



Canine Distemper Virus Infection in Shelter Dogs Presenting Clinical Signs: Prevalence and Risk Factors

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ABSTRACT

Canine distemper virus (CDV) infection is an important disease effecting dogs in worldwide. The aim of this study was to investigate the prevalence of canine distemper virus (CDV) infection, which is a globally important disease, in dogs with clinical signs in an animal shelter in Erzurum and the risk factors associated with age and gender. 119 unvaccinated dogs of various ages living in the Erzurum animal shelter were included in this study (74 females and 45 males). Each animal's clinical symptoms were recorded, and then blood samples, nasal, ocular, and rectal swab samples were taken. The canine distemper virus (CDV) was then assessed using RT-PCR. The analysis of conjunctival, nasal, and rectal swabs as well as blood samples revealed that 18 out of 119 dogs (15.1%) tested positive for CDV. With 13 of the 74 female dogs and 5 of the 45 male dogs testing positive, the results indicated no significant correlation ($p=0.434$) between gender and CDV prevalence. Ten (19.6%) of the 51 dogs under a year-old and eight (11.8%) of the 68 dogs over a year-old tested positive for CDV ($p=0.303$). Clinically, CDV PCR results were statistically significant in 31% of dogs with a cough ($p=0.014$) and in 27.9% of dogs with ocular discharge ($p=0.006$). As a result, the total prevalence rate was 15.1% in dogs presenting one of the clinical signs of distemper in a crowded animal shelter. One of the leading risk factors may be related to the housing conditions in the animal shelter. Effective preventive measures should be implemented in the management of the transmission.

Keywords: Distemper virus, Prevalence, Prevention, Risk factors.

ÖZ

Klinik Belirtiler Gösteren Barınak Köpeklerinde Canine Distemper Virüs Enfeksiyonu: Prevalans ve Risk Faktörleri

Canine distemper virus (CDV) enfeksiyonu tüm dünyada köpekleri etkileyen önemli bir hastalıktır. Bu çalışmanın amacı, Erzurum hayvan barınağında klinik belirtileri olan köpeklerde küresel olarak önemli bir hastalık olan canine distemper virus (CDV) enfeksiyonunun prevalansını ve yaş ve cinsiyet ile ilişkili risk faktörlerini araştırmaktır. Erzurum hayvan barınağında yaşayan çeşitli yaşlarda 119 aşısız köpek bu çalışmaya dahil edildi (74 dişi ve 45 erkek). Her hayvanın klinik semptomları kaydedildi ve ardından kan örnekleri, burun, oküler ve rektal sürüntü örnekleri alındı. Canine distemper virus (CDV), RT-PCR kullanılarak değerlendirildi. Konjonktival, nazal ve rektal sürüntü örneklerinin yanı sıra kan örneklerinin analizi, 119 köpekten 18'inin (%15.1) CDV için pozitif olduğunu ortaya koymuştur. Test edilen 74 dişi köpekten 13'ü ve 45 erkek köpekten 5'i pozitif çıkarken, sonuçlar cinsiyet ile CDV prevalansı arasında anlamlı bir korelasyon ($p=0.434$) olmadığını göstermiştir. Bir yaşından küçük 51 köpeğin onunda (%19.6) ve bir yaşından büyük 68 köpeğin sekizinde (%11.8) CDV testi pozitif çıkmıştır ($p=0.303$). Klinik olarak, CDV PCR sonuçları öksürüğü olan köpeklerin %31'inde ($p=0.014$) ve göz akıntısı olan köpeklerin %27.9'unda ($p=0.006$) istatistiksel olarak anlamlı bulunmuştur. Sonuç olarak, kalabalık bir hayvan barınağında distemper klinik belirtilerinden birini gösteren köpeklerde toplam prevalans oranı %15.1'dir. Önde gelen risk faktörlerinden biri, hayvan barınağındaki barınma koşullarıyla ilişkili olabilir. Bulaşmanın yönetiminde etkili önleyici tedbirler uygulanmalıdır.

Anahtar Kelimeler: Distemper virus, Korunma, Prevalans, Risk faktörleri.



