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RESEARCH ARTICLE

Relationship Between Viral Load and Reticulocyte/Lymphocyte, Neutrophil/Lymphocyte and Platelet/Lymphocyte Ratios in Dogs with Parvoviral Enteritis

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A B S T R A C T

Canine parvovirus is a viral disease that infects dogs and various animal groups. In hemogram analysis, there is panleukopenia, lymphopenia, neutropenia, and less commonly monocytosis. In recent years, different hematological parameters have been used to evaluate bacterial, viral, and neoplastic disease severity and course. Among these, there are several studies on neutrophil/lymphocyte (NEU/LYM) and platelet/lymphocyte (PLT/LYM) ratios to evaluate the immune system response to inflammation in human and veterinary medicine due to their low cost and easy applicability. The cut-off index (COI) in automated analyzers and immunoassays is a qualitative test that does not give antigen concentration, but studies have shown that it indicates pathogen load. This study investigated the relationship between viral load and reticulocyte/lymphocyte (RET/LYM), NEU/LYM, and PLT/LYM ratios in dogs with parvoviral enteritis. A total of 120 dogs were included in the study: 60 dogs diagnosed with parvoviral enteritis formed the study group, while 60 clinically healthy dogs constituted the control group. Viral loads of dogs with parvoviral enteritis were measured by COI. Complete blood counts were measured of all the animals. RET/LYM, NEU/LYM, and PLT/LYM ratios were statistically significantly higher in the study group than in the control group. COI was significantly positively correlated with the RET/LYM and NEU/LYM ratios. These ratios were new candidates for easy-to-use, cost-effective, objective, and non-invasive prognostic markers in dogs with canine parvoviral enteritis. In addition, since there was a positive correlation between the COI, an indicator of pathogen load, and the RET/LYM and NEU/LYM ratios. It was concluded that these ratios would also play an important role in determining the disease severity.

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INTRODUCTION

Canine parvovirus (CPV) is a viral disease that infects dogs and various animal groups. Parvoviruses (Parvoviridae) are small, non-enveloped, single-stranded DNA viruses that cause infections in various mammalian species (Goddard and Leisewitz, 2010). The agent can cause high-mortality infections in dogs of all ages, especially those under 12 months of age (Cardillo *et al.*, 2020; Muñoz *et al.*, 2021). CPV is a disease that spreads very quickly and has a high mortality rate (Özkanlar *et al.*, 2024).

The general clinical symptoms of the disease include depression, anorexia, abdominal pain, vomiting, bloody and foul-smelling diarrhea, and dehydration due to fluid loss (Yogesh, 2022; Ekinci *et al.,* 2024). It has been determined that acute cases last about 1 week, and clinical symptoms peak on days 2-5 of the disease. Factors such as immune deficiencies, living in overcrowded environments, sudden dietary changes, early weaning, and the presence of enteric parasites increase the susceptibility to parvovirus infections in puppies (McClure *et al.,* 2013). In hemogram analysis, there is panleukopenia, lymphopenia, neutropenia, and, less commonly, monocytosis. In infection, fibrinous necrotic enteritis and, in some cases, catarrhal or hemorrhagic enteritis are noted (Mihaela-Anca *et al.,* 2023). Diagnosis of the disease depends on the determination of viral load in stool or oropharyngeal swabs using a combination of clinical and pathological abnormalities, including enzyme-linked immunosorbent

assay (ELISA), polymerase chain reaction (PCR), electron microscopy, hemagglutination, and virus isolation (Ulas *et al.*, 2023).

In recent years, the neutrophil/lymphocyte ratio (NEU/LYM) has gained increasing attention as a simple yet informative marker in various infectious, inflammatory, and neoplastic conditions (Ekici *et al.*, 2023). Among these, there are several studies on NEU/LYM and platelet/lymphocyte (PLT/LYM) ratios to evaluate the immune system response to inflammation in human and veterinary medicine due to its low cost and easy applicability (Benvenuti *et al.*, 2020; Muñoz *et al.*, 2022; Pekmezci *et al.*, 2022). The NEU/LYM, a low-cost and straightforward biomarker, reflects the balance between innate and adaptive immune responses. The main determinants of the NEU/LYM value, endogenous catecholamines, and cortisol levels in the blood indirectly impact this value (Buonacera *et al.*, 2022). Changes in blood levels of catecholamines, such as epinephrine, can lead to leukocytosis and lymphopenia, while elevated cortisol levels can cause neutrophilia and lymphopenia (Chapman, 2018). In various diseases, NEU/LYM has been reported to increase as the severity of the disease increases (Furutate *et al.*, 2016; Zahorec, 2021). Reticulocytes are non-nucleated, immature erythrocytes with lengthy RNA molecules inside of them. After spending around two days in the bone marrow, reticulocytes are discharged into the bloodstream and undergo a two-day differentiation process to become adult erythrocytes (Choi *et al.*, 2022). Reticulocytosis refers to an increase in the number of reticulocytes in the blood. It is a quantitative indicator used to differentiate regenerative anemia, where erythrocyte production is increased, from non-regenerative anemia. Since reticulocytes are recently produced cells, reticulocyte indices are early indicators of anemia in humans and dogs, and reticulocyte indices may be affected during inflammatory processes at an earlier stage than indices associated with mature red blood cells in hemorrhagic diseases such as parvoviral enteritis (Meléndez *et al.*, 2015).

