Calcium and magnesium abnormalities in dogs with parvoviral enteritis

By

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Submitted in fulfilment of the requirements for the degree Master of Science (Veterinary Science) in the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria

October 2024

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Abstract

Canine parvoviral enteritis (CPE) is a common cause of acute, life-threatening enteritis in young dogs. While gastrointestinal disturbances and immunosuppression are the most recognised sequelae of CPE, a less apparent systemic inflammatory response syndrome (SIRS) also develops in many dogs.

Calcium and magnesium abnormalities are increasingly recognised in the critical care setting. Ionised hypocalcaemia (iHypoCa) is well documented among humans with sepsis, and it has been associated with an increased duration in hospital stay in dogs with CPE, as well as mortality in dogs evaluated in an emergency room or intensive care setting. Moreover, critical illness has been identified as the most common cause of iHypoCa in dogs.

Hypomagnesaemia is a common occurrence in critically ill people and animals, but it has not been associated with outcome in dogs infected with CPE. A significant correlation has been found between serum calcium and magnesium concentrations in dogs with hypomagnesaemia.

The objective of this study were a) to determine the association between the development of iHypoCa and total hypomagnesaemia, and sepsis, and b) to investigate whether ionised calcium (iCa) or total magnesium (tMg) is associated with mortality in dogs with CPE.

Sixty-four client-owned dogs 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 dogs were used as controls.

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). Ionised calcium concentrations of non-survivors were significantly higher compared to survivors on day two, but not on any other days. Dogs that were hypercalcaemic on day two were also significantly more likely to die than normocalcaemic dogs after adjusting for multiple comparisons (OR = 10.7; 95% CI: 1.7-71). Ionised 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 non-survivors, nor were they associated with the development of sepsis on any day.

In summary, dogs with CPE had lower iCa and higher tMg compared to healthy dogs on admission, and the iCa concentrations of non-survivors were significantly higher on day two compared to survivors.

Results of this study provide insight into calcium homeostasis in critically ill young dogs with CPE.

May I never see in the patient anything but a fellow creature in pain. May I never consider him merely a vessel of disease. Grant me the strength, time and opportunity always to correct what I have acquired, always to extend its domain; for knowledge is immense and the spirit of man can extend indefinitely to enrich itself daily with new requirements."

— Excerpt from the Oath of Maimonides

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Acknowledgements

I would like to express my appreciation to the following individuals for their invaluable support and assistance. This project would not have been possible without them.

Firstly, to my supervisor Prof Johan Schoeman. Thank you for the many cups of coffee, support and guidance throughout this journey. You foster curiosity and a love for learning and have become a mentor in both career and life.

To my co-workers:

> Dr Anri Celliers, thank you for your time, dedication and picking up all the 'small, but important' things. Your comments and suggestions were vital to the completion of this project!
>
> Prof Richard Mellanby, thank you for your continued support, kind words and encouragement.
>
> Prof Peter Thompson, thank you for doing (and re-doing) the statistical analysis and always being open to answering all my questions.

Dr Joe Hanekom, thank you for your interest and advice on the writing of this dissertation, as well as regularly checking in to see how I'm doing.

To my best friend and fellow master's student, Emma – thank you for all the late-night calls, being my sounding board and making sure my sanity stays intact. Walking this journey with you made all the difference.

Lastly, thank you to my mom, Sandra, and my dad, Ian, for your encouragement, understanding, and providing me with every opportunity to help me pursue my dreams. You believe in me more than I believe in myself.

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List of Abbreviations

AKI	Acute kidney injury
ALT	Alanine aminotransferase
Ca	Calcium
CaSR	Calcium sensing receptor
CBC	Complete blood count
CPE	Canine parvoviral enteritis
CPV	Canine parvovirus
CRP	C-reactive protein
EM	Electron microscopy
iCa	Ionised calcium
iHypoCa	Ionised hypocalcaemia
Interleukin	IL
iMg	Ionised magnesium
Mg	Magnesium
LPS	Lipopolysaccharide
OVAH	Onderstepoort Veterinary Academic Hospital
PTH	Parathyroid hormone
SIRS	Systemic inflammatory response syndrome
tCa	Total calcium
tMg	Total magnesium
TNF	Tumour necrosis factor
TSP	Total serum protein

Summary

Calcium and magnesium abnormalities are increasingly recognised in the critical care setting. Hypocalcaemia has been documented in dogs with canine parvoviral enteritis (CPE). In addition, hypomagnesaemia has been linked to the development of hypocalcaemia. Given that CPE is still an important cause of mortality in dogs worldwide, and calcium and magnesium homeostasis in critical illness is not fully defined, we investigated the relationship between ionised calcium (iCa), total magnesium (tMg) and sepsis in dogs with CPE by collecting daily serial measurements of calcium and magnesium.

Ionised hypocalcaemia is an important electrolyte disturbance that is well documented in humans with sepsis. It has been associated with an increased duration of hospitalisation and mortality. Moreover, critical illness has been identified as the most common cause of ionised hypocalcaemia in dogs. Hypomagnesaemia is a common occurrence in critically ill people and animals, but it has not been associated with outcome in dogs with CPE. A significant correlation has been found between total serum calcium and total magnesium concentrations in dogs with hypomagnesaemia.

Hypocalcaemia has been documented in dogs with CPE infection, however its association with the development of sepsis has not been reported. The relationship between hypocalcaemia, hypomagnesaemia, and the development of sepsis in CPE has also not been investigated. A better understanding of calcium and magnesium regulation may help guide diagnostic and treatment strategies.

The objectives of this study were a) to determine the association between ionised hypocalcaemia and total hypomagnesaemia and the development of sepsis; and b) to investigate whether serum ionised calcium and/or total magnesium concentrations are associated with mortality in dogs with CPE.

Sixty-four client-owned dogs with CPE were enrolled in a prospective cohort study. Serum iCa and tMg were measured daily from admission until death or discharge. Fifteen healthy client-owned dogs were used as controls.

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; $P < .01$). Ionised calcium concentrations of non-survivors were significantly higher compared to survivors ($P =$

0.038) on day two, but not on any other days. Dogs that were hypercalcaemic on day two were also significantly more likely to die than normocalcaemic dogs after adjusting for multiple comparisons (OR = 10.7; 95%CI: 1.7 - 71; *P* = 0.031). Ionised 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; *P* < .05). Yet, tMg concentration was not significantly different between survivors and non-survivors, nor was it associated with the development of sepsis on any day.

In conclusion, dogs with CPE had lower iCa and higher tMg compared to healthy dogs on admission, and higher iCa on day two of hospitalisation was associated with increased odds of mortality.

Results of this study provide insight into calcium homeostasis in critically ill young dogs with CPE.

Chapter 1: Introduction

1.1 Background

Canine parvoviral enteritis (CPE) is a common cause of severe acute, life-threatening enteritis in young dogs.[1] Despite the extensive availability of vaccination, infection with canine parvovirus (CPV) remains associated with high morbidity rates.[2,3] While gastrointestinal disturbances and immunosuppression are the most the most recognised sequelae of CPE, a less apparent systemic inflammatory response syndrome (SIRS) also develops in many dogs.[3] Sepsis has been defined as the development of SIRS secondary to an identifiable infection.[4,5] Additionally, dogs with CPE that meet at least three SIRS criteria upon admission have greater odds of non-survival.[6]

The importance of reliable biomarkers for the prognostication of dogs with CPE, lies in their potential to help determine disease severity, duration of hospitalisation, and prognosis of patients, while also guiding decisions on treatment options and euthanasia.[7] Multiple studies have investigated potential prognostic indicators in dogs affected by CPE including endocrine[8,9], haematological[10], and biochemical variables[6,11].

