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

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

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Editor Md Shamsuddin Sultan Khan, And accepted by the Editorial Board April 07, 2024 (received for review Jan 28, 2024)

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).

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Please cite this article.
Mohammed Ali Hussein, Mawlood Abbas Ali Al-Graibawi. (2024). A Rapid Diagnosis Development of Canine Enteric Coronavirus Inflammation, Journal of Angiotherapy, 8(4), 1-8, 9521

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 is 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). 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, inappetance, 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 appetence	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)

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

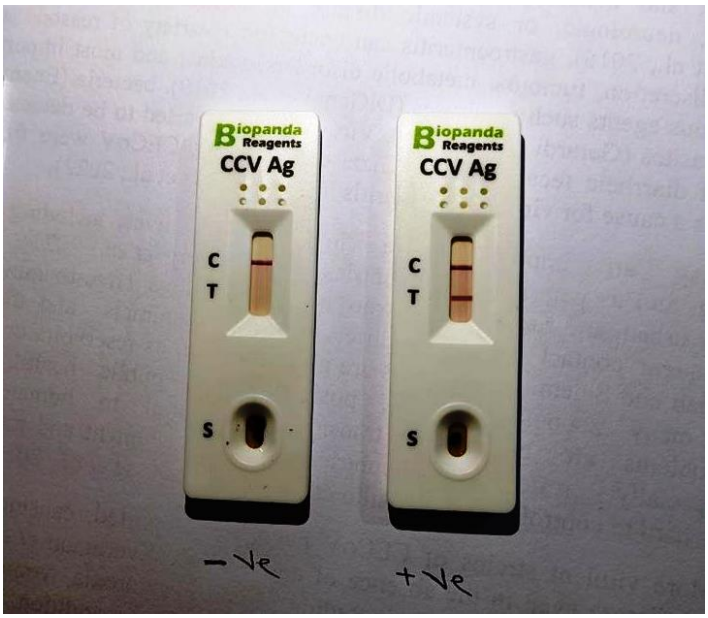

Figure 1. Negative and positive CECoV by lateral flow immunochromtagraphy

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 serprevalence 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, Tekelioglu *et al.,* (2021) found the seropositivity 19.8% for CECoV in local dogs breed by ELISA test in Turky, Avcı *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. Avcı *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, specifity 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 orginating 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. Avcı *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 lymphophenia, hypoproteinemia, hypoalbuminemia, mild anemia and trombocytophenia 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.

Acknowledgment

Authors were grateful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Alfano, F., Fusco, G., Mari, V., et al., 2020. Circulation of pantropic canine coronavirus in autochthonous and imported dogs, Italy. Transbound. Emerg. Dis. https://doi.org/ 10.1111/tbed.13542.

AL-Mutar, H. A. H. (2020). The evolution of Genetic Molecular Map and phylogenetic tree of Coronavirus (COVID-19). The Iraqi Journal of Veterinary Medicine, 44(2), 56–70.

Avcı O, Levent O, Yapici Orhan, Hasircioglu S, Simsek A. (2016). Canine coronavirus infection in dogs in Türkiye: Virological and serological evidence. Indian Journal of Animal Research.

Avci, O., Yavru, S., Kale, M. and Dik, I.: (2015) Determination of presence of canine coronavirus in shelter dogs. Eurasian. J. Vet. Sci. 31: 184-187.

Awad, R. A., S. Ali Hassan, A. G. Attallah & W. K. B. Khalil, 2020. Epidemiology and molecular diagnosis of canine coronavirus in Egypt: evaluation of different tests used for its diagnosis. Bulg. J. Vet. Med., 23, No 4, 467-477.

Awadallah MAI, Salem LMA.(2015) Zoonotic enteric parasites transmitted from dogs in Egypt with special concern to Toxocara canisinfection.Vet World., 8(8):946-957.

Badawi N.M, Yousif A.A.(2020) Babesia canis spp. in dogs in Baghdad Province, Iraq: First molecular identification and clinical and epidemiological study. Vet World. 13(3):579-585.

Buller H, Adam K, Bard A, Bruce A, (Ray) Chan KW, Hinchliffe S, Morgans L, Rees G, Reyher KK. (2020). Veterinary Diagnostic Practice and the Use of Rapid Tests in Antimicrobial Stewardship on UK Livestock Farms. Frontiers in Veterinary Science, 7: 765.

Castro TX, Cubel Garcia Rde C, Gonçalves LP, Costa EM, Marcello GC, Labarthe NV, Mendes-de-Almeida F.(2013) Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. Can Vet J. Sep;54(9):885-8.

