



A Rapid Diagnosis Development of Canine Enteric Coronavirus Inflammation

Mohammed Ali Hussein ^{1,2*}, Mawlood Abbas Ali Al-Graibawi ¹

Abstract

Background: Canine Enteric Coronavirus (CECoV) is a significant viral pathogen causing gastrointestinal disturbances in dogs worldwide. However, data regarding CECoV in Iraq remains scarce. This study aimed to investigate CECoV IgG antibodies using ELISA and detect CECoV antigens in feces using lateral flow immunochromatography test. **Methods:** A total of 211 blood and fecal samples were collected from 161 dogs with diarrhea and 50 apparently healthy dogs (control group) in Baghdad, Iraq. Clinical signs were recorded, and samples were analyzed for CECoV IgG antibodies and antigens. Hematological parameters were also assessed. **Results and Discussion:** Clinical examination revealed gastrointestinal disturbances in 76.3% of dogs, with diverse clinical signs observed. ELISA results showed 21.8% seropositivity for CECoV IgG antibodies, with none detected in apparently healthy dogs. Lateral flow immunochromatography identified CECoV antigens in 7.58% of fecal swabs. Hematological changes included leukopenia, lymphopenia, and increased PCV and MCV values. These findings align with previous studies and suggest the ongoing prevalence and clinical significance of CECoV infection in Iraqi dogs. **Conclusion:** The combined use of ELISA and lateral flow

immunochromatography tests can facilitate the diagnosis of CECoV infection in canine practice. This study provides valuable insights into the epidemiology and clinical impact of CECoV in Iraq, contributing to the global understanding of this viral pathogen.

Keywords: Dog, Canine enteric coronavirus (CECoV), ELISA, immunochromatography, Zoonotic disease, Public health

Introduction

Coronaviruses are spherical, enveloped, single-stranded, positive-sense RNA viruses within the family Coronaviridae. They infect humans as well as many other mammalian and avian species, generally causing variably severe intestinal, respiratory, neurologic, or systemic disease syndromes (Giannitti et al., 2015; Gnirs et al., 2016). Gastroenteritis can occur for a variety of reasons such as dietary indiscretion, tumors, metabolic disorders, toxins (Owain & Yousif, 2018), and most importantly, infectious agents such as viruses (DiGangi et al., 2019), bacteria (Yousif et al., 2016; Yousif et al., 2017; Salih & Yousif, 2018a, 2018b; Enany et al., 2021), and parasites (Gerardi et al., 2018; Faraj et al., 2018; Badawi & Yousif, 2020).

Viruses were reported to be detected in up to 60% of diarrheic dogs (Gizzi et al., 2014; Mansour & Hasso, 2022). Canine enteric coronavirus (CECoV) is frequently reported as a cause of viral gastroenteritis in dogs (Dema et al., 2022). More virulent strains of CECoV have been reported, causing significant intestinal disease even in the absence of co-infection (Evermann et al., 2005), as well as fatal systemic disease, including lethargy, anorexia, vomiting, bloody diarrhea, ataxia, and seizures (Alfano et al., 2020).

Significance | This study elucidated the prevalence of CECoV in Baghdad, Iraq, highlighting its zoonotic potential and emphasizing the importance of surveillance and diagnostic tools.

*Correspondence. Mohammed Ali Hussein, University of Baghdad, College of Veterinary Medicine, Department of Internal and Preventive Medicine, Iraq.
E-mail: Mohammedalani@uofallujah.edu.iq

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Author Affiliation.

¹ University of Baghdad, College of Veterinary Medicine, Department of Internal and Preventive Medicine, Iraq.

² University of Fallujah, College of Veterinary Medicine, Department of Internal and Preventive Medicine, Iraq.

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Canine enteric coronavirus (CECoV) spreads from domestic dogs to wild carnivores (Molnar et al., 2014). It has been reported in foxes and raccoons in China (Ma et al., 2005) and in wolf populations in several European countries (Molnar et al., 2014; Rosa et al., 2020).

Dogs play an important role in people's lives, including hunting, protecting, and serving as pets in many countries (Warembourg et al., 2021). They are owned by urban and rural families, and many stray dogs (free-roaming habits) have frequent contact with wildlife, livestock animals, and the public (Awadallah & Salem, 2015). Dogs are also significant as reservoirs or definitive hosts for many zoonotic diseases, posing considerable public health and economic problems due to their transmission potential to humans, especially in developing countries where the management and movement of dogs are difficult to control (Monge-Maillo et al., 2015; Tadesse et al., 2020).