INTRODUCTION

The canine distemper virus (*Morbillivirus canis*, CDV), a member of the *Morbillivirus* genus within the *Paramyxoviridae* family of negative-sense, single-stranded, non-segmented RNA viruses, is the cause of canine distemper (CD) in dogs (Appel and Gillespie 1972). The highly contagious virus known as CDV is widespread and can be fatal to domestic dogs (Dorji et al. 2020). While some studies (Temilade et al. 2015; Joshi et al. 2022; McDermott et al. 2023; Mousafarkhani et al. 2023) have indicated that female dogs are more likely to get distemper than males, the majority (Gemma et al. 1996; McCaw et al. 1998; Headley and Graça 2000; Eghafona et al. 2007; Costa et al. 2019) have found no discernible differences between the genders. The age of the host, immunity, type of virus, organ system afflicted, and the existence of secondary infections with other viruses and bacteria all influence the clinical manifestations of distemper (Leisewitz et al. 2001). The disease is less severe or asymptomatic, particularly in older dogs and dogs with partial immunity. Distemper in puppies has a higher death rate and a more severe, prolonged course (Sellon and Vahlenkamp 2017). A multisystemic disease, distemper presents with symptoms of the nervous system, genitalia, urogenital tract, respiratory system, and gastrointestinal tract (Budaszewski et al. 2014; da Fontoura et al. 2016; Tuzcu et al. 2021). The disease manifests clinically as lethargy, anorexia, rhinitis, purulent conjunctivitis, diphasic fever, severe gastrointestinal abnormalities, bronchopneumonia, vesiculopustular lesions, and myoclonus (Yarım and Yağcı 2006; Çalışkan and Burgu 2007; Headley et al. 2012).

The aim of this study was to investigate the prevalence of canine distemper disease by isolating nucleic acid fragments in dogs presenting one of the clinical signs of the disease, and to evaluate the risk factors, such as age and gender, in an animal shelter. This study is the first study to our knowledge to examine the prevalence of distemper disease in dogs with clinical signs and the risk factors associated with age and gender in the province of Erzurum, Türkiye.

MATERIAL AND METHODS

Ethical Statement

This study was approved by Atatürk University Animal Experiments Local Ethics Committee with decision number 2022/33.

Animal Materials

In this study, 119 mixed-breed dogs of different ages and genders, not vaccinated against CDV, showed clinical signs of distemper (nasal and/or ocular discharge, cough, nervous system findings, diarrhea and skin problems) in Erzurum Animal Shelter between May and July 2024 were used. For every dog that was sampled, data was gathered. No exclusion criteria were applied in this study. A minimum of one of the following clinical symptoms had to be present in order for the case to be included: coughing, diarrhea, cachexia, dehydration, weakness, lymphadenopathy, pale mucous membranes, fever, ocular discharge, serous or purulent nasal discharge, nasal hyperkeratosis, and hyperkeratosis of the foot pad. Teeth were used to estimate age, with under- and over-one-year olds being divided into two categories. The age and gender information of dogs included in the study is presented in Table 1.

Table 1: Age and sex of dogs included in the study.

<1 year old		>1 year old	
Female	Male	Female	Male
28	23	46	22

Samples

1.5 ml of blood was drawn from the *vena cephalica antebrachii* into tubes containing ethylene diamine tetra acetic acid (Becton Dickinson Co., USA) after the dogs' age, gender, and clinical signs were noted. Using sterile swabs, conjunctival, nasal, and rectal samples were obtained from the dogs. A cold chain was used to transport the specimens to the lab.

Virological Analysis

Blood samples collected in EDTA tubes were centrifuged at 2500 rpm for 10 minutes. The leukocyte and plasma layers were collected with an automated pipette and transferred to 2 ml eppendorf tubes. Swab samples were diluted with PBS (phosphate buffer saline) and then centrifuged at 3000 rpm for 5 minutes to collect the supernatants. The pre-prepared blood and swab samples were subjected to nucleic acid isolation. The GeneJET Viral DNA/RNA Purification Kit (Thermo Fisher Scientific, USA) was used according to the manufacturer's recommendations. The obtained nucleic acid suspension was converted into complementary DNA (cDNA) by RT-PCR. The First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used for this purpose. PCR was performed with the primers specific for the haemagglutinin (H) gene of CDV using cDNA samples as templates. Primers and PCR cycles were performed according to the conditions reported by Trebbien et al. (2014). The primer sequences used in the PCR process and the properties of these primers are shown in Table 2. A CDV sample previously sequenced was used as a positive control. The amplicons obtained after PCR were first subjected to agarose gel electrophoresis and then to UV gel imaging, and DNA bands of size 654 base pairs (bp) size were evaluated as CDV positive (Figure 1).

