Comparison of Serum and Salivary CRP Levels with Leukocyte Parameters in Dogs with Canine Parvoviral Enteritis

Gaye ÖLMEZ[1], Ceren ÇAKAR[2], Mahmut TURAN[3], Erdem GÜLERSOY[4*]

[1]Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Surgery, Burdur, Türkiye.
[2]Fırat University, Faculty of Veterinary Medicine, Department of Surgery, Elazığ, Türkiye.
[3]Harran University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, Şanlıurfa, Türkiye.
[4]Harran University, Faculty of Veterinary Medicine Department of Internal Medicine, Şanlıurfa, Türkiye.
[*]Corresponding author: egulersoy@harran.edu.tr

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Abstract

Aim to study: Canine parvovirus (CPV) is a highly contagious enteric pathogen with high morbidity and mortality in untreated dogs, particularly puppies, though immunocompromised adults are also vulnerable. Due to nonspecific clinical signs, reliable biomarkers are crucial for diagnosis and severity assessment. This study investigated the correlation between serum and salivary C-reactive protein (CRP) levels and their association with complete blood count (CBC) parameters in dogs with parvoviral enteritis (PVE), while also evaluating saliva as a less invasive diagnostic alternative.

Material and methods: A total of 20 dogs (10 healthy controls, 10 with PVE) underwent physical and laboratory examinations, including CBC and serum and salivary CRP measurements.

Results: In the PVE group, WBC, monocyte, granulocyte, MCH, and MCHC levels were significantly lower ($P < 0.05$), whereas serum and salivary CRP levels were markedly elevated ($P < 0.000$). ROC analysis demonstrated the outstanding diagnostic performance of serum and salivary CRP levels (AUC > 0.900). Moreover, a very strong positive correlation was observed between serum and salivary CRP concentrations ($r = 0.912$).

Conclusion: These findings suggest that salivary CRP, like serum CRP, may serve as a valuable biomarker for assessing disease severity in CPV especially in cases where blood collection is challenging. However, further studies are needed to confirm its diagnostic and prognostic reliability, considering factors such as potential dilution effect and variation in salivary flow rate.

Keywords: Biomarker, CRP, dog, saliva, serum.

Köpek Parvoviral Enteritli Köpeklerde Serum ve Salya CRP Düzeylerinin Lökosit Parametreleriyle Karşılaştırılması

Öz

Çalışmanın amacı: Köpek parvovirüsü (CPV), özellikle yavru köpeklerde olmak üzere, tedavi edilmeyen köpeklerde yüksek morbidite ve mortaliteye neden olan, bağışıklık sistemi zayıflamış yetişkin köpeklerin de risk altında olduğu, oldukça bulaşıcı bir enterik patojendir. Klinik belirtilerin spesifik olmaması nedeniyle, güvenilir biyobelirteçler tanı ve hastalık şiddetinin değerlendirilmesi açısından büyük önem taşır. Bu çalışmada, parvoviral enterit (PVE) tanısı konan köpeklerde serum ve salyadaki C-reaktif protein (CRP) düzeyleri ile tam kan sayımı (CBC) parametreleri arasındaki ilişki incelenmiş; ayrıca salyanın daha az invaziv bir tanısal alternatif olarak değerlendirilmesi amaçlanmıştır.

Materyal ve yöntemler: Toplam 20 köpek (10 sağlıklı kontrol, 10 PVE'li) CBC ve serum ve salya CRP ölçümleri de dahil olmak üzere fiziksel ve laboratuvar muayenelerine tabi tutuldu.

Bulgular: PVE grubunda WBC, monosit, granülosit, MCH ve MCHC düzeyleri anlamlı derecede düşüktü ($P < 0.05$), buna karşın serum ve salya CRP düzeyleri önemli ölçüde yüksekti ($P < 0.000$). ROC analizi, serum ve salya CRP düzeylerinin olağanüstü tanı performansını gösterdi (AUC > 0.900). Ayrıca, serum ve salya CRP konsantrasyonları arasında çok güçlü pozitif korelasyon gözlendi ($r = 0,912$).

Sonuç: Bu bulgular, serum CRP gibi salya CRP düzeyinin de kan alımının zor olduğu CPV vakalarında hastalık şiddetini değerlendirmek için değerli bir biyobelirteç görevi görebileceğini göstermektedir. Fakat, dilüsyon ve salya akış hızı gibi faktörler göz önünde bulundurularak tanısal ve prognostik güvenilirliğini doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Biyobelirteç, CRP, köpek, salya, serum.

