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Investigation of Distempervirus and Parvovirus Infections in Dogs

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ABSTRACT

CDV and CPV are significant viral agents that frequently cause fatal infections in both domestic and wild dogs. In this study, a total of 95 animals, including both healthy individuals and those exhibiting gastrointestinal and neurological symptoms, aged between 6 and 24 months, were serologically examined for CDV and CPV infections using the ELISA test. According to the manufacturer's instructions, the assay range for CDV was specified as 0.7 ng/ml to 200 ng/ml, with a sensitivity value of 0.665 ng/ml. Among the total 95 animals, 48 (50.52%) with good general health had antibody titers ranging between 7 and 20, while 9 (9.47%) had titers between 50 and 85. In animals showing lethargy, diarrhea, vomiting, and neurological symptoms, 22 (23.15%) had titers between 7 and 20, 10 (10.52%) between 20 and 35, 12 (12.63%) between 35 and 50, and 4 (4.2%) between 50 and 85. In terms of CPV antibodies, 88 (92.63%) were found to be positive. In conclusion, the study data indicate the necessity of developing and strictly implementing strategies to combat CDV and CPV infections. Further studies are required to investigate the genetic variability of these viruses, the effectiveness of vaccine-induced antibodies in protecting against local strains, and the pathogenesis of the diseases.

INTRODUCTION

Since its discovery in the 1730s, Distemper infection has been observed in Europe and many other parts of the world (Uhl et al., 2019; Saltık and Kale, 2023). Canine Distemper Virus (CDV), which belongs to the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, and genus *Morbillivirus*, is an enveloped, single-stranded RNA virus that primarily infects carnivores but can also cause infections in a wide range of animal species (ICTV, 2024a). Distemper infection, which leads to a multisystemic disease and often results in mixed infections in dogs, is commonly known as "canine distemper" among the public. In general, CDV has an affinity for epithelial tissue, and the initial clinical manifestation is a severe respiratory problem. Additionally, simultaneous or subsequent symptoms affecting the central nervous system and gastrointestinal tract may develop (Saltık and Kale, 2020; Zhao and Ren, 2022; Saltık and Atlı, 2023). The symptoms in animals vary depending on the duration of viral persistence in the body, viral strain, secondary bacterial infections, the host's age, and its immune response (Skyes and Vandevelde, 2021). CDV is typically transmitted through droplet infection, direct contact, or aerosol routes (Shin et al., 2022).

Canine parvovirus (CPV) has been known since the late 1970s and, despite intensive vaccination efforts, remains one of the leading causes of acute gastroenteritis and mortality in puppies worldwide (Decaro et al., 2020; Saltık and Koç 2024). Shortly after its first detection in 1978, CPV-2 reached pandemic proportions, and new antigenic variants, later named CPV-2a, CPV-2b, and CPV-2c, emerged (Grecco et al., 2024). Canine parvovirus (CPV), a DNA virus, belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, and genus *Protoparvovirus* (ICTV, 2024b). The transmission of CPV-2 occurs primarily through the oronasal route, direct contact, or exposure to contaminated feces. Clinical signs include lethargy, anorexia, vomiting, fever, and severe diarrhea, which may be bloody or non-bloody. The rapid progression of this disease can lead to fatal outcomes in immunocompromised animals (de Oliveira Santana et al., 2022).

In this study, due to the increasing number of pet animals in the Konya region, the aim was to determine the

epidemiological status of distemper and parvovirus infections, which pose a significant threat to canine health.

MATERIALS AND METHODS

Materials

In this study, a total of 95 unvaccinated animals, both healthy and symptomatic, aged between 6 and 24 months, brought to private clinics in the Konya, İzmit, and Antalya regions, were serologically examined for CDV and CPV infections. Gastrointestinal and neurological problems were commonly observed in the dogs. The clinical findings recorded in the dogs are listed in Table 1.

Among the sampled dogs, 50.52% (48/95) were in good general condition, 44.21% (42/95) exhibited lethargy, 16.84% (16/95) showed neurological symptoms, 38.94% (37/95) had diarrhea as a gastrointestinal issue, 12.63% (12/95) experienced vomiting, and 20% (19/95) suffered from cachexia.

When evaluating clinical findings, some dogs exhibited a single symptom, while others presented with two or even three symptoms simultaneously.

Table 1. Clinical findings in sampled dogs

Clinical findings	Positive/n	%
General Condition Good	48/95	50.52
Weakness	42/95	44.21
Tics	16/95	16.84
Diarrhea	37/95	38.94
Vomiting	12/95	12.63
Cachexia	19/95	20

Preparation of Blood Serum Samples

Blood samples were collected from all dogs via the cephalic vein (vena cephalica antebrachii) into biochemistry tubes (BD Vacutainer®). The collected blood samples were centrifuged at 3,000 rpm for 10 minutes.