Rapidly available and easily accessible biomarkers that can be measured on admission include haematocrit and blood glucose concentration, with anaemia and hypoglycaemia associated with decreased survival rates.[11] Additionally, lack of leukocyte cytopenia 24 hours after admission, specifically a normal total white blood cell count and lymphocyte count, has a positive predictive value of 100% for survival.[10] Hypoalbuminaemia and lymphopenia on admission are both associated with increased duration of hospitalisation.[6]

Ionised hypocalcaemia (iHypoCa) is an important electrolyte disturbance that is commonly reported in humans with sepsis, and it is increasingly recognised in critically ill animals.[12,13] The incidence of iHypoCa ranges from 16% in dogs with critical illness to 24% in dogs that meet two or more SIRS criteria.[5,14] Ionised hypocalcaemia has also been associated with prolonged hospitalisation periods in dogs with CPE[15], and mortality in dogs assessed in emergency or intensive care settings.[16]

Moreover, critical illness is the most common cause of iHypoCa in dogs.[17] In this regard, there are several proposed pathomechanisms for the development of hypocalcaemia in critically ill patients. These include acquired or relative hypoparathyroidism, vitamin D deficiency or resistance, and hypomagnesaemia.[12]

Hypomagnesaemia is a common occurrence in critically ill patients[18], but it has not been associated with outcome in dogs diagnosed with CPE[19,20]. Magnesium (Mg) depletion has been associated with reduced parathyroid hormone (PTH) production and impaired skeletal responsiveness to PTH.[18] A significant positive correlation has also been found between total calcium (tCa) and total magnesium (tMg) concentration in dogs with hypomagnesaemia.[21]

Hypocalcaemia has been noted in dogs with CPE[15], however its association with the development of sepsis has not been reported. The relationship between hypocalcaemia, hypomagnesaemia, and the development of sepsis in CPE has also not been investigated. A better understanding of calcium and magnesium regulation may help guide diagnostic and treatment strategies.

1.2 Aims and Objectives

Aim:

To investigate the relationship between and consequences of calcium and magnesium dysregulation in dogs with CPE

Objectives:

To determine the association between ionised hypocalcaemia and total hypomagnesaemia and the development of sepsis.

To investigate if serum ionised calcium and/or total magnesium concentrations are associated with mortality in dogs with CPE.

1.3 Hypotheses

Primary hypothesis:

H_0: Ionised hypocalcaemia and total hypomagnesaemia are not associated with the development of sepsis

H_1: Ionised hypocalcaemia and/or hypomagnesaemia are associated with the development of sepsis

Secondary hypothesis:

H_0: Ionised calcium and total magnesium concentrations are not associated with mortality in dogs with CPE

H_1: Ionised calcium and total magnesium concentrations are associated with mortality in dogs with CPE

1.4 Benefits arising from the study

Canine parvoviral enteritis is still prevalent in young dogs despite widespread vaccine availability. Hypocalcaemia has been documented in dogs with CPE however, its association with the development of sepsis has not been reported. Hypomagnesaemia has been linked to the development of hypocalcaemia. The relationship between hypocalcaemia, hypomagnesaemia, and the development of sepsis in CPE has not been investigated. The results of this study may aid in understanding of the mechanisms behind the development of hypocalcaemia and hypomagnesaemia in critically ill patients, and it could have implications for the monitoring and treatment of CPE.

Chapter 2: Literature review

2.1 Canine parvoviral enteritis

Since its first appearance in the late 1970s CPE has been a common cause of severe acute enteritis in young dogs.[1] Despite extensive accessibility to vaccination, CPE remains associated with high morbidity rates.[2,3]

Dogs of any age, sex, or breed, can present with CPE, but young dogs between 6 weeks and 6 months of age are affected most severely. Older animals are usually immune to the virus because of either immunisation or natural infection, whereas younger dogs are protected against the virus by maternal antibodies.[3] Risk factors for developing severe CPE include younger age, purebred dogs and low body weight.[7] An increased incidence of clinical disease and fatalities have been found in summer months.[22,23]

Infection with CPV is acquired through oronasal exposure to fomites or via faecal-oral transmission.[24] Following exposure viral replication initially occurs in the oropharynx and local lymphoid tissue. Within two to five days post-infection viraemia is marked and haematogenous spread of the virus takes place.[1,3,24] The virus targets fast dividing cells, affecting the intestinal epithelium, lymphoid tissue and bone marrow the most severely.[3] The rate of cell turnover has a direct effect on the severity of the disease. Factors such as a change in diet, changes in bacterial flora during weaning, or simultaneous infection with canine coronavirus can contribute to an increased cell turnover rate that favours viral replication, subsequently increasing disease severity.[1,24] In the intestinal tract destruction of the germinal epithelium of the intestinal crypts by the virus causes the intestinal villi to collapse and subsequently decreases the intestines' absorptive capacity.[1,25] Lymphopaenia, and in severe cases panleukopaenia develop secondary to the destruction of lymphoblasts in the lymphoid tissue and myeloblasts in the bone marrow.[1,25]

In addition to acute enteritis, myocardial failure has also been associated with CPE.[1,26] Myocarditis occurred more frequently in infected animals before widespread vaccination, but it can still occur today.[24,27] Although myocarditis is now infrequently seen in dogs with CPE, a recent study identified myocarditis in 67% and myocardial fibrosis in 58% of dogs with CPE.[27]

The first clinical signs of CPE are very non-specific and include lethargy, fever, and anorexia, followed by vomiting and diarrhoea[1,3].= On physical examination, abdominal pain due to acute gastroenteritis or intussusception may be evident.[24]

2.2 Sepsis in CPE infections

While gastrointestinal upset and immunosuppression are the clearest sequelae of CPV infection, a less apparent systemic inflammatory response occurs in many dogs. Although CPE is a viral disease, mortality is associated with bacterial translocation from the damaged intestines into the bloodstream, the development of SIRS, and sepsis.[28-30]

When the gastrointestinal barrier is compromised bacteria can gain access to the systemic circulation.[31] Multiple bacteria including *Escherichia coli*, *Salmonella* spp., *Clostridium perfringes* and *Clostridium difficile* have been documented in dogs with CPE.[32-34] *Escherichia coli* is a gram-negative bacterium which contains endotoxin, also known as lipopolysaccharide (LPS) in its outer membrane. Studies have found measurable quantities of endotoxin in the blood of dogs infected with CPV.[35,36] Endotoxin triggers an inflammatory cascade and subsequently stimulates the release of pro-inflammatory cytokines.[29] Translocated bacteria can incite a systemic inflammatory response either directly or via the release of toxins.[31]

2.3 Diagnosis of sepsis

The latest scientific consensus defines sepsis as organ dysfunction due to a dysregulated hist response to infection.[37,38] In 1991, a set of criteria were proposed to help identify systemic inflammatory response syndrome (SIRS).[39] Since the first adaptation of the SIRS criteria to animals[40], they have been modified several times and the cut-off values vary slightly among studies.

A survey in 2006 determined the median values of the different SIRS criteria in use by clinicians managing sepsis in small animals.[41] Veterinarians typically responded that a dog needs to meet two or more of the following criteria to be diagnosed with SIRS: heart rate > 150 beats per min, respiratory rate > 30 breaths per min, temperature > 39.4°C or <37.2°C, and a white blood cell count > 19 000 cells/μL or <5 000 cells/μL. The SIRS criteria are very sensitive, but have a low specificity.[42]

This has led to many practitioners requiring that 3 of the 4 SIRS criteria need to be met before diagnosing a dog with SIRS.[41]

2.4 The role of calcium

Calcium is an essential element in many cellular processes, including coagulation neuromuscular transmission, bone metabolism, enzymatic reactions and vasomotor tone.[12]

Circulating calcium can be found in three different forms: ionised, protein bound, and complexed (e.g., with citrate, lactate or bicarbonate), with ionised calcium (iCa) being the most metabolically active component.[43] The concentration of albumin and other proteins, acid-base status, and the availability of chelators can influence the fraction of calcium that is either ionised, bound, or complexed.[43]

Serum calcium concentration is regulated by PTH, calcitriol and calcitonin.[12,43] In response to decreased serum ionised calcium concentration the parathyroid secretes PTH. In turn, PTH stimulates osteoclasts to increase bone resorption and to release calcium, and it increases the reabsorption of calcium in the kidneys.[12,43] Parathyroid hormone also stimulates the formation of calcitriol from 1,25-hydroxycholecalciferol. Calcitriol acts on the digestive tract and increases calcium absorption across the enterocytes and into the bloodstream.[12,43]