ELISA, antibodies, surgical diagnosis are widely used for serological profiling of CECov, with most established methods using structural proteins as the antigen because these proteins are highly conserved and immunogenic (Zhou et al., 2020, Pugazhandhi et al. 2022, Hana et al. 2024, Jayanthi et al. 2024, Moniruddin et al. 2023, Sasi et al. 2021, Zahra et al. 2022). The immunochromatography assay is the most common rapid field diagnostic method used in clinical practice. The specificity and sensitivity of Rapid CCV Ag Test kits were found to be 98.8% and 100%, respectively (Soma et al., 2001; Pratelli, 2002).

Numerous studies on coronaviruses have been conducted in Iraq, focusing on humans (AL-Mutar, 2020; Jassim, 2021; Mahmood et al., 2021; Ghazzi et al., 2023) and animals (Salah et al., 2020). However, prior to this study, no data were available concerning CECov in Iraq. This study investigates CECov IgG antibodies based on ELISA and the detection of CECov antigens in feces using the lateral flow immunochromatography test.

Materials and Methods

Sample Collection

A total of 211 blood samples, along with fecal samples, were collected from 161 dogs with diarrhea and from 50 apparently healthy dogs (control group). These samples were obtained from the Baghdad Veterinary Hospital and private veterinary clinics in Baghdad, Iraq, over a period of 16 months from February 2022 to June 2023. Dogs were examined clinically, and clinical signs were recorded.

Blood Sample Collection

Blood was collected aseptically via puncture of the cephalic vein (Yagi & Holowaychuk, 2016). The collected blood was divided into two tubes:

One tube with anticoagulant for complete blood count (CBC).

One tube without anticoagulant for serum isolation.

Transportation and Storage

The samples were transported to the Biotechnology and Environmental Center at the University of Fallujah in an ice box. The sera were stored at -20°C until use.

Serum Analysis

Serum samples were analyzed for IgG using commercial ELISA Kits (MyBioSource, USA and EIA-2482, DRG®, USA) according to the manufacturer's instructions. A serum sample was scored positive if the optical density (OD) was higher than 2.5 times the OD of the negative control.

Antigen Detection

Rectal swabs were used for antigen detection by lateral flow immunochromatography test, following the manufacturer's instructions (Biopanda Reagents Ltd, UK).

Hematological Analysis

Hematological changes were detected using a veterinary hematology analyzer device (Guilin Biotoo Medical Technology Co., Ltd, China).

Ethical Approval

The study was approved by the Committee of Ethics Research of the Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq (No. 441 on 21/2/2022).

Results and discussion

Canine viral gastroenteritis remains an ongoing problem in animal practice despite the availability of vaccines (Gizzi *et al.*, 2014; Pesavento and Murphy, 2014; Radford *et al.*, 2021). CECov considered a significant viral disease in the dog population due to its ability to evolve into variants with altered tissue tropism and pathogenicity (Parkhe and Verma, 2021).

The clinical examination of 211 dogs revealed that 161 (76.3%) with gastrointestinal disturbances manifested by diarrhea, hemorrhagic diarrhea, vomiting, inappetence, pallor mucus membrane, dehydration, lethargy, ocular discharge and fever, these diverse clinical signs depending on severity of infection for each case (table 1).

Clinical signs of dogs in this study coincide with clinical signs reported by other studies. Pratelli, (2006) reported that the CECov cause anorexia, vomiting, dehydration, soft to fluidly diarrhea, Decaro and Buonavoglia, (2008) mentioned that the CECov infection is generally restricted to the alimentary tract, leading to the onset of clinical signs of gastroenteritis including loss of appetite, vomiting, fluid diarrhea and dehydration. CECov cause mild-to-severe enteritis in dogs, fatalities can occur from co-infection with other pathogens specially canine parvovirus (Smith *et al.*, 2022). The severity of enteric disease in dogs increases when infected with pantropic strain of CECov, the clinical signs thus include gastrointestinal distress, hemorrhagic diarrhea, along with neurological signs (Decaro *et al.*, 2008; Decaro *et al.*, 2013).