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Introduction

Canine parvoviral enteritis (CPE), caused by three variants of canine parvovirus type 2 (CPV-2), is a major cause of morbidity and mortality in dogs worldwide (Goddard and Leisewitz 2010). While severe disease primarily affects dogs younger than six months, adults with inadequate immunity may also be susceptible (Marcovich et al. 2012). The primary pathogenic mechanism of CPV-2 infection is the virus-induced destruction of rapidly dividing cells, including crypt intestinal epithelial cells, thymus, lymph nodes, and bone marrow precursor cells (Decaro et al. 2005). Consequently, disruption of the intestinal mucosal barrier leads to villous atrophy, malabsorption, and severe leukopenia (mainly neutropenia and/or lymphopenia), resulting in profuse diarrhea and vomiting, severe dehydration/hypovolemia, metabolic acidosis or alkalosis, bacterial translocation with coliform septicemia and endotoxemia, systemic inflammatory response syndrome (SIRS), hypercoagulability, multiorgan dysfunction, and death (Kalli et al. 2010). Due to clinical similarities with other acute gastrointestinal disorders, diagnosing CPE requires a combination of clinical and clinicopathological findings, specific biomarker assessments, and detection of viral antigen or polymerase chain reaction (PCR)-based amplification of viral DNA in stool samples (Sykes 2014).

A biomarker is generally defined as an objectively measured indicator of a biological state, used to assess physiological or pathological processes. In CPV infection, biomarkers can aid in diagnosing the disease, estimating hospital stay duration, evaluating disease severity, and predicting prognosis. Additionally, they can support clinical decision-making regarding treatment options or euthanasia (Schoeman et al. 2013). Blood biomarkers serve as direct indicators of physiological and pathological changes; however, blood collection is invasive and requires trained personnel (Rayment et al. 2015). Saliva sampling, on the other hand, is a simple, non-invasive technique that enables real-time tracking of transient physiological changes. Because of these benefits, saliva analysis is being used more and more to evaluate metabolic health in addition to behavioral changes (Dreschel et al. 2014).

When inflammatory situations such as infection, inflammation, and surgical damage occur, blood proteins known as acute phase proteins (APPs) undergo at least a 25% decrease in concentration (Murata et al. 2004). Cytokines serve as messengers between the site of damage or inflammation and hepatocytes, causing the synthesis of APPs and initiating the acute phase response. Activated leukocytes release these proinflammatory cytokines in response to tissue injury or bacterial toxins. Alpha-1-acid glycoprotein, haptoglobin, C-reactive protein (CRP), serum ceruloplasmin, fibrinogen, and serum amyloid A (SAA) are examples of positive or negative APPs, and their levels rise or fall in response to inflammation, respectively (Eckersall and Bell 2010). CRP, which is commonly tested in medical laboratories and extensively researched as a prognostic marker in severe sepsis, is a crucial APP in both humans and dogs (Gebhardt et al. 2009). Serum, whole blood, bodily cavities, and saliva are among the biological fluids in which canine CRP can be determined (Parra et al. 2005). Salivary CRP levels can be affected by diseases like gingivitis and periodontitis, even though the saliva sample is noninvasive. Similarly, although it is usually regarded as clinically robust, serum CRP levels can be impacted by a number of factors, including glucocorticoid treatment, hemolysis, lipemia, eating status, gender, and diurnal variation (Martínez-Subiela and Cerón 2005). Thus, this study aims to investigate the correlation between serum CRP concentration, an established diagnostic and prognostic marker in CPE cases

(Ok et al. 2015), and salivary CRP levels, alongside complete blood count (CBC) parameters. Additionally, the study highlights the clinical relevance of saliva as an alternative diagnostic fluid, potentially reducing the need for frequent blood sampling and minimizing stress in affected animals.

Material and Methods

Animals

Between July 2024 and January 2025, 36 healthy dogs and 52 dogs with clinical indications suggestive of CPE were brought to the Harran University Veterinary Faculty Animal Hospital for diagnosis, treatment, immunization, and general health examinations.

Physical Examinations

Following a detailed anamnesis, clinical evaluations were performed on all dogs, including measurements of gingival capillary refill time (CRT), respiratory rate, pulse, and rectal body temperature. Additionally, palpable lymph nodes were assessed, and lung and heart auscultations were conducted. Stool samples were collected directly from the rectum using sterile swabs for rapid diagnostic test kit analysis and microscopic native stool examination (for Toxocara canis, Ancylostoma caninum, and Coccidia spp.).

Collection of Blood Samples

Following physical examinations, venous blood samples (3-5 mL) were collected from all dogs deemed suitable for inclusion in the study through aseptic vena cephalica venipuncture under minimal stress restraint. A portion of the blood (1-2 mL) was placed in a K_3EDTA tube for complete blood count (CBC), while the remaining sample (2-3 mL) was placed in a gel serum tube without anticoagulant for serum CRP measurement. Blood samples were collected once on the day of admission to the hospital as part of this study.

Collection of Saliva Samples

For the measurement of salivary CRP, saliva samples (1-2 mL) were collected directly into sterile Eppendorf tubes by placing dental cotton thread in the animal's mouth in the form of a bit, then inducing a chewing movement, as previously described (Parra et al. 2005, Dreschel and Granger 2009). Saliva samples were collected once on the day of hospital admission for this study.