Decaro N, Buonavoglia C. An update on canine coronaviruses: viral evolution and pathobiology. Vet Microbiol. (2008) 132:221– 34.

Decaro N, Campolo M, Lorusso A, Desario C, Mari V, Colaianni ML, Elia G, Martella V, Buonavoglia C.(2008). Experimental infection of dogs with a novel strain of canine coronavirus causing systemic disease and lymphopenia. Vet Microbiol. Apr 30;128(3-4):253-60.

Decaro N, Cordonnier N, Demeter Z (2013). European surveillance for pantropic canine coronavirus. J Clin Microbiol. 51:83–88.

Dema A., Tallapally M R., Ganji V K., Buddala B., Kodi H., Ramidi A., Yella N R. and Putty K. (2022). A Comprehensive Molecular Survey of Viral Pathogens Associated with Canine Gastroenteritis, 02, Preprint (Version 1) available at Research Square.

DiGangi BA, Dingman PA, Grijalva CJ (2019) Prevalence and risk factors for the presence of serum antibodies against canine distemper, canine parvovirus, and canine adenovirus in communities in mainland Ecuador. Vet Immunol Immunopathol.

Elia G, Fiermonte G, Pratelli A, Martella V, Camero M, Cirone F and Buonavoglia C. (2003). Recombinant M protein-based ELISA test for detection of antibodies to canine coronavirus. Journal of Virological Methods 109.139_/142

Enany M, Wahdan A, El-Metwaly M et al (2021) Bacterial Causes of Hemorrhagic Gastroenteritis in Dogs and Cats with Detection of Some Virulence and β-lactamase Resistance Genes in Escherichia coli and Salmonella by Multiplex PCR. Suez Canal Vet Med Journal SCVMJ 0:0–0.

Evermann, J.F., Abbott, J.R., Han, S (2005). Canine coronavirus-associated puppy mortality without evidence of concurrent canine parvovirus infection. J. Vet. Diagn. Invest. 17 (6), 610–614.

Faraj, A. A., Shaimaa, A. M., Shahla, R. F. (2018). Prevalence of Cryptosporidium spp. in feces of 160 domestic dogs in Baghdad, Iraq. Online Journal of Veterinary Research. Volume 22 (7):563-567.

Gerardi F, Santaniello A, Del Prete L et al (2018) Parasitic infections in dogs involved in animal-assisted interventions. Ital J Anim Sci 17:269–272.

Ghazzi, J. J., Fadhil , H. Y., & Aufi , I. M. (2023). Impact of SARS-COV-2 Variants on the Infection Severity among Iraqi Patients. Iraqi Journal of Veterinary Science, 64(7), 3263–3272.

Giannitti, F.; Diab, S.; Mete, A.; Stanton, J.B.; Fielding, L.; Crossley, B.; Sverlow, K.; Fish, S.; Mapes, S.; Scott, L.; et al. Necrotizing Enteritis and Hyperammonemic Encephalopathy Associated with Equine Coronavirus Infection in Equids. Vet. Pathol. 2015, 52, 1148–1156.

Gizzi ABDR, Oliveira ST, Leutenegger CM et al (2014) Presence of infectious agents and co-infections in diarrheic dogs determined with a real-time polymerase chain reaction-based panel. BMC Vet Res 10:23.

Gnirs, K.; Quinton, J.F.; Dally, C.; Nicolier, A.; Ruel, Y. Cerebral pyogranuloma associated with systemic coronavirus infection in a ferret. J. Small Anim. Pract. 2016, 57, 36–39.

Gur S, Gencay A Dogan N. (2008). A Serologic Investigation for Canine Corona Virus Infection in Individually Reared Dogs in Central Anatolia. Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 5(2): 67-71.

Hasan M. S., Yousif A.A. and Alwan M. J. (2016). Detection of Virulent Genes in E. coli O157:H7 Isolated from Puppies and Adult Dogs by Polymerase Chain Reaction. Research Journal for Veterinary Practitioners. 4 (1) 1-6.

Jassim, H. S. (2021). Efficacy of Immune System Challenges with Tiny Enemy COVID-19. The Iraqi Journal of Veterinary Medicine, 44(1), 75–79.

Kaneshima, T., Hohdatsu, T., Satoh, K., Takano, T., Motokawa, K. and Koyama, H. (2006). The prevalence of a group 2 coronavirus in dogs in Japan. J. Vet. Med. Sci. 68: 21-25..

