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Seroepidemiological and Clinicopathological Investigation of Canine Coronavirus Infection in Dogs, in Türkiye

Bilge Kaan Tekelioğlu[1,a,*]

[1]*Faculty of Ceyhan Veterinary Medicine, Çukurova University, 01930 Ceyhan, Adana, Türkiye*
[*]*Corresponding author*

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ABSTRACT

Domestic and wild dogs of all ages and breeds are susceptible to Canine Coronavirus (CCoV) infections and be seen in Türkiye and amongst world. CCoV has recently been declared a zoonotic disease agent and the eighth pathogenic human coronavirus. This study was conducted on 143 naturally infected dogs with gastroenteritis which were not vaccinated against CCoV in Türkiye in 2015-2020. The data of dogs were analyzed seroepidemiologically, clinicopathologically and statistically. CCOV antibodies in serum and CCOV antigens in stool were detected by ELISA and lateral immunochromatography. The rising CCoV IgG antibody titers were detected at all dogs and were as follows; <10 ng/L in 3 (2%), 10-20 ng/L in 18 (13%), 20-30 ng/L in 16 (11%), 30- 40 ng/L in 14 (%10), 40-64 ng/L in 11 (8%) and >64 ng/L in 81 (81%) dogs. CCOV and Canine Parvovirus (CPV) antigen were detected together in the stool of the 41 (28.7%) dogs. As a result, it was concluded that the CCOV agent is in circulation among dogs living in Türkiye. CCOV and CPV can cause co-infections and increased mortality. Although infection can be seen in dogs of all ages, it can be seen more frequently in dogs younger than 1 year of age, and especially in dogs younger than 6 months, and can cause enteritis, low hemoglobin, erythropenia, lymphopenia, leukopenia, thrombocytopenia, and hypoproteinemia.

[a] *ktekelioglu@cu.edu.tr* *https://orcid.org/0000-0001-6727-3175*

Introduction

Domestic and wild canines of all ages and breeds are susceptible to CCoV infection (Chitwood et al., 2015; Haake et al., 2020; Rawland et al., 2021; Tekelioglu et al., 2021; Watts et al., 2016). The disease is seen in Türkiye and across the world (Aktutay et al., 2020; Gur et al., 2008; Haake et al., 2020; Tekelioglu et al., 2021 Yesilbag et al., 2004). International Committee on the Taxonomy of Viruses (ICTV) listed the coronaviruses in the family of *Coronaviridae* from the *Nidovirales* order. CoVs are a large family of enveloped, single-stranded, positive-sense RNA viruses and are classified into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gamacoronavirus*, and *Deltacoronavirus*. Canine Coronaviruses (CCoVs) include 3 subtypes of viruses as Canine Coronavirus type 1 (CCoV-1), Canine Coronavirus type 2 (CCoV-2) and Canine Respiratory Coronavirus (CRCoV) (Decaro et al. 2007; Erles and Brownlie, 2008; Kanchima et al. 2006). CCoV-1 and CCoV-2 are listed in the Alphacoronavirus genus, and CRCoV in the Betacoranvirus genus by ICTV taxonomy (Decaro and Buonavoglia, 2008; El-Wahed and Truyen 2021; Haake et al., 2020; Woo et al., 2010; ICTV, 2020; Ün 2020; Zang et al., 2020). CoVs have an unusually large genome of ~30 kb compared to other known viruses. The surfaces of viruses are equipped with pointed protrusions called 'Spike' (S). Bats, birds, and rodents in the wild are known reservoirs of CoVs, and due to their genetic variation suitable for mutation and recombination, they have the ability to cause infections of varying severity with increased virulence, multiple tissue and organ tropism, and an expanding host range, with the emergence of new viral strains (Hasöksüz et al., 2020; Scepanski et al. 2019; Tekelioglu et.al., 2015, 2020; 2021; Ün 2020). CCoV as an emerging infection is more closely related to feline coronavirus (FCoV) and transmissible gastroenteritis virus (TGEV) of pigs and ferrets (Licitra et al., 2014). The CCoV virus interacts with the APN receptor of the host cell to bind and enters into the host cell (Fehr and Perlman, 2015).