Table 1. Clinical signs detected in examined dogs

Parameter	No.	Percentage
Diarrhea	161	100%
Hemorrhagic diarrhea	28	17.4%
Vomiting	141	87.6%
In appetite	161	100%
Pallor mucous membrane	161	100%
Dehydration	134	83.22%
Lethargy	122	75.8%
Ocular discharge	67	41.6%
Fever	18	11.2%

Table 2. number and percentage of sera positive for CECov IgG in ELISA test

Animals	No. of positive (%)	No. of Negative (%)
Diarrheic dogs	46 (21.8)	115 (54.5)*
Healthy dogs	0 (%)	50 (23.7)
Total 211 (100%)	46 (21.8)	165 (78.2)

* $p \leq 0.05$ $\chi^2_{25.06}$

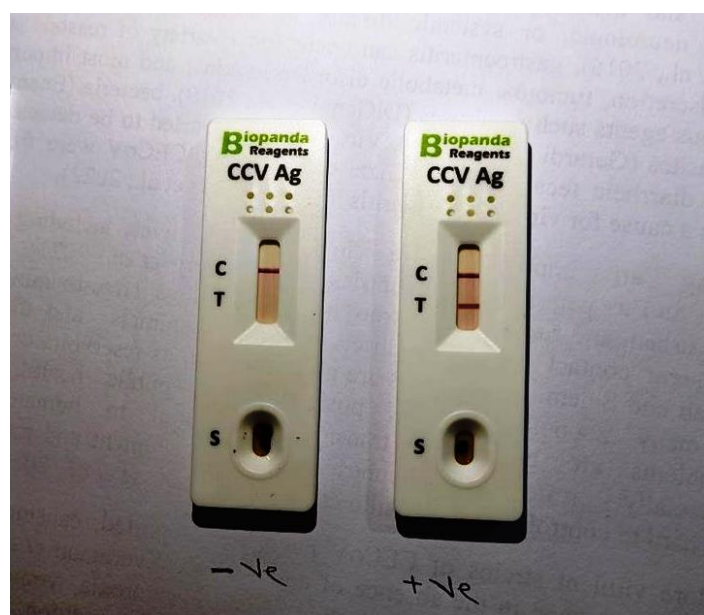
**Figure 1.** Negative and positive CECov by lateral flow immunochromatography

Table 3. Hematological profile of dogs in this study

Parameters	Apparently Healthy Dogs	Dogs with CECov (Mean ± SE)
WBC 10 ⁹ /L	18.9±0.8a	15.4±3.8b
LYM%	5.5±0.25a	1.6±1.7b
RBC 10 ⁶ /μL	7.1 ± 0.4a	5.4 ± 0.32b
MCH pg	22.4±0.9	19.54±4.2
MCHC g/L	324±4.7	293.6±18.6
RDW-CV %	12±0.43	10.4±2.4
PLT 10 ⁹ /L	428±3.2	398±26.5
HGB g/L	13.1±0.62a	8.24±1.39b
PCV	42± 1.63a	44.3±7.3b

Since a clinical diagnosis is not definitive, several laboratory methods have been developed to detect CECoV in the feces of infected dogs such as polymerase chain reaction (PCR), hemagglutinin (HA), ELISA, Immunofluorescence assay (IFA), Immunoperoxidase, though these tests are more sensitive, specific and more reproducible, but these tests can be expensive and generally take time to be analyzed by a specialized laboratory (Mosallanejad *et al.*, 2008). Since it is difficult to cultivate CECoV, the development of sensitive ELISA methods made it easy to investigate the epidemiological aspects of the disease by serological means (Naylor *et al.*, 2001; Pratelli *et al.*, 2002; Elia *et al.*, 2003; Yeşilbağ *et al.*, 2004), Results of ELISA in the present study revealed significant difference between diarrheic and healthy dogs 21.8% (46/211) were positive for CECoV IgG antibodies, all the apparently healthy dogs were seronegative (table 2).

Numerous studies reported the ELISA is a sensitive test for detecting the rising antibody titers against CECoV (Pratelli *et al.*, 2002 and 2004; Rowland *et al.*, 2021), the indirect ELISA test for anti-CECoV antibody was chosen in this study because it is quick, practical and specific, and is therefore widely used today for antibody screening (Yilmaz, 2017), the seroprevalence of CECoV antibodies in different dog populations throughout the world has been found to ranging from 15.8% to 95.1% (Naylor *et al.*, 2001; Pratelli *et al.*, 2002; Kaneshima *et al.*, 2006; Gur *et al.*, 2008; Decaro *et al.*, 2013; Avci *et al.*, 2015).

The CECoV IgG seropositivity results obtained in this study are similar to the results reported by previous studies, Tekelioğlu *et al.*, (2021) found the seropositivity 19.8% for CECoV in local dogs breed by ELISA test in Turkey, Avci *et al.*, (2016) record the first evidence for CECoV infection in dogs in two Turkish provinces by use indirect ELISA in various breeds and age groups dogs (24.46%). Rowland *et al.*, (2021) reported rising in antibodies from 0 to >1280 over a two week in two zoological collections in the United Kingdom between 2009 and 2017 in 19 bush dogs, in Australia the prevalence of CECoV antibody was 15.8% and 40.8% in the open and kenneled populations (Naylor *et al.*, 2001) respectively.

