J S Afr Vet Assoc. 2022;93(1):1-7
https://doi.org/10.36303/JSAVA.2022.93.1.496

J S Afr Vet Assoc
ISSN 2224-9435 EISSN 1019-9128

ORIGINAL RESEARCH

Circulating markers of endothelial activation in canine parvoviral enteritis

BK Atkinson, A Goddard, M Engelbrecht, S Pretorius, P Pazzi

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, South Africa
Corresponding author, email: *drbroganka@gmail.com*

Canine parvovirus (CPV) is a common cause of enteritis, immune suppression and systemic inflammation in young dogs. Endothelial markers, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), and molecules that upregulate their expression, such as high mobility group box 1 protein (HMGB-1), provide insight into the state of the endothelium during inflammation.

This study aimed to determine if circulating concentrations of ICAM-1, VCAM-1 and HMGB-1 were altered in CPV enteritis compared to healthy controls, and whether a correlation existed between these molecules and the degree of inflammation.

Thirty dogs with naturally occurring CPV enteritis and ten control dogs were included. Physical examinations, complete blood count and C-reactive protein (CRP) measurements were performed on all dogs at presentation. The concentrations of ICAM-1, VCAM-1 and HMGB-1 were measured using commercially available canine-specific enzyme-linked immunosorbent assay (ELISA) kits.

In dogs with CPV enteritis, ICAM-1 concentrations were significantly lower (median: 5.9 [IQR: 4.3–8.3]) and CRP higher (134 [IQR: 85–195]) compared to controls (8.0 [IQR: 6.9–10.3], $p = 0.008$; 1 [IQR: 0–7], $p < 0.001$). No significant difference was found for VCAM-1 and HMGB-1. A strong correlation was identified between VCAM-1 and segmented neutrophil count ($r = 0.612$, $p < 0.001$).

Despite the presence of systemic inflammation in CPV enteritis, evidenced by high CRP concentrations, our results suggest circulating concentrations of ICAM-1, VCAM-1 and HMGB-1 failed to show an increase. Endothelial activation with subsequent leukocyte adhesion and transmigration through the endothelium may be affected in CPV enteritis and these findings require further investigation.

Keywords: canine parvovirus, ICAM-1, VCAM-1, HMGB-1, leukocytes

Introduction

Canine parvovirus (CPV) is a common cause of enteritis, immune suppression and systemic inflammation with a high rate of morbidity and mortality in unvaccinated young dogs around the world. The causative agent, CPV type 2, is transmitted through the faecal-oral route or indirectly through contaminated fomites (Goddard & Leisewitz 2010). Once exposed, the virus replicates in lymphoid tissue of the oropharynx before haematogenous dissemination to tissues with high cellular turnover rates, such as the gastrointestinal tract (GIT), bone marrow, thymus and lymph nodes (Goddard & Leisewitz 2010). Within these tissues, there is viral replication and tissue destruction resulting in compromised GIT barrier and leukopenia (Goddard et al. 2008; Meunier et al. 1985; Mohr et al. 2003). Eventually infection leads to compromised immune function, bacterial translocation from the GIT and sepsis (Isogai et al. 1989; Otto et al. 1997). Studies have demonstrated evidence of circulating endotoxin and increased inflammatory markers during CPV infection (Isogai et al. 1989; McClure et al. 2013; Otto et al. 1997). The positive acute phase protein, C-reactive protein (CRP), is one marker of inflammation which increases during CPV enteritis (Black et al. 2004; McClure et al. 2013).

The vascular endothelium is involved in the regulation of vascular tone, haemostasis, leukocyte transmigration and is also essential to the systemic inflammatory response (Liao 2013). Dysfunctional endothelial responses may contribute to morbidity in critical disease (Hildebrand et al. 2005). Increased endothelial biomarkers have been identified in human sepsis, malaria and dengue fever, and were even predictive of disease severity and mortality in some cases (Page & Liles 2013). Endothelial adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), as well as molecules that influence their expression such as high mobility group box 1 protein (HMGB-1) provide insight into the state of the endothelium during systemic inflammation (Fiuza et al. 2003; Page & Liles 2013). There is a dearth of research into endothelial function in small animals. Two studies investigating ICAM-1 in dogs with *Babesia canis* infection reported contradicting results between infected and healthy control dogs on the day of admission (Baric Rafaj et al. 2013; Kules et al. 2017). The first study found increased circulating ICAM-1 concentrations in *Babesia* infected dogs compared to control dogs (Baric Rafaj et al. 2013); however, the second study failed to identify a difference (Kules et al. 2017). The second study did, however, report that both VCAM-1 and HMGB-1 were increased in the *Babesia* infected dogs at admission (Kules et al. 2017). Another study reported that being overweight or obese did not appear to influence circulating concentrations of ICAM-1 and HMGB-1 in dogs (Baric Rafaj et al. 2017). Endothelial adhesion molecule expression is upregulated by pro-inflammatory cytokines, endotoxin, tissue damage and cell death (Liao 2013), all of which are present in CPV enteritis (Isogai et al. 1989; Meunier et al. 1985; Otto et al. 1997; Sahinduran et al. 2016).

