Animal Health Prod and Hyg (2025) 14 (2) : 1 - 8

Animal Health, Production and Hygiene

www.dergipark.or.tr/tr/pub/aduveterinary

Research Article

Investigation of Canine Parvovirus Infection in Stray Dogs in Alanya Region

Orhan YAPICI[1], Irmak DİK[1], Sibel YAVRU[1], Oğuzhan AVCI[1], Oya BULUT[2], Mehmet KALE[3], Rüveyde GÜLBAHÇE[1*]

[1]Selçuk University, Faculty of Veterinary, Virology Department, Konya, Türkiye
[2]Dokuz Eylül University, Faculty of Medicine, Medical Microbiology Department, İzmir, Türkiye
[3]Mehmet Akif Ersoy University, Faculty of Veterinary, Virology Department, Burdur, Türkiye

ABSTRACT

Canine Parvovirus (CPV) is a highly contagious viral disease of domestic and wild dogs and is associated with high morbidity and mortality, particularly in puppies. Early and accurate diagnosis is essential for preventing secondary infections and controlling transmission. This study aimed to determine the prevalence and seroprevalence of CPV infection in unvaccinated dogs aged 0–12 months in the Alanya region and to compare the diagnostic performance of the immunochromatographic (IC) test and the Enzyme-Linked Immunosorbent Assay (ELISA). Fecal samples were analyzed using ELISA and IC kits, while serum samples were tested for CPV-specific IgG antibodies. Among 100 dogs examined (47 percent female, 45 percent male, 8 percent unrecorded), 49 percent were positive for CPV-2 antigen by the IC test, whereas 17 percent were positive by ELISA. The higher number of IC-positive animals suggests that the test may offer practical advantages in field conditions due to its rapid and user-friendly nature. Serological testing showed that 42 dogs were antibody-positive; of these, 42.8 percent had high IgG titers and 57.1 percent had low titers. Statistical analysis indicated significant differences in antibody titers among age groups (P<0.001), while no significant difference was found between genders (P>0.05). This study provides the first combined antigen and antibody data on CPV infection in unvaccinated puppies in the Alanya region. The findings highlight the role of reliable diagnostic tools and timely vaccination in disease control. Furthermore, comparison of diagnostic methods demonstrated lower sensitivity for the IC test relative to ELISA, emphasizing the need for careful selection of diagnostic approaches in clinical practice and epidemiological surveys.
Keywords: *CPV, immunity, sensitivity, specificity, prevalence.*

Alanya İlçesindeki Sahipsiz Köpeklerde Canine Parvovirus Enfeksiyonunun Araştırılması

ÖZET

Köpek Parvovirusu (CPV), evcil ve yabani köpekleri etkileyen, özellikle yavru köpeklerde yüksek morbidite ve mortalite ile karakterize, oldukça bulaşıcı bir viral hastalıktır. Erken ve doğru tanı, ikincil enfeksiyonların önlenmesi ve bulaşmanın kontrol altına alınması açısından kritik önem taşır. Bu çalışma, Alanya bölgesinde 0–12 aylık aşılanmamış köpeklerde CPV enfeksiyonunun prevalans ve seroprevalansını belirlemeyi ve immünokromatografik (IC) test ile Enzim Bağlantılı İmmünosorbent Analizi'nin (ELISA) tanısal performansını karşılaştırmayı amaçlamıştır. Dışkı örnekleri ELISA ve IC kitleri kullanılarak, serum örnekleri ise CPV'ye özgü IgG antikorları açısından analiz edilmiştir. İncelenen 100 köpeğin (yüzde 47 dişi, yüzde 45 erkek, yüzde 8 kaydı olmayan) yüzde 49'u IC testi ile CPV-2 antijeni açısından pozitif bulunurken, yüzde 17'si ELISA ile pozitif olarak belirlenmiştir. IC testi ile daha fazla pozitif hayvan tespit edilmesi, bu testin hızlı ve kullanıcı dostu yapısı nedeniyle saha koşullarında pratik avantajlar sağlayabileceğini düşündürmektedir. Serolojik incelemede 42 köpek antikor pozitif bulunmuş; bunların % 42,8'inin yüksek, % 57,1'inin düşük IgG titresine sahip olduğu belirlenmiştir. İstatistiksel analiz, yaş grupları arasında antikor titreleri açısından anlamlı farklılıklar olduğunu göstermiştir (P<0.001). Buna karşılık, cinsiyetler arasında anlamlı bir fark saptanmamıştır (P>0.05). Bu çalışma, Alanya bölgesindeki aşılanmamış yavru köpeklerde CPV enfeksiyonuna ilişkin hem antijen hem de antikor düzeylerindeki ilk kapsamlı verileri sunmaktadır. Elde edilen bulgular, hastalığın kontrolünde güvenilir tanı yöntemleri ve zamanında aşılama programlarının önemini vurgulamaktadır. Ayrıca, tanı yöntemlerinin karşılaştırılması IC testinin ELISA'ya göre daha düşük duyarlılığa sahip olduğunu ortaya koymuş, bu durumun klinik uygulamalar ve epidemiyolojik çalışmalar için uygun tanı yönteminin seçilmesinde dikkate alınması gerektiğini göstermiştir.
Anahtar Kelimeler: *CPV, immünite, sensitive, spesifite, prevalans*

***Corresponding author**: Rüveyde Gülbahçe, Selcuk University, Faculty of Veterinary, Virology Department, Konya, Türkiye. gulbahceruveyde@gmail.com*
Received Date: 25.03.2025 - Accepted Date: 26.09.2025
DOI: 10.53913/aduveterinary.1665037

Introduction

Canine parvovirus type 2 (CPV-2) is a highly contagious and potentially fatal virus, particularly affecting unvaccinated puppies. Since its emergence in the late 1970s as a mutation of the feline panleukopenia virus, CPV-2 has spread globally and, despite extensive vaccination programs implemented in many countries, remains a leading cause of acute gastroenteritis in dogs (Khatri et al., 2017; Decaro et al., 2020). CPV-2 belongs to the *Parvoviridae* family and its 5.3 kb single-stranded DNA genome encodes structural and non-structural proteins. Of these, VP2 is the major capsid protein responsible for host interaction, antigenicity, and viral evolution. Mutations in the VP2 gene have led to antigenic variants: CPV-2a, CPV-2b, and CPV-2c, which co-circulate globally. CPV-2 infection is most common in puppies aged 6–12 weeks, and maternally derived antibodies provide early protection. However, these antibodies can interfere with vaccination, necessitating strategic immunization schedules (Desario et al., 2005; Giraldo-Ramirez et al., 2020).