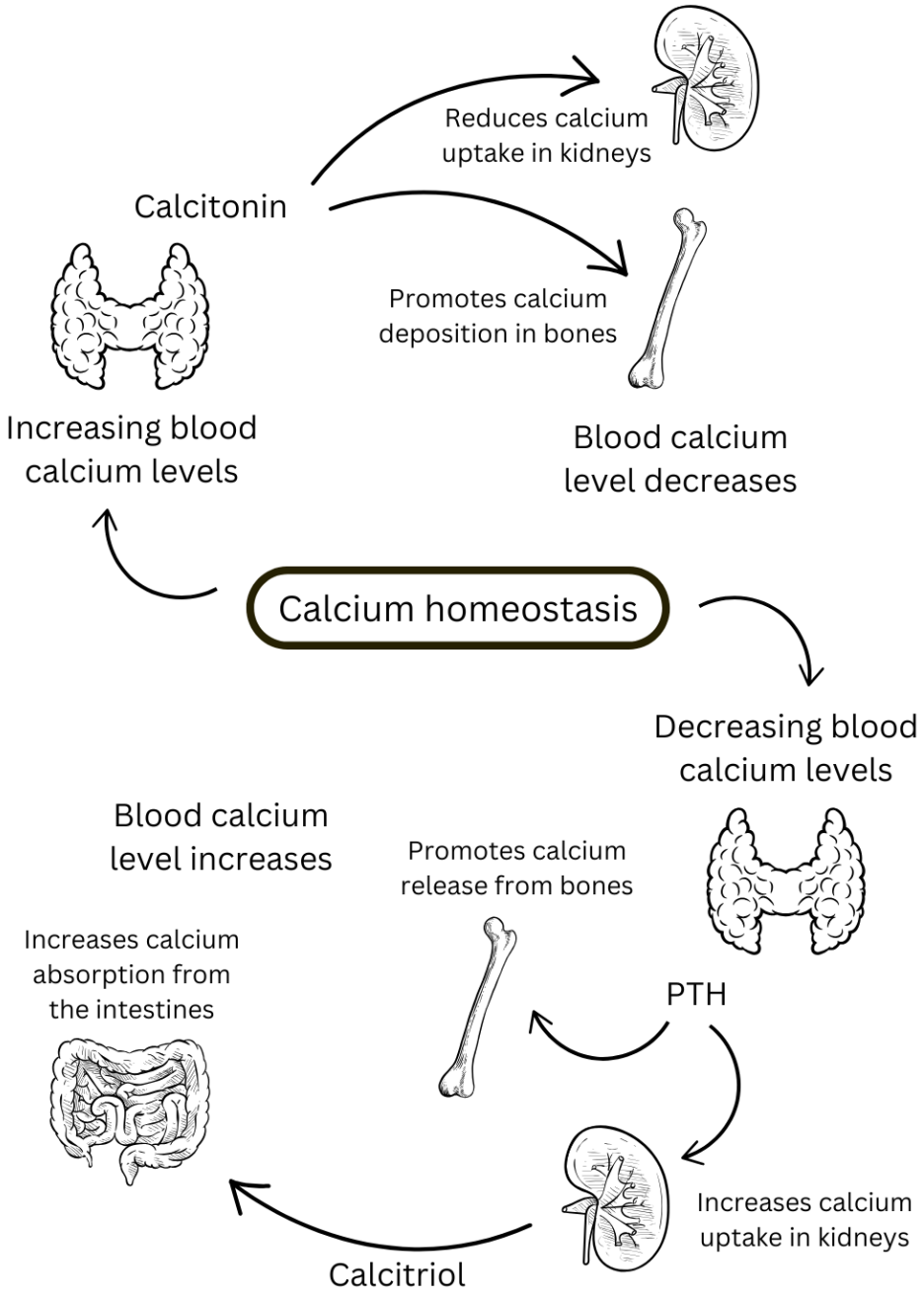

Figure 1 - Calcium homeostasis

Ionised hypocalcaemia is an important electrolyte disturbance that is common in humans with sepsis, and increasingly recognised in critically ill animals.[12,13] The incidence of iHypoCa ranges from 16% in dogs with critical illness to 24% in dogs that meet two or more SIRS criteria.[5,14] Ionised hypocalcaemia has also been associated with prolonged hospitalisation in dogs with CPE[5,14], and mortality in dogs assessed in emergency or intensive care settings[16].

Moreover, critical illness is the most frequent cause of iHypoCa in dogs.[17] Some proposed mechanisms for the development of hypocalcaemia in critical illness include acquired or relative hypoparathyroidism, vitamin D deficiency or resistance, and hypomagnesaemia, but there is often no clear underlying pathomechanism.[12]

The upregulation of calcium sensing receptors (CaSR) by inflammatory cytokines is the leading hypothesis on the development low or inappropriately low PTH conncentrations in critically ill patients.[44] The CaSR aid Ca homeostasis directly by regulating Ca excretion in the kidneys and indirectly by controlling PTH release from the parathyroid glands.[44] Particularly interleukin 1β (IL-1 β) and IL-6 have been shown to upregulate CaSR expression in the parathyroid glands, thyroid, and kidneys[45], leading to decreased production of PTH and increased excretion of Ca in urine.

There is increasing evidence that vitamin D levels are inversely linked to inflammatory markers such as circulating pro-inflammatory cytokines and acute phase proteins. However, the exact mechanisms behind this relationship remain unclear.[46] Multiple studies have demonstrated an inverse relationship between inflammatory biomarkers and vitamin D levels. In healthy dogs decreased vitamin D concentrations have been negatively associated with C-reactive protein.[47] Additionally, dogs with babesiosis, *Spirocerca lupi infections,* dogs with sepsis, and critically ill dogs all have significantly lower vitamin D concentrations compared to healthy control dogs.[48-50] However, chronic inflammation has also been reported to result in a dysregulated vitamin D metabolism and hypercalcaemia[51] and it is thought to be due to dysregulated production of 1,25 dihydroxyvitamin D by activated macrophages.[52]

As previously discussed, sepsis is common in dogs with CPE. Hypocalcaemia, based on total calcium (tCa) concentration, has been described in 34% of dogs with CPE.[6] In another study, utilising venous blood gas to measure iCa, hypocalcaemia 24 hours and 48 hours after admission was associated with significantly increased duration of hospitalisation.[15]

Ionised calcium concentration should be measured to accurately assess calcium status, particularly if a patient is critically ill, has acid-base abnormalities, altered

protein concentrations, renal disease, or thyroid disease.[53-55] When interpreting iCa concentrations, it is essential to consider that dogs normally have higher iCa concentrations than adults.[56] This is likely due to normal bone growth.[57]

Proper collection and handling of samples are essential for the accurate determination of iCa concentration.[58] Ionised calcium measurements can be affected by pH, storage conditions and air exposure. An acidic pH promotes the dissociation of calcium from proteins, leading to higher iCa levels. Conversely, an alkaline pH encourages calcium to bind to proteins, reducing iCa levels. Aerobic collection of samples, which exposes serum to air and a subsequent loss of carbon dioxide, raises the pH and decreases iCa concentrations.[58] A recent study however reported that although iCa concentrations decreased in samples stored under aerobic conditions for either 30 or 90 days at -80°C, it was clinically insignificant.[59] In vivo pH abnormalities have also been reported to affect iCa concetrations. An increase in iCa concetrations have been reported in dogs with induced acidosis.[60]

2.5 The role of magnesium

Like calcium, magnesium is also found in ionised, protein bound, and complexed forms within plasma. It is required for PTH synthesis and secretion and thus influences calcium homeostasis.[43] Additionally, it plays an essential role in ion flux across cell and mitochondrial membranes, the depolarisation of myocardial cells, activation of T-cells and vascular endothelial contractility.[18] Magnesium concentrations are dependent on intestinal and renal magnesium absorption, as well as renal magnesium excretion.[18]

Hypomagnesaemia is a common occurrence in humans and dogs with critical illness[18], but it has not been associated with outcome in dogs with CPE[19,20]. Magnesium depletion has been associated with reduced PTH secretion and impaired skeletal responsiveness to PTH, and a positive correlation has been found between tCa and tMg concentration in dogs with hypomagnesaemia.[21]

Although the hormonal regulation of calcium absorption and mobilization from tissues has been well-studied, the mechanisms controlling systemic magnesium regulation remain largely unclear.[61] Magnesium is affected similarly to calcium by PTH and vitamin D.[62] Furtermore, CaSR also senses systemic and local changes

magnesium concentrations in addition to calcium, resulting in increased or decreased calcium or magnesium absorption.