Sepsis, particularly that associated with coliform septicaemia, is a well-defined complication of CPV enteritis and is often a key role

player in the eventual death of severely affected dogs (Isogai et al. 1989; Mohr et al. 2003; Prittie 2004). The endothelium and its dysfunction may be an important component in the progression of CPV enteritis given the prominent role of sepsis and systemic inflammation in the pathophysiology of this disease. Research into endothelial function in critical diseases such as CPV enteritis would not only improve our understanding of endothelial activation and the consequential effect it has on leukocyte migration and vascular barrier function but may also allow for triage and prognostication in patients (Lee & Liles 2011). To the authors' knowledge, endothelial activation during CPV infection has not been investigated and the aims of this study were to determine whether alterations in circulating endothelial markers of activation are present in CPV enteritis compared to healthy controls, as well as enhance our understanding of the disease pathogenesis and the state of endothelial activation in relation to the inflammatory response. The objectives of this study were firstly to determine if circulating concentrations of endothelial adhesion molecules were altered in CPV enteritis at presentation. Secondly, we aimed to ascertain if correlations between endothelial adhesion molecules, markers of inflammation or leukocyte changes exist. We hypothesised that circulating concentrations of endothelial adhesion molecules would be increased in dogs with CPV enteritis, and that the degree of increase would correlate with the degree of inflammation. The results of this study may provide meaningful insight into the level of endothelial activation, subsequent leukocyte transmigration and disease pathogenesis in CPV enteritis.

Materials and methods

Animals

This was a prospective observational clinical study in which dogs with CPV enteritis were compared with healthy control dogs at presentation. The CPV group were client owned dogs, between 6 weeks and 12 months of age, with clinical signs consistent with natural CPV infection, including lethargy, anorexia, vomiting, diarrhoea and dehydration. Based on clinical suspicion, dogs were tested for CPV using the faecal CPV enzyme-linked immunosorbent assay (ELISA) test (IDEXX SNAP Parvo Test, Netherlands) and confirmed with faecal electron microscopy. Dogs were excluded if they received treatment for CPV enteritis within the preceding 7 days. The healthy control group consisted of dogs that presented for routine procedures (vaccinations and sterilisation) and were aged matched to the CPV group. Control dogs were excluded if there was any history of illness for the preceding 14 days or if CPV was identified on faecal electron microscopy. For both groups, dogs had to weigh more than 3 kg. Dogs receiving medication known to influence inflammation or with comorbidities identifiable on clinical examination or blood smear evaluation, with the exception of gastrointestinal helminths, were excluded.

Data and sample collection

A history, clinical examination, peripheral blood smear and faecal flotation were performed at presentation. A faecal sample was collected from all dogs and a CPV ELISA snap test was performed on the CPV group. Faecal samples from both groups were stored at 2–8 ^{0}C and electron microscopy was performed within 72 hours of collection to confirm/exclude infection with CPV. Blood was collected atraumatically via jugular venepuncture, using the Vacutainer blood collection system, into ethylenediaminetetraacetic acid (EDTA) and serum vacutainer tubes (Beckton Dickinson Vacutainer Systems, UK). A complete blood count (CBC) (Advia 2120i, Siemens, Germany) and central blood smear were performed within 30 minutes of collection. The EDTA and serum samples were then centrifuged. When possible, serum samples were refrigerated at 2–8 ^{0}C and used to measure CRP (canine-specific immunoturbidimetric CRP method, Cobas Integra 400 plus analyser) within 24 hours of collection. When analysis within 24 hours was not possible, the serum and EDTA plasma were stored at -80 ^{0}C. Batch measurement of CRP was then performed at the end of the study period. Freezing and storage of serum does not significantly influence CRP concentrations (Aziz et al. 2003; Hillstrom et al. 2014).

Endothelial adhesion molecule evaluation

Thawed EDTA plasma was used to measure ICAM-1, VCAM-1 and HMGB-1 using canine-specific ELISA sandwich enzyme immunoassays (USCN Life Science, Wuhan, China) (Baric Rafaj et al. 2013; Kules et al. 2017). Studies have reported no effect of freezing and thawing of samples when running these assays (Kavsak et al. 2008; Wang et al. 2015). The ELISA analyses included plate preparation and assay procedures performed according to the manufacturer's recommended protocol. The colour change of the enzyme-substrate reaction was measured spectrophotometrically at a wavelength of 450 nm (Thermo Scientific Multiskan™ FC Microplate Photometer, Thermo Fischer Scientific). The concentrations of the ICAM-1, VCAM-1 and HMGB-1 were determined by comparing the optical density of the samples to the standard curves. The detection ranges for the ICAM-1, VCAM-1 and HMGB-1 assays were 1.56–100 ng/ml, 3.12–200 ng/ml and 6.25–400 pg/ml, respectively. The concentrations read from the standard curve were multiplied by the dilution factor for VCAM-1 and HMGB-1. The intra-assay coefficient of variance was less than 10% and the inter-assay coefficient of variance less than 12% for all three immunoassays.