Rapid Diagnostic Test Kit Applications

Rapid diagnostic tests were conducted on all canines who qualified for the study in order to confirm CPV infection and rule out any concomitant conditions (Gülersoy et al. 2022, Dik et al. 2024). Specifically, the Anigen Rapid Canine Distemper Virus and Canine Adenovirus (CDV/CAV) Antigen Test Kit (relative sensitivity: 93.10% and 96.10%, relative specificity: 97.50% and 98%, respectively) was used to exclude CDV and CAV infections. Additionally, the Anigen Rapid CPV/CCV Antigen Test Kit (relative sensitivity: 100% and 93.1%, relative specificity: 98.80% and 97.50%, respectively) was used to confirm CPV and exclude canine coronavirus (CCV). Tests were performed using appropriate body fluids/materials in accordance with the manufacturer's instructions.

Laboratory Examinations and Biomarker Measurements

Leukocytes (WBC), lymphocytes (Lym), monocytes (Mon), granulocytes (Gra), erythrocytes (RBC), mean corpuscular volume (MCV), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte distribution width (RDW), and hemoglobin (Hb) levels were among the parameters that were measured from the blood samples as part of the

complete blood count (CBC). These measurements were performed using a whole blood autoanalyzer (POCH 100i Sysmex, Japan) in the central laboratory of Harran University Veterinary Faculty Animal Hospital within 15 minutes of blood collection. Venous blood samples for serum CRP measurement were centrifuged at 2000 g for 10 minutes, and the supernatant was transferred into Eppendorf tubes. These samples were stored at -80°C for approximately three months until biomarker measurement. Serum CRP levels were quantified using canine-specific commercial ELISA kits (BT Lab, Canine CRP ELISA kits, China), following the manufacturer's instructions. The detection range of the ELISA kit was 0.10 – 156 ng/mL, with a sensitivity of 0.05 ng/mL, intra-assay precision ≤ 8%, inter-assay precision ≤ 12%, and a specification of 96T. The coefficient of variation was calculated as 0.1 (10%). The same procedures and canine-specific ELISA kits were applied for measuring salivary CRP levels.

Inclusion/Exclusion Criteria and Forming Groups

Dogs with additional diseases detected via rapid diagnostic tests, endoparasites or protozoa in fecal exams, or oral conditions like stomatitis, gingivitis, or periodontitis were excluded from the study (Parra et al. 2005). Additionally, dogs that had been vaccinated within the past week or had received medications and/or supplements, such as antibiotics, non-steroidal anti-inflammatory drugs, or vitamin-mineral supplements, were also excluded (Gülersoy et al. 2022). As a result, of the 88 dogs that visited the animal hospital during the specified date range, 10 dogs that were diagnosed with only CPV infection based on all physical and laboratory examinations were included in the PVE group, and 10 healthy dogs with normal physical and laboratory findings and no clinical symptoms were included in the Control group.

Statistical Analysis

Data were analyzed using SPSS 27.00 (SPSS for Windows®) statistical software. The Shapiro-Wilk test (or one-sample Kolmogorov-Smirnov test) was applied to assess the normality of the data, and based on the results, parametric or nonparametric tests were selected for further analysis. Parametric data were evaluated as mean ± SD using the Mann-Whitney U test and Kruskal-Wallis test. Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic cut-off values for the measured parameters found to be useful in the differential diagnosis of CPV infection, based on comparisons of CBC and serum/saliva CRP parameters. Additionally, relationships between the investigated parameters were assessed using Spearman correlation analysis. The area under the curve (AUC), standard error, P value, cut-off value, sensitivity, and specificity were determined within the scope of ROC analysis. In this context, an AUC >0.700, P value < 0.05, and sensitivity and specificity > 70% were considered significant. Likelihood ratio (LR) was calculated for each cut-off threshold, and the highest LR was accepted as the optimal cut-off point. AUC values <0.600 were considered no discrimination (inability to diagnose patients with or without the disease), 0.7-0.8 as acceptable discrimination, 0.8-0.9 as perfect discrimination, and >0.9 as exceptional discrimination. Correlation coefficients were interpreted as follows: 0.40-0.69 as moderate correlation, 0.70-0.89 as strong correlation, and 0.90-1.00 as very strong correlation. Statistical significance was accepted as $P < 0.05$ for all analyses.

Results

Animals

All dogs included in the study were owned, fed commercial dry food, and aged between 2 and 6

months. In the PVE group (n=10), 7 were male, 3 were female, 8 were crossbred, and 2 were Golden Retrievers. In the Control group (n=10), 6 were male, 4 were female, 4 were crossbred, 3 were Cocker Spaniels, and 3 were Golden Retrievers. It was noted that 3 dogs in the PVE group had received antiparasitic treatment, while none of the dogs had been vaccinated. Similarly, the dogs in the control group were also unvaccinated, with 6 having received antiparasitic treatment only once. According to the anamnesis, the duration of symptoms in the patient group ranged from 2 to 5 days. Furthermore, no treatment had been administered to the diseased dogs prior to their admission to the hospital.