The virus spreads rapidly among susceptible puppies, whereas domestic dogs generally have lower exposure rates compared to stray dogs. The primary sources of infection are the feces of infected dogs found in shelters, parks, and kennels (Khatri et al., 2017; Nandi et al., 2019). Transmission primarily occurs via the fecal-oral route or through contact with contaminated surfaces, although direct contact with infected dogs also significantly contributes to viral spread (Tuteja et al., 2022). The incubation period of CPV-2 is typically 4–5 days; however, in experimental infections, clinical signs may appear as early as 3 days post-exposure. CPV-2 causes clinical signs such as fever, anorexia, diarrhea (bloody or non-bloody), vomiting, depression, lethargy, and leukopenia. Additionally, CPV-2 can lead to myocardial damage (Ogbu et al., 2017; Ahmed Ismail and Hanedan, 2024). The virus is extremely stable in the environment and exhibits high resistance to disinfectants (Ogbu et al., 2017).

Vaccination with modified live virus (MLV) vaccines remains the cornerstone of CPV-2 prevention and is recommended by international veterinary associations. Nevertheless, stray and shelter dogs, which often remain unvaccinated, continue to represent a high-risk population (Lechner et al., 2010; Decaro et al., 2020).

Accurate diagnosis of CPV-2 is essential for disease management. Common diagnostic methods include ELISA, immunochromatographic (IC) tests, and PCR-based techniques (Desario et al., 2005; Khatri et al., 2017). Due to their speed and practicality, ELISA and IC tests are widely used in routine diagnosis. However, IC tests, while rapid and economical, have been reported to show variable sensitivity (Macêdo et al., 2022). ELISA offers greater sensitivity and is also useful for measuring IgG antibody levels, providing insights into immune protection and vaccine responses (Lambe et al., 2016). Compared to traditional diagnostic methods such as hemaglutination and virus isolation, PCR demonstrates significantly higher sensitivity. In particular, quantitative real-time PCR (qPCR) is regarded as the gold standard for the diagnosis of CPV infections. Nevertheless, the requirement for specialized laboratory conditions to perform qPCR may lead to delays in diagnosis (WalterWeingärtner et al., 2021). Serological evaluation of CPV-specific IgG titers contributes to determining the immune status of individual dogs as well as canine populations, thereby guiding vaccination strategies. Although an antibody titer of 1:80 is generally considered protective, the overall immunity also depends on cellular immune responses and the dynamics of viral exposure (Litster et al., 2012; Decaro et al., 2020).

Treatment of canine parvovirus infections is generally symptomatic, with maintenance of electrolyte and fluid balance being essential. Supportive therapy remains the cornerstone, as there is no specific antiviral treatment available (Khatri et al., 2017). Considering the high mortality rate in young dogs (0–12 months) and the widespread occurrence of parvoviral enteritis in the region, this study primarily aims to determine the prevalence and seroprevalence of parvovirus infection in unvaccinated dogs in the Alanya region and to compare the diagnostic performance of two commonly used methods: Enzyme-Linked Immunosorbent Assay (ELISA) and immunochromatographic (IC) tests. The results provide current data on CPV prevalence and contribute to evaluating the reliability of diagnostic tools.

Materials and Methods

Procurement of Samples

For this study, fecal and blood serum samples were collected from dogs suspected of parvovirus infection that brought to a private clinic in Alanya. These dogs exhibited gastroenteritis symptoms such as lethargy, vomiting, and diarrhea during clinical examination. To determine the optimal sample size for the study and ensure sufficient statistical power to detect the effect, a total of 100 unvaccinated dogs aged 0–12 months were sampled (83 of them showed symptoms, while 13 had a good general condition). Of the 100 dogs examined, 47 were female, 45 were male, and the sex of 8 dogs (8%) was not recorded. Additionally, in this study, microscopic examination for gastrointestinal parasites was not performed because all sampled dogs exhibited clinical symptoms (lethargy, vomiting, and diarrhea) consistent with canine parvoviral enteritis, and the study was specifically designed to investigate CPV infection. The technical limitations encountered during sample processing were also explicitly acknowledged and taken into consideration in the interpretation of the results. This study was approved by the Selcuk University Faculty of Veterinary Medicine Experimental Animals Production and Research Center (SÜVDAMEK) Ethics Committee (Ethics approval number 2025/38).

Collection of Fecal and Blood Serum Samples

Fecal samples were collected from dogs suspected of parvovirus infection using rectal swabs. The rectal swabs were placed in 2 ml of Phosphate Buffered Saline containing 25.000 U/ml penicillin and 20 mg/ml streptomycin. In the laboratory, swab tubes were vortexed, and the buffer solution was transferred into 1.5 ml DNase and RNase-free tubes. Samples were stored at -20 °C until further analysis.

Blood samples were collected from the dogs into 2 ml serum tubes without anticoagulant. The tubes were transported to Selcuk University Veterinary Faculty Virology Department laboratory while maintaining the cold chain. After centrifugation (Hettich Universal 320R, Germany) at 3000 rpm for 10 min, the serum from the supernatant was transferred to eppendorf tubes and stored at -20 °C until analysis.

Application of Canine Parvovirus Antigen (IC) Test

The test kit (RIDX-CPV Ag Test Kit, RIDX™, Korea, cat no: CGM-CPG-11) includes fecal diluent, Pasteur pipettes, and a test cassette. Fecal samples collected from the dogs were diluted with the diluent provided in the kit. Using the Pasteur pipette, 3-4 drops of the diluted sample were added to the sample well marked "S" on the test cassette. After an incubation period of 5-10 minutes, the results were interpreted visually based on the presence of indicator line. During the interpretation of the test, the formation of a single line in the "C" section

of the cassette indicated that the kit was functioning properly, and it was considered a sign that the tested fecal sample was negative for CPV-2 Ag. The presence of lines in both the "C" and "T" sections of the cassette was interpreted as a positive result.