Hypomagnesaemia has been documented in 22% of dogs with hypocalcaemia. The same study also found that gastrointestinal disease was the disease process most commonly occurring with concurrent iHypoCa and hypomagnesaemia.[63] Magnesium deficiency has also been linked to increased myocardial vulnerability to injury.[64] As mentioned earlier, myocardial damage due to CPV infection is underreported.[27]

Measuring ionised magnesium (iMg) is preferred to tMg, because it constitutes the most biologically active component of circulating Mg and therefore represents total body magnesium conncentrations the best.[18,65] However, measuring tMg levels remains the easiest and most available option for assessing magnesium status.[66] When interpreting magnesium concentrations, it essential to point out that dogs have lower iMg concentrations than those reported in older animals.[56]

Chapter 3: Materials and Methods

3.1 Study design

This prospective cohort study was performed in 64 client-owned dogs admitted to the Onderstepoort Veterinary Academic Hospital (OVAH) for severe CPE from January to March 2006. Fifteen age-matched client-owned dogs that were healthy and presented for 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 odds ratio (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 before patients were enrolled into 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 dogs have previously been published[9,67].

The diagnosis of CPE was suspected based on the dogs' history, presenting complaint and clinical examination, and verified by detection of CPV particles in faeces via electron microscopy. Additionally, to be considered eligible for the study patients had to meet the following criteria:

- A body weight of >3 kg
- Admitted to the OVAH for treatment
- No blood-borne parasites detected on capillary blood smear evaluation
- Tested negative for canine distemper virus on electron microscopy
- Did not receive any treatment for CPE prior to admission

Upon admission, a blood smear was made from blood collected from the tip of the ear and examined under a light microscope. Faeces was also collected. A faecal flotation, faecal smear and faecal wet preparation were performed and also examined under a light microscope.

All the CPE dogs were treated according to OVAH institutional standards and as determined by the attending clinician. This included intravenous Ringer's lactate fluid therapy, antibiotics, antiemetics and prokinetic drugs, electrolyte replacement, blood or plasma transfusions if required, deworming, and enteral feeding. Dogs were fed

a commercial diet (A/D, Hill's). A nasogastric tube was placed, and early enteral nutrition was implemented if a dog did not eat by itself within one day of admission.

Dogs presenting for routine vaccinations were included in the control group if they were considered to be clinically healthy based upon clinical examination findings (temperature, pulse, respiratory rate, mucous membrane colour, capillary refill time, and abdominal palpation) and routine laboratory testing (complete blood count and serum biochemistry).

3.2 Experimental procedure

Upon admission to the OVAH and before administering any treatment, a clinical examination was performed on each patient. The following data were recorded:

- Signalment (age, sex, and breed)
- Date and time of admission
- Vaccination status
- Time and date of last meal
- Number of days depressed, anorectic and vomiting before admission
- Number of vomiting episodes per day and description of vomitus
- Duration and description of diarrhoea
- Habitus
- Temperature
- Pulse quality, rate and rhythm
- Respiratory rate and effort
- Mucous membrane colour and capillary refill time
- Percentage dehydration
- Presence of oral ulcerations
- Presence of abnormal lung sounds
- Abdominal palpation findings
- Blood glucose concentration
- Blood lactate concentration

Blood samples were collected at admission on day one, and every subsequent morning between 08:00 am and 11:00 am until either death, euthanasia or discharge. History, clinical examination and blood sample collection from the control

dogs were performed in the consulting room. Blood was collected via jugular venipuncture into serum tubes. The samples were allowed to clot at room temperature before centrifugation. After centrifugation the serum was collected and stored at -80°C until analysis, to ensure accuracy of iCa measurements[59]. Samples were stored for the duration of the study period and analysed in a single batch at the end of the study. Ionised calcium was adjusted to pH 7.40. As the study was part of a larger project it was not designed with the accurate measurement of iCa in mind. The pH was thus corrected to account for the aerobic handling of samples.

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

Ionised calcium and tMg measurements were classified as hypo-, normo-, hypercalcaemia and hypo-, normo- and hypermagnesaemia. The reference interval (RI) for iCa was set at 1.30 - 1.55 mmol/L to account for age-specific variation[56]. The RI for tMg was set at 0.6 - 1.2 mmol/L based on institutional RI (Onderstepoort Veterinary Clinical Pathology Laboratory). The CPE dogs were then classified based on outcome (fulfilment of 3 SIRS criteria, and non-survival vs survival). The following criteria were used to define SIRS:

- heart rate > 150 beats per minute
- respiratory rate > 30 breaths per minute
- temperature > 39.4°C or < 37.2°C
- white blood cell count > 19 x 10^9 cells/L or < 5 x 10^9 cells/L[25]

Dogs were assigned a SIRS score out of 4 based on how many SIRS criteria were met upon admission. The dogs were then re-evaluated daily before collection of blood samples 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 dogs diagnosed with parvovirus infection that met the SIRS criteria, were classified as having sepsis by definition.

3.3 Statistical analysis

Data were analysed using commercially available statistical software (Stata 16, StataCorp, College Station, TX). The Shapiro-Wilk test and evaluation of histograms were used to assess continuous data for normality. Differences in median between nonsurvivor and survivor groups were analyzed by use of the Mann-Whitney U test. Mean concentrations of iCa and tMg were compared between CPE and control groups on day one using Student's *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 one. The associations of hypo- and hypercalcaemia (vs normocalcaemia) on each day with mortality, and of hypo- and hypermagnesaemia (vs normomagnesaemia), were assessed using odds ratios and Fisher's exact test. The associations of hypo- and hypercalcaemia, and hypo- and hypermagnesaemia on day one with the development of sepsis on any day were assessed using Fisher's exact tests. The associations of hypo- and hypercalcaemia, and hypo- and hypermagnesaemia with the presence of sepsis using all daily data were assessed using mixed-effects multiple logistic regression. For all tests, significance was set at $P < .05$.

Chapter 4 – Results

Study population

Sixty-four dogs with CPE and 15 healthy control dogs were included in the study (**Table 1**). Dogs with CPE comprised of 33 (52%) intact males and 31 (48%) intact females. There were 52 (81%) survivors and 12 (19%) non-survivors. Of the 12 non-survivors only one was euthanised when the dog became agonal. Non-survivors (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 = 0.017). Healthy control dogs comprised of 10 (66%) intact male and 5 (33%) intact females. On day one, 3 (20%) of the control dogs, 13 (26%) of the survivors, and 6 (50%) of the non-survivors met three or more SIRS criteria (**Table 2**). One survivor (2%) and two non-survivors (17%) received whole blood transfusions as part of their treatment protocol. The survivor received the transfusion on day 1 and the non-survivors on day 1 and 4 respectively. Eight survivors (15%) received fresh frozen plasma transfusions: two on day 3, four on day 4, one on day 5, and one on day 6. Two non-survivors (17%) received fresh frozen plasma on day 2 and day 3 respectively. No dogs received any intravenous or oral calcium supplementation, nor were they administered any medication that could have impacted calcium and magnesium absorption.

Ionised calcium

Ionised calcium values were available for 56 dogs on admission (day one). Mean iCa concentrations of the CPE group on admission were significantly lower compared to the control group (P < .001). On day two, iCa concentrations of non-survivors were higher compared to survivors (P = 0.038). Moreover, dogs with hypercalcaemia on day two were more likely to die than those with normocalcaemia (OR = 10.7; 95%CI: 1.7-71; P = 0.031; **Table 3**). In contrast, iCa was not significantly different between survivors and non-survivors on any other days (**Figure 2**). Calcium concentrations were also not associated with the development of sepsis on any day.