Physical Examination Findings

Clinical findings in the PVE group included anorexia (present in 10 out of 10 dogs), vomiting (8 out of 10 dogs), lethargy (10 out of 10 dogs), and diarrhea (7 out of 10 dogs with bloody diarrhea, 3 out of 10 with mucous diarrhea). Palpable lymph node evaluation revealed enlargement and a slight temperature increase, particularly in the submandibular (4 out of 10 dogs) and popliteal lymph nodes (3 out of 10 dogs). Respiratory and heart rates were significantly higher in the PVE group compared to the control group ($P<0.001$). However, CRT and body temperature did not show statistically significant differences between the groups. Physical examination findings are presented in Table 1.

Laboratory Examination and Biomarker Measurement Results

CBC analysis results revealed that WBC, Mon, Gra, MCH, and MCHC levels were significantly lower in the PVE group compared to the Control group ($P<0.020$). In contrast, serum CRP and salivary CRP levels were significantly higher in the PVE group compared to the Control group ($P<0.000$). The CBC and serum/salivary CRP measurement results are presented in Table 2.

Table 1. Physical examination findings

Parameters	Control Group (n =10) mean±SD	PVE Group (n =10) mean±SD	P value
RR (breath/sec)	35.9±6.82	73.4±16.62	0.000
HR (beat/sec)	79.5±8.68	108.8±20.26	0.001
CRT (sec)	2.6±0.51	2.8±0.78	0.511
Temp (°C)	38.1±0.3	38.21±0.81	0.694

RR: Respiratory Rate, HR: Heart rate, CRT: Capillary refill time (gingival), Temp: Body temperature (rectal), Sec: Second

Table 2. CBC and serum/saliva CRP measurement results

Parameters	Control Group (n =10) mean±SD	PVE Group (n =10) mean±SD	P value
WBC (m/mm^3)	14.73±2.86	6.07±4.38	0.000
Lym (m/mm^3)	4.08±1.41	2.84±1.91	0.114
Mon (m/mm^3)	0.97±0.55	0.27±0.25	0.020
Gra (m/mm^3)	9.68±2.39	2.98±2.48	0.000
RBC (M/mm^3)	6.86±0.78	7.53±1.34	0.189
MCV (fl)	64.46±6.39	69.39±4.05	0.055
Hct (%)	45.71±6.07	52.02±7.85	0.060
MCH (pg)	23.07±1.75	18.88±1.93	0.000
MCHC (g/dL)	33.67±4.22	27.36±3.13	0.001
RDW	10.21±1.23	10.78±0.97	0.273
Hb (g/dL)	15.51±1.78	14.31±3.06	0.300
Serum CRP (ng/mL)	3.19±2.09	23.80±13.75	0.000
Saliva CRP (ng/mL)	0.82±0.65	3.75±2.06	0.000

WBC: Leukocyte, Lym: Lymphocyte, Mon: Monocyte, Gra: Granulocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, Hct: Hematocrit, MCH: Mean hemoglobin amount, MCHC: Mean hemoglobin concentration, RDW: Reticulocyte distribution ratio, Hb: Hemoglobin, CRP: C-reactive protein

Correlation Analysis Results

As a result of the correlation analysis performed between the leukocyte parameters, serum, and salivary CRP levels, a moderate negative correlation (r=-0.571) was found between serum CRP and granulocyte count, while a very strong positive correlation (r= 0.912) was observed between serum CRP and salivary CRP. The correlation results of the leukocyte and serum/saliva CRP levels are presented in Table 3.

Table 3. Correlation between leukocyte parameters and serum/saliva CRP levels

Parameters	**WBC**	**Lym**	**Mon**	**Gra**	**Serum CRP**	**Saliva CRP**
WBC	1.000	0.731**	0.831**	0.932**	-0.429	-0.314
Lym		1.000	0.596**	0.514*	-0.182	-0.130
Mon			1.000	0.822**	-0.409	-0.265
Gra				1.000	-0.571**	-0.460*
Serum CRP					1.000	0.912**
Saliva CRP						1.000

WBC: Leukocyte, Lym: Lymphocyte, Mon: Monocyte, Gra: Granulocyte, CRP: C-reactive protein, **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

ROC Analysis Results

As a result of the ROC analysis conducted to evaluate the diagnostic performance of leukocyte parameters, serum, and salivary CRP levels in distinguishing the disease, it was found that leukocyte parameters did not exhibit any discriminatory ability. In contrast, serum and salivary CRP levels demonstrated exceptional discriminatory power (AUC=1.000 and AUC=0.985). The ROC analysis results for leukocyte and serum/salivary CRP levels are presented in Table 4, and the ROC curves are shown in Figure 1.