Indirect ELISA

The prepared blood serum samples were analyzed for the presence of antibodies against CDV and CPV. For this purpose, commercially available Sunred ELISA (Cat No. SRB0543) and Agrolabo ELISA (Cat No. 27224096) test kits were used, following the manufacturer's instructions.

Statistical Analysis

CDV antibody titers and CPV antibody positivity were examined based on the gender and age distribution of the 95 animals included in the study. The animals were grouped into two categories based on gender (male and female) and three age groups (11-15 months, 16-20 months).

RESULTS

According to the manufacturer's instructions, the test result range was 0.7 ng/ml to 200 ng/ml, with a sensitivity value of 0.665 ng/ml. Among the 95 animals: 48 (50.52%) in good general condition had CDV antibody titers between 7-20, while 9 (9.47%) had titers between 50-85. In animals with symptoms such as lethargy, diarrhea, vomiting, and neurological signs: 22 (23.15%) had antibody titers between 7-20, 10 (10.52%) between 20-35, 12 (12.63%) between 35-50, 4 (4.2%) between 50-85. The range of values observed among the sampled animals is presented in the table below. Regarding CPV antibody positivity, 56 animals in good general condition and 32 animals showing gastrointestinal clinical signs were found to be positive.

Table 2. Relationship between clinical findings of animals and CDV antibody concentration

Clinical Finding	Calculated CDV Ab concentrations (CC)	Number of Animals
General Condition is good	7-30	48
	50-85	9
Weakness, Diarrhea, Nervous symptoms	7-20	22
	20-35	5
	35-50	7
	50-85	4

Statistics

No statistically significant difference was found between CDV and CPV infections and age or gender.

Table 3. The effect of age on CDV antibody titer values in dogs (ANOVA test)

					Concentration					
					7-35		36-50		51-85	
Age	N	%	Mean±SE	P	N	%	N	%	N	%
11-15 months	42	44,2	1,30±0,68		34	81	3	7,1	5	11,9
16-20 months	35	36,8	1,45±0,81		26	74,3	2	5,7	7	20,0
21-24 months	18	18,9	1,27±0,57	0.583	14	77,8	3	16,7	1	5,6
Total	95	100	1,35±0,71		74	77,9	8	8,4	13	13,7

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 4. The effect of gender on CDV antibody titer values in dogs (Independent t-test)

					Concentration					
					7-35		36-50		51-85	
Gender	N	%	Mean±SE	P	N	%	N	%	N	%
Male	41	43,2	1,34±0,72		33	80,5	2	4,9	6	14,6
Female	54	56,8	1,37±0,70	0.846	41	75,9	6	11,1	7	13,0
Total	95	100	1,35±0,71		74	77,9	8	8,4	13	13,7

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 5. The effect of age on CPV antibody titer values in dogs (ANOVA)

					Positive		Negative	
Age	**N**	**%**	**Mean±SE**	**P**	**N**	**%**	**N**	**%**
11-15 months	42	44,2	1,07±0,26		39	92,9	3	7,1
16-20 months	35	36,8	1,14±0,35		31	88,6	4	11,4
21-24 months	18	18,9	1,05±0,23	0.473	18	100	-	-
Total	95	100	1,09±0,29		88	92,6	7	7,4

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

Table 6. The effect of gender on CPV antibody titer values in dogs (Independent t-test)

					Positive		Negative	
Gender	**N**	**%**	**Mean±SE**	**P**	**N**	**%**	**N**	**%**
Male	41	43,2	1,12±0,33		37	90,2	4	9,8
Female	54	56,8	1,07±0,26	0.435	51	94,4	3	5,6
Toplam	95	100	1,09±0,29		88	92,6	7	7,4

N: Number, %: Percentage, SE: Standard Error (SE), p<0.05: The difference between groups is significant.

DISCUSSION AND CONCLUSION

This study reports the results of research on the seroepidemiology of distemper virus and parvovirus infections in dogs in Turkey. Both infections cause contagious and fatal diseases in unprotected domestic and wild carnivores worldwide (Kimpston et al., 2022).