CCoV have a high incidence in the canine population, usually causing self-limiting infections accompanied by mild enteritis with a high morbidity and low mortality

course. Co-infections with CPV, Canine Distemper Virus (CDV) and Canine Adenovirus (CAV) are encountered and increase the severity and mortality (Decaro and Buonavoglia 2011; El Wahed and Truyen 2021; Tekelioglu et al., 2021; Zapulli et al., 2020). CCoV-IIa is a pantropic virus and leads systemic infections, and it has been determined that it can located in other tissues and organs, including the lungs and brain, outside the intestines (Alfano et al., 2020; Day et al., 2020; Timurkan et al. 2021). It has also been reported that a type of CCoV-IIa can produce a generalized form of SARS-CoV-like diseases (Decaro and Buonavoglia 2011; Pratelli et al., 2022; Priestnall 2020). SARS CoV-2 exposure to dogs was reported (Dilepan et al., 2021). Among the diagnostic methods, electron microscopy, virus isolation, serum neutralization test, ELISA, lateral immunochromatography, conventional, nested and real time (RT) reverse transcription PCR methods are used (Aktutay-Yoldar et al., 2020; Decaro et al., 2007; Gan et al., 2021; Perera et al., 2021; Tekelioglu et al., 2021; Wang et al., 2017).

Severe Acute Respiratory Syndrome-causing coronaviruses SARS-CoV-1 and SARS-CoV-2 were originated from an animal reservoir and cross the species barrier, confirming the potential threat of animal coronaviruses to the human population (Hasoksuz 2020, Zang et al., 2020; Zapulli et al., 2020). This has led to a surge of interest in coronavirus research in all species. The World Health Organization (WHO) announced in the pre-COVID-19 years that it expects pandemics and a large number of affected, sick and dying people worldwide due to virus-related pandemics (Tekelioglu 2016). It has been determined that new canine-feline recombinant alphacoronaviruses of CCoV origin isolated and identified from human pneumonia infections in recent years presented high genetic similarity with each other (HuCCoV_Z19Haiti and CCoV-HuPn-2018). The findings indicate the possibility of transmission from dogs to humans and have been described as capable of causing human upper respiratory tract infections (Lednicky et al., 2021; Vlasova et al., 2021). To date, seven different pathogenic corona viruses have been identified, four of which are endemic to humans and cause diseases similar to colds and flu. This novel canine-feline recombinant alfacoronavirus has been reported as the eight pathogenic human coronaviruses to cause human disease (Vlasova et al., 2021).

The aim of this study is to better understanding the seroepidemiology and clinicopathology of CCoV infections among dogs, which gained importance after clearly demonstrated that SARS-CoV-2 infects dogs as reverse zoonosis and CCoV is declared zoonotic and as eight pathogenic coronaviruses of human.

Material and Methods

Study Area and Sampling

Samplings were done from naturally infected dogs between 2015-2020 years. In total of 143 ill dogs, not vaccinated against CCoV were consulted by the Faculty of Veterinary Medicine (n=41), virology department and local veterinarians (n=102) in Adana, Istanbul, Osmaniye and Rize provinces in Türkiye. The dogs had signs of gastroenteritis and age, breed, gender, clinical and laboratory findings were recorded. Blood samples were collected from jugular vein of the animals into the K_2EDTA anticoagulant 5 ml sample tubes for hematology and gelatinous serum sampling tubes for biochemical analyses. Samples were kept in a cold chain; hematology were done immediately in the same day. Serological samples were coagulated at the room temperature for 15 minutes, then centrifuged at 2500 rpm for 20 minute to obtain the serum, and stored at -20°C until examined as the producer of the ELISA kit recommended. Stool samples were collected in disposable sample collection tubes supplied with the kit and kept in a cold chain until examined and immediately tested in the same day.

