact on multiple cellular processes.[16]

Ionized calcium concentrations were decreased in puppies with CPE, whereas tMg concentrations were higher compared to controls. The development of calcium and Mg disorders can share similar pathomechanisms, including malabsorption, intestinal losses, altered distribution, and abnormalities of vitamin D metabolism.[13,18,39] Hypovitaminosis D, iHypoCa, and secondary hyperparathyroidism have all been described in dogs with protein-losing enteropathies (PLE).[40,41] Malabsorption is widely considered to be the most significant cause for the development of hypovitaminosis D in PLE; however, its development is most likely multifactorial.[42] The development of villous atrophy and subsequent loss of absorptive capacity in puppies with CPE[2] could potentially cause a similar malabsorptive state. Other causes include reduced dietary intake of vitamin D, increased vitamin D metabolism, and ongoing systemic inflammation.[42] Additionally, critically ill dogs, dogs with sepsis, and dogs with babesiosis have significantly lower vitamin D concentrations compared to healthy control dogs.[43,44]

The most common etiology for both hypomagnesemia and iHypoCa in dogs is gastrointestinal diseases,[45] and both have been well documented in dogs with PLE.[46–48] Since Mg is needed to produce 1,25-dihydroxyvitamin D in the renal tubules, concurrent hypomagnesemia may reduce the availability of 1,25-dihydroxyvitamin D and consequently affect intestinal absorption of Ca.[47] Ionized Mg concentrations have also been correlated with iCa, PTH, and 25-hydroxyvitamin D concentrations.[49] Measuring ionized Mg is preferred to tMg because it represents the most biologically active component of circulating Mg and is therefore the best representation of total body Mg levels.[18] Although low tMg levels reflect total body depletion, normal tMg levels can exist in the presence of ionized hypomagnesemia. While taking this into consideration, measuring tMg levels still remains the easiest and most available option for assessing Mg status.[50] Accordingly, a limitation of this study included the use of tMg instead of ionized Mg.

Puppies differ physiologically from adults, and age-related variations in hematological, biochemical, and electrolyte values have been reported.[24,31] Both iCa and tCa are higher in puppies compared to adults,[24,31] whereas ionized Mg was reported to be lower in puppies compared to adults.[24] Total Mg ranges have not been compared between puppies and adults; however, a strong correlation between ionized Mg and tMg have been found in healthy adult dogs.[51] The control group in our study confirmed these findings. More than half of the control puppies were still considered hypercalcemic even with a higher RI to account for age-specific variation. This raises concerns regarding the RIs used such that caution should be taken in interpreting puppies as hypercalcemic in this study. Similarly, all of the control puppies were either hypomagnesemic or at the low end of the RI. These findings suggest that age-related variations may be larger than previously reported and pose a limitation with regard to interpreting whether or not these puppies truly had iCa and tMg concentrations outside of RIs.

Neither iCa or tMg concentrations were associated with the development of sepsis in this study. This is in contrast with previous findings where iHypoCa and hypomagnesemia have been well documented in critically ill and septic animals.[16,19,20] However, the currently recognized SIRS criteria where 2 out of 4 criteria must be met has a very low specificity.[52] Additionally, excitable puppies frequently have elevated heart and respiratory rates, which further decreases the specificity of these SIRS criteria. Notably, 20% of the control puppies in our study met the SIRS criteria; however, none of them satisfied the criteria on WBC count. Since CPE is generally characterized by low to severely low WBC counts,[10] this also played a role in decreasing the specificity of the SIRS criteria. Median WBC counts in puppies with CPE are significantly lower in nonsurvivors compared to survivors.[10] By applying more stringent criteria of meeting 3 out of 4 SIRS criteria in puppies with CPE, an association has been found with greater odds of nonsurvival,[7] and dogs meeting 3 or more SIRS criteria tended to have iHypoCa.[14] However, this study found no such associations.

Another limitation of this study is the small sample size as well as the small number of nonsurvivors. Consequently, a type II error may have resulted in low power to detect associations of iCa and tMg with outcomes in puppies with CPE. Therefor caution

should be used when applying the results to other populations of critically ill dogs.

In summary, on admission, puppies with CPE had lower iCa and higher tMg compared to healthy puppies, and calcium may play a role in the outcome of puppies with CPE. This study provides insight into calcium homeostasis in critically ill young dogs with parvoviral enteritis. Future studies investigating the role of calcium and Mg are warranted to confirm these findings.