Canine Parvovirus Ag and Ab ELISA:

The test (Agrolabo SpA, Italy, cat no:27224032, 27224096) was performed according to the manufacturer's instructions. For both Ag and Ab detection, the optical density (OD) values were measured for each well of the microplate using a spectrophotometer [ELISA reader (Rayto RT-2100C, China)] at wavelengths of 405 nm and 450 nm, respectively. The obtained absorbance values were used to determine the presence of CPV Ag in each fecal sample and the presence and titers of CPV Ab (Ig G) in each serum sample, according to the formula provided below.

Validity and Interpretation of CPV Ag ELISA for Fecal Samples

OD Positive Control / OD Negative Control > 10

Cut off = OD Positive Control x 0.2

Positive results: Samples with an OD higher than the cut-off value

Negative results: Samples with an OD lower than the cut-off value

The validity and interpretation of CPV Ab (Ig G) ELISA for serum samples

Positive control: OD > 1.2; Negative control: OD < 0.15

The S/P ratio (sample OD/positive control OD) value should be calculated. According to the manufacturer's instructions, positive samples are those with an S/P ratio greater than 0.15. Negative samples are those with an S/P ratio less than 0.15.

The titer of the samples is calculated as follows. The value of 98.39 indicated by the manufacturer is considered the minimum protective titer.

Titer value = 54 (e^4x)

e = base of the natural logarithm (2.718282), x = the S/P ratio of the sample.

Statistical Analysis

All data were statistically analyzed using GraphPad Prism version 9.0. To evaluate differences in antibody titers between different age groups, a one-way analysis of variance (One-way ANOVA) was performed. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) post-hoc test was used for multiple comparisons. To compare antibody levels between male and female dogs, an unpaired t-test was applied. A P value less than P<0.001 was considered statistically significant in all tests.

Additionally, the McNemar test was applied to assess the level of agreement between the ELISA and immunochromatographic (IC) test results for the same animals, and to determine whether there was a statistically significant difference between paired dependent samples. A P value less than P<0.001 was considered statistically significant in all analyses.

Results

Canine Parvovirus Antigen IC Test

Out of the 100 dog fecal samples examined with the IC test, 49 samples (49%) were found to be positive for CPV-2 Ag, while 51 (51%) tested negative (Figure 1).

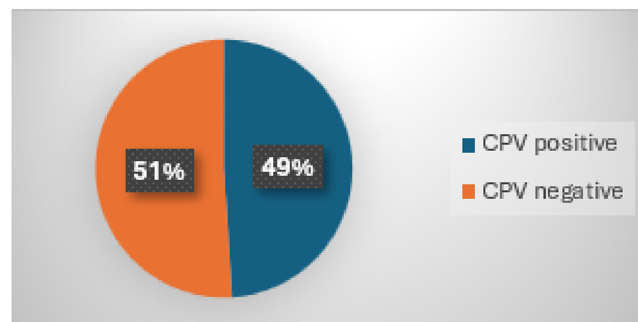

Figure 1. CPV IC test results

ELISA CPV-2 Ag

Fecal samples from 100 dogs were analysed using the ELISA Ag test to detect the presence of CPV-2 Ag. After evaluation, CPV-2 Ag was detected in 17 samples (17%), while 83 samples (83%) tested negative (Figure 2).

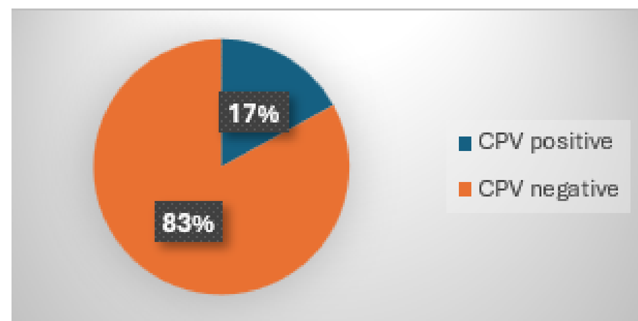

Figure 2. CPV-2 Ag ELISA results.

ELISA Ab (IgG)

Serum samples from 100 dogs were analyzed for presence CPV antibodies using the ELISA IgG Ab kit (Agrolabo SpA, Italy). The test was performed according to the manufacturer's instructions. After evaluation, CPV antibodies were detected in 42 samples (42%), while 58 samples (58%) were found to be negative for CPV antibodies. Among the 42 dogs considered antibody-positive, 18 (42.8%) had high levels of CPV IgG antibody titers, whereas 24 (57.1%) had low levels of CPV IgG antibody titers.

Protective antibody titers were classified based on the age distribution of the dogs. In 5 dogs aged 0-3 months, titers above 18,000 were found; in 13 dogs aged 3-6 months, titers ranging from 6,000 to 18,000; in 8 dogs aged 6-9 months, titers ranging from 1,000 to 4,000; and in 16 dogs aged 9-12 months, titers ranging from 100 to 800 were detected (Figure 3).

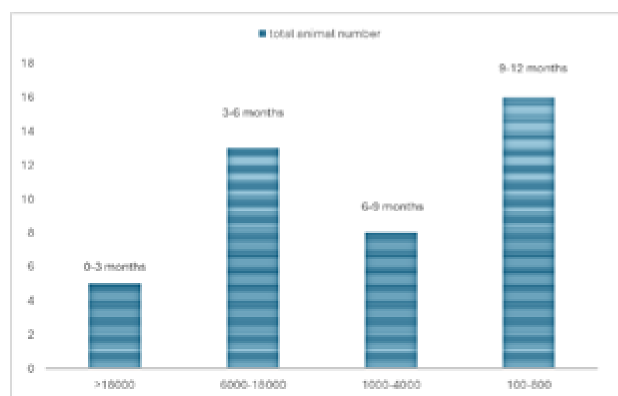

Figure 3. Distribution of CPV-2 antibody titer values in dogs according to age months

Statistics

The relationship between age and immunity level against CPV was statistically evaluated. The results revealed a significant effect of age on immunity, particularly in dogs younger than 6 months. Antibody titers in dogs aged 0–6 months were found to be very high (P<0.001). By the 6th month, titers significantly decreased (P<0.001). No significant change in titer values was observed during the 6–12 month period (Table 1). Additionally, the relationship between gender and immunity level against CPV was statistically evaluated. The results showed no significant difference (P=0.612) between gender and immunity levels (Table 2). It was concluded that gender did not affect the antibody titer values.