Ma, G.G., Lu, C.P (2005). Two genotypes of Canine coronavirus simultaneously detected in the fecal samples of healthy foxes and raccoon dogs. Wei Sheng Wu Xue Bao 45 (2), 305–308.

Mahmood, Z. S., Fadhil, H. Y., and Ad'hiah, A. H (2021). Estimation of Hematological Parameters of Disease Severity in Iraqi Patients with COVID-19. Iraqi Journal of Science, 62(10), 3487–3496.

Mansour, K. A., Hasso, S. A. (2022). Molecular Detection of Canine Distemper Virus in Dogs in Baghdad Province, Iraq. The Iraqi Journal of Veterinary Medicine, 45(2), 46–50.

Marinaro M, Mari V, Bellacicco AL, Tarsitano E, Elia G, Losurdo M, Rezza G, Buonavoglia C, Decaro N (2010). Prolonged depletion of circulating CD4+ T lymphocytes and acute monocytosis after pantropic canine coronavirus infection in dogs. Virus Res. 152(1-2):73-8.

Molnar, B., Duchamp, C., M¨ostl, K., Diehl, P.A., Betschart, B., 2014. Comparative survey of canine parvovirus, canine distemper virus and canine enteric coronavirus infection in free-ranging wolves of central Italy and south-eastern France. Eur. J. Wildl. Res 60 (4), 613–624.

Monge-Maillo B, López-Vélez R, NormanFF, Ferrere-González F, Martínez-Pérez Á, Pérez-Molina JA. Screening of imported infectious diseases among asymptomatic Sub-Saharan African and Latin American immigrants: a public health challenge. Am J Trop Med Hyg. 2015; 92 (4):848-856.

Mosallanejad B, Ghorbanpoor N M, Avizeh R, Nikoosiar J M. 2008. Antigenic detection of Canine Coronavirus in diarrheic dogs in Ahvaz. Int.J.Vet.Res. 2,1:81-85.

Naylor, M.J., Monckton, R.P., Lehrbach, P.R., Deane, E.M., 2001b. Canine coronavirus in Australian dogs. Aust. Vet. J. 79, 116–119.

Owain M S., and Yousif A. A (2018). Effect of lead with or without thiamine and calcium EDTA on hematology in young dogs. Online Journal of Veterinary Research. Volume 22 (6):434-443.

Parkhe P and Verma S (2021) Evolution, Interspecies Transmission, and Zoonotic Significance of Animal Coronaviruses. Front. Vet. Sci. 8:719834.

Pesavento PA, Murphy BG (2014) Common and emerging infectious diseases in the animal shelter. Vet Pathol 51:478–491.

Pratelli A, Tinelli A, Decaro N, Martella V, Camero M, Tempesta M, Martini M, Carmichael LE, Buonavoglia C. Safety and efficacy of a modified-live canine coronavirus vaccine in dogs. Vet Microbiol. 2004 Mar 26;99(1):43-9.

Pratelli, A., 2006. Genetic evolution of canine coronavirus and recent advances in prophylaxis. The Veterinary Research 37, 191–200.

Pratelli, A., Elia, G., Martella, V., Palmieri, A., Cirone, F., Tinelli, A., Corrente, M. and Buonavoglia, C. 2002. Prevalence of canine coronavirus antibodies by an enzyme-linked immunosorbent assay in dogs in the south of Italy. J. Virol. Methods 102: 67–71.

Radford AD, Singleton DA, Jewell C et al (2021) Outbreak of severe vomiting in dogs associated with a canine enteric coronavirus, United Kingdom. Emerg Infect Dis 27:517.

Rosa, G.M., Santos, N., Grøndahl-Rosado, R., et al., 2020. Unveiling patterns of viral pathogen infection in free-ranging carnivores of northern Portugal using a complementary methodological approach. Comp. Immunol. Microbiol Infect. Dis. 69, 101432.

Rowland H, Holding E, Falces PM, Wissink-Argilaga N, Stidworthy MF, Denk D, Weir W, Krumrie S, Dunbar D, Hopper JS. 2021. Canine coronavirus subtype 2a associated with outbreaks of fatal diarrhoea in bush dog (Speothos venaticus) groups, Fatale Diarrhoe bei Waldhunden (Speothos venaticus) durch Canines Coronavirus Subtyp 2a. Schweizer Archiv fur Tierheilkunde, 164(10): 661–671.

Salah, H. A.; Fadhil, H. Y.; Alhamdani, F. G. (2020) "multiplex sybr green assay for coronavirus detection using fast real-time RT-PCR", iraqi journal of agricultural sciences, 51(2), pp. 556–564.