Cut-off index (COI) is a parameter often used to determine the accuracy of a test kit, especially serological tests. COI is a cut-off value used to determine whether a test is positive or negative. This value indicates a certain threshold level required for the test to be accurate. Although the COI in Automatic Analysis and Immunity Testing Devices is a qualitative test that does not give the antigen concentration, studies have shown that it is an indicator of pathogen load (Poon *et al.*, 2021; Walker *et al.*, 2021; Urrutikoetxea-Gutierrez *et al.*, 2023). The COI value is critical in obtaining accurate results, especially at low pathogen load levels (Rabaan *et al.*, 2021).

This study aimed to determine whether there is a significant relationship between parvoviral viral load and hematological ratios—specifically reticulocyte-to-lymphocyte (RET/LYM), neutrophil-to-lymphocyte (NEU/LYM), and platelet-to-lymphocyte (PLR)—in dogs with parvoviral enteritis.

MATERIALS AND METHODS

Animal selection and Groups: This study was conducted on a total of 120 client-owned dogs that were brought to Ondokuz Mayıs University Animal Hospital. The study group comprised 60 parvoviral enteritis diagnosed dogs representing various breeds, including 30 females and 30 males. The distribution of breeds within the study group was as follows: mixed breed (n=5), Doberman (n=6), Rottweiler (n=6), Doberman Pinscher (n=6), Pomeranian (n=8), German Shepherd (n=8), Terrier (n=10) and Kangal Turkish Shepherd (n=11). These dogs were aged between 2 and 4 months and had not received vaccinations against canine parvovirus (CPV) or other common infectious pathogens. The control group consisted of 60 healthy dogs, including 30 females and 30 males, aged between 2 and 4 months. The breed distribution within the control group, the same in the study group, was as follows: mixed breed (n=5), Doberman (n=6), Rottweiler (n=6), Doberman Pinscher (n=6), Pomeranian (n=8), German Shepherd (n=8), Terrier (n=10) and Kangal Turkish Shepherd (n=11). These dogs were presented for routine examination before their first vaccination. The dogs, which was owned pets, came from various provinces of the Black Sea Region in Turkey, specifically Samsun (n=18), Amasya (n=10), Giresun (n=8), Sinop (n=6), Çorum (n=7), Ordu (n=6), and Trabzon (n=5). A power analysis was conducted prior to the study, and based on the results, a total of 120 animals (60 per group) were included to ensure adequate statistical power (Fig. 1).

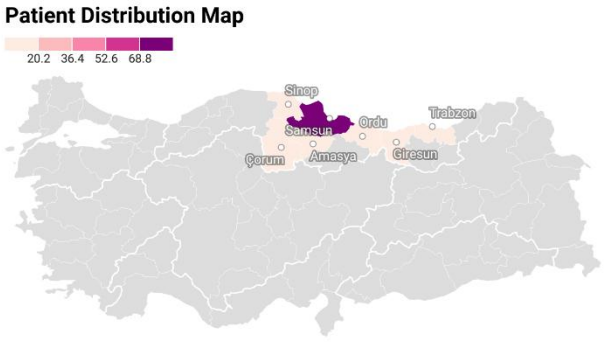

Fig. 1: Patient density across the provinces.

Study design

Diagnosis of CPV: All dogs in the study group exhibited clinical signs consistent with parvoviral enteritis, including acute hemorrhagic diarrhea, vomiting, lethargy, dehydration, and pyrexia. The diagnosis was confirmed using ELISA-based fecal antigen test kits, which detected the presence of CPV antigens. Only dogs that tested positive for CPV and exhibited no concurrent infections were included in the study to ensure diagnostic specificity. Prior to inclusion, all dogs underwent fecal flotation and antigen-based testing to screen for common enteric pathogens. Dogs testing positive for canine coronavirus (CCoV), *Giardia* spp., *Toxocara canis*, and/or Cystoisospora spp. were excluded from the study.

Physical examination findings: Before the study was included, all dogs in the study and control groups underwent a comprehensive and standardized physical examination. The body temperature of each dog was measured using a digital thermometer (rectal temperature). Fever is a common sign of systemic infections, including parvoviral enteritis. Dogs with a temperature exceeding

39.5°C were classified as febrile, indicative of an active infectious process. The submandibular and popliteal lymph nodes were palpated to detect any signs of lymphadenopathy. Enlargement of lymph nodes was an important clinical sign of systemic inflammation or viral infections like parvoviral enteritis. In dogs from the control group, lymph nodes were found to be of standard size with no palpable enlargement, indicating an absence of ongoing infection or inflammation.

Abdominal tenderness or pain was evaluated via gentle palpation, as gastrointestinal distress is a prominent feature in dogs suffering from parvoviral enteritis. Heart and respiratory rates were measured to assess each dog's cardiovascular and respiratory status. Tachycardia and tachypnea were noted in the study group as common findings in dogs with parvoviral enteritis, reflecting the stress caused by dehydration and systemic infection. Mucous membranes were examined for any signs of pallor, cyanosis, or hyperemia, and the capillary refill time (CRT) was also assessed. A CRT longer than 2 seconds was considered abnormal and indicative of dehydration or circulatory compromise. Clinical signs such as vomiting, lethargy, and diarrhea were carefully recorded. Any signs of systemic infection, including shock or fever, were closely monitored, especially in the study group of dogs showing symptoms of parvoviral enteritis. The hydration status was assessed by examining skin turgor, eye appearance, and mucous membrane moisture. Dehydration was classified as mild, moderate, or severe based on clinical parameters and observation. All dogs included in the study were housed under similar environmental conditions before sample collection to eliminate potential confounding variables. In addition, medical history, breed predisposition, and previous health records were reviewed to exclude pre-existing conditions that could influence hematological parameters.