Statistical analyses

Statistical analysis was performed using SPSS Statistics 25.0. The distribution of the data from the CBC, CRP, ICAM-1, VCAM-1 and HMGB-1 were tested for normality using the Shapiro–Wilk test. The data had a non-normal distribution and comparison of all variables between the two groups was analysed using the Mann–Whitney U test. The chi-square test was used to compare breed and sex between groups. Spearman's rank correlation was used to measure the strength of associations between all continuous variables within the CPV group. Descriptive data are presented as median and interquartile range (IQR). $P < 0.05$ was considered significant.

Results

The demographic characteristics of the CPV and control groups can be found in Table I. There were no significant differences for sex, age or breed between the two groups.

Table I: Demographic characteristics of the CPV and control groups

Demographic information	CPV group (n = 30) Median (IQR)	Control group (n = 10) Median (IQR)
Age (Months)	4 (3–6)	4 (3–6)
Gender		
Male	21	6
Female	9	4
Breed		
Jack Russel Terrier	1	
Cross breed	9	2
Pekingese	1	1
Labrador Retriever	6	1
Pitbull Terrier	6	1
St Bernard	1	
Husky	1	2
Dachshund	1	1
Ridgeback	1	
Boerboel	1	
Yorkshire Terrier	1	
Fox Terrier	1	
Golden Retriever		2

IQR – interquartile range, CPV – canine parvovirus

Table II: Haematology, inflammatory and circulating endothelial adhesion molecule variables in the CPV and control groups

Variable	Group	Measurement Median (IQR)	p-value
Haemoglobin	Control CPV	135.50 g/L (119–138) 130.00 g/L (114–156)	0.901
Red cell count	Control CPV	5.67 x 10^{12}/L (5.31–6.33) 5.99 x 10^{12}/L (5.30–6.96)	0.390
Haematocrit	Control CPV	0.40 L/L (0.37–0.41) 0.38 L/L (0.36–0.45)	0.913
White cell count	Control CPV	12.43 x 10^9/L (10.22–14.01) 5.89 x 10^9/L (3.19–7.85)	0.001*
Segmented neutrophils	Control CPV	6.97 x 10^9/L (5.57–7.87) 4.14 x 10^9/L (2.1–6.24)	0.034*
Band neutrophils	Control CPV	0 x 10^9/L (0–0) 0.09 x 10^9/L (0–0.25)	0.038*
Lymphocytes	Control CPV	4.1 x 10^9/L (1.99–5.25) 0.67 x 10^9/L (0.41–0.97)	< 0.001*
Monocytes	Control CPV	0.81 x 10^9/L (0.68–1.08) 0.32 x 10^9/L (0.20–0.55)	< 0.001*
Eosinophils	Control CPV	0.42 x 10^9/L (0.21–0.79) 0.05 x 10^9/L (0–0.16)	< 0.001*
Platelets	Control CPV	416 x 10^9/L (292–506) 340 x 10^9/L (258–386)	0.070
CRP	Control CPV	1 mg/l (0–7) 134 mg/l (85–195)	< 0.001*
ICAM-1	Control CPV	8.02 ng/ml (6.93–10.33) 5.86 ng/ml (4.28–8.27)	0.008*
VCAM-1	Control CPV	410 ng/ml (363–452) 459.5 ng/ml (363–505)	0.267
HMGB-1	Control CPV	86.5 pg/ml (80–87) 91.5 pg/ml (84–101)	0.150

* Significant differences
IQR – interquartile range, CPV – canine parvovirus, ICAM-1 – intercellular adhesion molecule 1, VCAM-1 – vascular cell adhesion molecule 1, HMGB-1 – high mobility group box protein 1

The leukocyte (p = 0.001), mature neutrophil (p = 0.034), lymphocyte (p < 0.001) and monocyte (p < 0.001) concentrations were significantly lower in the CPV group compared to the control group (Table II). The erythrocyte variables and the platelet

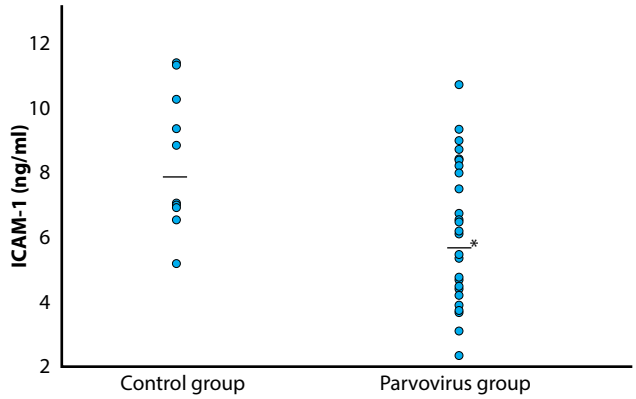

Figure 1: ICAM-1 concentrations at presentation for CPV enteritis. Black line represents the median for each group. * Significantly lower than the control group.