In this study, the specificity and sensitivity of the IC test, as compared to the ELISA, were found to be 48.19% and 35.29%, respectively. McNemar test was applied to evaluate the agreement between the ELISA and IC test results for the same animals and to determine whether there was a statistically significant difference between paired dependent samples. As a result of the analysis, the chi-square (χ^2) value was calculated as 18.96, with p-value of ≈13.3281×10^-6. Since P<0.001, a statistically significant difference was observed between the ELISA and rapid test results (Table 3).

Discussion

Rapid and reliable diagnosis is essential for isolating infected animals and providing appropriate treatment to dogs with life-threatening infections (WalterWeingärtner et al., 2021). Recently, IC or ELISA-based methods are commonly used for diagnosing viruses that cause gastroenteritis. These methods are widely used due to their practicality and low cost (Topcu et al., 2023). IC tests for detecting CPV-2 Ag are commercially available due to the large amounts of CPV shed in their feces of infected dogs (WalterWeingärtner et al., 2021). However, the sensitivity of these methods may vary depending on the commercial kits used (Topcu et al., 2023). For instance, in a study, the sensitivity and specificity of ELISA and IC tests, commonly used in diagnosing viruses that cause gastroenteritis, were calculated using RT-qPCR as a reference. The sensitivity and specificity were determined to be 88%-100% for ELISA and 90%-100% for IC tests, respectively (Ibrahim et al., 2015). Bagali et al. (2023) and Dhiman et al. (2015) compared the sensitivity of the IC method with ELISA in their studies and determined it as 98.20% and 95.24%, respectively. Similarly, the specificity was found to be 100% and 97.47%, respectively. Additionally, Dik and Simsek (2021) compared IC tests and PCR in 100 fecal samples from diarrheic dogs, finding the sensitivity of IC to be

Table 1. The effect of age on immunity level in dogs

Month	n	Mean	Std. Error	P value
0-3	5	14851.51[a]	1108.08	<0.001
3-6	13	4995.21[b]	1099.81	
6-9	45	524.93[c]	214.00	
9-12	29	80.50[c]	9.28	
General	92	1795.12	415.78	

Different letters (a, b, c) indicate groups that differ significantly from each other (P<0.001). Groups that share the same letter do not show a statistically significant difference

Table 2. The effect of gender on immunity level in dogs of different age groups

Gender	n	Mean	Std. Error	P value
Male	45	1577.66	561.33	0.612
Female	47	2003.34	615.58	

Table 3. Specificity, sensitivity, and McNemar test results of the IC test compared with ELISA

Test	Result (n/100)	
IC Test	49/100	
ELISA CPV-2 Ag	17/100	
ELISA Ab	42/100	
	IC Test Positive	**IC Test Negative**
ELISA Positive	6[a]	11[c]
ELISA Negative	43[b]	40[d]

Specificity (%) = 48.19 , Sensitivity (%) = 35.29, McNemar test, $(x)^2 = 18.96$, P<0.001

a = True Positive (TP), b = False Positive (FP), c = False Negative (FN), d = True Negative (TN). Sensitivity (%) = TP/(TP+FN) × 100, Specificity (%) = TN/(TN+FP) × 100, McNemar test; $(x)^2=(b-c)^2/(b+c)$

36.36% and specificity to be 100%. Similarly, Dik et al. (2023) evaluated the sensitivity and specificity of CPV detection by comparing IC testing and PCR in 50 fecal samples collected from unvaccinated dogs aged 0–12 months. CPV was detected in 2 fresh fecal samples using the IC test and in 29 samples using PCR. Upon evaluation of the results, the sensitivity of the IC test for CPV detection was found to be notably low at 6.89%, whereas its specificity remained high at 100%. In the present study, the sensitivity and specificity of IC were found to be 35.29% and 48.19%, respectively, when compared with ELISA. Moreover, the data obtained showed a statistically significant difference according to the McNemar statistical test (P< 0.001) (Table 3). It was observed that these data were similar to the results of the study conducted by Dik and Simsek (2021) (Table 3). While the IC test may yield higher positive results in the early stages of infection due to a potentially lower detection threshold, its lower sensitivity and specificity raise concerns regarding the reliability of such results. The lower positivity rate observed with the ELISA test may be attributed to its ability to detect antigen only above a certain threshold level. However, the accuracy of these positive findings cannot be definitively interpreted without confirmation using PCR, which is considered the gold standard.

In addition to their low sensitivity, IC tests have raised concerns regarding their ability to detect CPV-2 subtypes (Decaro et al., 2010). The genetic and antigenic diversity of the virus makes it challenging for a single test format to detect all variants with the same efficiency. This presents a significant challenge, particularly in diagnosing newly emerging or mutated CPV-2 variants. Additionally, the use of only a single monoclonal antibody (mAb) for capture and detection in IC tests can negatively impact test sensitivity (Sharma et al., 2018). Although monoclonal antibodies offer high specificity, they bind to a single epitope, making them insensitive to minor antigenic changes in the virus. This increases the likelihood of false-negative results, thus reducing the reliability of the test. While IC tests are useful for screening due to their rapid and practical application, their low sensitivity and limitations in detecting different CPV-2 subtypes highlight the need for confirmation with more sensitive tests, such as PCR, for a definitive diagnosis (Suzuki et al., 2020). Another diagnostic method for confirming CPV infection, virus isolation, is more sensitive, but it is labor-intensive and time-consuming, making it not a preferred method for routine diagnoses (Dik and Simsek, 2021).