Laboratory analysis

Compelete blood count and hematological ratios: Blood samples were collected from all dogs via the cephalic vein (vena cephalica antebrachii) using sterile syringes, ensuring the aseptic technique was maintained throughout the process. The samples were immediately transferred into blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, preventing coagulation and ensuring that the blood cells remain viable for subsequent analysis. To ensure the accuracy of the results, blood samples were processed without delay and analyzed immediately upon collection, thus minimizing any potential degradation of the cellular components. Hematological parameters were analyzed using the Mindray® Bc60r Vet Hi-End Laser & Fluorescent Hemogram Analyzer, a state-of-the-art device designed specifically for veterinary hematological assessments. This analyzer employs advanced laser scattering and fluorescent techniques to differentiate and quantify various blood cell populations accurately. Laser scattering is particularly effective for measuring cells' size, complexity, and internal structures. At the same time, fluorescence technology allows for precise identification and counting of cell types based on their specific fluorescence characteristics. The hematological parameters assessed in this study included the total leukocyte count, which measures the overall white blood cell count; the neutrophil count, which is crucial for evaluating immune response and inflammation; the lymphocyte count, which plays a significant role in the adaptive immune response; the platelet count, which is important for assessing coagulation status and vascular health; and the reticulocyte count, which reflects the bone marrow's response to anemia or blood loss. These parameters were carefully recorded for each dog to evaluate the hematological profile of viral load and the clinical status of parvoviral enteritis. To ensure consistency and minimize potential operator error, all analyses were performed by trained laboratory technicians following standardized protocols for each parameter. The quality control measures included using internal and external quality control samples, which were run alongside the study samples to verify the accuracy and reliability of the hematological results. By processing the blood samples promptly, the study ensured that the results accurately reflected the dogs' hematological status at the time of collection, allowing for precise correlations with the viral load and other clinical findings. This prompt analysis is crucial in studies investigating viral infections like canine parvoviral enteritis, where the immune response dynamics can change rapidly.

Diagnostic imaging

Radiography: The radiographic procedures were conducted without anesthesia, and radiographic images were acquired in anterolateral and ventrodorsal positions using an 838 UHF 100 Digital Radiography System. Abdominal radiography is a commonly utilized diagnostic tool for evaluating the gastrointestinal system in dogs suspected of having parvoviral enteritis. Radiographic evaluation is also valuable for ruling out the presence of gastrointestinal foreign bodies, which may mimic the clinical signs of parvoviral infection. For this reason, abdominal radiography was performed on all dogs included in the study.

Ultrasonography: Abdominal ultrasonographic examinations were performed using a Mindray Vetus 9 color Doppler ultrasound system equipped with a microconvex probe. The dogs were positioned in dorsal recumbency on a specialized ultrasound table, and no anesthesia was administered during the procedure. Abdominal ultrasonography is a valuable adjunctive tool in assessing dogs with suspected parvoviral enteritis; however, the findings are generally nonspecific. The abdominal ultrasonography is critical in identifying secondary complications, such as intestinal intussusception, which may arise due to altered intestinal motility and wall thickening and often require surgical intervention. For these reasons, all dogs in the study underwent abdominal ultrasonographic evaluation.

Fecal parvovirus antigen ELISA: Viral loads of CPV-positive dogs were quantified using the Vcheck V200 (Bionote®, USA) Automatic Veterinary Analysis and Immunity Testing Device. The Vcheck V200 device employs a unique immunoassay-based method rather than traditional PCR-based techniques. This device quantifies the presence of CPV antigens in the samples, accurately measuring the viral load based on the COI values. The COI

method utilized by the Vcheck V200 quantifies viral load by assessing the inhibitory effect of CPV-specific antibodies in the sample during an enzyme-linked immunosorbent assay (ELISA)-like process. Essentially, the device measures the inhibition of an enzyme reaction that occurs when CPV antigens are present. The amount of enzyme inhibition correlates with the viral concentration in the sample. The COI values are directly proportional to the virus in the sample, making this method a reliable marker for viral load in parvoviral enteritis. Higher COI values were interpreted as indicative of greater viral antigen load. This method provides a non-PCR alternative for viral quantification, which can be advantageous in clinical settings where rapid testing and ease of use are important. Viral antigen levels were evaluated using a commercially available ELISA-based rapid test kit, and results were expressed as COI values. The COI method was preferred over qPCR due to its lower cost, shorter processing time, and suitability for routine clinical use in veterinary practice. Unlike PCR, which amplifies the target DNA or RNA, the COI method relies on detecting viral antigens. This approach ensures that the viral load measurement is based on a direct interaction between the sample and the virus, making it a straightforward and reliable method for assessing infection intensity. Standardized protocols for sample handling, storage, and processing were strictly followed to minimize variability and ensure the reliability of the data. The Vcheck V200 device was calibrated regularly, and each analysis was conducted following the manufacturer's guidelines to ensure consistency and accuracy across all samples. The results obtained from the Vcheck V200 device were used to correlate the viral load with clinical outcomes, including the severity of parvoviral enteritis and the immune response, as indicated by the hematological parameters. This device's ability to quantify viral load provides important insights into the progression of the disease, and its use in clinical diagnostics facilitates the monitoring of infection intensity in affected dogs.