Table 1 - Signalment and clinical data (day one) of the control group, and the survivor and non-survivor groups of dogs with CPE

Variable	**Control Group** **n = 15**	**Survivors** **n = 52**	**Non-survivors** **n = 12**
Age (months)	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 hospitalisation (days)		4.62 ± 2.77	2.58 ± 1.44[b]
Haematocrit (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 compared to survivors (*P* = 0.017)

[b] Significantly different compared to survivors (P = 0.003)

Table 2 - Summary of dogs meeting three or more SIRS criteria in the control group and the survivor and non-survivor groups of dogs with CPE

	Day 1 (Admission) **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%)
Non-survivors	6/12 (50%)	4/8 (50%)	2/5 (40%)	1/3 (33%)

Table 3 - Ionised calcium data in the control group and the survivor and non-survivor groups of dogs with CPE

Analyte categories	**Day 1 (Admission)** **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
Non-survivors	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%)
Non-survivors	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%)
Non-survivors	0/12 (0%)	3/6 (50%)	1/4 (25%)	0/2 (0%)

[a] Significantly higher compared to survivor group at $P < 0.05$.

*Ionised calcium measurements were not available for 8 dogs

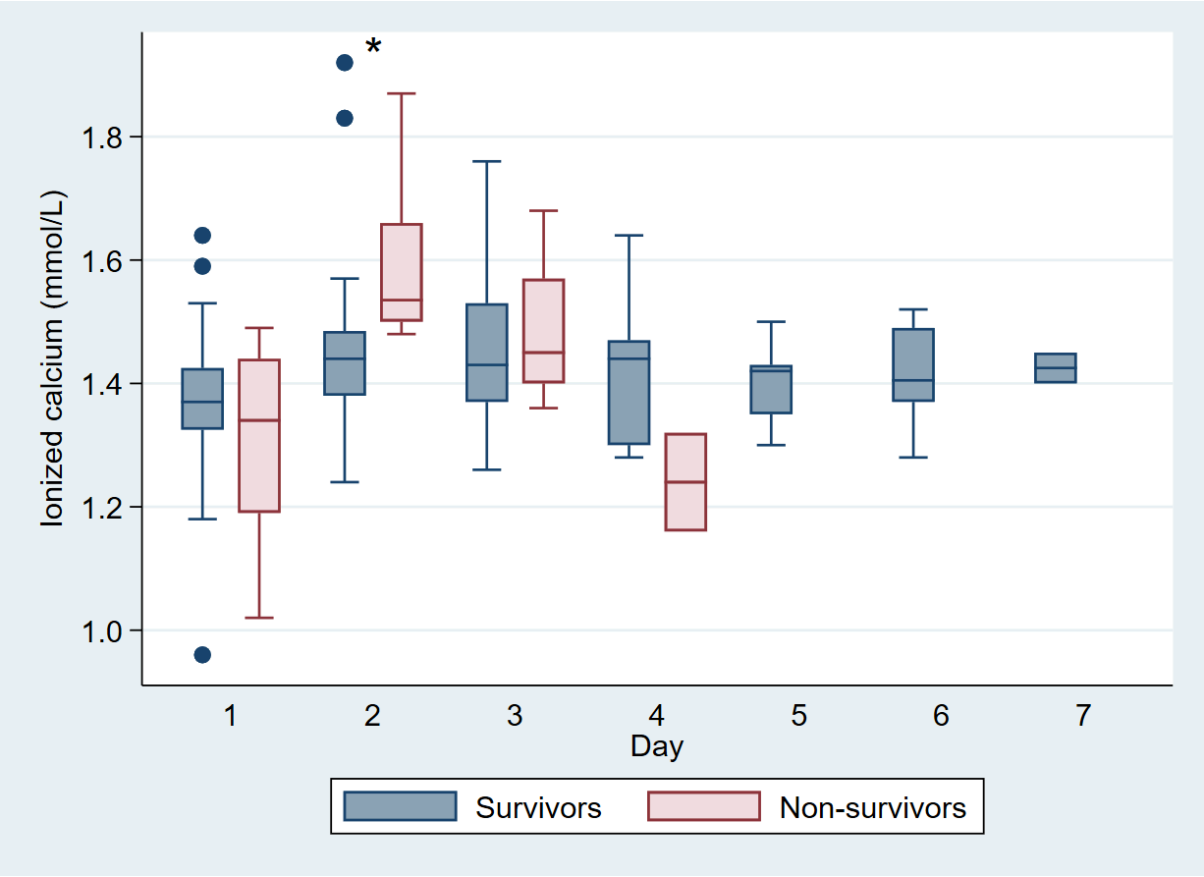

Figure 2 - Serial daily serum ionised calcium measurements in dogs with CPE. Horizontal lines indicate the median, box extends from 25th (Q1) to 75th (Q3) quartile, whiskers indicate fence values (lowest value ≥ Q1 – (1.5 x IQR) and highest value ≤ Q3 + (1.5 x IQR)) and dots show outliers.

* Ionised calcium concentrations of non-survivors were significantly higher compared to survivors at P = 0.038

Total magnesium

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 hypermagnesaemia was similar between the CPE and control groups (**Table 4**). Total Mg concentrations were not significantly different between survivors and non-survivors on any day (**Figure 3**), nor were they associated with mortality or the development of sepsis on any day.

Concurrent calcium and magnesium abnormalities

Two dogs in the control group had concurrent ionised hypercalcaemia and hypomagnesaemia. Six other dogs with ionised hypercalcaemia all had low normal magnesium values. In contrast, the remaining four control dogs with hypomagnesaemia were normocalcaemic. In the CPE survivor group two dogs had concurrent ionised hypercalcaemia and hypomagnesaemia upon admission. The dogs were siblings admitted on the same day. In the non-survivor group one dog had concurrent iHypoCa and hypermagnesaemia upon admission. On day two there was one dog in the survivor group with iHypoCa and hypomagnesaemia. On days three and four there were no concurrent iCa or tMg abnormalities in any dogs.

Table 4 - Total magnesium data in the control group, and the survivor and non-survivor groups of dogs with CPE

Analyte categories	**Day 1 (Admission)** **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
Non-survivors	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%)
Non-survivors	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%)
Non-survivors	1/12 (8%)	0/8 (0%)	0/5 (0%)	0/3 (0%)