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References

1. Pollock RVH, Coyne MJ. Canine parvovirus. *Vet Clin North Am Small Anim Pract.* 1993;23(3):555–568. doi:10.1016/S0195-5616(93)50305-4
2. Prittie J. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *J Vet Emerg Crit Care.* 2004;14(3):167–176. doi:10.1111/j.1534-6935.2004.04020.x
3. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Chest.* 1992;101(6):1644–1655. doi:10.1378/chest.101.6.1644
4. Purvis D, Kirby R. Systemic inflammatory response syndrome: septic shock. *Vet Clin North Am Small Anim Pract.* 1994;24(6):1225–1247. doi:10.1016/S0195-5616(94)50136-0
5. de Laforcade AA, Freeman LA, Shaw SP, Brooks MB, Rozanski EA, Rush JE. Hemostatic changes in dogs with naturally occurring sepsis. *J Vet Intern Med.* 2003;17(5):674–679. doi:10.1111/j.1939-1676.2003.tb02499.x
6. Luschini MA, Fletcher DJ, Schoeffler GL. Incidence of ionized hypocalcemia in septic dogs and its association with morbidity and mortality: 58 cases (2006-2007). *J Vet Emerg Crit Care.* 2010;20(4):406–412. doi:10.1111/j.1476-4431.2010.00553.x
7. Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Res Vet Sci.* 2010;89(2):174–178. doi:10.1016/j.rvsc.2010.02.013
8. Schoeman JP, Goddard A, Herrtage ME. Serum cortisol and thyroxine concentrations as predictors of death in critically ill puppies with parvoviral diarrhea. *J Am Vet Med Assoc.* 2007;231(10):1534–1539. doi:10.2460/javma.231.10.1534
9. Schoeman JP, Herrtage ME. Serum thyrotropin, thyroxine and free thyroxine concentrations as predictors of mortality in critically ill puppies with parvovirus infection: a model for human paediatric critical illness? *Microbes Infect.* 2008;10(2):203–207. doi:10.1016/j.micinf.2007.11.002
10. Goddard A, Leisewitz AL, Christopher MM, Duncan NM, Becker PJ. Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. *J Vet Intern Med.* 2008;22(2):309–316. doi:10.1111/j.1939-1676.2008.0073.x
11. Chalifoux NV, Parker SE, Cosford KL. Prognostic indicators at presentation for canine parvoviral enteritis: 322 cases (2001-2018). *J Vet Emerg Crit Care.* 2021;31(3):402–413. doi:10.1111/vec.13052
12. Dhupa N, Proulx J. Hypocalcemia and hypomagnesemia. *Vet Clin North Am Small Anim Pract.* 1998;28(3):587–608. doi:10.1016/S0195-5616(98)50057-5
13. Holowaychuk MK. Hypocalcemia of critical illness in dogs and cats. *Vet Clin North Am Small Anim Pract.* 2013;43(6):1299–1317. doi:10.1016/j.cvsm.2013.07.008
14. Holowaychuk MK, Hansen BD, DeFrancesco TC, Marks SL. Ionized hypocalcemia in critically ill dogs. *J Vet Intern Med.* 2009;23(3):509–513. doi:10.1111/j.1939-1676.2009.0280.x