Statistical analysis: Data were analyzed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, N.Y., USA). The normality of data distribution was assessed using the Shapiro–Wilk test. In addition, visual inspection of histograms, Q–Q plots, and numerical evaluation of skewness and kurtosis values were performed to support the assumption of normality. Accordingly, while WBC, LYM, PLT, and RET values had a normal distribution, NEU, NEU/LYM, PLT/LYM, and RET/LYM values did not have a normal distribution. In normally distributed data, before using the independent t-test, the two groups were assessed for equality of variances. For this purpose, Levene's test was used to determine whether the standard deviation of the control group was expected to be the same as the standard deviation of the study group. Since the PLT and RET values had a normal distribution and also showed equality of variance, the independent t-test was used. Although the WBC and LYM values had a normal distribution, it did not show a homogeneous distribution, so the Welch t-test was used. A non-parametric Levene's test was used to verify the equality of variances in samples with non-normal distribution. As a result of this test, it was determined that all non-parametric data had variance equality. Therefore, the Mann-Whitney U test was applied to data that did not have a normal distribution. Spearman rank correlation was also performed to obtain the relationship between hematological parameters and COI. Means and standard deviation were obtained for the continuous variables when normally distributed. Also, median and interquartile range were obtained when the distribution was non-normal. P values less than 0.05 were considered significant.

RESULTS

This study evaluated age and body weight results for parvoviral enteritis in healthy dogs. WBC, NEU, LYM, PLT, and RET results were assessed using hematological analyses. NEU/LYM, PLT/LYM, and RET/LYM results were discussed using hematological ratios. Radiographic and ultrasonographic findings of dogs with PVE are included. Finally, viral load (COI) results were evaluated in the study group of dogs with parvoviral enteritis.

Animal demographics: The age and body weight of the dogs in both the control and study groups were compared. The control group had an average age of 2.6±0.95 months, while the study group had an average age of 2.5±0.97 months. The difference in age between the two groups was not statistically significant (P>0.05). Regarding body weight, the control group had an average weight of 10.6±4.1kg, and the study group had an average weight of 11.54±5.2kg. Similar to age, the difference in body weight between the two groups was also not statistically significant (P>0.05). These findings indicate that both groups were comparable in terms of age and body weight, ensuring that these variables did not influence the outcomes of the study (Table 1) (P>0.05).

Table 1: Demographic data of dogs in the study and control groups

Groups	Age (month)	Body Weight (kg)
Control (n=60)	2.6±0.95	10.6±4.1
Study (n=60)	2.5±0.97	11.54±5.2
P Value	>0.05	>0.05

Compelete blood count and hematological ratios: A comprehensive comparison of hematological parameters was made between the control and study groups and significant differences were observed in several parameters. White Blood Cell Count (WBC) was significantly higher in the control group ($8.01\pm1.07\times10^9$/L) compared to the study group ($4.57\pm0.57\times10^9$/L) and there was a statistical difference (P<0.001). This indicates a significant decrease in the WBC count in dogs with parvoviral enteritis. For neutrophils (NEU), the mean count of the control group was $4.98\pm0.76\times10^9$/L while the study group exhibited a significantly lower count of $4.03\pm0.96\times10^9$/L (P<0.01). Lymphocyte (LYM) count was also significantly decreased in the study group ($0.47\pm0.21\times10^9$/L) compared to the control group ($2.78\pm0.68\times10^9$/L) (P<0.001), indicating significant lymphopenia in the parvoviral enteritis group. NEU/LYM was significantly higher in the study group (8.58±3.38) compared to the control group (1.79±0.81), and this difference was statistically significant (P<0.001). This finding suggests a disproportionate increase in neutrophils compared to lymphocytes, a common feature of acute viral infections. In terms of Platelet Count (PLT), the mean platelet count ($350.61\pm73.94\times10^3$/L) in the study group was

higher than that in the control group (291.85±66.19×10^3/L), and the difference was significant (P<0.01). PLT/LYM was significantly higher in the study group (745.83 ± 413.16) than in the control group (105.92±56.45) (P<0.001). Reticulocyte Count (RET) (40.01±9.54 × 10^9/L) in the study group was significantly higher than that in the control group (22.25±7.93×10^9/L), and the difference was highly significant (P<0.001). This suggests a regenerative response to anemia, a common consequence of parvoviral enteritis. Finally, the Reticulocyte/Lymphocyte ratio (RET/LYM) was significantly increased in the study group (83.07±48.45) compared to the control group (8.00±4.72) and was found to be statistically significant (P<0.001) (Table 2). This finding further emphasizes the high reticulocyte count in the presence of lymphopenia. These results indicate significant changes in hematological parameters in dogs affected by parvoviral enteritis, with significant changes in white blood cell populations, platelet counts, and reticulocyte dynamics.