The prevalence of CPV in various countries around the world has been reported as follows: in Greece, 58.1% in 116 dogs with diarrhea (Godsall et al., 2010); in the United Kingdom, 58% in 355 dogs with diarrhea (Godsall et al., 2010); in South America, 70.42% in 71 dogs with acute diarrhea (Duque-Garcia et al., 2017); in Egypt, 84% in 50 dogs with diarrhea (Elbaz et al., 2021); in North Central Nigeria, 45% in 320 dogs with gastroenteritis; in China, 55.7% in 61 dogs with parvoviral clinical signs (Hao et al., 2020); in Tunisia, 32.14% in 168 dogs with diarrhea (Tagorti, 2018); in Portugal, 77.5% in 209 dogs with parvoviral clinical signs (Miranda et al., 2015); and in India, 40.85% in dogs with diarrhea (Behera et al., 2015). In Türkiye, in the province of Bursa, the CPV prevalence in 60 dogs with hemorrhagic diarrhea was 35% (Yılmaz et al., 2005), and in the province of Kars, it was 76.3% in 93 dogs with diarrhea (Yılmaz, 2020). In the study conducted by Temizkan and Temizkan (2023), the prevalence of CPV in unvaccinated dogs in Yozgat province was determined by PCR to be 86.27% (44/51) overall, with 100% (26/26) in puppies and 72% (18/25) in adult dogs. In another study, fecal samples were collected from 83 dogs housed in an animal shelter and 17 dogs admitted to an animal hospital in the Erzurum region, all exhibiting clinical signs of CPV infection. Of the 100 dogs examined using a rapid test, 40 (40%) tested positive for CPV antigen, while 60 (60%) were negative (Ahmed Ismail and Hanedan, 2024). In the study by Dik et al. (2023), the CPV positivity rate in unvaccinated dogs aged 0–12 months in the Konya region was reported as 58% (29/50) based on PCR analysis. In the study conducted in the Alanya region, among the 100 dog fecal samples tested using IC tests and ELISA, 49 (49%) were positive for CPV-2 Ag by IC test, and 17 (17%) were positive by ELISA (Figure 1, Figure 2). The results obtained from this study are similar to the studies of Godsall et al. (2010), Hao et al. (2020), Tagorti (2018), and Behera et al. (2015), but differ from those reported by Duque-Garcia et al. (2017), Elbaz et al. (2021), and Miranda et al. (2015). These findings suggest that the presence of CPV in dogs is widespread both in Türkiye and globally.

Dogs that recover from a natural CPV-2 infection develop life long immunity (Schultz et al., 2010). Vaccination remains the primary method for controlling the disease and modified live virus (MLV) vaccines providing long-lasting immunity (Rota et al., 2019). The longest duration of antibody persistence following the first MLV vaccination was found to be 10 years in dogs kept in natural environments (Schultz et al., 2010), and a case of a 14-year-old female English Setter was reported to still have protective immunity 13 years after its first and only vaccination, with a protective antibody titre remaining even 10 years after the last vaccine (Riedl et al., 2015). One of the primary methods of protection against CPV infection is the antibodies from the mother through colostrum (Decaro et al., 2005). These maternal antibodies protect newborns during the first weeks of life, which can delay the timing of vaccination (Rota et al., 2019). Maternal immunity prevents the virus from infecting young animals by neutralizing it before viremia begins, thus inhibiting the colonization of the intestinal epithelium (Decaro et al., 2005). Recent studies have shown that maternal-derived antibodies (MDA) can offer protection against CPV-2 infection for up to 15-20 weeks (Riedl et al., 2015; Larson et al., 2018). Maternal antibodies begin to decline between 8 and 12 weeks of age, influenced by various factors such as the mother's vaccination status, colostrum quality, intake and absorption, the size and strength of the newborn, and the timing of the intestinal barrier closure, thus allowing the development of active immunity (Dall'Ara et al., 2023). However, since the presence of maternal antibodies appears to be a significant obstacle in vaccination strategies against CPV, it is crucial to measure maternal antibody levels and plan the optimal vaccination time (Kumari et al., 2022). In parallel, studies by Waner et al. (1996), Nandi and Kumar (2010), Mila et al. (2014) and Decaro et al. (2020) have also reported that vaccination failures may occur in cases where maternal antibody levels are high. These findings further emphasize the importance of accurately determining the optimal timing for vaccination (Gülbahçe et al., 2025).

CPV-2 antibody titres can be used to assess whether dogs are protected from infection and to measure IgG antibody levels against various diseases (Rota et al., 2019). This provides veterinarians with the opportunity to monitor antibody titres on an annual basis and detect disease trends over time. Additionally, it can be used for the early detection of dogs that may have lost their immunity to specific diseases (Babalola et al., 2016). Since neutralizing CPV-2 antibody titres are indicators of protection against infection, dogs with seropositive titres above the cutoff value are considered to be protected from infection (Rota et al., 2019). In this study, the minimum protective titre

was considered to be the value of 98.39 as indicated by the manufacturer.

In a study conducted in Nigeria, among 48 vaccinated dogs with age ranges between 7 weeks and 7 years, high levels of CPV antibody titers were detected in 38 (79.2%) dogs, while low levels were found in 10 (20.8%) dogs. The results provided evidence that vaccinated dogs maintain protective antibody titres (Babalola et al., 2016). In a study by Böhm et al. (2004) in the UK, protective antibodies against CPV infection were detected in 94.4% (135 out of 143) of 143 adult dogs that had not been vaccinated for at least 3 years. Titres above 128 were chosen as the protective titre based on studies conducted at the Glasgow Canine Infectious Disease Research Unit. According to this, 8 dogs had antibody titres at the borderline, but none had low or undetectable titers. This was attributed to high levels of natural CPV exposure. In a study covering different regions of Andhra Pradesh, the seroprevalence of canine parvovirus infection was evaluated using the ELISA test on 542 serum samples from both vaccinated and unvaccinated dogs. CPV-specific antibodies were detected in 451 out of 542 serum samples (83.21%). The antibody titres for CPV ranged from 419 to 2337. Vaccinated animals (94.65%) showed significantly higher antibody titers compared to unvaccinated animals (72.50%) (Kumari et al., 2022). The difference in CPV antibody detection between vaccinated and unvaccinated dogs by ELISA was found to be statistically significant. Yang et al. (2010) conducted a seroepidemiological study on stray dogs using the hemagglutination inhibition test in South Korea. It was estimated that 26.2% of the dogs had antibody titers above 1:5120, and the overall positive rate for CPV-2 antibodies in the tested dogs was approximately 93.8%. Devajani Deka et al. (2015) reported higher antibody titers in unvaccinated dogs (66.67%) compared to vaccinated dogs (33.82%) using the ELISA test. They attributed the high antibody levels in unvaccinated animals to acquired immunity from natural infection. In the current study, 42 dogs that tested positive for antibodies based on the cutoff value had CPV Ig G antibody titers. High levels of CPV Ig G antibody titers were found in 18 dogs (42.8%), while 24 dogs (57.1%) had low levels. Additionally, the protective antibody titers from the 42 unvaccinated dogs (42%) were classified according to age distribution. In 5 dogs aged 0-3 months, titers were above 18.000; in 13 dogs aged 3-6 months, titers ranged from 6.000 to 18.000; in 8 dogs aged 6-9 months, titres ranged from 1.000 to 4.000; and in 16 dogs aged 9-12 months, titres ranged from 100 to 800 (Figure 3).