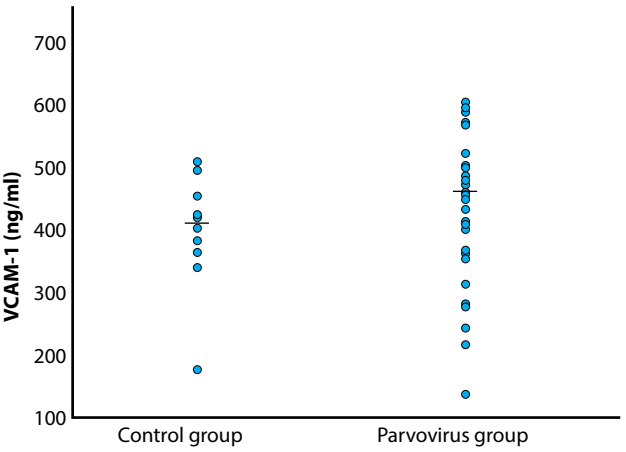

Figure 2: VCAM-1 concentrations at presentation for CPV enteritis. Black line represents the median for each group.

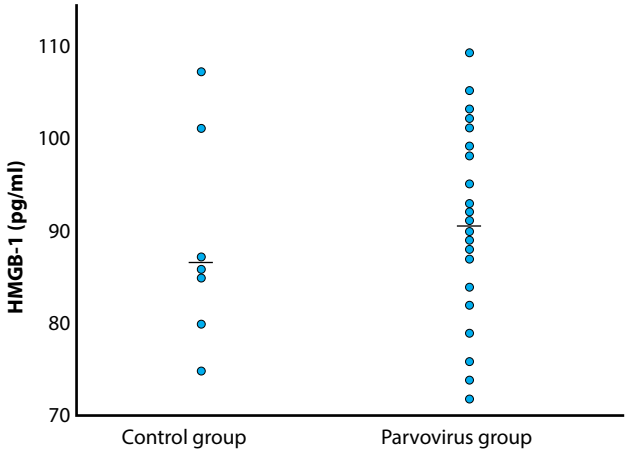

Figure 3: HMGB-1 concentrations at presentation for CPV enteritis. Black line represents the median for each group.

concentrations were not significantly different between the two groups. The CRP concentration was significantly increased (p < 0.001) in the CPV group compared to the control group (Table II).

All the dogs in the study had plasma concentrations of ICAM-1, VCAM-1 and HMGB-1 within the detection limit of the assays used and the results may be found in Table II and Figures 1–3. The CPV group had a significantly lower ICAM-1 concentration

compared to the control group ($p = 0.008$). No significant differences were identified between the CPV and control groups for VCAM-1 and HMGB-1 concentrations.

The VCAM-1 concentration was positively correlated with the mature neutrophil concentration ($r = 0.612$, $p < 0.001$) in the CPV group.

Discussion

This is the first study to evaluate circulating endothelial adhesion molecule concentrations in dogs with CPV enteritis. Our study found that despite the presence of marked systemic inflammation, as indicated by high CRP concentrations, there was limited release of soluble ICAM-1 and VCAM-1 from the endothelium during the course of this disease. Moreover, the concentrations of HMGB-1, which influences the release of ICAM-1 and VCAM-1, were not increased in dogs with CPV enteritis at presentation. This was an unexpected finding as CPV is associated with marked systemic inflammation and sepsis (Isogai et al. 1989; Kocaturk et al. 2010; McClure et al. 2013; Otto et al. 1997), both of which are known to trigger endothelial activation and the release of soluble endothelial adhesion molecules in septic states in humans (Page & Liles 2013).

CRP, a recognised marker of systemic inflammation and a positive acute phase protein produced by the liver, is known to increase in CPV enteritis (Kocaturk et al. 2010; McClure et al. 2013). The serum CRP concentration was significantly increased in the CPV group at presentation compared to the control group. Increases in serum CRP concentration occur in response to pro-inflammatory cytokines such as interleukin (IL)-6 and IL-1β, and serial measurements may be a useful monitoring tool along with other parameters in CPV enteritis (Black et al. 2004; McClure et al. 2013). The increase in serum CRP during CPV enteritis is thought to be due to the tissue damage and systemic inflammatory response associated with the viral infection and associated sepsis (McClure et al. 2013).

Markers of endothelial activation, such as circulating endothelial adhesion molecules, ICAM-1 and VCAM-1, have demonstrated increased concentrations in human sepsis, malaria and dengue fever (Page & Liles 2013). However, these findings may be inconsistent, making endothelial markers unreliable diagnostic or prognostic markers in human medicine (Page & Liles 2013). Despite this, endothelial markers provide important insight into the dynamic response of the endothelium during systemic inflammation and sepsis (Page & Liles 2013). The only research available on changes in circulating ICAM-1 and VCAM-1 concentrations in an inflammatory condition affecting canine patients was performed in dogs naturally infected with *B. canis* and reported increased VCAM-1 concentrations, but ICAM-1 concentrations were conflicting between the studies (Baric Rafaj et al. 2013; Kules et al. 2017). In our study, we found significantly lower soluble ICAM-1 concentrations in dogs with CPV enteritis compared to healthy dogs at presentation, and no difference in the concentrations of VCAM-1 between groups.