Upon examination of the box and whisker plot of the NEU/LYM ratio (Fig. 2), the study group exhibits a substantially higher median NEU/LYM ratio (8.18), indicating a marked central tendency, compared to the control group, which has a median value of 1.58. This suggests that individuals in the study group have significantly elevated NEU/LYM ratios. The interquartile range (IQR) of variability, represented by the height of the box to determine the distribution, is significantly wider in the study group, indicating more significant variability in NEU/LYM ratios. In contrast, the control group exhibits a narrower IQR, suggesting a more homogeneous distribution of NEU/LYM values. Minimum and Maximum Values of the study group (Whiskers) exhibit a broader range of values, with whiskers extending approximately from 4 to 14, reflecting a higher degree of dispersion. Conversely, the control group has a much more restricted range, with whiskers from approximately 1 to 3, demonstrating a more constrained distribution. When examined for outliers, some outliers (indicated by circles and asterisks) are observed in the control group, suggesting that a few individuals have NEU/LYM ratios that deviate markedly from the typical range within this cohort. No apparent outliers are present in the study group, implying that the distribution of NEU/LYM ratios, although broad, follows a more consistent pattern.

When looking at the box and whisker plot of the PLT/LYM (Fig. 3), the median values with the central tendency in the study group demonstrated a markedly higher median PLT/LYM ratio, approximately 800–900, in contrast to the control group, which exhibits a substantially lower median value (around 100–200). This suggests a significantly increased PLT/LYM ratio in the study group compared to healthy controls. The study group displays a broad interquartile range (IQR), indicating substantial variability in PLT/LYM ratios. The control group, however, has a narrower IQR, suggesting a more consistent and less variable distribution of values. In the study group, the whiskers extend across a wide range, from approximately 100 to over 1500, highlighting significant dispersion in PLT/LYM ratios. The control group has shorter whiskers, with values clustering between 50 and 300, indicating a more homogeneous distribution of PLT/LYM ratios. The control group contains multiple outliers, represented by circles, which indicate individual cases with higher PLT/LYM ratios than the majority of the group. Despite its wide distribution, the study group does not show clear outliers, suggesting that elevated PLT/LYM ratios are a consistent characteristic in this group.

The study group exhibits a substantially higher median RET/LYM ratio (approximately 50–60) than the control group, which has a much lower median value (around 5–10). This indicates a significant increase in RET/LYM ratios among affected individuals compared to healthy controls. The interquartile range (IQR) is considerably wider in the study group, suggesting more significant variability in RET/LYM ratios. The control group, in contrast, displays a narrower IQR, indicating a more consistent and homogeneous distribution. The study group has whiskers extending from approximately 10 to 150, reflecting high dispersion in RET/LYM values. Conversely, the control group has shorter whiskers, with values clustering between 0 and 20, indicating a more stable and uniform distribution (Fig. 4).

Diagnostic imaging

Radiography: Radiographic evaluation in dogs with parvoviral enteritis (PVE) commonly revealed gas-filled, distended intestinal loops suggestive of paralytic ileus due to intestinal inflammation. Mild intestinal wall thickening and reduced peristaltic activity were also noted. No evidence of mechanical obstruction, foreign bodies, or other gastrointestinal complications was detected. These findings supported the clinical diagnosis and helped exclude alternative causes of gastrointestinal signs.

Ultrasonography: Ultrasonographic examination frequently demonstrated intestinal wall thickening and loss of normal layering, consistent with inflammatory changes. Dilated, fluid-filled intestinal loops and markedly reduced motility were observed, indicating ileus and mucosal dysfunction. Hyperechoic intraluminal content, likely representing necrotic debris, and mild mesenteric lymphadenopathy were also noted. These findings supported the diagnosis of PVE and excluded complications such as intussusception or perforation.

Fecal parvovirus antigen ELISA: The viral load in the study group (Fig. 5) was assessed based on the COI values, which indicate the concentration of CPV in the samples. The COI values in the study group exhibited a significant range, with a mean value of 15.67±12.70. The median COI value was 12, while the minimum and maximum values were 2 and 54.78, respectively. This wide range reflects the variability in viral load among the affected dogs, with some showing relatively low levels of CPV infection and others exhibiting very high viral concentrations. The observed variability in COI values highlights the differing intensity of infection in individual cases, which may influence the clinical severity and progression of parvoviral enteritis. COI values were significantly positively correlated with NEU/LYM (r=0.546, P=0.001) and RET/LYM (r=0.629, P=0.001) ratio (Fig. 6, 7). However, no statistically significant difference was found between the COI value and PLT/LYM (r=0.057, P=0.76). Demographic data of COI value is presented in Table 3.

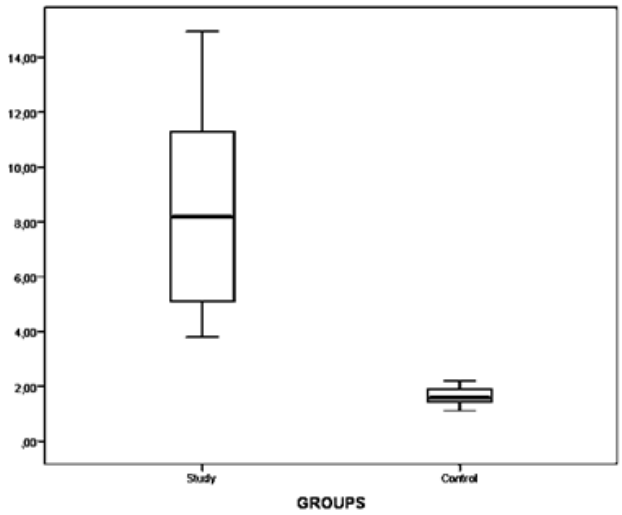

Fig. 2: Box and whiskers plot representation of the NEU/LYM ratio of the study and control groups. For each plot, the box represents the interquartile range, the horizontal line in each box represents the median, and the whiskers denote the range. Note that a statistically significant difference was observed between the groups (***P<0.001).