Dogs with antibody titres below the cutoff value were considered to be at high risk of developing clinical disease if exposed to the field virus. Dogs older than 6 months with specific antibodies against CPV are likely to have been exposed to CPV through natural infection rather than vaccination, as they would not develop immunity through vaccination. In the 0-6 month age group, the high prevalence of protective antibody titers was attributed to the presence of maternal antibodies acquired through colostrum, and no clinical signs were observed in these dogs. Significant clinical signs of CPV infection were observed only in dogs older than 6 months with low Ig G titers. In these dogs, the gradual decrease in protective antibody titers was thought to weaken their protection against infection. In such cases, the use of the ELISA test would be helpful in determining the optimal time for vaccination. Despite their low antibody titers, these dogs may still be protected due to their immunological memory (Babalola et al., 2016). This study also evaluated the relationship between the dogs' immunity status against Canine Parvovirus and factors such as age and sex. The results showed a significant statistical difference between age and immunity status, whereas no significant difference was found between sex and immunity status (Table 1, Table 2). Similarly, a study by Babalola et al. (2016) reported no significant difference between sex and immunity level against CPV, but did find a significant relationship between age and immunity, particularly in dogs under 1 year old. However, Twark and Dodds (2000) and Eghafona et al. (2007) reported that age did not show a significant relationship with CPV serum antibody titres. The data from the current study were similar to those of Babalola et al. (2016), but contradicted the earlier studies by Twark and Dodds (2000) and Eghafona et al. (2007). This discrepancy may be attributed to the fact that Twark and Dodds (2000) and Eghafona et al. (2007) focused on a population of adult dogs over 10 years old, in which CPV infections were less prevalent, and variations in antibody titres might have been caused by differences in vaccination status or frequency. Furthermore, age-related immunosenescence, environmental exposure, breed-specific immune responses, and differences in sample sizes or diagnostic techniques used across studies may have also contributed to the variation in findings. These factors highlight the complexity of interpreting seroprevalence data and emphasize the need for age-and context-specific approaches in evaluating CPV immunity.

Conclusion

This study provided a comparative evaluation of the diagnostic performance of ELISA and IC tests in detecting CPV. The findings indicate that the IC test retains its value as a practical and cost-effective screening tool for use in field conditions. However, considering the limitations of both methods, it is recommended that they be supported by more sensitive techniques, such as PCR, particularly in cases requiring a definitive diagnosis. In addition, regular monitoring of antibody titers in dogs that have reached a certain age is crucial for accurately determining vaccination timing and ensuring the effectiveness of preventive healthcare. This study presents important data on CPV infections in the Alanya region, including virus prevalence, seroprevalence, and the reliability of diagnostic tests. The results underscore the need for implementing regular vaccination programs, adopting effective diagnostic approaches, and improving hygiene conditions to protect the local dog population. Moreover, the widespread use of rapid and reliable diagnostic tests in veterinary clinics plays a critical role in the early detection and control of CPV infections.

Acknowledgments

This work was supported by Selcuk University Scientific Research Projects (Project No: 21212010).

Author contribution statement

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest

The authors declare that they have no conflict of interest in this study.

References

Ahmed Ismail, W.F., & Hanedan, B. (2024). Investigation of prevalence and risk factors of Parvovirus infection in dogs in Erzurum province, Turkey. *Eurasian Journal of Veterinary Sciences,* 40(1) 16-23. https://doi.org/10.15312/EurasianJVetSci.2024.421

Babalola, E.T., Ijaopo, O.K., & Okonko, I.O. (2016). Evaluation of immunity and seropositivity of IgG antibodies to canine parvoviruses in vaccinated and unvaccinated dogs in Abeokuta, Nigeria. *Journal of Immunoassay and Immunochemistry*, 37(1), 16-28. https://doi.org/10.1080/15321819.2015.1040159

Bagali, S., Kakhandaki, L., Karigoudar, R., Wavare, S., Shahapur, P.R., & Patil, M.M. (2023). Comparative analysis of enzyme-linked immunosorbent assay and immunochromatography for rotavirus and adenovirus detection in children below five years with acute gastroenteritis. *.Journal of Laboratory Physicians*, 15(1), 110-116. https://doi.org/10.1055/s-0042-1757234

Behera, M., Panda, S.K., Sahoo, P.K., Acharya, A.P., Patra, R.C., Das, S., & Pati, S. (2015). Epidemiological study of canine parvovirus infection in and around Bhubaneswar, Odisha, India. *Veterinary World*, 8(1), 33-37. https://doi.org/10.14202/vetworld.2015.33-37

Böhm, M., Herrtage, M. E., Thompson, H., Weir, A., Hasted, A.M., & Maxwell, N.S. (2004). Serum antibody titres to canine parvovirus, adenovirus, and distemper virus in dogs in the UK which had not been vaccinated for at least three years. *Veterinary Record*, 154(15), 457-463. https://doi.org/10.1136/vr.154.15.457

Dall'Ara, P., Lauzi, S., Zambarbieri, J., Servida, F., Barbieri, L., Rosenthal, R., … & Filipe, J. (2023). Prevalence of serum antibody titers against core vaccine antigens in Italian dogs. *Life*, 13(2), 587. https://doi.org/10.3390/life13020587