Investigation of Anti-CCoV IgG

A canine species-specific ELISA kits is used to assay the Canine Coronavirus IgG in the sample of canine's serum, blood plasma, and other related tissue liquid obtained from a commercial producer (SunRed Biotechnology Company, China). The ELISA kit is based on the principle of double-antibody sandwich technique to detect Canine (Coronaviruses IgG) for research purposes. The assay sensitivity and range are 0.465 ng/L and 0.5 ng/L – 100 ng/L respectively. The test was performed in accordance with the user's manual as recommended by the manufacturer. Blank wells without any sample and CCoV IgG antibody were used as negative control. 50 µl of Streptavidin-HRP without CCoV IgG antibody was added to the standard wells. 40 µl samples were added to the test wells, and then both CCoV IgG -antibody 10µl and Streptavidin-HRP 50µl were added. After incubation at 37°C for 60 minutes, washing was done with the wash concentrate and again after 10 minutes of incubation at 37°C away from light, the reaction was stopped and it was observed that the blue color changed to yellow immediately. Final measurement: blank well calculated as zero, the optical density (OD) was measured under 450 nm wavelength within 15min after adding the stop solution by an ELISA reader device (BioTek ELX800, USA). According to standards' concentration and the corresponding OD values, the standard curve linear regression equation was calculated, and then OD values of the sample on the regression equation to calculate the corresponding sample's concentration were applied.

Measurements of ELISA Results

CurveExpert Professional (ver.2.6.5) was used to make calculations and quantitative measurements by comparing the optical densities of the samples with the 2-fold dilutions of the standard solution provided by the ELISA test kit.

Validity

Optical densities of the samples were calculated, the amount of substance corresponding to the optical density of each sample on the curve was calculated, and optical density measurements were confirmed by retrospective control.

Investigation of CCOV Ag and CPV Ag

Stool samples collected by rectal swabs from sick dogs were analyzed by a commercial lateral immunochromatography test (Fassisi ParCo, Fassisi, Germany). The tests are manufactured to simultaneously detect Canine Coronavirus (CCoV Ag) and Canine

Parvovirus antigens (CPV Ag). The sensitivity and specificity of CCoV Ag has 99.99% and 97.50% while the CPV Ag has 93.33% sensitivity and 99.99% specificity respectively. Collected samples were examined in accordance with the manufacturer's instructions.

Hematological Analyses

Hematology was performed to calculate Leukocyte (WBC), lymphocyte (LYM), erythrocyte (RBC), hemoglobin (HGB), and platelets (HCT) using veterinary specific auto analyzer devices (Mindray-Vet, China) and its kits on the same day immediately.

Biochemical Analyses

Biochemistry was performed to calculate serum albumin (ALB), globulin (GLB), total protein (TP) and ALB/GLB ratio using veterinary specific auto analyzer device (VetScan, Abaxis, USA, FUJICHEM, Japan) and its kits on the same day immediately.

Data Statistical Analyses

Data were analyzed by statistical analyze software program (IBM SPSS Statistics 2020). Clinicopathological data were evaluated by estimates of combined categories and sub-groups of the variables predicted to be effective. Group statistics and independent samples tests were done for examining the differences in the mean values of the dependent variable associated with the effect of the controlled independent variables, after taking into account the influence of the uncontrolled independent variables. For clinical, hematological, and biochemical findings of infected dogs, odds ratios and grand mean, standard deviations and p value ($P>0.05$) were calculated. Approximately unbiased estimates of prevalence were calculated by assuming known values for the Se and Sp tests using Levene's test for equality of variances and Student's t test for equality of means (Greiner and Gardner 2000).

Table 1. Seroepidemiological data of breed, gender, age and CCoV-CPV co-infection status.