Statistical Analysis

The prevalence of distemper infection in dogs was calculated as the number of CDV positive dogs divided by the number of dogs tested for CDV in percent. To determine the dependence of positive rates on age, gender, and clinical signs, Fisher's Exact test was used to determine the dependence of positive rates on age, gender, and clinical signs. The statistical analysis was performed using the IBM SPSS 22 package program.

RESULTS

Age and Gender of the Dogs

The research material comprised of samples collected from a total of 119 unvaccinated dogs, comprising 74 females (62%) and 45 males (38%) at the Erzurum animal shelter. The prevalence of CDV among dogs was found to be 15.1% (18/119). Of the 119 dogs, 51 were less than one-year-old and 68 were older than 1 year. In the study, 17.6% (13/74) female dogs and 11.1% (5/45) male dogs were positive for CDV. In contrast, out of the 68 dogs that were older than one year, 11.8% (8/18) were found to be CDV-positive. The age and gender information of the CDV-positive dogs included in the study is presented in Table 3.

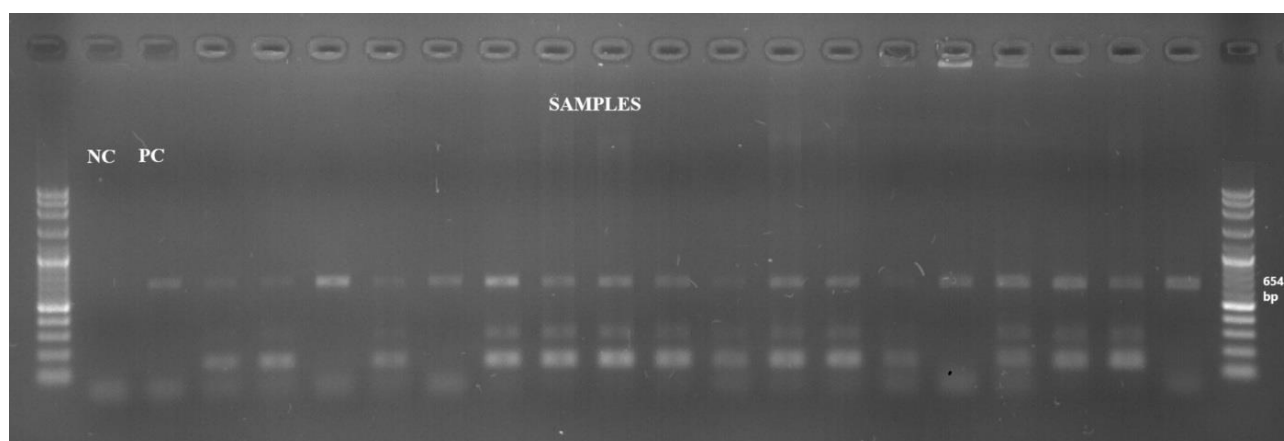


Figure 1: CDV positive DNA fragments under UV light. NC: negative control, PC: positive control, bp: base pair.

Table 2: Primers used in the study, target gene region and product size.

Region	Primers	Primer sequences F/R (5'-3')	Length	Reference
H-Gen	Zhao2010fwd 7711rev	F: TTAGGGCTCAGGTAGTCCA R: TGAGATCAAAGACATGGA	654bp	(Trebien et al., 2014)

Table 3: Age and sex of CDV positive dogs.