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

Decaro, N., Desario, C., Beall, M.J., Cavalli, A., Campolo, M., DiMarco, A., … & Buonavoglia, C. (2010). Detection of canine parvovirus type 2c by a commercially available in-house rapid test. *The Veterinary Journal*, *184*(3), 373-375. https://doi.org/10.1016/j.tvjl.2009.04.006

Decaro, N., Buonavoglia, C., & Barrs, V. (2020). Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Veterinary Microbiology*, 247, 108760. https://doi.org/10.1016/j.vetmic.2020.108760

Devajani Deka, D., Phukan, A., Sarma, D.K., Chakraborty, A.K., & Das, S. (2015). Canine parvovirus infection in immunized and nonimmunized diarrhoeic dogs. *Indian Journal of Veterinary Pathology*, 39, 125-128. https://doi.org/10.5958/0973-970X.2015.00029.2

Dhiman, S., Devi, B., Singh, K., & Devi, P. (2015). Comparison of enzyme-linked immunosorbent assay and immunochromatography for rotavirus detection in children below five years with acute gastroenteritis. *Journal of Clinical Diagnostical Research*, 9(9), 6-9. https://doi.org/10.7860/JCDR/2015/14049.6425

Dik, I., & Simsek, A. (2021). Comparison of different diagnostic methods in detection of canine parvovirus infection. *Eurasian Journal of Veterinary Sciences*, 37(2), 76-81. https://doi.org/10.15312/EurasianJVetSci.2021.329

Dik, I., Aslım, H. P., & Bulut, O. (2023). Evaluation of the Performance of Diagnostic Methods of Canine Parvovirus-2 and Canine Enteric Coronavirus Infections under Different Storage Conditions and Determination of Molecular Characterization. *Eurasian Journal of Veterinary Sciences*, 39(4), 156-163. https://doi.org/10.15312/ EurasianJVetSci.2023.412

Duque-García, Y., Echeverri-Zuluaga, M., Trejos-Suarez, J., & Ruiz-Saenz, J. (2017). Prevalence and molecular epidemiology of canine parvovirus 2 in diarrheic dogs in Colombia, South America: A possible new CPV-2a is emerging? *Veterinary Microbiology*, 201, 56-61. https://doi.org/10.1016/j.vetmic.2016.12.039

Eghafona, N.O., Jacob, J., & Yah, S.C. (2007). Evaluation of post-vaccination immunity to canine distemper and parvoviruses in Benin City, Nigeria. *African Journal of Biotechnology*, 6(16). https://doi.org/10.5897/AJB2007.000-2286

Elbaz, E., El-Tholoth, M., Elfadl, E.A.A., & Mosad, S.M. (2021). Molecular investigation on the presence of canine parvovirus in Egypt. *Comparative Immunology, Microbiology and Infectious Diseases*, 74, 101576. https://doi.org/10.1016/j.cimid.2020.101576

Giraldo-Ramirez, S., Rendon-Marin, S., & Ruiz-Saenz, J. (2020). Phylogenetic, evolutionary, and structural analysis of canine parvovirus (CPV-2) antigenic variants circulating in Colombia. *Viruses*, 12(5), 500. https://doi.org/10.3390/v12050500

Godsall, S.A., Clegg, S.R., Stavisky, J.H., Radford, A.D. & Pinchbeck G. (2010). Epidemiology of canine parvovirus and coronavirus in dogs presented with severe diarrhoea to PDSA PetAid hospitals. *Veterinary Record*, 167(6), 196-201. https://doi.org/10.1136/vr.c3095

Gülbahçe, R., Palancı, H.S., Dik, I., Baş, T., & Bulut, O. (2025). Evaluation of Canine parvovirus–2 case observed in a Toy Poodle breed dog. *Journal of Istanbul Veterinary Sciences*, *9*(1), 23-29. https://doi.org/10.30704/http-www-jivs-net.1600129

Hao, X., He, Y., Wang, C., Xiao, W., Liu, R., Xiao, X., … & Li, S. (2020). The increasing prevalence of CPV-2c in domestic dogs in China. *PeerJ*, 8, e9869. https://doi.org/10.7717/peerj.9869

Ibrahim, S.B., El-Bialy, A.A., Mohammed, M.S., El-Sheikh, A.O., Elhewala, A., & Bahgat, S. (2015). Detection of rotavirus in children with acute gastroenteritis in Zagazig University Hospitals. *Egyptian Electronic Physician*, 7(5), 1227-1233. https://doi.org/10.14661/1227

Khatri, R., Poonam, M.H., & Minakshi, P. (2017). Epidemiology, pathogenesis, diagnosis and treatment of canine parvovirus disease in dogs: A mini review abstract. *Journal of Veterinary Science & Medical Diagnosis*, 6(3). https://doi.org/10.4172/2325-9590.1000233

Kumari, G.D., Pushpa, R.R., Subramanyam, K.V., Rao, T.S., & Satheesh, K. (2022). Seroprevalence of parvovirus infections from vaccinated and unvaccinated dogs of Andhra Pradesh. *Qeios*. https://doi.org/10.32388/OVF6LF

Lambe, U., Guray, M., Bansal, N., Kumar, P., Joshi, V.G., Khatri, R., … & Prasad, G. (2016). Canine parvovirus—An insight into diagnostic aspect. *Journal of Experimental Biology*, 4(3S), 23-30. http://dx.doi.org/10.18006/2016.4(3S).279.290

Larson, H., de Figueiredo, A., Karafillakis, E., & Rawal, M. (2018). State of vaccine confidence in the EU 2018. *Luxembourg: Publications Office of the European Union*, *10*(2018), 241099. https://doi.org/10.2875/241099

Lechner, E.S., Crawford, P.C., Levy, J.K., Edinboro, C.H., Dubovi, E.J., & Caligiuri, R. (2010). Prevalence of protective antibody titers for canine distemper virus and canine parvovirus in dogs entering a Florida animal shelter. *Journal of the American Veterinary Medical Association*, 236(12), 1317-1321. https://doi.org/10.2460/javma.236.12.1317

Litster, A., Pressler, B., Volpe, A., & Dubovi, E. (2012). Accuracy of a point-of-care ELISA test kit for predicting the presence of protective canine parvovirus and canine distemper virus antibody concentrations in dogs. *The Veterinary Journal*, 193(2), 363-366. https://doi.org/10.1016/j.tvjl.2012.01.027