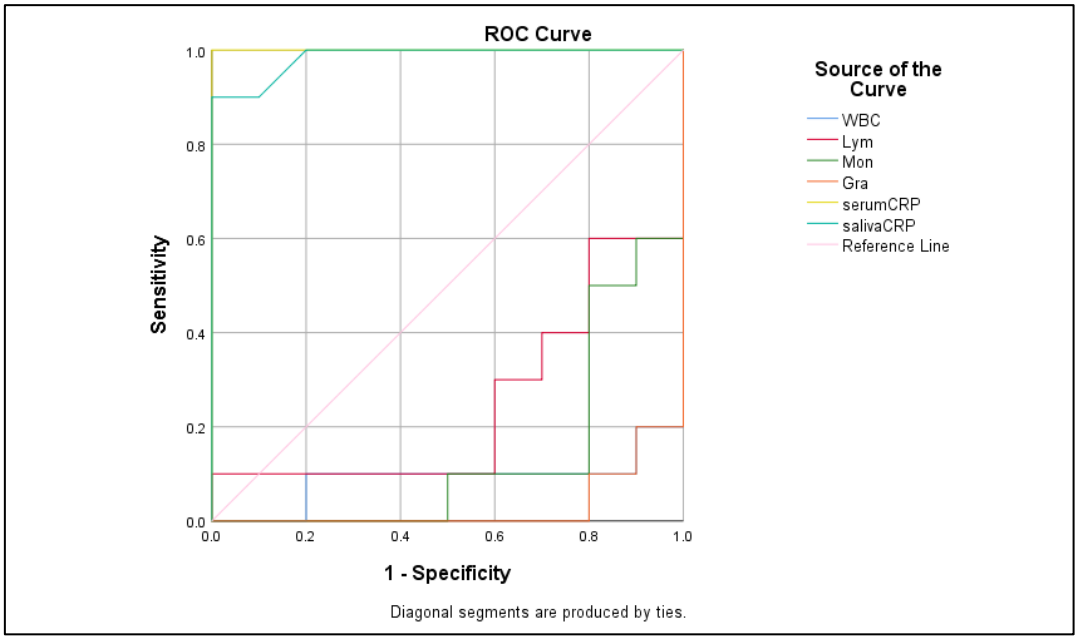

Figure 1. ROC curves of the investigated parameters

Discussion

In this study, the physical examination, CBC, and serum and salivary CRP levels of dogs with CPE were evaluated. The results showed elevated respiratory and pulse rates in diseased dogs, while CBC parameters such as WBC, monocytes, granulocytes, MCH, and MCHC were lower, consistent with previous reports. Serum and salivary CRP levels were significantly higher in dogs with CPE compared to healthy controls. The ROC analysis demonstrated that serum and salivary CRP measurements had outstanding diagnostic efficacy compared to routine parameters. In conclusion, while the diagnostic value of serum CRP levels in dogs with CPE, as well as their prognostic utility in determining hospitalization duration and treatment response, has been well-documented, this study suggests that salivary CRP may also have similar diagnostic and potentially prognostic significance (r=0.912, based on the correlation analysis). However, the prognostic value could not be fully assessed in this study, as clinical outcomes of the patient dogs were not evaluated. Therefore, in the absence of oral cavity disease, salivary CRP can be incorporated into routine diagnostic and prognostic panels due to its noninvasive nature, ease of measurement, and rapid changes. Given its potential for both diagnosis and prognosis, salivary CRP could enhance the management of CPE in dogs, offering a more accessible and reliable tool for clinicians. However, further studies with serial measurements and larger sample sizes are essential to fully establish its role.

Despite the availability of effective vaccines, canine parvovirus (CPV) continues to be a major cause of enteritis globally (Goddard and

Leisewitz, 2010). Dogs between the ages of six weeks and six months are the main victims of the disease, which can cause serious systemic illness and a high death rate in vulnerable groups. Research has shown that some breeds, such as Labrador Retrievers, Rottweilers, and Doberman Pinschers, are more likely to develop severe CPV enteritis (Kalli et al., 2010; Mylonakis et al., 2016). Although the precise origins of this propensity are yet unknown, potential contributing variables include immunological deficits, genetic predisposition, and clotting disorders (Sykes, 2014). In the present study, no specific breeds with a known predisposition were selected according to the inclusion/exclusion criteria. The PVE group included 8 crossbreeds and 2 Golden Retrievers, while the control group comprised 4 crossbreeds, 3 Cocker Spaniels, and 3 Golden Retrievers. This approach aimed to minimize the impact of severe systemic disease and sepsis on the serum and salivary CRP levels, as well as the CBC parameters (Franco-Martinez et al., 2018).