<1 year old		>1 year old	
Female	Male	Female	Male
7	3	6	2

Clinical Symptoms of the Dogs

The study included dogs that showed clinical symptoms that were consistent with having a distemper infection. The most common findings in CDV positive dogs were ocular discharge and cough, followed by serous-mucopurulent nasal discharge, hyperkeratosis of the foot pad, swelling of the lymph nodes, diarrhea-dehydration and nasal hyperkeratosis. While serous-mucopurulent nasal discharge was the most common finding in patients younger than one year, ocular discharge was the most common finding in patients older than one year. The clinical signs of all dogs are presented in Table 4, and the clinical signs of CDV-positive dogs are presented in Table 5 (Figure 2).



Figure 2: Clinical signs of CDV positive dogs. A: Purulent nasal discharge, B: Nasal hyperkeratosis, C: Ocular discharge, D-E: Ocular discharge and nasal hyperkeratosis, F: Hyperkeratosis of the foot pads.

Table 4: Clinical findings observed in the dogs.

Clinical finding	<1 year-old		>1 year-old	
	Female	Male	Female	Male
Ocular discharge	9	5	18	11
Serous-mucopurulent nasal discharge	12	6	11	8
Nasal hyperkeratosis	6	2	17	6
Hyperkeratosis of the foot pad	0	0	13	6
Coughing	10	7	8	4
Diarrhea-Dehydration	4	9	3	2
Swelling of the lymph nodes	6	6	19	9

Table 5: Clinical signs in CDV positive dogs.

Clinical finding	<1 year-old		>1 year-old	
	Female	Male	Female	Male
Ocular discharge	4	1	6	1
Serous-mucopurulent nasal discharge	5	1	0	1
Nasal hyperkeratosis	0	0	2	0
Hyperkeratosis of the foot pad	0	0	5	0
Coughing	4	1	3	1
Diarrhea-Dehydration	1	0	2	0
Swelling of the lymph nodes	1	1	2	0

Analysis results indicated that CDV infection was not significantly associated with age or gender ($p>0.05$). Nonetheless, the clinical manifestations of ocular discharge ($p=0.006$) and coughing ($p=0.014$) were found to be significantly correlated with canine distemper. According to statistical analysis, there was no significant association between CDV infection and serous-mucopurulent nasal discharge, nasal hyperkeratosis, hyperkeratosis of the foot pad, diarrhea with dehydration, or lymphadenopathy.

DISCUSSION AND CONCLUSION

Distemper is a common disease all over the world, but its prevalence is low in some countries due to widespread vaccination and high utilization rates of veterinary services by dog owners. Although vaccination programs for canine companions in Turkey are routinely conducted by owners, free-ranging dogs are not vaccinated, rendering both vaccinated and unvaccinated dogs susceptible to diseases. In Pakistan, CDV was detected in 22.22% of 45 dogs exhibiting respiratory symptoms using RT-PCR analysis (Shabbir et al. 2010). 40.2% in 386 dogs with or without clinical signs suggestive of CDV infection by nested RT-PCR technique in seven states of Brazil (Budaszewski et al. 2014). In Poland, the virus was identified in 22% of 224 dogs with clinical signs using the direct immunofluorescence technique (Jóźwik and Frymus 2002). In Ahvaz, Iran, 17.52% of 97 clinically healthy dogs tested positive by indirect immunofluorescence (Avizeh et al. 2007). In Turkey (Ankara, Muğla, and Istanbul), 9.03% of 609 healthy dogs were seropositive as determined by the virus neutralization technique (Gencay et al. 2004). In Nepal, CDV was detected in 17% of 163 randomly selected dogs using commercial ELISA kits (Sadaula et al. 2022), while in Wenzhou, China, 28.5% of 2406 dogs tested positive with rapid diagnostic test kits (Luo et al. 2017). According to RT-PCR analysis of swab and blood samples taken from dogs with clinical signs, 15.1% (18/119) were found to be CDV-positive. The high prevalence of infection in dogs with clinical signs may be related to the fact that dogs are in close contact with each other in the shelter environment and crowded living conditions increase the development of clinical signs.