Macêdo, J.V., Frias, I.A., Oliveira, M.D., Zanghelini, F., & Andrade, C.A. (2022). A systematic review and meta-analysis on the accuracy of rapid immunochromatographic tests for dengue diagnosis. *European Journal of Clinical Microbiology & Infectious Diseases*, *41*(9), 1191-1201. https://doi.org/10.1007/s10096-022-04485-6

Mila, H., Grellet, A., Desario, C., Feugier, A., Decaro, N., Buonavoglia, C., & Chastant-Maillard, S. (2014). Protection against canine parvovirus type 2 infection in puppies by colostrum-derived antibodies. *Journal*

of Nutritional Science, 3, e54. https://doi.org/10.1017/jns.2014.57

Miranda, C., Carvalheira, J., Parrish, C.R., & Thompson, G. (2015). Factors affecting the occurrence of canine parvovirus in dogs. *Veterinary Microbiology*, 180(1-2), 59-64. https://doi.org/10.1016/j.vetmic.2015.08.002

Nandi, S., & Kumar, M. (2010). Canine parvovirus: Current perspective. *Indian Journal of Virology*, 21(1), 31-44. https://doi.org/10.1007/s13337-010-0007-y

Nandi, S., Sharma, G.K., Gupta, V., Deol, P., Chander, V., De, U., & Gupta, V. (2019). Global scenario of canine parvovirus mutants: epidemiology, diagnostics and immunoprophylactic agents. *JSM Veterinary Medicine and Research*, 2, 12.

Ogbu, K., Anene, B., Nweze, N., Okoro, J., Danladi, M., & Ochai, S. (2017). Canine parvovirus: A review. *Journal of Veterinary and Animal Sciences*, 44(1), 1-10

Riedl, M., Truyen, U., Reese, S., & Hartmann, K. (2015). Prevalence of antibodies to canine parvovirus and reaction to vaccination in client-owned, healthy dogs. *Veterinary Record*, 177(23), 597-599. https://doi.org/10.1136/vr.103271

Rota, A., Dogliero, A., Muratore, E., Pregel, P., Del Carro, A., & Masoero, L. (2019). Serological survey of canine parvovirus 2 antibody titres in breeding kennels in northern Italy. *BMC Veterinary Research*, 15, 1-5.

Schultz, R.D., Thiel, B., Mukhtar, E., Sharp, P., & Larson, L.J. (2010). Age and long-term protective immunity in dogs and cats. *Journal of Comparative Pathology*, 142(1), S102–S108. https://doi.org/10.1016/j.jcpa.2009.10.009

Sharma, C., Singh, M., Upmanyu, V., Chander, V., Verma, S., Chakrovarty, S., … & Gupta, V. K. (2018). Development and evaluation of a gold nanoparticle-based immunochromatographic strip test for the detection of canine parvovirus. *Archives of Virology*, 163, 2359-2368. https://doi.org/ 10.1007/s00705-018-3846-2

Suzuki, K., Huits, R., Phadungsombat, J., Tuekprakhon, A., Nakayama, E.E., Van Den Berg, R., & Shioda, T. (2020). Promising application of monoclonal antibody against chikungunya virus E1-antigen across genotypes in immunochromatographic rapid diagnostic tests. *Virology Journal*, *17*, 1-11. https://doi.org/10.1186/s12985-020-01364-4

Tagorti, G. (2018). Prevalence of canine parvovirus infection in Grand Tunis, Tunisia. *Journal of Advanced Veterinary and Animal Research*, 5(1), 93-97. https://doi.org/10.5455/javar.2018.e251

Temizkan, M.C., & Temizkan, S. (2023). Canine parvovirus in Turkey: first whole-genome sequences, strain distribution, and prevalence. *Viruses*, 15(4), 957. https://doi.org/10.3390/v15040957

Topcu, M., Köroglu, M., Aydemir, O., Aydemir, Y., Altındis, M., & Elmas, B. (2023). Investigation of Viruses Causing Gastroenteritis by Immunochromatographic Tests and Multiplex PZR Method. *Value in Health Sciences*, 13(1), 1-5. https://doi.org/10.33631/sabd.1073789

Tuteja, D., Banu, K., & Mondal, B. (2022). Canine parvovirology–A brief updated review on structural biology, occurrence, pathogenesis, clinical diagnosis, treatment and prevention. *Comparative Immunology, Microbiology and Infectious Diseases*, 82, 101765. https://doi.org/10.1016/j.cimid.2022.101765

Twark, L., & Dodds, W.J. (2000). Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. *Journal of the American Veterinary Medical Association*, 217(7), 1021-1024. https://doi.org/10.2460/javma.2000.217.1021

Walter-Weingärtner, J., Bergmann, M., Weber, K., Truyen, U., Muresan, C., & Hartmann, K. (2021). Comparison of eight commercially available faecal point-of-care tests for detection of canine parvovirus antigen. *Viruses*, 13(10), 2080. https://doi.org/10.3390/v13102080

Waner, T., Naveh, A., Wudovsky, I., & Carmichael, L.E. (1996). Assessment of maternal antibody decay and response to canine parvovirus vaccination using a clinic-based enzyme-linked immunosorbent assay. *Journal of Veterinary Diagnostic Investigation*, 8, 427 -432

Yang, D.K., Yoon, S.S., Byun, J.W., Lee, K.W., Oh, Y.I., & Song, J.Y. (2010). Serological survey for canine parvovirus type 2a (CPV-2a) in the stray dogs in South Korea. *Journal of Bacteriology and Virology*, 40(2), 77-81. https://doi.org/10.4167/jbv.2010.40.2.77

Yılmaz, V. (2020). Virological survey of canine parvovirus infection in Kars shepherd dogs in Kars Province, Turkey. *Advances in Animal and Veterinary Sciences*, 8(7), 687-691. https://doi.org/10.17582/journal.aavs/2020/8.7.687.691

Yılmaz, Z., Pratelli, A., & Torun, S. (2005). Distribution of antigen types of canine parvovirus type 2 in dogs with hemorrhagic enteritis in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 29(4), 1073-1076