

REVIEW ARTICLE

# Seroprevalence of Canine Parvovirus and Coronavirus Infection in Dogs with Enteritis in Van Province, Turkey

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## Abstract

In this study, CPV and CCoV agents were investigated in blood serum samples obtained from dogs exhibiting gastroenteritis symptoms such as vomiting, bloody diarrhea, dehydration, and fever in the Van region using the ELISA method. For this purpose, blood sera were obtained from 90 dogs aged 0-6 months showing gastroenteritis symptoms at the Van Municipality animal shelter and animal hospital using appropriate methods. As a result of examining the obtained blood sera using the ELISA method, CPV was detected in 4.44% (4/90) of the samples, and CCoV was detected in 7.78% (7/90). The rate at which both agents were detected together was determined to be 6.67% (6/90). This study revealed the presence of CPV and CCoV infections in dogs in the Van region, albeit at a low rate.

**Keywords:** Parvovirus, Enteritis, Coronavirus, Dog, ELISA.

## 1. Introduction

Enteritis is a frequently reported health problem in companion animals, particularly in dogs, and is characterized by rapid progression that may become fatal in young or untreated animals. This multi-etiological syndrome can arise from various infectious agents. Among these, viral etiologies are considered the most common pathogens in canines worldwide (1,2,3). Canine parvovirus (CPV) and canine coronavirus (CCoV) are the causative agents of most common viral pathogens that responsible for severe and life-threatening outbreaks in dogs (4). CPV belongs to Parvoviridae family and was first recognized in 1978 and is characterized by its highly infection rate of dividing cells of gastrointestinal tract and lymphoid tissue leading to symptoms such as lethargy, myocarditis, hemorrhagic gastroenteritis, fever, vomiting and often causes death if remained untreated (5,6). CPV infection is typically more severe in puppies and dogs under one year of age than in adults. In very young pups, CPV-2 may cause a cardiac form characterized by myocarditis,

which can result in sudden death. Early detection of infection, along with isolation of affected animals and prevention of secondary infections in kennels and shelters, is essential to limit viral transmission (6,14). CCoV is an enveloped RNA virus associated with enteric disease in dogs. Infection is generally confined to the gastrointestinal tract and is often mild or asymptomatic; however, clinical signs may be exacerbated in cases of co-infection with canine parvovirus or other enteric pathogens (4, 7, 8, 9). Clinical signs of CCoV is characterized with depression, bloody diarrhea and vomiting. Death can occur due to dehydration within 2 days if animal is not treated (7). Clinical signs and postmortem findings alone are insufficient for the definitive diagnosis of canine enteric viral infections, as canine coronavirus and canine parvovirus may produce identical disease courses (11, 12).

A range of serological and virological methods has been employed for the detection of canine coronavirus and canine parvovirus, including enzyme-linked immunosorbent assay (ELISA), serum neutralization,

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immunofluorescence, virus isolation, and molecular techniques such as polymerase chain reaction (PCR). Among these, ELISA is widely used due to their practicality, rapid turnaround, and reliability in detecting viral antigens or antibodies in clinical samples (6,10,13). In this study, a serological survey was conducted on dogs with enteritis in Van province using ELISA.

## 2. Materials and methods

### 2.1 Animals

The study was carried out on 90 animals who was presented to Animal Hospital Clinic and kept in breeding kennels in animal shelter in Van. Of these dogs, following the clinical examination 90 were diagnosed with gastroenteritis include vomiting, inappetence, lethargy and bloody or normal diarrhea. The ethical approval for the study was obtained from the Animal Research Ethics Committee of Van Yuzuncu Yil University on the use of animals (Approval date: 24th June 2021, Decision Number: 2021/06-41).