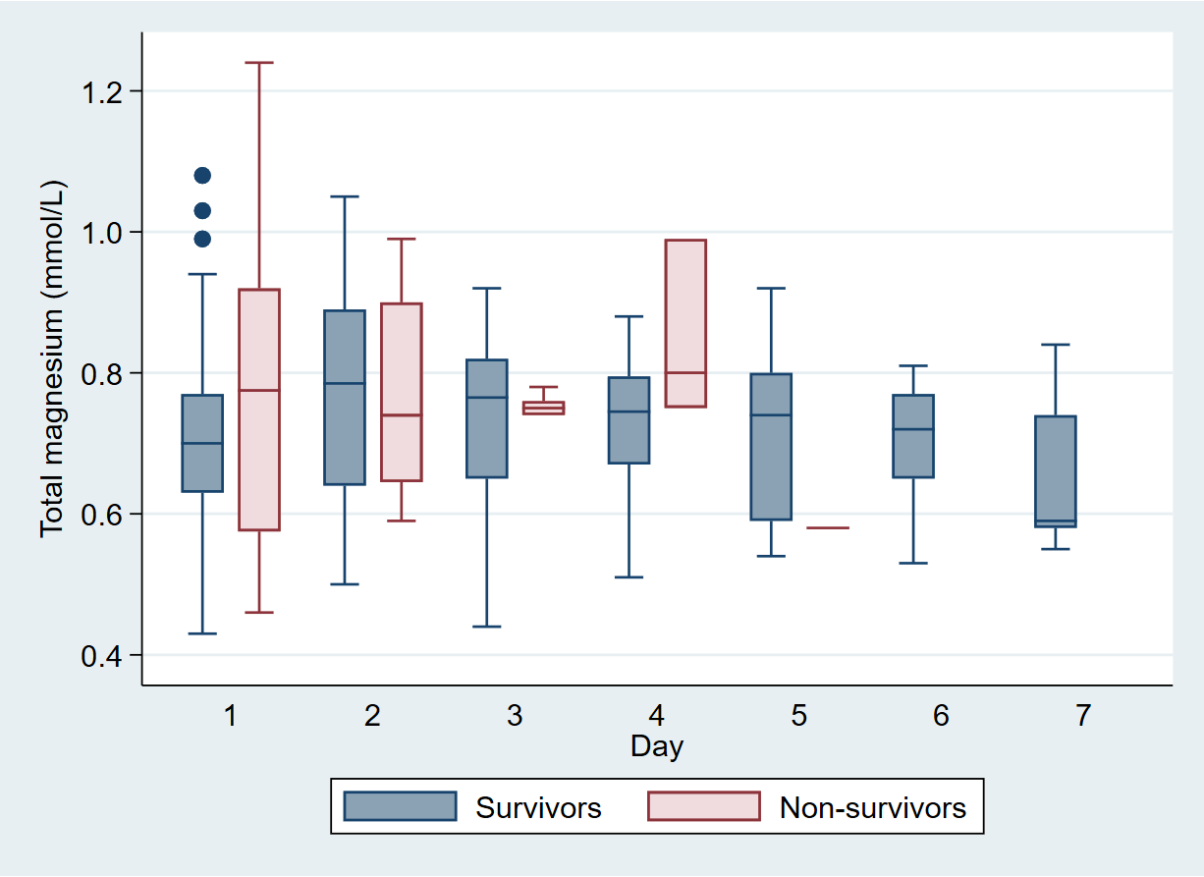

Figure 3 - Serial daily serum total magnesium measurements in dogs with CPE. Horizontal lines indicate the median, box extends from 25th (Q1) to 75th (Q3) quartile, whiskers indicate fence values (lowest value ≥ Q1 – (1.5 x IQR) and highest value ≤ Q3 + (1.5 x IQR)) and dots show outliers.

Chapter 5 – Discussion

In this study, iCa concentrations of non-survivors on day two were significantly higher compared to survivors, and dogs that were hypercalcaemic on day two were more likely to die than normocalcaemic dogs. A previous study in dogs demonstrated that iCa and outcome have a non-linear U-shaped relationship, as progressively abnormal concentrations, be it higher or lower, have been associated with increased fatality rates.[16] For example, cats with septic peritonitis that failed to normalise their iCa during hospitalisation had decreased survival rates.[68] Additionally, it has also been suggested that 24 hours after admission is the best time to prognosticate dogs with CPE.[7] A severe persistent leukopenia and lymphopenia, persistently elevated tumour necrosis factor (TNF) and/or C-reactive protein (CRP) concentrations, hypocholesterolaemia, severe hypothyroxinaemia, and elevated serum cortisol concentration have all been associated with a poor prognosis if present 24 hours after admission.[7]

Only 5% of the dogs with CPE were hypercalcaemic on day one, however that increased to 14% on day two and 19% on day three. Pathological causes of hypercalcaemia include malignancy, primary hyperparathyroidism, renal injury, hypoadrenocorticism, osteolysis, hypervitaminosis D and granulomatous disease.[17] Organ dysfunction, including acute kidney injury (AKI), is known to develop secondary to sepsis.[69] It may have played a role in the calcium abnormalities observed in the dogs with CPE, however hypocalcaemia is more commonly observed compared to hypercalcaemia in patients with AKI.[70,71] A rapid decline in glomerular filtration rate may result in increased serum phosphorus concentrations, along with a corresponding drop in serum calcium concentrations.[72] Metabolic acidosis commonly occur in dogs with AKI.[70] Calcium increases in patients with metabolic acidosis[60] and a significant inverse correlation has been found between pH and hypercalcaemia in dogs with hypoadrenocorticism.[73] Metabolic acidosis is also the most frequent acid-base abnormality reported in dogs with CPE.[74]

The development of sepsis induced AKI has several proposed mechanisms including ischaemia and reperfusion injury, inflammation, oxidative stress, renal tubular dysfunction and sublethal cell injury.[69] Evidence of AKI has been reported in dogs with CPE, however serum urea and creatinine are not sensitive indicators of early kidney injury. Subsequently AKI often remains undetected.[75] Additionally, a once off serum

creatinine is not considered a sensitive or specific marker for the diagnosis of early renal injury[76], dogs have lower creatinine concentrations than adults[77], creatinine production is decreased in sepsis[78], and dogs with CPE receive aggressive fluid therapy as part of their treatment, potentially diluting serum creatinine. Moreover, hypercalcaemia has been described in humans with SIRS, AKI and liver injury.[79] Extra-renal activation of 25-hydroxyvitamin D 1α-hydroxylase, the enzyme that converts 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3, in the liver may be responsible for the increase in calcium levels.[79] Recently, a study that investigated sepsis in dogs with CPE reported hepatic dysfunction as the most frequently occurring organ dysfunction.[80]

The prevalence of iHypoCa in dogs with CPE on presentation in our study was 18%. This is comparable to ranges reported from 16% in critically ill dogs to 24% in dogs that meet two or more SIRS criteria[5,14], but lower compared to a previous study that reported the prevalence of hypocalcaemia as 34% in dogs with CPE.[6] 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 hypocalcaemia and underestimate normocalcaemia in critically ill dogs with hypoalbuminaemia.[55] 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.[81,82]

Although tMg was not associated with outcome in this study, a recent study found a significant association between tMg on presentation and survival in dogs with CPE; for every 0.1 mmol/L increase in tMg concentration on admission, dogs had 2.50 lower odds of survival.[11] This discrepancy can potentially be ascribed to the small number of non-survivors in this study. Hypomagnesaemia has been the main focus related to magnesium regulation in critically ill people and animals[18], however hypermagnesaemia has also been associated with a negative outcome.[83] The most common aetiologies reported for the development of hypermagnesaemia are renal failure and iatrogenic causes[18], and an increase in magnesium concentrations has been associated with non-survival in dogs with acute kidney injury.[84] Although hypomagnesaemia was more common in critically ill dogs, hypermagnesaemic dogs were 2.6 times more likely die compared to dogs that had normal Mg levels[83]. In the

same study, hypomagnesaemia was associated with an increased incidence of concurrent hypokalaemia and hyponatraemia.[83] 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]

Ionised calcium concentrations were decreased in dogs with CPE, while tMg concentrations were higher compared to controls. The development of calcium and magnesium disorders can share similar pathomechanisms including malabsorption, intestinal losses, changes in distribution, as well as abnormalities in the metabolism of vitamin D.[12,18,85] Hypovitaminosis D, iHypoCa, and secondary hyperparathyroidism have all been described in dogs with protein-losing enteropathies (PLE).[86,87] Malabsorption is widely considered to be the most significant cause for the occurence of low vitamin D levels in PLE, however there are likely multiple factors contributing to its development.[88] Development of villous atrophy and subsequent loss of absorptive capacity in dogs with CPE could potentially cause a similar malabsorptive state. Other potential causes include increased vitamin D metabolism, reduced dietary intake and ongoing systemic inflammation.[88] As dogs do not synthesise vitamin D in their skin they are dependent on dietary intake in order to meet their vitamin D requirements.[89] After absorption of vitamin D into the bloodstream it is transported to the liver where hydroxylation occurs, resulting in 25(OH)D, also known as calcidiol.[90] Although 25(OH)D has a long biological half-life of 10 days to three weeks, the half-life of 1,25-dihydroxyvitamin D is only three to six hours.[91] Additionally, dogs with sepsis and critically ill dogs all have significantly lower 25(OH)D concentrations compared to healthy control dogs[49] and experimental induction of inflammation in dogs decreases 25(OH)D.[92]

The most common aetiology for both hypomagnesaemia and iHypoCa in dogs is gastrointestinal disease[63], and both have been well documented in dogs with PLE.[93-95] A severe decrease in Mg concentrations suppresses PTH release by interfering with magnesium-dependent enzymes necessary for PTH exocytosis. It is needed for the last hydroxylation step required to produce 1,25-dihydroxyvitamin D3[88] Since Mg is needed to produce 1,25-dihydroxyvitamin D3, concurrent hypomagnesaemia may reduce the availability of 1,25-dihydroxyvitamin D3, and consequently affect calcium absorption in the intestines.[94] Ionised Mg concentrations have also been correlated with iCa, PTH and 25-hydroxyvitamin D concentrations.[96] Measuring iMg is preferred

to tMg, because it contitutes the most biologically active component of circulating Mg and therefore represents total body Mg levels the best.[18,82] Although low tMg levels reflect total body depletion, normal tMg levels can exist in the presence of ionised hypomagnesaemia. While taking this into consideration, measuring tMg levels still remains the easiest and most available option for assessing Mg status.[66] Accordingly, a limitation of this study included the usage of tMg instead of iMg.