Due to the reduced maternal antibodies from their mothers, distemper is more prevalent in dogs aged 3-6 months. In contrast, in susceptible and isolated dog populations, the disease is severe and widespread, affecting dogs of all ages (Sykes and Vandevelde 2021). In this study, it was observed that 55.6% (10/18) of the CDV-positive dogs were under one year of age, whereas 44.4% (8/18) were over one year of age. Although the proportion of CDV-positive dogs was higher among those under one year, the disparity was not statistically significant ($p=0.303$). Similarly, Avizeh et al. (2007) conducted an examination of the incidence of CDV in 97 unvaccinated dogs older than 6 months and concluded that the disease was not influenced by age or gender. In another study, it was reported that in the age distribution of 56 dogs diagnosed with distemper, 40 were younger than 6 months, 10 were younger than 12 months and 6 were under 2 years of age (Ghoke and Thorat 2020). According to another research, it was reported that the age of CDV-positive dogs was between 2 months and 3 years and most of the dogs (68.88%) were younger than 18 months (Shabbir et al. 2010). In a different study, it was found that 40 of the 56 dogs with a distemper diagnosis were under 6 months old, 10 were under 12 months old, and 6 were under 2 years old (Ghoke and Thorat 2020). Another study found that the majority of CDV-positive dogs (68.88%) were under the age of 18 months, and that the age range of these dogs was between two months and three years (Shabbir et al. 2010). According to Jóźwik and Frymus (2002), 72% of CDV-positive dogs were younger than a year-old, and 28% of the dogs were between the ages of three and six months. According to Jóźwik and Frymus (2002), 72% of CDV-positive dogs were younger than a year-old, and 28% of the dogs were between the ages of three and six months. Adult dogs are more commonly

carriers of the disease, while young dogs often experience acute progression, leading to rapid death. In the anamnesis obtained from the shelter authorities, it was stated that death cases were seen in young dogs showing distemper symptoms. This may be considered as one of the possible reasons why there was no statistically significant difference between age groups in this study. It has been reported that gender is not important in the incidence of distemper (Costa et al. 2019). Headley and Graça (2000) found that there was no difference in susceptibility to CDV infection between males and females in their study of CDV-positive dogs. In another study of 62 dogs with distemper, it was reported that gender did not affect the prevalence of distemper (Gemma et al. 1996). In this study, 17.6% of female dogs (13/74) and 11.11% of male dogs (5/45) were CDV-positive. The present findings lend further support to the conclusions of earlier studies, which demonstrated that positivity for canine distemper virus is not contingent upon sex ($p>0.005$).

Distemper is a multisystemic disease that can affect the respiratory, gastrointestinal, genital, urogenital, and nervous systems may be observed. In this study, dogs with symptoms such as ocular discharge, serous- mucopurulent nasal discharge, nasal hyperkeratosis, hyperkeratosis on the foot pads, coughing, diarrhea, dehydration and swollen lymph nodes were included in the study. It was determined that a statistically significant percentage of 27.9% ($p=0.006$) of dogs with ocular discharge, and 31% ($p=0.014$) of dogs with cough were positive for CDV. Although other symptoms were positive for CDV, they were not statistically significant ($p>0.05$). In a study conducted on 56 dogs diagnosed with distemper, it was reported that 75% (42/56) of dogs showed gastrointestinal system symptoms with respiratory system symptoms, and 25% had cutaneous form symptoms with neurological complications (Ghoke and Thorat 2020). Elia et al. (2015) included two dogs with distemper in their study to examine the virologic and serologic findings of CDV. It was reported that one of the dogs displayed respiratory symptoms such as ocular and nasal discharge and cough, whereas the other dog displayed fever, lethargy, nasal discharge, conjunctivitis, and nasal hyperkeratosis. Değirmençay (2023) reported that the most common clinical findings in 19 of 24 dogs with distemper were nasal discharge. Other clinical findings included ocular discharge (16/24), high fever (14/24), cough, diarrhea, dehydration (12/24), skin problems (9/24), death (9/24), poor general condition (4/24), vomiting, and loss of appetite (2/24). In our study, it was observed that the most prevalent symptoms in CDV-positive dogs were ocular discharge, and cough (respiratory system symptoms), as per previous studies.