### 2.2 Obtaining Samples And Data Collection

Before obtaining samples, age, gender and breed data were recorded for each dog. Clinical examination forms were filled and signed by each animal owner. The dogs were properly handled for clinical examination. Blood samples were extracted from V. cephalica into anticoagulant-free tubes. The samples were centrifuged at 3000 rpm (Rotofix 32 ® Hettich,

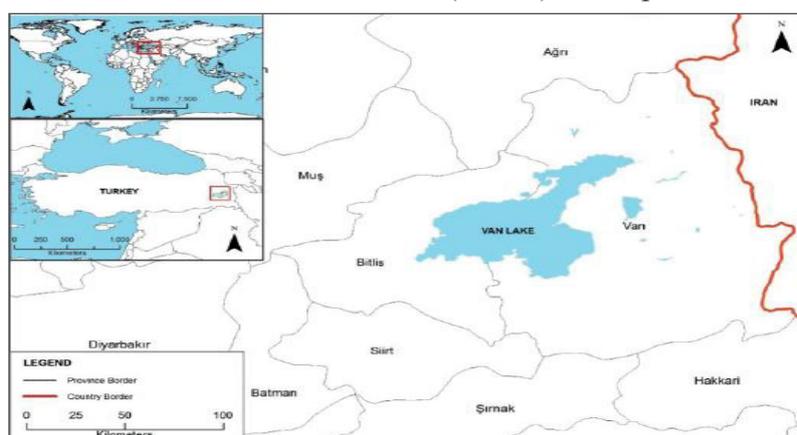
Germany) for 15 min and the sera were extracted. Obtained serum samples were moved to microtubes and kept in -20 °C until analyzed.

### 2.3 Serological Analysis- DAS Elisa

Antibodies to CCV and CPV were determined by enzyme-linked immunoassay using commercial kits and following the manufacturer's instructions (LOT: 20220110 SinoGeneClon Co., Ltd. China). Shortly, 10 µl of analytes were added to the wells and incubated for 30 minutes at 37° C then washed with wash solution. Following the wash procedure, 50 µl of HRP-conjugate were added each well and incubated for 30 minutes at 37° C. Unbound conjugate was separated by washing and 100 µl substrate solution were added to the each well then incubated at 25 °C for 15 minutes. After incubation, the enzymatic reaction was stopped by adding 50 µl of stop solution. The test was assessed using automatic ELISA reader (DASS®, Italy) at 450 nm filter. ELISA results were analyzed on the basis of optical densities values of samples using positive and negative control wells. A sample was scored positive; if the sample OD (optical density) ≥ Cut Off (Calculate Critical) and a sample was scored negative if the sample OD <Cut off.

## 3. Results

In this study, a total of 73 (81.11%) of the 90 dogs tested negative for both CPV and CCoV. Of the remaining 17 (18.89%) dogs, 4 (4.44%) tested positive for CPV, and 7 (7.78%) tested positive for CCoV, and 6 (6.67%) tested positive for both CPV and CCoV.



**Figure 1.** Geographical position of the Van province

## 4. Discussion

CCoV and CPV are the most important factors that play a role in infectious gastroenteritis in dogs worldwide. CPV occurs in dogs aged 0-6 months with clinical symptoms of severe hemorrhagic gastroenteritis, vomiting, diarrhea, loss of appetite and myocarditis. The disease is very contagious and

lethal, and it is seen in unvaccinated dogs mostly (15, 25). Meanwhile, CCoV is an infection that occurs in dogs along with enteritis, and it is characterized with symptoms of vomiting, diarrhea and lethargy appearing as mixed infections with CPV. Especially when CCoV is mixed with CPV agents, the clinical condition gets worse (11, 17, 18, 22). In the diagnosis

of these two diseases, there are more than one serological diagnostic methods other than clinical symptoms. For this purpose, methods such as PCR, ELISA, virus neutralization are utilized (11, 16). In the studies done, ELISA stands out as the most preferred method due to its reliability and high sensitivity (21, 20, 19, 18).