Dogs differ physiologically from adults and age-related variations in haematological, biochemical and electrolyte values have been reported.[56,77,97] Both iCa and tCa are higher in dogs compared to adults[56,77], whereas iMg was reported to be lower in dogs compared to adults.[56] Total magnesium ranges have not been compared between dogs and adults, however a strong correlation between ionised and tMg have been found in healthy adult dogs.[98] The control group in our study confirmed these findings. More than half of the control dogs were still considered hypercalcaemic 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 dogs as hypercalcaemic in this study. Similarly, all of the control dogs were either hypomagnesaemic or at the low end of the RI. These findings suggest that age-related variations may be larger than previously reported and poses a limitation with regards to interpreting whether or not these dogs truly had iCa and tMg concentrations outside of RIs.

In this study neither iCa or tMg concentrations were associated with the development of sepsis. This is in contrast with previous findings where iHypoCa and hypomagnesaemia have been well documented in critically ill and septic patients.[16,19,20] However, the currently recognised SIRS criteria where two out of four criteria must be met has a very low specificity.[42] Additionally, it has been noted that excitable dogs frequently have elevated heart and respiratory rates which further decreases the specificity of these SIRS criteria.[99] Consequently in this study three out of four SIRS criteria had to be met to increase specificity as to only include dogs that were truly septic. Notably, 20% of the control dogs in our study met the SIRS criteria however, none of them satisfied the criteria on white blood cell count. Since CPE is generally characterised by low to severely low white blood cell counts[10], this also played a role in decreasing the specificity of the SIRS criteria. Median white blood cell counts in dogs with CPE are significantly lower in non-survivors compared to survivors.[10] By applying more stringent criteria of meeting three out of four SIRS

criteria in dogs with CPE an association has been found with greater odds of non-survival[6], and dogs meeting three 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 non-survivors. Consequently, a type II error may have resulted in low power to detect associations of iCa and tMg with outcomes in dogs with CPE. Therefor caution should be used when applying the results to other populations of critically ill dogs. Additionally samples were collected under aerobic conditions and stored before analysis, which could have caused a false decrease in iCa measurements.

Chapter 6 – Conclusion

In summary, this study aimed to describe serial calcium and magnesium concentrations in dogs with severe CPE and to compare the results to healthy control dogs.

On admission dogs with CPE had significantly lower iCa and higher tMg compared to healthy dogs. Additionally, calcium may play a role in the outcome of dogs with CPE. Higher iCa a day after initiation of treatment was associated with increased odds of mortality. This correlates with previous studies that found that prognostication of dogs with CPE should ideally be done 24 hours after starting intensive therapy.

This study provides insight into calcium homeostasis in critically ill young dogs with parvoviral enteritis. Future studies investigating the correlation between calcium, magnesium, PTH and 1,25-dihydroxyvitamin D concentrations are warranted to further investigate calcium homeostasis in critically ill patients.

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Appendix A: Research ethics certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee 15 August 2022

LETTER OF APPROVAL

Ethics Reference No	**REC092-22**
Protocol Title	**Ionised calcium and magnesium, and their association with outcome and sepsis in puppies with parvoviral enteritis**
Principal Investigator	**Miss A Mouton**
Supervisors	**Prof JP Schoeman**

Dear Miss A Mouton,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC092-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals:** FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

We wish you the best with your research.

Yours sincerely

Mrs. MR Watson-Kriek
Chairperson (acting): Research Ethics Committee

100 YEARS OF VETERINARY EDUCATION

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Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disaense tša Bongakadiruiwa

Appendix B: Data collection and consent forms – Parvoviral enteritis group

PARVOVIRAL DIARRHOEA

CASE .....

JP SCHOEMAN
HPA axis in Parvo

Information sheet on canine parvoviral diarrhoea

From the clinical examination and laboratory tests so far performed on your dog, it seems most likely that he/she is suffering from a viral infection, called canine parvovirus or so called "cat flu". This virus causes damage to the intestines such that the normal intestinal lining is lost, bleeding occurs and food cannot be absorbed.

It has been advised that your dog should be admitted to the Onderstepoort Isolation unit for intensive care treatment. Your dog will be treated with intravenous fluids (drips containing glucose and various salts), antibiotics, drugs that suppress nausea and vomiting, deworming, and if needed, blood- or plasma transfusions.

In this study we would like to test the daily function of your dog's adrenal glands by taking 1.5 ml of blood, injecting a substance called ACTH (which is routinely used to test the function of these glands in dogs and people) and then taking 1 ml of blood 1 hour later. Urine will be either expressed or taken from the bladder every 2nd day by needle puncture.

This study will cost you no more money than it would usually cost to treat your dog. We are paying for all the additional blood and faecal tests performed on your puppy.

This study has been approved by the Animal Use and Care Committee of the University of Pretoria.

A better understanding of the disease process may lead to better treatment of clinical cases.

If you have any questions, please discuss the matter with the clinician on duty or myself.

Your decision whether or not to allow samples to be drawn from your dog will in no way influence the handling of this case, or cases which you may present at the Veterinary Hospital in future.

Sincerely

Prof JP Schoeman
Department of Companion Animal Clinical Studies
Faculty of Veterinary Sciences
University of Pretoria
Onderstepoort
0110
Tel 012-5298261

FORM FOR INFORMED CONSENT

(To be completed by the patient's owner)

Title of the research project: Adrenal gland function in canine parvoviral diarrhoea.

Encircle Yes or No where necessary

1. Have you read the information sheet on canine parvoviral diarrhoea?
   Yes No
2. Have you had the opportunity to ask questions about the research project? Yes No
3. Have you received satisfactory answers to your questions?
   Yes No
4. Have you received enough information about this study
   Yes No
5. Supply the name of the person to whom you have spoken to :

   .....
6. Do you grant consent that blood and urine can be drawn from your dog? Yes No

Name of owner: .....

Signature: .....

Name of witness: .....

Signature: .....

Date: .....

Inligtingsblad oor parvovirusinfeksie in honde

Vanuit die kliniese ondersoek en laboratoriumtoetse sover uitgevoer op u hond, lyk dit asof u hond aan 'n virusinfeksie lei, genaamd hond parvovirus of sogenaamde "katgriep". Hierdie virus veroorsaak skade aan die dermkanaal, so erg dat die normale dermvoering verlore gaan, bleeding voorkom en kos nie meer geabsorbeer kan word nie.

Dit word aanbeveel dat u hond opgeneem moet word na die Onderstepoort Isolasie-eenheid vir intensiewe behandeling. U hond sal behandel word met binne-aarse vloeistowwe (drips wat glukose en verskeie soute bevat), antibiotika, middels wat naarheid en vomisie onderdruk, ontwurming, en indien nodig, bloed- of plasma oortapping.

In hierdie studie wil ons graag die daaglikse funksie van u hond se adrenaalkliere toets deur 1.5 ml bloed *te* trek, 'n substans genaamd ACTH in te spuit (wat gewoonlik gebruik word om die funksie van hierdie kliere in honde en mense te toets) en dan 1 ml bloed een uur later te trek. Urine sal ook elke tweede dag of uitgedruk word of met 'n naald direk van die blaas getrek word.