15. Chalifoux NV, Burgess HJ, Cosford KL. The association between serial point-of-care test results and hospitalization time in canine parvovirus infection (2003-2015). *Can Vet J.* 2019;60(7):725–730.
16. Goggs R, De Rosa S, Fletcher DJ. Electrolyte disturbances are associated with non-survival in dogs - a multivariable analysis. *Front Vet Sci.* 2017;4:135. doi:10.3389/fvets.2017.00135
17. Coady M, Fletcher DJ, Goggs R. Severity of ionized hypercalcemia and hypocalcemia is associated with etiology in dogs and cats. *Front Vet Sci.* 2019;6:276. doi:10.3389/fvets.2019.00276
18. Humphrey S, Kirby R, Rudloff E. Magnesium physiology and clinical therapy in veterinary critical care. *J Vet Emerg Crit Care.* 2015;25(2):210–225. doi:10.1111/vec.12253
19. Kocaturk M, Martinez S, Eralp O, Tvarijonaviciute A, Ceron J, Yilmaz Z. Tei index (myocardial performance index) and cardiac biomarkers in dogs with parvoviral enteritis. *Res Vet Sci.* 2012;92(1):24–29. doi:10.1016/j.rvsc.2010.10.018
20. Mann FA, Boon GD, Wagner-Mann CC, Ruben DS, Harrington DP. Ionized and total magnesium concentrations in blood from dogs with naturally acquired parvoviral enteritis. *J Am Vet Med Assoc.* 1998;212(9):1398–1401. doi:10.2460/javma.1998.212.09.1398
21. Levi J, Massry SG, Coburn JW, Llach F, Kleeman CR. Hypocalcemia in magnesium-depleted dogs: evidence for reduced responsiveness to parathyroid-hormone and relative failure of parathyroid-gland function. *Metabolism.* 1974;23(4):323–335. doi:10.1016/0026-0495(74)90050-X
22. Dossin O, Rupassara SI, Weng H-Y, Williams DA, Garlick PJ, Schoeman JP. Effect of parvoviral enteritis on plasma citrulline concentration in dogs. *J Vet Intern Med.* 2011;25(2):215–221. doi:10.1111/j.1939-1676.2010.0671.x
23. Mazaki-Tovi M, Topol S, Aroch I. Effect of pH and storage conditions on measured ionised calcium concentration in dogs and cats. *Vet Rec.* 2020;187(9):e72. doi:10.1136/vr.105900
24. O'Brien MA, McMichael MA, Le Boedec K, Lees G. Reference intervals and age-related changes for venous biochemical, hematological, electrolytic, and blood gas variables using a point of care analyzer in 68 puppies. *J Vet Emerg Crit Care.* 2014;24(3):291–301. doi:10.1111/vec.12162
25. Otto CM. Sepsis in veterinary patients: what do we know and where can we go? *J Vet Emerg Crit Care.* 2007;17(4):329–332. doi:10.1111/j.1476-4431.2007.00253.x
26. Kellett-Gregory LM, Boller EM, Brown DC, Silverstein DC. Ionized calcium concentrations in cats with septic peritonitis: 55 cases (1990-2008). *J Vet Emerg Crit Care.* 2010;20(4):398–405. doi:10.1111/j.1476-4431.2010.00562.x
27. Schoeman JP, Goddard A, Leisewitz AL. Biomarkers in canine parvovirus enteritis. *N Z Vet J.* 2013;61(4):217–222. doi:10.1080/00480169.2013.776451