There are studies that examine the CCoV and CPV incidences in dogs that are characterized by enteritis symptoms both in the world and Türkiye. CPV was isolated in India (23) in 1982 for the first time, and CCoV was first isolated in Germany in 1971 (7). In a study (4) carried out at an animal shelter in Portugal, it was found that the presence rate of CPV and CCoV as mixed agents were 4.6% in fecal samples collected from 240 animals by using ELISA method, and CPV and CCoV seropositivity rates were 6.2% and 9.2% respectively. In a study by Sakulwira et al. (13) conducted in Thailand in 2003, the feces of 70 dogs were examined by using PCR and it was found that there was a positivity rate of 62.8% for CPV and 12.8% for CCoV. In a study conducted by Pratelli et al (24) in Italy in 2002 using ELISA method on 109 dogs, 19 samples tested positive for CCoV. In another study in which CPV was investigated by using PCR in 125 dogs in Brazil (26), a 40% rate of positivity was found. In a study by Naylor et al. (27), CCoV incidence was investigated by using ELISA method in 1396 dogs that show gastroenteritis symptoms in Australia, an 85% of positivity was detected. Takano et al. (22) found a 7.4% positivity rate in their study in which CCoV was investigated by using PCR in Japan.

In Türkiye, CPV was first detected in 1961 by Berkin et al. (28). Afterwards, Özkul et al. detected CPV agent for the first time in 2002 with PCR, which is a molecular method. Using the ELISA method in dogs showing gastroenteritis symptoms in Burdur region, Yıldırım (15) detected CPV agent in 4 of 117 (3.42%) animals. In another study by Yapıcı et al. (19), blood serum of 100 stray and unvaccinated dogs in Alanya region were analyzed by using the ELISA method, and CPV positivity was found in 17 (17%) of them. Gülbahçe et al. (18) examined the blood serum of 90 dogs that show gastroenteritis symptoms by using the ELISA method for CPV agents, and it was found that the serum antigen titers were above 700 in 7 samples, and were above 500 in 34 samples. In a study conducted by Dik and Şimşek (6) in 2021, CPV agent was investigated by using ELISA method, and positivity was found in 16 (16%) samples. In Türkiye, there are numerous studies regarding coronavirus

prevalence. In a study by Gür et al. (30) carried out in 2008, they investigated CCoV seroprevalence in 87 dogs, and found positivity in 84 (96.5%) samples. In another study by Yılmaz (21) done in 2017, CCoV presence was investigated using ELISA among 102 healthy Kars shepherd dogs. As a result of the study, positivity was found in 77 (75.49%) samples. In the study conducted by Avcı et al. (11), they found CCoV positivity in 46 dogs out of 188 (24.46%) that show gastroenteritis symptoms. The same researchers (11) found in 2015 that there was CCoV positivity in 91 dogs out of 121 (75.20%) that show gastroenteritis symptoms. Gür and Civelek (31), detected positivity in 57 dogs out of 59 (96.6%) that show gastroenteritis symptoms at a dog shelter in Bursa in 2007, after investigating CCoV infection using ELISA method.

Studies conducted by numerous researchers around the world and in Türkiye demonstrate that the incidence rates of CPV and CCoV infections differ both in Türkiye and in the world. A positivity rate from 6% to 85% is detected for CPV infection in studies done in various countries. Seroprevalence studies carried out in different countries for CCoV infection demonstrate rates ranging from 7% to 85%. In Türkiye, studies show results similar to the positivity rates found in other countries in terms of CPV and CCoV infections. In our study, CPV had a positivity rate of 4.4%, while CCoV had 6.67%. The findings of this study are in accordance with the other findings of researchers (4, 15, 22). However, our findings are found to be lower than the findings of other studies (11, 21, 26, 30, 31).

## 5. Conclusion

We believe that the reasons why our findings were low can be that the conditions of animal care and feeding is good, vaccinations are followed regularly, and adequate hygiene and disinfection are provided. As a result, no matter how low the level of the agent detected in this study, it should be taken into account that CPV and CCoV infections can be seen in dogs with gastroenteritis symptoms in Van province both as single and mixed infections. We believe that this study provides a good source for other studies that can be carried out in Van province.

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## Conflict of Interest

The authors have declared no conflict of interest.

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