During the first two days of infection, CPV replicates in the lymphoid tissues and oropharynx. CPV is directed to its target tissues after viremia, which usually happens between days three and five. Symptoms such bloody diarrhea, vomiting, anorexia, lethargy, severe dehydration, abrupt collapse, and mortality appear between days 5 and 7 after fecal-oral transmission (Ceron et al., 2005; Ok et al., 2015; Gülersoy et al., 2022). In the present study, the symptom duration in dogs with CPE ranged from 2 to 5 days (3.5±1.08 days). Clinical findings observed during physical examination included anorexia (10/10 dogs), vomiting (8/10 dogs), lethargy (10/10 dogs), and diarrhea (7/10 dogs with bloody diarrhea, 3/10 with mucous). These findings align with CPV's hematogenous spread to the intestinal mucosa and its replication in the intestinal crypt epithelium, which is consistent with the virus's pathogenesis and the observed symptom duration in affected dogs (Mylonakis et al., 2016; Schoeman et al., 2013).

Excessive destruction of lymphoblasts in lymphoid tissues and myeloblasts in the bone marrow leads to lymphopenia, followed by severe panleukopenia. Vomiting and bloody diarrhea result from viral replication in germinal epithelial cells in the intestinal crypts, causing destruction and necrosis of the epithelium. Abdominal pain and intussusception may also occur in some cases. Systemic inflammatory response syndrome (SIRS) is commonly observed (Kalli et al., 2010; Schoeman et al., 2013). Sepsis, defined as coliform septicemia from bacterial translocation in damaged intestinal epithelium, is a critical factor in CPV pathogenesis (Sykes, 2014; Ok et al., 2015). Although clinical signs like vomiting, diarrhea, and lethargy are nonspecific, hematological findings are more consistent. Low hemoglobin, hematocrit, MCV, MCH, and MCHC levels in CPV infection are linked to disruptions in iron recovery and metabolism in erythroid precursor cells. Lymphopenia in the acute phase and subsequent leukopenia, followed by lymphocytosis and leukocytosis, are common (Mylonakis et al., 2016; Gülersoy et al., 2022). In the current study, lower WBC, monocyte, granulocyte, MCH, and MCHC levels were observed in CPE dogs compared to healthy controls (Table 2). These altered hematological parameters, along with elevated respiratory and pulse rates, may reflect CPV's pantropic nature, its rapid replication in the gastrointestinal tract, lymphoid, and bone marrow tissues, and the severe systemic response due to coliform septicemia and immunosuppression (Ok et al., 2015). While routine CBC parameters are valuable for differential diagnosis and assessing general health, their effectiveness is influenced by demographic factors and clinical findings. Therefore, the investigation of additional biomarkers with diagnostic and prognostic

potential is essential (Schoeman et al., 2013; Dik et al., 2024).

Biomarkers, measurable indicators of physiological or pathological conditions, can enhance disease suspicion, determine severity, and predict hospitalization duration in the context of CPV (Franco-Martínez et al., 2018). Additionally, biomarkers allow monitoring treatment response and may help decide whether euthanasia is necessary. Certain biomarkers have gained prominence in CPV research to elucidate complex virus-host interactions (Parra et al., 2015). Key clinicopathological markers include hematological parameters like WBC and granulocytes, coagulopathic parameters like antithrombin, and biochemical markers like albumin and magnesium. Acute phase proteins (APPs), such as SAA, haptoglobin, and CRP, increase within hours of infection (Ok et al., 2015). APPs, produced in response to proinflammatory cytokines, are reported to be 8 times more sensitive than WBC count for detecting disease. In previous studies, serum CRP levels in puppies with CPE were associated with survival rate (Ceron, 2005), and while all APPs are elevated in CPE, CRP has a higher predictive potential for mortality (Malin and Witkowska-Piłaszewicz, 2022). In the present study, serum CRP concentrations in CPE dogs were significantly higher than in healthy dogs, with levels more than 7 times greater ($P<0.000$, Table 2). These elevated CRP levels reflect the acute phase response and proinflammatory cytokine release associated with bacterial translocation, coliform septicemia, and endotoxemia, key elements in CPV pathogenesis (Ok et al., 2015; Malin and Witkowska-Piłaszewicz, 2022). Serum CRP levels in healthy dogs are typically <10 ng/mL, but can increase by up to 50-100 times within 4-24 hours of inflammatory stimuli. In the present study, the serum CRP concentration of 23.80±13.75 ng/mL in CPE dogs was correlated with the duration of symptoms (3.5±1.08 days), as these dogs received no treatment before hospitalization (Kalli et al., 2010; Goddard and Leisewitz, 2010; Sykes, 2014).