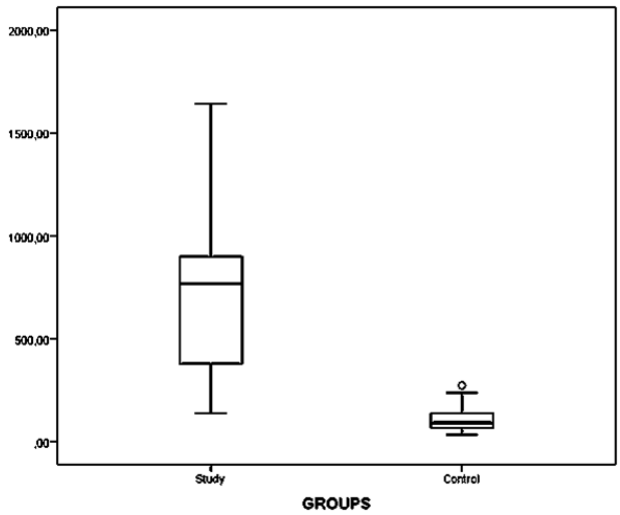

Fig. 3: Box and whisker plot of the PLT/LYM ratio of the study and control groups. For each plot, the box represents the interquartile range, the horizontal line in each box represents the median, and the whiskers denote the range. Note that a statistically significant difference was observed between the groups (***P<0.001).

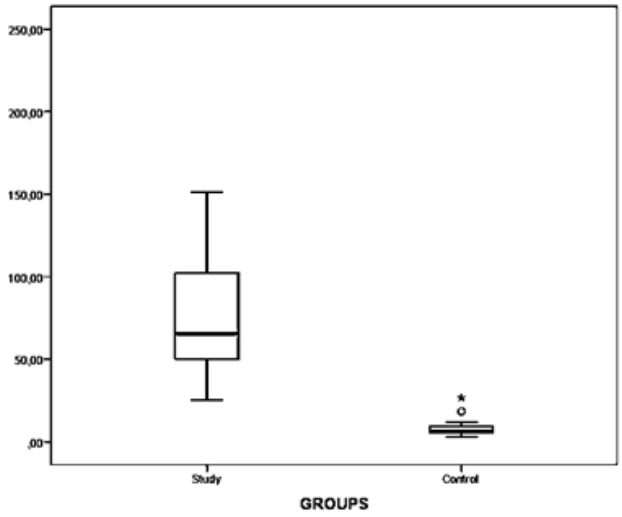

Fig. 4: Box and whisker plot of the RET/LYM ratio of the study and control groups. For each plot, the box represents the interquartile range, the horizontal line in each box represents the median, and the whiskers denote the range. Note that a statistically significant difference was observed between the groups (***P<0.001).

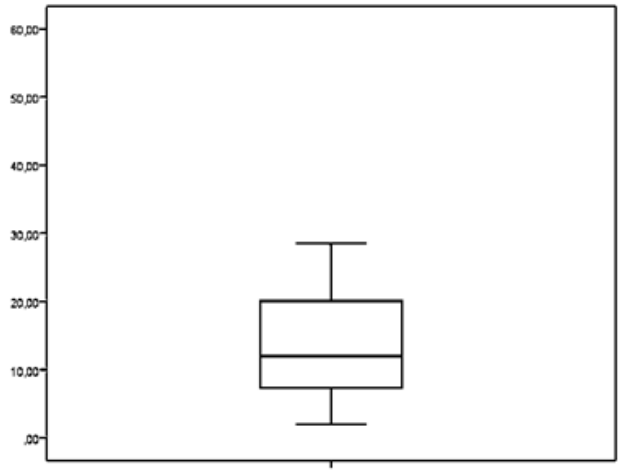

Fig. 5: Box and whisker plot of the COI value of the study group. For each plot, the box represents the interquartile range, the horizontal line in each box represents the median, and the whiskers denote the range.

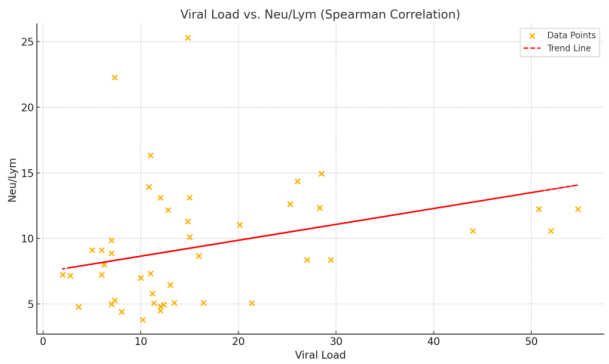

Fig. 6: Scatter plot showing the correlation between viral load and neutrophil-to-lymphocyte ratio (NEU/LYM) in dogs with parvoviral enteritis. Data points represent individual cases. The red dashed line indicates the trend line. Spearman's rank correlation coefficient was used for statistical analysis.