The endothelial adhesion molecule, ICAM-1, is an inducible cell surface adhesion glycoprotein expressed on the surface of endothelial cells, monocytes and several other cell lines (Hubbard & Rothlein 2000; Roebuck & Finnegan 1999). It is upregulated in inflammatory conditions by cytokines such as TNFα, IL-1β, IL-6, INFγ and HMGB-1 released by neutrophils, lymphocytes and monocytes (Fiuza et al. 2003; Hubbard & Rothlein 2000; Roebuck & Finnegan 1999). This adhesion molecule plays an important role in the transmigration of leukocytes through the endothelium, acts as a co-stimulator in antigen receptor activation of T and B lymphocytes and is involved in signal transduction pathways across cellular membranes (Hubbard & Rothlein 2000; Roebuck & Finnegan 1999). A member of the immunoglobulin superfamily, VCAM-1, is a cellular adhesion molecule expressed by endothelial cells and is also involved in leukocyte transmigration (Iademarco et al. 1995; McHale et al. 1999). Upregulation of VCAM-1 expression is triggered by increases in cytokines such as TNFα, IL-1 and IL-4 as well as HMGB-1 (Fiuza et al. 2003; Iademarco et al. 1995; McHale et al. 1999). Soluble circulating endothelial adhesion molecule concentrations present during inflammatory and septic conditions are the result of increased cellular expression and the proteolytic activity of sheddases responsible for releasing these molecules from the cell surface (Zonneveld et al. 2014). Important sheddases known to cleave endothelial adhesion molecules from the cell surface include neutrophil elastase, involved in the release of both ICAM-1 and VCAM-1, as well as matrix metalloproteinase 9 involved in the release of ICAM-1 (Vandooren et al. 2013; Zonneveld et al. 2014). The lack of increased circulating concentrations of ICAM-1 and VCAM-1 in dogs with CPV enteritis may be explained by a combination of severe leukopenia seen in dogs with CPV enteritis, increased endogenous glucocorticoid concentrations or a time-dependent transient suppression of the release of soluble endothelial adhesion molecules (Goddard et al. 2008; Schoeman et al. 2007; Zonneveld et al. 2014).

The leukopenia seen in CPV enteritis is caused by a number of factors including myeloid precursor toxicity and degeneration (Boosinger et al. 1982; Goddard et al. 2008). There is also a marked increase in the demand for polymorphonuclear leukocytes, neutrophils in particular, due to severe GIT inflammation and compromised gut barrier predisposing to bacterial translocation (Goddard et al. 2008; Goddard & Leisewitz 2010).

In our study, the median segmented neutrophil count in the CPV group was significantly lower than the control group. Neutrophils are key players in the immune response and produce cytokines known to influence endothelial activation, such as TNFα and IL-1β, in addition to producing sheddases (Cassatella 1995; Zonneveld et al. 2014). Chemotherapy in people has been reported to result in decreased soluble ICAM-1 concentrations during the neutropaenic phase with recovery of ICAM-1 concentrations to normal levels when the neutrophil counts normalise (Mills et al. 2004; Wang et al. 2000). The positive correlation found between VCAM-1 and the segmented neutrophil count in our study may support the impact neutropenia has on this endothelial adhesion molecule. CPV infection causes lysis of lymphocytes in both the thymus and lymph node germinal centres, increases endogenous cortisol concentrations leading to redistribution and sequestration of lymphocytes in lymphoid tissues, and causes loss of lymphocyte-rich lymph from the inflamed, compromised GIT (Goddard et al. 2008; Goddard & Leisewitz

2010; Macartney et al. 1984; Schoeman et al. 2007). All of this culminates in a marked lymphopenia in dogs affected with CPV enteritis. Lymphocyte counts were significantly lower in the CPV group compared to the control group in our study. Antigen-activated T and B lymphocytes produce a number of cytokines including IL-4, IL-6, INFγ and TNFα, which are involved in endothelial activation and the expression of ICAM-1 and VCAM-1 (Lund 2008; Vazquez et al. 2015). Monocytes/macrophages are one of the most potent producers of cytokines influencing the endothelium such as TNFα, IL-1(α/β), IL-6, and HMGB-1 (Magna & Pisetsky 2014; Munoz et al. 1991; Wang et al. 2004; Zonneveld et al. 2014). The monocytopenia identified in this and previous CPV enteritis studies is probably the result of bone marrow suppression and increased migration to the inflamed GIT (Goddard et al. 2008). Although a number of cytokines, including TNFα, IL-1β, IL-6 and INFγ, have demonstrated increases in dogs with CPV enteritis, these changes have been inconsistent between studies (Otto et al. 1997; Sahinduran et al. 2016) and the concentrations required to effectively stimulate the endothelium are unknown. Therefore, the neutropenia, lymphopenia and monocytopenia may have contributed to the unexpected lack of increased circulating ICAM-1 and VCAM-1 concentrations through inadequate production of stimulating cytokines and proteolytic sheddases.

Endogenous glucocorticoid concentrations are known to be increased in CPV infection and glucocorticoids downregulate the transcription factor nuclear factor-kappa B (NF-κB) (Schoeman et al. 2007; Wissink et al. 1998). The expression of numerous genes implicated in the immune and inflammatory responses are modulated by NF-κB (Wissink et al. 1998). Genes encoding for the production of ICAM-1 and VCAM-1 form part of those targeted by NF-κB and suppression of this transcription factor by endogenous glucocorticoids may subsequently reduce the expression of these endothelial adhesion molecules (Iademarco et al. 1995; Roebuck & Finnegan 1999; Schoeman et al. 2007; Wissink et al. 1998).