The standard procedure for assessing immunity against canine distemper is to experimentally infect vaccinated animals with a virulent strain. However, an alternative method widely used is the measurement of serum antibody titers (McCaw et al., 1998; Carmichael, 1999; Meazzi et al., 2022; Gonzalez et al., 2023). Nevertheless, the antibody titers considered protective vary between laboratories and methodologies. Meazzi et al., (2022) reported a protective titer of ≥1:32 in a neutralization test. Rima et al., (1991) stated that dogs with neutralizing antibody titers above 50 in an SN test survived distemper. Carmichael (1999) found that a neutralizing antibody titer of ≥1:80 in vaccinated Beagle dogs kept in isolation indicated immunity against CDV. McCaw et al., (1998) considered an SN antibody titer of ≥1:96 in vaccinated dogs to be protective.

ELISA is a preferred method due to its high sensitivity and specificity (Fan et al., 2013). In this study, the ELISA test was used to serologically evaluate distemper infection in animals. Inder et al., (2021), in a study comparing SN tests and ELISA, found a correlation between the two methods. They reported that ELISA titer values provided uncertain levels of protection on day 25 in vaccinated puppies and dropped to a non-protective titer (≤30 AU) by day 45. They emphasized that maternal antibodies play a crucial role in preparing puppies against canine distemper, as maternal immunity is considered the primary reason for vaccine failure in young dogs.

If serum antibody titers reach high levels within 8-9 days after infection, the virus disappears from the lymphatic and other tissues, and the infection remains subclinical or mild. However, if the immune response is weak or delayed, CDV spreads to multiple tissues, leading to an acute or chronic disease with high mortality (Jóźwik, 2004). The results of this study confirm the role of humoral immunity in disease recovery.

Among 12 dogs with lethargy, vomiting, and neurological symptoms, high CDV serum antibody titers 35-50 Calculated CDV Ab concentrations (CC) were detected (Table 1, Table 2). These animals were suspected to be in the acute phase of infection. Additionally, four dogs with clinical symptoms and a strong serological response (50-85 CC) exhibited muscle twitching.

It is known that Feline Leukemia Virus (FeLV) (Cong et al., 2016), Toxoplasma gondii (Simion et al., 2019), and Feline Immunodeficiency Virus (FIV) (Mauler et al., 2014) can cause muscle twitching. If an animal does not receive adequate nutrition, neurological problems may develop depending on the duration and severity of the deficiency. Neurological symptoms can be caused by deficiencies in vitamins B1, B6, B12, and C, Omega-3 fatty acids, hypocalcemia, hyponatremia, hypochloremia, hyperphosphatemia, vitamin D deficiency, and taurine deficiency (Kumar et al., 2024). It is suspected that these four dogs had recovered from distemper but suffered from a secondary infection or vitamin deficiency.

Respiratory, intestinal and dermatological signs are known to appear 10 days after epithelial localisation of distemper virus infection. Symptoms such as purulent nasal discharge, cough, dyspnoea, pneumonia, diarrhoea, vomiting and dermal pustules are often exacerbated by secondary bacterial infections. Hyperkeratosis of the soles of the feet and nose and enamel hypoplasia are typical (Saltık and Kale, 2020; Saltık and Atlı, 2023). Signs of infection can be observed in dogs surviving CDV subclinical or subacute infections (Martella et al., 2008). It was found that 10 animals with slightly higher CDV antibody titre (20-35 CC) and clinical signs of diarrhoea, vomiting and cough were animals with increased antibody production as a result of infection. It was reported that neurological signs such as circling, head tilt, eye tremor, partial or complete paralysis, convulsions and dementia could be seen 20 days after infection. Involuntary sudden twitching or contraction of muscles is considered as a typical example of CDV infection (Green and Appel, 1990). Neurological clinical findings were present in 39 of the sampled animals. Among these, 9 of them (antibody titre 50-85CC) had only neurological symptoms despite their good general condition. Considering the high antibody titres of these animals, in parallel with Jozwik et al., (2004) we determined that these animals were in the peak period of antibody in the 3rd week after infection. Jozwik et al., (2004) in their study, when they tested with Immuno Peroxidase Monolayer Assay (IPMA) in dogs in which CDV was detected by RT PCR, they determined that those with high antibody titres (1280) recovered, those

with relatively medium titres (640, 320) had localised twitching in their muscles, and those with lower titres (40,10,5) died.