Die studie sal u niks meer kos as wat dit u in elk geval sou kos om u hond te behandel nie. Ons sal vir alle addisionele bloed- en fekale toetse wat gedoen word, betaal.

Hierdie studie is goedgekeur deur die Dieregebruikskomittee van die Universiteit van Pretoria.

Deur die siekte beter te verstaan, sal kliniese gevalle beter behandel kan word.

Bespreek die aangeleentheid gerus met die klinikus aan diens of myself, indien u enige vrae het.

U besluit om bloed- en urinemonsters van u dier te laat trek al dan nie, sal op geen manier die behandeling van die geval, of enige ander gevalle wat u na die Dierehospitaal in die toekoms mag bring, beinvloed nie.

Die uwe

Prof JP Schoeman
Departement Geselskapsdier Kliniese Studies
Fakulteit Veeartsenykunde
Universiteit van Pretoria
Onderstepoort
Tel 0125298261

VORM VIR INGELIGTE TOESTEMMING

(Om deur die pasient se eienaar ingevul te word.)

Titel van die navorsingsprojek: Bynierfunksie in hond parvovirus

Omkring Ja of Nee, waar toepaslik

1. Het u die inligtingsblad oor parvovirus infeksie van honde gelees?
   Ja Nee
2. Het u die geleentheid gehad om oor die navorsings projek uit te vra?
   Ja Nee
3. Is u vrae bevredigend
   beantwoord? Ja Nee
4. Voel u dat u voldoende ingelig is oor die navorsingsprojek?
   Ja Nee
5. Verskaf asseblief die naam van die persoon met wie u gepraat het:

   .....
6. Verleen u toestemming dat bloed- en urienemonsters van u hond getrek word? Ja Nee

Naam van eienaar: ..... .

Handtekening: .....

Naam van getuie: .....

Handtekening: .....

Datum: .....

Clinical Examination (Admission)

Client Name: ..... Patient Number: .....
Patient Name: .....
Breed: .....
Sex: ..... Age: ..... Weight: .....
Vaccination Dates: .....
Date of Admission: .....
Time of Admission: .....
Time of last meal: .....

Number of days depressed	1	2	3	>3
Number of days vomiting	1	2	3	>3
Vomiting episodes per day	1	2	3	>3
Description of vomitus				
Number of days diarrhoea	1	2	3	>3
Diarrhoea episodes per day	1	2	3	>3
Description of diarrhoea				
Habitus	1+	2+	3+	4+
Lo Dehydrated	0-5%	5%	10%	>10%
Mucosae	Moist		Dry	
	Pale	Pink	Congested	
Oral ulcerations	Yes		No	
Pulse quality	Weak	Strong	Water hammer	
Pulse rhythm	Regular		Irregular	
Depth of respiration	Normal	Laboured	Shallow	
Abnormal lung sounds	Yes		No	
If yes, describe				
Abdominal paloation	Tense		Easily palpable	
	Painful		Not painful	
	Thickened gut loops		Fluid filled gut loops	
	Gas in intestines		Intussusception	
Blood smear	Parasites		Leukopenia	
	Thrombocytopenia		Reticulocytes present	
Faecal flotation				
Faecal wet preparation				

Clinical scoring assessment:

Patient-number:

Date:

Day number: 1(admission), 2, 3, 4, 5, 6, 7, 8, 9, 10, 11. (Encircle choice)

Temp: Pulse: Resp:

Weight: **Encircle the applicable choice under 1 - 8 below:**

1) Habitus	1	Collapsed / moribund
	2	Severe depression
	3	Mild-to-moderate depression
	4	Normal
2) Appetite:	1	No interest in food
	2	Voluntarily eats small amounts of food offered
	3	Voluntarily eats moderate amounts of food offered (but not normal)
	4	Normal
3) Vomition:	1	Severe (>6 times per 12h)
	2	Moderate (3-5 times per 12h)
	3	Mild (l-2 times per 12h)
	4	Absent
4) Faecal consistency:	1	Watery diarrhoea, bloody
	2	Watery diarrhoea, not bloody
	3	Soft
	4	Well-formed
7) Mucous membranes	1	Congested
	2	Pale
	3	Normal
8) Capillary Refill Time	I	> 2 seconds
	2	< 1 second
	3	1-2 seconds

Blood glucose mmol/1

Blood lactate mmol/1

ACTH dose: **C = ……** **C+ = ……**

Patient Outcome

Patient number: ....

SNAP test positive for parvovirus .....

E.M. Positive for parvovirus? .....

E.M. Positive for Coronavirus? .....

Died / Recovered

Date died / recovered: .....

Time died / recovered: .....

Days to recovery / death? .....

Complications developed? .....

If so, describe .....

.....

……

……

.....

Appendix B: Data collection and consent forms – Control group

PARVOVIRAL DIARRHOEA (CONTROL GROUP)

CASE .....

JP SCHOEMAN
HPA axis in Parvo

Information sheet on the healthy control group to compare with dogs suffering from canine parvoviral diarrhoea

We have recently tested the function of the endocrine system in dogs with parvoviral diarrhoea. We are now conducting the same test on a group of healthy dogs.

In the control group we would like to take 5 ml of blood (1 teaspoon), inject a substance called ACTH (which is routinely used to test the function of these glands in dogs and people) and then take 2 ml of blood 1 hour later. Urine will be either expressed or taken from the bladder by needle puncture. Blood pressure will be taken by placing a cuff around your dog's leg and reading measurements off the machine

As a compensation for participating in the study we will perform the clinical examination and vaccination of your dog FREE OF CHARGE.

This study has been approved by the Animal Use and Care Committee of the University of Pretoria.

A better understanding of the disease process may lead to better treatment of clinical cases.

If you have any questions, please discuss the matter with the clinician on duty or myself.

Your decision whether or not to allow samples to be drawn from your dog will in no way influence the handling of this case, or cases which you may present at the Veterinary Hospital in future.

Sincerely

Prof JP Schoeman
Department of Companion Animal Clinical Studies
Faculty of Veterinary Sciences
University of Pretoria
Onderstepoort
0110
Tel 012-5298261

FORM FOR INFORMED CONSENT

Title of the research project: Adrenal gland function in canine parvoviral diarrhea (Control group).

Encircle Yes or No where necessary

1. Have you read the information sheet?
   Yes No
2. Have you had the opportunity to ask questions about the research project? Yes No
3. Have you received satisfactory answers to your questions?
   Yes No
4. Supply the name of the person to whom you have spoken to :
   ……
5. Do you grant consent that blood and urine can be drawn from your dog?
   Yes No

Name of owner: .....

Signature: .....

Name of witness: .....

Signature: .....

Date: .....

Client Name: ..... Patient Number: .....
Patient Name: .....
Breed: .....
Sex: ..... Age: ..... Weight: .....
Vaccination Dates: .....
Date of Sampling: .....
Time of Sampling: .....
Time of last meal: .....

Temp: Pulse: Resp:

1) Habitus	3	Mild-to-moderate depression
	4	Normal
2) Appetite	3	Voluntarily eats moderate amounts of food offered
	4	Normal
3) Vomition	3	Mild (1-2 times per 12h)
	4	Absent
4) Faecal consistency	3	Soft
	4	Well-formed
7) Mucous membranes	1	Congested
	2	Pale
	3	Normal
8) Capillary Refill Time	1	> 2 seconds
	2	< 1 second
	3	1-2 seconds

Blood glucose **……mmol/1**

Blood lactate **……mmol/1**

ACTH dose: **C = ……** **C+ = ……**

Appendix C: Presentations and publications

Presentations:

- Mouton A, Celliers A, Thompson PN, Mellanby RJ, Schoeman JP. 2024. Serial ionized calcium and total magnesium in puppies with parvoviral enteritis. [Poster]. World Veterinary Association Congress, 16-19 April, Cape Town.

Publications:

- Mouton A, Celliers A, Thompson PN, Mellanby RJ, Schoeman JP. Calcium and magnesium abnormalities in puppies with parvoviral enteritis. Accepted for publication by the American Journal of Veterinary Research.