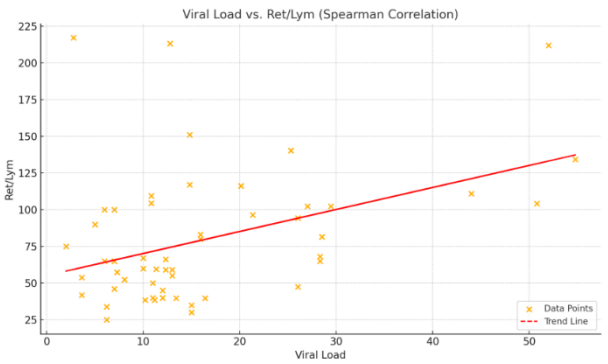

Fig. 7: Scatter plot showing the correlation between viral load and reticulocyte-to-lymphocyte ratio (RET/LYM) in dogs with parvoviral enteritis. Each dot represents an individual case. The trend line was added for visual interpretation. Correlation was assessed using Spearman's rank test.

Table 2: Hematological parameter findings of dogs in the control and study groups

Hematologic Parameters	Control Group (n=60)			Study Group (n=60)			P-value
	Mean±SD	Median	Interquartile range	Mean±SD	Median	Interquartile range	
WBC(10^9/L)	8.01±1.07			4.57±0.57			<0.001***
NEU (10^9/L)	4.98±0.76	5.1	1.45	4.03±0.96	4.08	1.43	<0.01**
LYM (10^9/L)	2.78±0.68			0.47±0.21			<0.001***
NEU/LYM	1.79±0.81	1.58	0.51	8.58±3.38	8.18	6.46	<0.001***
PLT (10^3/L)	291.85±66.19			350.61±73.94			<0.01**
PLT/LYM	105.92±56.45	90.39	72.26	745.83±413.16	766.65	555.71	<0.001***
RET (10^9/L)	22.25±7.93			40.01±9.54			<0.001***
RET/LYM	8.00±4.72	6.26	4.52	83.07±48.45	65.56	54.06	<0.001***

Data are expressed as means ± SD; statistical significance: **P<0.01, ***P<0.001.

Table 3: Demographic data of COI values in dogs belonging to the study group

Viral Load	Study Group (n=60)			
	Mean±SD	Median	Min	Max
COI Value	15.67±12.70	12	2	54.78

DISCUSSION

Canine parvoviral enteritis (PVE) remains one of the most prevalent and clinically significant viral diseases in veterinary medicine, affecting dogs regardless of breed or sex. Since its emergence in 1978, the canine parvovirus (CPV) has undergone several antigenic mutations, resulting in the persistence of new and more virulent variants with enhanced environmental resistance and host infectivity (Goddard & Leisewitz, 2010). These evolutionary adaptations contribute substantially to the continued global prevalence of the disease despite widespread vaccination efforts. Numerous studies have identified the highest incidence of PVE in puppies between two and six months of age, with the majority of cases reported in animals under six months (Mylonakis *et al.*, 2016; Sarpong *et al.*, 2017; Chalifoux *et al.*, 2021). This age-dependent susceptibility is thought to reflect both immature immune system function and the waning of maternally derived antibodies, which may create a critical window of vulnerability. In agreement with previous literature, the age of affected dogs in the present study ranged from 2 to 4 months, supporting the consensus that this population is at highest risk. Clinically, PVE is characterized by a constellation of signs, including severe depression, anorexia, abdominal pain, persistent vomiting, hemorrhagic and malodorous diarrhea, and progressive dehydration secondary to fluid and electrolyte losses (Mazzaferro, 2020; Yogesh, 2022). In the current study, all dogs diagnosed with parvoviral enteritis exhibited these hallmark clinical manifestations, confirming the consistency of the disease presentation and reinforcing the reliability of clinical assessment in suspected cases. Moreover, the combination of gastrointestinal signs with rapid clinical deterioration underscores the critical importance of early diagnosis and supportive care.

Hematological analysis plays a pivotal role in assessing the systemic impact of parvoviral enteritis. Among the most consistent laboratory abnormalities reported in canine PVE are leukopenia and, in particular, lymphopenia, both of which reflect the cytopathic effect of CPV on rapidly dividing hematopoietic progenitor cells in the bone marrow and lymphoid tissues (Alves *et al.*, 2019). These hematologic alterations serve as diagnostic markers and correlate with disease severity and prognosis. In this study, affected dogs displayed hematological changes in line with those reported in prior literature, suggesting significant immune suppression and marrow involvement during the acute phase of infection. The NEU/LYM has emerged as a sensitive and accessible biomarker of systemic inflammation and immune activation in both human and veterinary medicine (Zahorec, 2021; Wang *et al.*, 2023). Neutrophils represent the first line of defense against bacterial invasion, particularly in intestinal barrier disruption and secondary bacterial translocation, which are common complications in parvoviral infections (Mazzaferro, 2020). Their rapid mobilization to sites of inflammation and infection is accompanied by a transient depletion of circulating lymphocytes, which require more time to mount an adaptive response (Grossman & Paul, 2015). This temporal mismatch often results in a marked increase in the NEU/LYM, reflecting the acute innate immune response. The current study observed a positive correlation between NEU/LYM and viral load, as measured by COI values. This finding supports previous observations that elevated NEU/LYM ratios indicate disease severity and systemic immune activation.