Canine CRP concentrations can be measured in serum, whole blood, body cavity effusions, and saliva. When there are no oral cavity diseases like periodontitis or gingivitis, serum sample evaluation is easy and non-invasive. Saliva, being a non-invasive fluid, has gained attention in clinical applications, providing insights into conditions such as leishmaniasis, Helicobacter infection, and stress (Malin and Witkowska-Piłaszewicz, 2022). Saliva collection offers several advantages: it is safer, easier, pain-free, and causes less stress than other sample types like feces, which require labor-intensive pre-treatment. Saliva has been proposed as a useful diagnostic tool, especially in early disease detection and monitoring (Pay and Shaw, 2019). In veterinary medicine, dog saliva has been used to assess immune competence, detect rabies virus antigen, and monitor drug levels (Pay and Shaw, 2019; Malin and Witkowska-Piłaszewicz, 2022). Previous studies have shown elevated CRP concentrations in the saliva of diseased dogs compared to healthy animals, but regression coefficients between saliva and serum CRP concentrations were not high (Franco-Martinez et al., 2018; Malin and Witkowska-Piłaszewicz, 2022). Factors affecting saliva secretion or composition may account for this discrepancy. Salivary CRP assessment may be a valuable tool in clinical treatment, as evidenced by the consistently increased CRP levels in the saliva of dogs with various inflammatory illnesses compared to healthy dogs (Parra et al., 2015). In the present study, salivary CRP concentrations in the PVE group were significantly higher than in healthy dogs ($P < 0.000$, Table 2). Saliva consists of components such as saliva, salivary exocrine secretions, and gingival crevicular fluid (GCF), which is a serum exudate containing components like complement proteins, immunoglobulins, and

acute phase proteins such as CRP and SAA (Pay and Shaw, 2019; Malin and Witkowska-Piłaszewicz, 2022). The high CRP levels detected in the saliva of CPE dogs likely originate from GCF. While GCF reflects the blood plasma composition, salivary secretions dilute the sample, and saliva flow rate was not determined, which may explain the lower salivary CRP concentration compared to serum CRP (Franco-Martinez et al., 2018; Malin and Witkowska-Piłaszewicz, 2022). Previous studies have suggested that dehydration may lead to denser saliva and increased salivary osmolarity and its components, potentially affecting CRP concentrations. These limitations, along with the small sample size, should be taken into consideration, particularly given the lack of statistically significant differences between groups in parameters such as CRT, which is commonly used to assess dehydration (Walsh et al., 2004). Unlike previous studies, the present study examined the correlations between serum and salivary CRP concentrations and hematological parameters, using comparative ROC analyses (Tables 3 and 4). The results revealed outstanding diagnostic efficacy for both serum and salivary CRP in CPV infection (AUC=1.000 and AUC=0.985, respectively), with a strong positive correlation between serum and salivary CRP levels (r=0.912). However, further studies with a larger sample size and more precise saliva measurements are needed to confirm these findings.

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Ethical Statement

This study was approved by the Harran University Animal Experiments Local Ethics Committee (session number 2024/004, decision number 01-18). Additionally, informed consent was obtained from the owners of all animals involved in the study. No interventions that could cause harm to the animals were performed during the study.

Author Contributions

Investigation: GÖ. and EG.; Material and Methodology: CÇ. and MT.; Supervision: EG.; Visualization: GÖ., MT. and EG.; Writing-Original Draft: EG and GÖ.; Writing- review & Editing: EG., GÖ., CÇ. and MT.

Conflict of Interest

The authors declared that there is no conflict of interest.

Data Availabilty Statement

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

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None.

References

Ceron, J. J., Eckersall, P. D., & Martínez-Subiela, S. (2005). Acute phase proteins in dogs and cats: Current knowledge and future perspectives. *Veterinary Clinical Pathology, 34(2),* 85-99. https://doi.org/10.1111/j.1939-165x.2005.tb00019.x

Decaro, N., Campolo, M., Desario, C., Elia, G., Martella, V., Lorusso, E. & Buonavoglia, C. (2005). Maternally-derived antibodies in pups and protection from canine parvovirus infection. *Biologicals, 33*(4), 261-267. https://doi.org/10.1016/j.biologicals.2005.06.004

Dik, I., Gulersoy, E., & Simsek, A. (2024). Importance of biomarkers and cytokines in the prognosis of canine parvovirus infection. *Pakistan Veterinary Journal, 44*(3), 875-881. https://doi.org/10.29261/pakvetj/2024.214

Dreschel, N. A., Cobb, M., Iskandarani, K., & Chinchilli, V. M. (2014). A meta-analysis of salivary cortisol measurement in domestic canines. *Journal of Veterinary Behavior: Clinical Applications and Research, 9,* 11-12. https://doi.org/10.1016/j.domaniend.2016.04.003

Dreschel, N. A., & Granger, D. A. (2009). Methods of collection for salivary cortisol measurement in

dogs. *Hormones and Behavior, 55(1),* 163-168. https://doi.org/10.1016/j.yhbeh.2008.09.010

Eckersall, P. D., & Bell, R. (2010). Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal, 185(1),* 23-27. https://doi.org/10.1016/j.tvjl.2010.04.009