In parallel with Vandevelde and Zurbrigge, (2005) who found acute demyelination in the brain at day 20 post CDV infection, we interpreted that these animals with neural symptoms were approximately at day 20 post infection. Litster et al., (2012) determined antibody titres for CDV in ≥4-month-old dogs for 2 weeks following vaccination with modified live vaccine (MLV) and reported that animals still had negative antibody titres at 6-8 days post-vaccination, but turned positive at 13-15 days. Bergmann et al., (2021) reported that they detected the maximum antibody titre increase (≥4-fold titre increase) on the 28th day after vaccination. As a result of this study, it was determined that 12 animals (antibody titre 35-50CC) had acute infection and their antibody titres started to increase slowly. Because clinical findings were also present in these animals. Pardo et al., (1997) reported that clinical signs in CDV started at the earliest on the 9th day after exposure to the virus. It has been reported that CDV-infected dogs with high body temperature (above 39.5°C) become depressed and anorexic 3 or 4 days after infection. In another study, it was reported that all dogs, except those fatally infected, became clinically stable between 14 and 21 days (Appel et al., 1982). According to the antibody titres of the 12 dogs in the study, it was interpreted that it was around the 16th day after exposure to the agent (9+7). The 37 animals with clinical signs and low antibody titres (7-20CC) were thought to be in the peracute period at the onset of infection. The 78 animals with good general condition and low distemper antibody titre (7-20 CC) were thought to have been infected long ago and may not even have clinical signs. The results shown in Table 1 confirm that serological examination of a distemper patient may have a prognostic value.

High antibody titres are known to be associated with protective immunity. Therefore, the critical issue in a study of this nature is to define protective serum antibody titres against CDV. Böhm et al., (2004) stated that CPV HI titres equal to or higher than 1:128 are protective titre values, while another study pointed out that a cut-off titre of 1:80 was used (Waner et al., 2006). Böhm et al., (2004) chose a cut-off SN titre of 1:64 or higher, but SN titres between 1:16 and 1:32 have been shown to be protective in other studies (Olson et al., 1988; Coyne et al., 2001). In the present study, antibody titres of 50CC and above were found to be protective against the disease. Young dogs, especially newborns and recently weaned dogs, are generally more susceptible to CDV infection and there is a relationship between susceptibility and age (Headley and Graça, 2000). In this study, similar to Shabbir et al., (2010), antibody levels were found to be independent of age (Table 3). However, Martella et al., (2008) reported that CDV titre was higher in juveniles. The fact that there was no statistically significant difference between CDV antibody titre and age in the study was interpreted as the animals were not puppies and were at an age that could be considered adult (12-24 months). In addition, in parallel with Luo and Zhang, (2017); Costa et al., (2019); Tavakoli et al., (2021) no statistically significant difference was found between antibody titre and sex (Table 4). Similar results were reported by Brady et al., (2012); Hübner et al., (2010) that there was no relationship between gender and CPV (Table 6). On the contrary, Gamege et al., (2020) reported a negative correlation between age and infection. In the present study, in parallel with Mokhtari et al., (2018), the lack of a significant relationship between age and infection (Table 5) was thought to be due to variables such as sample size or severity of infection, status in the population and habitus of dogs. The severity of the disease, mortality and morbidity rates vary depending on the body's immune system and secondary infections. Especially in animal shelters with collective living conditions, a more favourable environment for the spread of the disease occurs (Şahna et al., 2008). Pets are also likely to come into contact with stray animals when walking on the street and meeting their toilet needs. Therefore, there is always a risk of catching the virus. Since stray animals are unvaccinated and the virus is constantly circulating in them, they are reservoirs and pose a potential risk for pets (Sayın and Erol, 2021). It is known that dogs from the urban population vaccinated with commercial modified live virus distemper vaccines have antibody titres indicating immunity for 2 years and then the antibody level decreases significantly (Jozwik et al., 2004). An unknown history of vaccination should raise suspicion that the dog is not protected against CPV and CDV and there should be a rush for basic vaccination. However, it is currently not logistically or economically feasible to vaccinate all stray dogs. It may therefore be advisable to keep dogs at home, especially in the first phase, and only release them after they have been vaccinated. In addition, control programmes need to be developed to prevent the spread of infectious diseases, such as the adoption and sterilisation of stray dogs and the improvement of hygiene and management of shelters.

In conclusion, the study data show that strategies to combat CDV and CPV infections should be developed and strictly implemented. For this, future studies on the epizootiology (incidence, prevalence, host characteristics) and virological characteristics (genetic variability of the virus, the success of antibodies against vaccinia virus in protecting against existing strains in the region, pathogenesis and the relationship between field isolates, etc.) of these viruses are needed.

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Ethical Declaration

This study, approved by Selcuk University Animal Experiments Local Ethics Committee (SÜVDAMEK) under decision number 2025/11.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: H.P.A., Design: H.P.A., I.D., Data Collection or Processing: H.P.A. Analysis or Interpretation: H.P.A., I.D., Literature Search: H.P.A., Writing: H.P.A., I.D.

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