Salih W. and Yousif A.A. (2018b). Prevalence of salmonellosis in domestic dogs. Online Journal of Veterinary Research. 22 (9):739-743.

Salih W. and Yousif A.A.(2018a). Molecular detection of Salmonella typhimurium isolated from canine feces by PCR. Advances in Animal and Veterinary Science. 6 (12) 542- 547.

Smith C S, Lenz M F, Caldwell K, Oakey J. 2022. Identification of a canine coronavirus in Australian racing Greyhounds. Journal of Veterinary Diagnostic Investigation 2022, Vol. 34(1) 77– 81.

Soma, T., Hara, M., Ishii, H., Yamamoto, S. (2001) Antibody testing against Canine Coronavirus by immunoperoxidase plaque staining. Vet. Res. Commun. 25: 327-336.

Sulehria, M U; Ahmad, S S; Ijaz, M; Mushtaq, M H; Khan, A Y; Ghaffar, A. (2020). Molecular evidence and hematological alterations associated with the occurrence of coronavirus in domestic dogs in Pakistan. Tropical Biomedicine 37(4): 963–972.

Tadesse M, Ayana D, Kumsa B, Fromsam F. Zoonotic helminth parasites of dog in Bishoftu Town, central Ethiopia: prevalence, dog owners' knowledge and control practice. J Ethiop Vet. 2020; 24(1): 93-115

Takano, T., Yamashita S., Murata-Ohkubo M., Satoh K., Doki T., and Hohdatsu T. Prevalence of canine coronavirus (CCoV) in dogs in Japan: detection of CCoV RNA and retrospective serological analysis. J. Vet. Med. Sci., 78(2): 341–345 (2016).

Tekelioglu BK, Yüceer HB, Akın B, Koç Ö, Çelik M, Kandır S, Baykal Çelik L, Gökçe M. 2021. Osmaniye İlinde Sahipsiz Köpeklerin Rehabilitasyonu ve Viral

Enfeksiyon Profilaksisi. Avrupa Bilim ve Teknoloji Dergisi, Ejosat, Özel Sayı 32: 956-966.

Tekelioğlu, B., K. (2022). Seroepidemiological and Clinicopathological Investigation of Canine Coronavirus Infection in Dogs, in Türkiye. Turkish Journal of Agriculture - Food Science and Technology, 10(10): 1884-1892.

Warembourg C, Wera E, Odoch T, Bulu PM, Berger-González M, Alvarez D, et al. Comparative study of free-roaming domestic dog management and roaming behavior acrossfour countries: Chad, Guatemala, Indonesia, and Uganda. Front Vet Sci. 2021; 8:A617900.

Yagi K., Holowaychuk M. K. (2016). Manual of Veterinary Transfusion Medicine and Blood Banking (First Edition). WILEY Blackwell. USA; pp; 199.

Yesilbag K, Yilmaz Z, Torun S, Pratelli A. 2004. Canine coronavirus infection in Turkish dog population. Journal of Veterinary Medicine, Infectious Diseases and Veterinary Public Health, 51(7): 353–355.

Yeşilbağ, K., Yilmaz, Z., Torun, S., Pratelli, A., 2004. Canine coronavirus infection in Turkish dog population. J. Vet. Med. B Infect. Dis Vet. Public Health 51, 353–355.

Yilmaz, V. (2017). Seroprevalence of Canine Coronavirus in Kars Shepherd Dogs in Kars Province, Turkey. Palestine Journal of Veterinary Medicine. Vol. 72 (1)

Yoon SJ, Seo KW, Song KH. 2018. Clinical evaluation of a rapid diagnostic test kit for detection of canine coronavirus, Korean Journal of Veterinary Research, 58: 27–31.

Yousif A. A., Hasan M S. and Alwan M J. (2016). Clinical and molecular study of E. coli O157:H7 isolated from Diarrheic and non-diarrheic dogs. MRVSA 5 (2), 1-10

Yousif A.A., Hasan M S., Najim T M. and Hussein M A. (2017). Detection of virulence and antimicrobial susceptibility testing of Escherichia coli O157:H7 isolated from pets and stray dogs. Journal of Entomology and Zoology Studies 5(4): 573-578

Zhou YH, Yin JB, Liu L, Yin XP, Li YM, Zhang ZD (2020). Prokaryotic expression of canine coronavirus N protein and preparation of its polyclonal antibodies. Chinese Animal Husbandry & Veterinary Medicine.