Finally, the unexpected decreased circulating concentrations of ICAM-1 and absence of increased VCAM-1 concentrations in dogs with CPV enteritis compared to healthy controls may only be a time-dependent transient event. A popular hypothesis in human medicine is that the release of endothelial adhesion molecules is a homeostatic process that serves to reduce the impact of systemic inflammation on bystander tissues (Zonneveld et al. 2014). Initially, leukocyte transmigration through the endothelium is promoted by the upregulation of ICAM-1 and VCAM-1 cell surface expression during inflammation (Zonneveld et al. 2014). Following this phase, adhesion molecules are then shed from the surface of endothelial cells through the activity of sheddases and these soluble adhesion molecules then bind to circulating leukocytes limiting leukocyte binding to the endothelial surface (Zonneveld et al. 2014). The reduced leukocyte transmigration then promotes the resolution of the inflammatory response (Zonneveld et al. 2014). As this is a dynamic process, there are a number of points during the evolution of the host inflammatory response where concentrations of soluble circulating endothelial adhesion molecules vary significantly (Zonneveld et al. 2014). During sepsis, insufficient shedding of adhesion molecules may promote excessive leukocyte transmigration and contribute to sustained tissue inflammation and systemic leukopenia (Zonneveld et al. 2014). In CPV enteritis, the question becomes whether our findings of reduced circulating endothelial adhesion molecules are due to reduced tissue expression, delayed release or a disruption of this process with aberrant shedding due to the progressive sepsis in these patients. As leukocyte numbers rebound and the production of cytokines and sheddases increase, shedding of endothelial adhesion molecules may be triggered in later stages of CPV enteritis, and further studies are needed to investigate the kinetics of these molecules during the course of the disease.

The nuclear and cytosolic protein HMGB-1 acts as a pro-inflammatory cytokine and is released from monocytes when stimulated by endotoxin, TNFα or IL-1β (Fiuza et al. 2003). It plays a role in upregulating the expression of ICAM-1 and VCAM-1, and there are reports of increased concentrations in a number of acute and chronic inflammatory conditions in humans, including sepsis (Magna & Pisetsky 2014; Wang et al. 2004). In the cell, HMGB-1 interacts with DNA and histones within the nucleus and therefore may also be released by cell death (Magna & Pisetsky 2014). In addition to influencing the endothelium, HMGB-1 also enhances the secretion of various cytokines from monocytes/macrophages (Fiuza et al. 2003). In canine babesiosis, increased concentrations of HMGB-1 were identified in dogs at presentation (Kules et al. 2017). Our study showed no difference in the concentration of HMGB-1 between the dogs with CPV enteritis and control dogs. The monocyte concentrations, the cellular source of a large proportion of circulating HMGB-1, were significantly lower in dogs with CPV enteritis at presentation compared to the healthy control dogs. In addition, leukopenia and insufficient concentrations of cytokines influencing the release of HMGB-1 may be an important contributor to the absence of an increase of HMGB-1 in dogs with CPV enteritis (Cassatella 1995; Goddard et al. 2008; Lund 2008; Vazquez et al. 2015). Finally, HMGB-1 demonstrates delayed kinetics in relation to increases in TNFα and increases may only be seen later in the course of disease (Wang et al. 2004).

Our study had some limitations. The small number of animals included in the study may have led to a failure to identify significant differences in circulating VCAM-1 and HMGB-1 concentrations between the CPV and control groups. The number of animals used for this study was, however, comparable to previous studies investigating similar parameters in the same population types (Otto et al. 1997; Sahinduran et al. 2016). Samples were only collected at a single time point, at presentation, limiting our ability to investigate the kinetics of these markers over time.

Conclusion

Our results identified no increase in, and even reduced circulating concentrations of endothelial adhesion molecules in dogs with CPV enteritis at presentation, based on the blood concentrations of ICAM-1 and VCAM-1. We also demonstrated a lack of at least one molecule influencing the expression of these adhesion molecules, HMGB-1. Our findings suggest the presence of altered endothelial activation during CPV enteritis. The effect

on adhesion and transmigration of leukocytes from circulation in CPV enteritis requires further investigation. Research into circulating endothelial adhesion molecule kinetics, their expression at tissue level and the cytokines that influence them over the course of CPV infection is required to clarify the role endothelial dysfunction plays in this disease.

Authors contributions

P Pazzi, BK Atkinson, A Goddard and S Pretorius conceived and planned the research. BK Atkinson and M Engelbrecht carried out the sample preparation and research data acquisition. P Pazzi, BK Atkinson and S Pretorius derived the models, analysed the data and contributed to the interpretation of the results. BK Atkinson took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgement

The authors would like to thank Dr Zandri Whitehead for her involvment in the initial conceptualisation of this project.

Conflict of interest

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Funding source

The authors received no financial support for the research, authorship, and/or publication of this article.

Data availability statement

The data that support the findings of this study are available from the corresponding author, B.K. Atkinson, upon reasonable request.

Disclaimer

This article was prepared by the author in her own personal capacity and the views in this article are the author's own and do not reflect the position of any institution.

Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Science (REC089-18) and the Animal Ethics Committee of the University of Pretoria (V090-19).