Franco-Martínez, L., Tvarijonaviciute, A., Horvatić, A., Guillemin, N., Cerón, J. J., Escribano, D., Eckersall, D., Kocatürk, M., Yilmaz, Z., Lamy, E., Martínez-Subiela, S., & Mrljak, V. (2018). Changes in salivary analytes in canine parvovirus: A high- resolution quantitative proteomic study . *Comparative Immunology, Microbiology and Infectious Diseases, 60,* 1-10. https://doi.org/10.1016/j.cimid.2018.09.011

Gebhardt, C., Hirschberger, J., Rau, S., Arndt, G., Krainer, K., Schweigert, F. J., Brunnberg, L., Kaspers, B. & Kohn, B. (2009). Use of C-reactive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. *Journal of Veterinary Emergency and Critical Care, 19(5),* 450-458. https://doi.org/10.1111/j.1476-4431.2009.00462.x

Goddard, A., & Leisewitz, A. L. (2010). Canine parvovirus. *Veterinary Clinics of North America: Small Animal Practice, 40*(6), 1041-1053. https://doi.org/10.1016/j.cvsm.2010.07.007

Gülersoy, E., Balıkçı, C., Günal, I., & Şahan, A. (2022). Evaluation of the efficacy of blood gases and hemogram parameters in the diagnosis of non-neurogenic distemper and parvoviral enteritis in dogs with acute gastroenteritis. *Revista Científica de la Facultad de Ciencias Veterinarias, 32(1), 1-8.* https://doi.org/10.52973/rcfcv-e32091

Kalli, I., Leontides, L. S., Mylonakis, M. E., Adamama-Moraitou, K., Rallis, T., & Koutinas, A. F. (2010). Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Research in Veterinary Science, 89*(2), 174-178. https://doi.org/10.1016/j.rvsc.2010.02.013

Malin, K., & Witkowska-Piłaszewicz, O. (2022). C-reactive protein as a diagnostic marker in dogs: A review. *Animals (Basel), 12*(20), 2888. https://doi.org/10.3390/ani12202888

Marcovich, J. E., Stucker, K. M., Carr, A. H., Harbison, C. E., Scarlett, J. M., & Parrish, C. R. (2012). Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. *Journal of the American Veterinary Medical Association, 241*(1), 66-72. https://doi.org/10.2460/javma.241.1.66

Martínez-Subiela, S., & Cerón, J. J. (2005). Effects of hemolysis, lipemia, hyperbilirubinaemia and anticoagulants in canine C-reactive protein, serum amyloid A, and ceruloplasmin assays. *Canadian Veterinary Journal, 46*, 625-629.

Murata, H., Shimada, N., & Yoshioka, M. (2004). Current research on acute phase proteins in veterinary diagnosis: An overview. *The Veterinary Journal, 168*, 28-40.

Mylonakis, M. E., Kalli, I., & Rallis, T. S. (2016). Canine parvoviral enteritis: An update on the clinical diagnosis, treatment, and prevention. *Veterinary Medicine (Auckland), 7*, 91-100. https://doi.org/10.2147/VMRR.S80971

Ok, M., Er, C., & Yildiz, R. (2015). Evaluation of acute phase proteins and cytokines in dogs with parvoviral enteritis. *Eurasian Journal of Veterinary Sciences, 31*(3), 143-147. https://doi.org/10.15312/EurasianJVetSci.2015310970

Parra, M. D., Tecles, F., Martínez-Subiela, S., & Cerón, J. J. (2005). C-reactive protein measurement in canine saliva. *Journal of Veterinary Diagnostic Investigation, 17(2),* 139-144. https://doi.org/10.1177/104063870501700207

Pay, J. B., & Shaw, A. M. (2019). Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. *Clinical Biochemistry, 68*, 1-8. https://doi.org/10.1016/j.clinbiochem.2019.04.006

Rayment, D. J., De Groef, B., Peters, R. A., & Marston, L. C. (2015). Applied personality assessment in domestic dogs: Limitations and caveats. *Applied Animal Behaviour Science, 163*, 1-18. https://doi.org/10.1016/j.applanim.2014.11.020

Schoeman, J. P., Goddard, A., & Leisewitz, A. L. (2013). Biomarkers in canine parvovirus enteritis. *New Zealand Veterinary Journal, 61*(4), 217-222. https://doi.org/10.1080/00480169.2013.776451

Sykes, J. E. (2014). Canine parvovirus infections and other viral enteritides. In J. E. Sykes (Ed.), *Canine and Feline Infectious Diseases* (1st ed., pp. 141-151). Elsevier.

Walsh, N. P., Montague, J. C., Callow, N., Rowlands, A. V. (2004). Saliva flow rate, total protein concentration and osmolality as potential markers of whole body hydration status during progressive acute dehydration in humans. *Arch Oral Biol, 49*(2), 149-54. https://doi.org/10.1016/j.archoralbio.2003.08.001.