Given its ease of calculation and availability from routine blood work, NEU/LYM may serve as a practical prognostic marker in clinical settings managing parvoviral enteritis. The PLT/LYM and RET/LYM were also elevated in dogs with parvoviral enteritis. PLT/LYM is influenced by cytokine-mediated megakaryocyte activation, particularly interleukin-6 (IL-6), which promotes thrombopoiesis in the context of systemic inflammation (Korniluk *et al.*, 2019; Corda *et al.*, 2023). Elevated platelet counts, especially of large, immature platelets, reflect bone marrow stimulation and a heightened inflammatory state, further amplifying the relevance of PLT/LYM in disease monitoring. Similarly, increased reticulocyte counts are consistent with compensatory erythropoietic activity in response to anemia, which may arise from hemorrhagic enteritis or bone marrow suppression (Carter, 2017; Sah & Rao, 2024). Reticulocytosis observed in infected dogs likely reflects the regenerative response to red blood cell loss and bone marrow stress secondary to parvoviral replication. Simultaneously, canine parvovirus targets rapidly dividing lymphoid and hematopoietic precursors, leading to lymphoid depletion and lymphopenia—both well-established features of parvoviral infection. The elevated RET/LYM ratio observed in this study may therefore serve as a dual indicator: reflecting hematopoietic compensation (via increased reticulocyte production) and immunosuppression (via reduced lymphocyte count). These results highlight the potential utility of RET/LYM as a supplementary marker alongside traditional indicators of inflammation. Its observed correlation with viral load further supports its relevance as a proxy measure for disease severity in canine parvoviral enteritis.

Radiographic and ultrasonographic imaging provided supportive diagnostic information in dogs with parvoviral enteritis (PVE). One of the most common radiographic findings was generalized small bowel distention with gas-filled loops, consistent with paralytic ileus resulting from viral-induced inflammation and intestinal dysmotility (Kanat and Ortatatlı, 2022; Novakov *et al*., 2024). Intestinal wall thickening was also observed, further supporting the presence of mucosal injury and inflammation (Tcygansky *et al*., 2021). No signs of mechanical obstruction or foreign bodies were detected, which helped exclude other differential diagnoses.

Ultrasonographically, intestinal wall thickening and loss of normal wall layering were common, indicating transmural inflammation and structural disruption (d'Anjou and Penninck, 2015). Reduced motility and the presence of dilated, fluid-filled loops suggested viral-induced ileus. Mesenteric lymphadenopathy, although nonspecific, was frequently observed and may reflect immune activation in response to intestinal infection (Parrish and Sykes, 2021). These findings were consistent with previous reports and helped reinforce the clinical diagnosis of PVE while excluding complications such as intussusception or perforation.

In recent years, increasing attention has been paid to COI's diagnostic and prognostic value, particularly in the context of antigen-based assays and immunoassay technologies. Traditionally used as a qualitative threshold to determine test positivity, the COI has recently been interpreted as a semi-quantitative marker reflecting antigenic burden or pathogen load (Habibzadeh *et al.,* 2016; Saegerman *et al.,* 2021). This conceptual shift is supported by multiple studies demonstrating a direct correlation between COI values and viral load in various infectious diseases, including canine parvoviral enteritis (Poon *et al.,* 2021; Urrutikoetxea-Gutierrez *et al.,* 2023; Walker *et al.,* 2021).

In our study, we observed a significant positive correlation between the COI and the NEU/LYM, PLT/LYM, and RET/LYM ratios, suggesting that these hematological markers are not only reflective of systemic inflammation but also proportional to viral replication intensity. Since automatic diagnostic platforms readily generate COI, its interpretation as a proxy for viral load may enhance clinical decision-making and risk stratification. In resource-limited or time-sensitive clinical settings, integrating COI values with routine blood parameters such as NEU/LYM and RET/LYM could enable rapid and accurate assessment of disease severity. The positive correlation between RET/LYM and COI opens an interesting avenue for future research. While reticulocyte counts are traditionally associated with regenerative anemia, their elevation in high COI values suggests a deeper relationship between hematopoietic response and viral replication. Further studies are warranted to explore the predictive value of RET/LYM as a standalone or combined marker for disease progression, therapeutic monitoring, and prognostication in CPV-infected dogs. Despite the informative findings of this study, certain limitations should be acknowledged. The cross-sectional nature of the data limits the ability to establish causality or observe dynamic changes in hematological ratios throughout disease and recovery. Additionally, the absence of viral quantification by molecular methods such as quantitative PCR restricts the ability to validate COI as an exact surrogate for viral load. Nevertheless, the consistency of observed correlations across multiple immune indicators enhances the robustness of the findings.

Conclusions: Consistent with previous studies, the NEU/LYM and PLT/LYM ratios were identified as promising candidates for prognostic markers in dogs with canine parvoviral enteritis. These ratios are particularly advantageous due to their ease of use, cost-effectiveness, objectivity, and non-invasive nature. Furthermore, beyond these established ratios, our study revealed that the RET/LYM ratio was significantly elevated in dogs suffering from parvoviral enteritis compared to healthy controls. This observation suggests that the RET/LYM ratio could serve as an additional, valuable prognostic marker for assessing the condition of dogs affected by this viral infection. Additionally, the positive correlation observed between the COI value, which indicates pathogen load, and both the RET/LYM and NEU/LYM ratios further emphasize the potential of these ratios in potentially indicate disease severity. These findings underline the importance of including these hematological ratios as part of a comprehensive diagnostic approach, enabling early identification and more accurate prognostication of canine parvoviral enteritis.

Ethics approval: The animal experiments conducted in this study were evaluated by the Ethics Committee for Laboratory Animals at Ondokuz Mayıs University (approval No. 2025/20).

Competing interests: The author declares no competing interests.

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