ORCID

BK Atkinson https://orcid.org/0000-0002-0609-8761
A Goddard https://orcid.org/0000-0002-8415-4802
M Engelbrecht https://orcid.org/0000-0002-9295-1967
S Pretorius https://orcid.org/0000-0003-2078-153X
P Pazzi https://orcid.org/0000-0002-5331-1422

References

Aziz, N., Fahey, J.L., Detels, R., Butch, A.W., 2003, Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol* 10, 652–657. https://doi.org/10.1128/CDLI.10.4.652-657.2003.

Baric Rafaj, R., Kules, J., Marinculic, A., et al., 2017, Plasma markers of inflammation and hemostatic and endothelial activity in naturally overweight and obese dogs. *BMC Vet Res* 13, 13. https://doi.org/10.1186/s12917-016-0929-8.

Baric Rafaj, R., Kules, J., Selanec, J., et al., 2013, Markers of coagulation activation, endothelial stimulation, and inflammation in dogs with babesiosis. *J Vet Intern Med* 27, 1172–1178. https://doi.org/10.1111/jvim.12146.

Black, S., Kushner, I., Samols, D., 2004, C-reactive protein. *J Biol Chem* 279, 48487–48490. https://doi.org/10.1074/jbc.R400025200.

Boosinger, T.R., Rebar, A.H., Denicola, D.B., Boon, G.D., 1982, Bone marrow alterations associated with canine parvoviral enteritis. *Vet Pathol* 19, 558–561. https://doi.org/10.1177/030098588201900513.

Cassatella, M.A., 1995, The production of cytokines by polymorphonuclear neutrophils. *Immunol Today* 16, 21–26. https://doi.org/10.1016/0167-5699(95)80066-2.

Fiuza, C., Bustin, M., Talwar, S., et al., 2003, Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 101, 2652–2660. https://doi.org/10.1182/blood-2002-05-1300.

Goddard, A.,Leisewitz, A.L., 2010, Canine parvovirus. *Vet Clin North Am Small Anim Pract* 40, 1041–1053. https://doi.org/10.1016/j.cvsm.2010.07.007.

Goddard, A., Leisewitz, A.L., Christopher, M.M., et al., 2008, Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. *J Vet Intern Med* 22, 309–316. https://doi.org/10.1111/j.1939-1676.2008.0073.x.

Hildebrand, F., Pape, H.C., Harwood, P., et al., 2005, Role of adhesion molecule ICAM in the pathogenesis of polymicrobial sepsis. *Exp Toxicol Pathol* 56, 281–290. https://doi.org/10.1016/j.etp.2004.09.004.

Hillstrom, A., Hagman, R., Tvedten, H., Kjelgaard-Hansen, M., 2014, Validation of a commercially available automated canine-specific immunoturbidimetric method for measuring canine C-reactive protein. *Vet Clin Pathol* 43, 235–243. https://doi.org/10.1111/vcp.12150.

Hubbard, A.K., Rothlein, R., 2000, Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades. *Free Radic Biol Med* 28, 1379–1386. https://doi.org/10.1016/S0891-5849(00)00223-9.

Iademarco, M.F., Barks, J.L., Dean, D.C., 1995, Regulation of vascular cell adhesion molecule-1 expression by IL-4 and TNF-alpha in cultured endothelial cells. *J Clin Invest* 95, 264–271. https://doi.org/10.1172/JCI117650.

Isogai, E., Isogai, H., Onuma, M., et al., 1989, *Escherichia coli* associated endotoxemia in dogs with parvovirus infection. *Nihon Juigaku Zasshi* 51, 597–606. https://doi.org/10.1292/jvms1939.51.597.

Kavsak, P.A., Ko, D.T., Newman, A.M., et al., 2008, "Upstream markers" provide for early identification of patients at high risk for myocardial necrosis and adverse outcomes. *Clin Chim Acta* 387, 133–138. https://doi.org/10.1016/j.cca.2007.09.023.

Kocaturk, M., Martinez, S., Eralp, O., et al., 2010, Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. *J Small Anim Pract* 51, 478–483. https://doi.org/10.1111/j.1748-5827.2010.00965.x.

Kules, J., Gotic, J., Mrljak, V., Baric Rafaj, R., 2017, Blood markers of fibrinolysis and endothelial activation in canine babesiosis. *BMC Vet Res* 13, 82. https://doi.org/10.1186/s12917-017-0995-6.

Lee, W.L., Liles, W.C., 2011, Endothelial activation, dysfunction and permeability during severe infections. *Curr Opin Hematol* 18, 191–196. https://doi.org/10.1097/MOH.0b013e328345a3d1.

Liao, J.K., 2013, Linking endothelial dysfunction with endothelial cell activation. *J Clin Invest* 123, 540–541. https://doi.org/10.1172/JCI66843.

Lund, F.E., 2008, Cytokine-producing B lymphocytes-key regulators of immunity. *Curr Opin Immunol* 20, 332–338. https://doi.org/10.1016/j.coi.2008.03.003.

Macartney, L., Mccandlish, I.A., Thompson, H., Cornwell, H.J., 1984, Canine parvovirus enteritis 1: Clinical, haematological and pathological features of experimental infection. *Vet Rec* 115, 201–210. https://doi.org/10.1136/vr.115.9.201.