This study covered three months of period from May to July since the present findings reflect the spring-to-summer transition period of the distemper virus prevalence. Previous studies reported that the prevalence of distemper disease may be due to immunosuppression associated with weather or temperature changes (Luo et al. 2017). In addition, studies have reported higher seropositivity against CDV in summer compared to winter (Luo et al. 2017; Dorji et al. 2020), while another study reported a significant increase in the number of cases in winter and a corresponding decrease in the warmer seasons (Headley and Graça 2000). Therefore, seasonal factors appear to effect on the prevalence of the disease.

Measles virus (MeV) and canine distemper virus (CDV) are recognised as the most highly infectious agents in the Paramyxoviridae family (de Vries et al. 2014). The fact that

CDV is notable not only for its high transmission rate but also for its capacity to cross between different species is a serious concern for both global health and conservation authorities, as it may be zoonotic. CDV has a broad host range, causing infections in both domestic and wild carnivores, as well as in various other wildlife species. This poses a particular threat to endangered wildlife populations (Martínez-Gutiérrez and Ruiz-Saenz 2016). In a study conducted by de Vries et al. (2014), it was suggested that following the achievement of global measles eradication, the increasing number of non-immune individuals may render CDV—a virus closely related to measles—a potential risk for humans. The same study observed partial protection against CDV in measles-vaccinated macaques, as evidenced by accelerated viral control and limited shedding from the upper respiratory tract. This suggests that zoonotic morbillivirus infections could potentially be controlled through measles vaccination. Although there is currently no direct evidence of CDV causing infections in humans, an in vitro study reported that CDV was capable of infecting and replicating in human osteoclast precursors, providing further support for the possible role of paramyxoviruses in the pathogenesis of Paget's disease (Selby et al. 2006). Therefore, CDV disease may be a potentially risk for veterinary public health and further studies should be directed for the zoonotic potential of the morbillivirus infections.

The dogs with CDV infection should be separated from the healthy dogs due to CDV infection may be contaminated by aerosol transmission, particularly in shelter environment suggesting a separated ventilation system. Canine distemper virus is sensitive to lipid solvents such as ether and most disinfectants, including phenols and quaternary ammonium compounds; these substances should be a part of cleaning and disinfection protocols, especially in crowded shelter environments. The prevalence rate obtained in this study indicates that CDV infection is an important risk factor for the population of stray dogs and veterinarians should prioritize CDV in the differential diagnosis list. Distemper should be considered in dogs with symptoms of respiratory system diseases such as cough, ocular and nasal discharge, and transmission control protocols should be developed accordingly. In addition, since animals in the shelter are more exposed to the risk of infectious diseases, a vaccination protocol developed under the supervision of the shelter veterinarian should be prepared (Newbury et al. 2010). Therefore, vaccination seems to be one of the most important methods to improve the collective immune status or population immunity necessary to prevent or reduce the transmission.

This study has several limitations. The sampling was limited only to shelter dogs (main source is stray dogs) showing clinical symptoms of CDV while owned dogs or stray dogs were not included in the study. This situation limits the evaluation of the obtained distemper prevalence and identified risk factors of the dog population. In addition, due to the fact that the data collection process was in a certain time interval, the seasonal distribution of the disease has not been evaluated. Furthermore, more epidemiological studies are needed to conduct throughout the year contributing to evaluate the prevalence of the distemper disease in different regions. In addition, the sample size of the study may be relatively small according to G*power (3.1.9.4) analysis (Faul et al. 2009). However, from a public health and preventive veterinary medicine perspective, it should be considered that even such small

differences may have significant effects on the spread of the disease.

In conclusion, this study demonstrates the existence of the CDV infection with a prevalence rate of 15.1% in dogs presenting one of the clinical symptoms, such as cough, ocular secretion, nasal discharge, in the animal shelter in the province of Erzurum. Diagnosis of CDV infection is crucial not only for effective disease management but also for the implementation of preventive measures and vaccination. It can be suggested therefore herein that early determination of the disease allows veterinarians to initiate appropriate interventions to limit viral transmission and to prevent future outbreaks, particularly in high-risk, such as animal shelters.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Idea / Concept: NU, KZ

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Data Collection and / or Processing: NU, KZ

Analysis and / or Interpretation: HA, MÖT, HBY, NU

Writing the Article: NU, KZ

Critical Review: HA, MÖT, NU

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