Whereas numerous studies recorded seropositivity higher than the results of the present study such as Pratelli *et al.*, (2002) who reported that the seropositivity of CECoV was 90.8% in Italy, Yesilbag *et al.* (2004) tested serum collected from 179 clinically healthy dogs from 7 Turkish provinces from private owners, municipality shelters, a breeding kennel and stray dogs using serum neutralization and ELISA methods and seropositivity was 62.5% and 74.3%, respectively. In a serological survey by Gur *et al.*, (2008) in apparently healthy purebred Akbaş, Turkish greyhounds and Kangal dogs using the ELISA technique, seropositivity was 94.7, 95.1 and 100%, respectively. Avci *et al.*, (2015) conducted a serological investigation of CECoV infection in dogs shelters in the province of Adana, including serum samples from 121 dogs, using

the ELISA, the seroprevalence was 75.2%. Takano *et al.*, (2016) reported that 77.7% of the dogs were seropositive for CECoV in a retrospective investigation during 1998 to 2006 in Japan. The differences in seropositivity rate were thought to be due to factors such as test methods, sampling animals and environment (Tekelioğlu, 2022).

The results of the lateral flow immuno-chromatographic assay showed that 7.58% (16/211) of fecal swabs were positive for CECoV antigen (figure 1).

Immunochromatography assay is the most common rapid field diagnostic method used in clinical practice, specificity and sensitivity of Rapid CCV Ag Test kits were found to be 98.8% and 100% respectively (Soma *et al.*, 2001; Pratelli, 2002; Awad *et al.*, 2020).

Yoon *et al.*, (2018) reported that CECoV rapid tests have high sensitivity and specificity and do not cross-react with other canine parvovirus and canine distemper virus antigen tests, the relative speed and simplicity of such test facilitate immediate treatment responses. The rapid tests are increasingly used among veterinarians in the diagnosis of the disease because of economic and rapid results in co-infections with more than one etiological agent in the fields (Tekelioğlu, 2022). It has been reported that rapid diagnosis of viral infection has a positive effect as it reduces the use of antibiotics and reduces the risk of antibiotic resistance and residue (Buller *et al.*, 2020).

Awad *et al.*, (2020) in Egypt found the infection rate with CECoV by using immunochromatographic test was 37% with no significant difference of sex and age of dogs. Sulehria *et al.*, (2020) found that 158 out of 450 (35.1%) fecal samples collected from symptomatic dogs originating from various pet-clinics and kennels in Pakistan were positive to CECoV by rapid test. In Iran Mosallanejad *et al.*, (2008) reported that the positive fecal swabs for CECoV was 3.45% German shepherd dogs aged less than 6 months. Avci *et al.*, (2015) conducted a virological investigation of CECoV infection in dogs shelters in the province of Adana, including fecal samples from 121 dogs, using the rapid test kit, the viroprevalence was 14.87%.

In the current study, hematological tests indicated significant decrease in total leukocyte count in infected dogs especially lymphopenia, increase in PCV and MCV values, with slight increase in platelet count compared with apparently healthy dogs (table 3).

Similar hematological profile tests were reported by other researchers, Tekelioğlu, (2022) mentioned that the dogs infected with CECoV had low hemoglobin, erythropenia, leukopenia, lymphopenia and thrombocytopenia in Turkey, Castro *et al.*, (2013) found hematological changes include lymphopenia, hypoproteinemia, hypoalbuminemia, mild anemia and trombocytopenia in CECoV infected dogs in Brazil, Sulehria *et al.*, (2020) in Pakistan recorded hematological changes such as erythropenia, thrombocytopenia, decreased hemoglobin and

albumin levels, another studies for canine coronavirus infection was associated with leukopenia, acute lymphopenia, and monocytosis in dogs experimentally infected with a pantropic CECoV (CB/05) strain, these data may suggest a different behavior of this agent in natural infection (Decaro *et al.*, 2008; Marinaro *et al.*, 2010).

Conclusion

In conclusion, the concurrent use of CECoV ELISA and lateral flow immunochromatography tests can effectively diagnose CECoV infection in dogs. The ELISA test demonstrated significant seropositivity among diarrheic dogs, while the lateral flow test confirmed CECoV antigen presence in fecal samples. These diagnostic methods are practical and reliable for use in clinical practice, facilitating timely and accurate detection of CECoV. The results underscore the importance of implementing these diagnostic tools to improve canine health management and control the spread of CECoV, especially in regions with high canine populations.

Author contributions

M.A.H. and M.A.A.G. developed the concept and the design of the study, analyzed the data, and wrote the draft of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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