Magna, M., Pisetsky, D.S., 2014, The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. *Mol Med* 20, 138–146. https://doi.org/10.2119/molmed.2013.00164.

Mcclure, V., Van Schoor, M., Thompson, P.N., et al., 2013, Evaluation of the use of serum C-reactive protein concentration to predict outcome in puppies infected with canine parvovirus. *J Am Vet Med Assoc* 243, 361–366. https://doi.org/10.2460/javma.243.3.361.

Mchale, J.F., Harari, O.A., Marshall, D., Haskard, D.O., 1999, TNF-alpha and IL-1 sequentially induce endothelial ICAM-1 and VCAM-1 expression in MRL/lpr lupus-prone mice. *J Immunol* 163, 3993–4000.

Meunier, P.C., Cooper, B.J., Appel, M.J., Slauson, D.O., 1985, Pathogenesis of canine parvovirus enteritis: the importance of viremia. *Vet Pathol* 22, 60–71. https://doi.org/10.1177/030098588502200110.

Mills, P.J., Parker, B., Jones, V., et al., 2004, The effects of standard anthracycline-based chemotherapy on soluble ICAM-1 and vascular endothelial growth factor levels in breast cancer. *Clin Cancer Res* 10, 4998–5003. https://doi.org/10.1158/1078-0432.CCR-0734-04.

Mohr, A.J., Leisewitz, A.L., Jacobson, L.S., et al., 2003, Effect of early enteral nutrition on intestinal permeability, intestinal protein loss, and outcome in dogs with severe parvoviral enteritis. *J Vet Intern Med* 17, 791–798. https://doi.org/10.1111/j.1939-1676.2003.tb02516.x.

Munoz, C., Carlet, J., Fitting, C., et al., 1991, Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest* 88, 1747–1754. https://doi.org/10.1172/JCI115493.

Otto, C.M., Drobatz, K.J., Soter, C., 1997, Endotoxemia and tumor necrosis factor activity in dogs with naturally occurring parvoviral enteritis. *J Vet Intern Med* 11, 65–70. https://doi.org/10.1111/j.1939-1676.1997.tb00075.x.

Page, A.V., Liles, W.C., 2013, Biomarkers of endothelial activation/dysfunction in infectious diseases. *Virulence* 4, 507–516. https://doi.org/10.4161/viru.24530.

Prittie, J., 2004, Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *Journal of Veterinary Emergency and Critical Care* 14, 167–176. https://doi.org/10.1111/j.1534-6935.2004.04020.x.

Roebuck, K.A., Finnegan, A., 1999, Regulation of intercellular adhesion molecule-1 (CD54) gene expression. *J Leukoc Biol* 66, 876–888. https://doi.org/10.1002/jlb.66.6.876.

Sahinduran, S., Albay, M.K., Karakurum, M.C., et al., 2016, Investigation of some cytokines, acute phase proteins and hepcidin levels before and after treatment in dogs with parvoviral gastroenteritis. *Pakistan Veterinary Journal* 36, 487–492.

Schoeman, J.P., Goddard, A.,Herrtage, M.E., 2007, Serum cortisol and thyroxine concentrations as predictors of death in critically ill puppies with parvoviral diarrhea. *J Am Vet Med Assoc* 231, 1534–1539. https://doi.org/10.2460/javma.231.10.1534.

Vandooren, J., Van Den Steen, P.E., Opdenakker, G., 2013, Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9): the next decade. *Crit Rev Biochem Mol Biol* 48, 222–272. https://doi.org/10.3109/10409238.2013.770819.

Vazquez, M.I., Catalan-Dibene, J., Zlotnik, A., 2015, B cells responses and cytokine production are regulated by their immune microenvironment. *Cytokine* 74, 318–326. https://doi.org/10.1016/j.cyto.2015.02.007.

Wang, H., Yang, H., Tracey, K.J., 2004, Extracellular role of HMGB1 in inflammation and sepsis. *J Intern Med* 255, 320–331. https://doi.org/10.1111/j.1365-2796.2003.01302.x.

Wang, J., Zhu, H.H., Xue, J.H., et al., 2015, Effects of storage conditions on the stability of serum CD163, NGAL, HMGB1 and MIP2. *Int J Clin Exp Pathol* 8, 4099–4105.

Wang, X., Clowes, C., Duarte, R., Pu, Q.Q., 2000, Serum ICAM-1 concentrations following conventional dose consolidation chemotherapy for acute myeloid leukemia and after high dose chemotherapy with autologous haematopoietic stem cell rescue. *Int J Oncol* 17, 591–5. https://doi.org/10.3892/ijo.17.3.591.

Wissink, S., Van Heerde, E.C., Van der Burg, B., Van der Saag, P.T., 1998, A dual mechanism mediates repression of NF-kappaB activity by glucocorticoids. *Mol Endocrinol* 12, 355–363. https://doi.org/10.1210/mend.12.3.0081.

Zonneveld, R., Martinelli, R., Shapiro, N.I., et al., 2014, Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. *Crit Care* 18, 204. https://doi.org/10.1186/cc13733.