28. Keir I, Kellum JA. Acute kidney injury in severe sepsis: pathophysiology, diagnosis, and treatment recommendations. *J Vet Emerg Crit Care.* 2015;25(2):200–209. doi:10.1111/vec.12297
29. van den Berg MP, Schoeman JP, Defauw P, et al. Assessment of acute kidney injury in canine parvovirus infection: comparison of kidney injury biomarkers with routine renal functional parameters. *Vet J.* 2018;242: 8–14. doi:10.1016/j.tvjl.2018.10.002
30. De Loor J, Daminet S, Smets P, Maddens B, Meyer E. Urinary biomarkers for acute kidney injury in dogs. *J Vet Intern Med.* 2013;27(5):998–1010. doi:10.1111/jvim.12155
31. Rortveit R, Saevik BK, Eggertsdóttir AV, et al. Age-related changes in hematologic and serum biochemical variables in dogs aged 16-60 days. *Vet Clin Pathol.* 2015;44(1): 47–57. doi:10.1111/vcp.12220
32. Doi K, Yuen PST, Eisner C, et al. Reduced production of creatinine limits its use as marker of kidney injury in sepsis. *J Am Soc Nephrol.* 2009;20(6):1217–1221. doi:10.1681/ASN.2008060617
33. Kazama JJ, Yamamoto T, Oya H, et al. A patient with severe hypercalcemia in multiple organ dysfunction syndrome: role of elevated circulating 1α,25(OH)2vitamin d levels. *J Bone Miner Res.* 2010;25(6):1455–1459. doi:10.1002/jbmr.18
34. Sharp CR, Kerl ME, Mann FA. A comparison of total calcium, corrected calcium, and ionized calcium concentrations as indicators of calcium homeostasis among hypoalbuminemic dogs requiring intensive care. *J Vet Emerg Crit Care.* 2009;19(6):571–578. doi:10.1111/j.1476-4431.2009.00485.x
35. Lebastard M, Cuq B, Sharman MJ, Danner J, Le Boedec K. Diagnostic performance of predicted ionized calcium in dogs with total hypercalcemia and total hypocalcemia. *Vet Clin Pathol.* 2021;50(4):515–524. doi:10.1111/vcp.13024
36. Schenck PA, Chew DJ. Prediction of serum ionized calcium concentration by use of serum total calcium concentration in dogs. *Am J Vet Res.* 2005;66(8):1330–1336. doi:10.2460/ajvr.2005.66.1330
37. Martin LG, Matteson VL, Wingfield WE, Pelt DRV, Hackett TB. Abnormalities of serum magnesium in critically ill dogs: incidence and implications. *J Vet Emerg Crit Care.* 1994;4(1):15–20. doi:10.1111/j.1476-4431.1994.tb00111.x
38. Troìa R, Gruarin M, Grisetti C, et al. Fractional excretion of electrolytes in volume-responsive and intrinsic acute kidney injury in dogs: diagnostic and prognostic implications. *J Vet Intern Med.* 2018;32(4):1372–1382. doi:10.1111/jvim.15146
39. Pepe J, Colangelo L, Biamonte F, et al. Diagnosis and management of hypocalcemia. *Endocrine.* 2020;69(3): 485–495. doi:10.1007/s12020-020-02324-2
40. Gow AG, Else R, Evans H, Berry JL, Herrtage ME, Mellanby RJ. Hypovitaminosis D in dogs with inflammatory bowel disease and hypoalbuminaemia. *J Small Anim Pract.* 2011;52(8):411–418. doi:10.1111/j.1748-5827.2011.01082.x
41. Mellanby RJ, Mellor PJ, Roulois A, et al. Hypocalcaemia associated with low serum vitamin D metabolite concentrations in two dogs with protein-losing enteropathies. *J Small Anim Pract.* 2005;46(7):345–351. doi:10.1111/j.1748-5827.2005.tb00331.x
42. Clarke KE, Hurst EA, Mellanby RJ. Vitamin D metabolism and disorders in dogs and cats. *J Small Anim Pract.* 2021;62(11):935–947. doi:10.1111/jsap.13401
43. Dvir E, Rosa C, Handel I, Mellanby RJ, Schoeman JP. Vitamin D status in dogs with babesiosis. *Onderstepoort J Vet Res.* 2019;86(1):e1–e5. doi:10.4102/ojvr.v86i1.1644
44. Jaffey JA, Backus RC, McDaniel KM, DeClue AE. Serum vitamin D concentrations in hospitalized critically ill dogs. *PLoS One.* 2018;13(3):e0194062. doi:10.1371/journal.pone.0194062
45. Woods GA, Oikonomidis IL, Gow AG, et al. Investigation of hypomagnesaemia prevalence and underlying aetiology in a hospitalised cohort of dogs with ionised hypocalcaemia. *Vet Rec.* 2021;189(9):e301.
46. Bush WW, Kimmel SE, Wosar MA, Jackson MW. Secondary hypoparathyroidism attributed to hypomagnesemia in a dog with protein-losing enteropathy. *J Am Vet Med Assoc.* 2001;219(12):1732–1734. doi:10.2460/javma.2001.219.1732
47. Kimmel SE, Waddell LS, Michel KE. Hypomagnesemia and hypocalcemia associated with protein-losing enteropathy in Yorkshire Terriers: five cases (1992-1998). *J Am Vet Med Assoc.* 2000;217(5):703–706. doi:10.2460/javma.2000.217.703
48. Woods GA, Willems A, Hurst E, Mellanby RJ. Epileptic seizure in a cocker spaniel associated with hypocalcaemia, hypovitaminosis D and a protein-losing enteropathy. *Vet Rec Case Rep.* 2019;7(2):e000813.
49. Jones C, Jablonski SA, Petroff BK, Langlois DK. Relationship between serum magnesium, calcium, and parathyroid concentrations in dogs with abnormally low serum 25-hydroxyvitamin d concentration and chronic or protein-losing enteropathy. *J Vet Intern Med.* 2023;37(1):101–109. doi:10.1111/jvim.16595
50. Cortes YE, Moses L. Magnesium disturbances in critically ill patients. *Compend Contin Educ Vet.* 2007;29(7):420–427.
51. Murray ME, Boiron L, Buriko Y, Drobatz K, Waddell LS. Total serum and ionized magnesium concentrations in healthy and hospitalized dogs. *J Vet Emerg Crit Care.* 2023;33(4):427–434. doi:10.1111/vec.13302
52. Hauptman JG, Walshaw R, Olivier NB. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. *Vet Surg.* 1997;26(5):393–397. doi:10.1111/j.1532-950X.1997.tb01699.x