Variable	Category	Frequency	Percentage	CCoV-CPV Co-infection	Percentage
Breed	Akita	1	0.7	-	-
	Anatolian Shepherd	4	2.8	4	9.8
	Bolognese	1	0.7	-	-
	Border Collie	2	1.4	-	-
	Boxer	1	0.7	-	-
	Bulldog	5	5.4	-	-
	Çatalburun	1	0.7	-	-
	Chiwawa	2	1.4	2	4.9
	Chow Chow	1	0.7	-	-
	Cocker Spaniel	5	4	2	4.9
	Doberman	2	1.4	-	--
	Golden Retriever	20	14	8	19.5
	German Shepherd	9	6	2	4.9
	Husky	2	1.4	-	-
	Jack Russell	2	1.4	-	-
	Keeshond	1	0.7	-	-
	King Charles Cavalier	7	5	2	4.9
	Labrador Retriever	10	6.9	1	2.4
	Mix Breed	38	26.6	15	36.6
	Pekingese	4	2.8	1	2.4
	Pincher	1	0.7	1	2.4
	Pit bull	1	0.7	1	2.4
	Pointer	2	1.4	-	-
	Poodle	1	0.7	-	-
	Pomerania	1	0.7	-	-
	Rottweiler	4	2.8	1	2.4
	Samoyed	1	0.7	-	-
	Setter	1	0.7	-	-
	Shih Tzu	1	0.7	-	-
	Spitz	2	1.4	1	2.4
	Terrier	10	6.9	-	-
Gender	Female	64	45	19	46.3
	Male	79	55	22	53.7
Age*	≤ 0.6	56	39.2	13	31.7
	≤1	83	58	22	54
	1-5	30	21	13	32
	6-10	16	11	5	12
	≥ 10	14	10	1	2
N		143	100	41	100

*Age category of sampled animals: ≤ 1 = Up to 1 year old. 1-5 = between 1 to 5 years old. 6-10 = between 6 to 10 years old. ≥ 10 = elder than 10 years old.

Table 2. Clinical signs, biochemical and hematological results of study population (n=143).

Variables	H. Enteritis	Fever	Low Hgb	Low Rbc	Low Wbc	High Wbc	Low Lym
n	76	21	47	45	22	19	26
%	53	122	33	31	15	13	18
Variables	High Lym	Low Plt	Low Tp	Low Alb	Low Glb	Low Plt	
n	21	50	53	122	22	53	
%	15	35	37	85	15	37	

Table 3. Descriptive statistics of hematological and biochemical results of CCoV infected dogs.

Means	Wbc	Lym	Rbc	Hgb	Alb	Glb	Alb/glb	Tp	Plt
Mean	11.19	3.99	6.12	13.69	2.15	3.81	0.62	5.96	249.47
Stdev	6.01	3.43	1.78	4.37	0.65	1.06	0.36	1.22	93.85
±2Stdev	23.20	10.84	9.68	22.42	3.45	5.92	1.33	8.40	437.17
±2Stdev	(0.83)	(2.87)	2.56	4.96	0.85	1.70	(0.09)	3.52	61.77

Reference Ranges: WBC: 6.0–17.0 X 10^3/µL, LYM: 2.3–6.2 X 10^3/µL, RBC: 4.4–8.5 X 10^6/µL, HGB: 12-18 g/dl, ALB: 2.6-4.0 g/dl, GLB: 2.1-3.7 g/dl, ALB/GLB: 0.6-2.0 g/dl, TP: 5.5-7.5 g/dl, PLT: 200-900 X 10^3/µL

Table 4. Descriptive statistics of vital status, gender, hemorrhagic enteritis and fever of CCoV infected dogs.

Living Status	Wbc*	Lym	Hgb*	Rbc*	Alb	Glb*	Alb/Glb	Tp*	Plt*
Alive	12.49	4.17	14.51	6.42	2.18	3.94	0.60	6.12	258.49
Dead*	5.29	3.14	9.97	4.74	2.00	3.20	0.69	5.20	208.31
p value	<0.0001	0.166	<0.0001	<0.0001	0.199	<0.006	>0.272	<0.0001	<0.014
Grand Mean	11.19	3.99	13.69	6.12	2.15	3.81	0.62	5.96	249.47
Gender Values	WBC	LYM	HGB	RBC	ALB	GLB	ALB/GLB	TP	PLT
FM	10.82	3.81	13.58	6.00	2.15	3.74	0.61	5.88	243.28
M	11.48	4.13	13.79	6.22	2.15	3.87	0.63	6.02	254.48
p value	0.516	0.587	0.776	0.470	0.961	0.441	0.768	0.488	0.480
Grand Mean	11.19	3.99	13.69	6.12	2.15	3.81	0.62	5.96	249.47
H.Enteritis Values	WBC	LYM	HGB*	RBC*	ALB*	GLB	ALB/GLB*	TP*	PLT
NO	10.57	4.45	14.59	6.47	2.43	3.92	0.69	6.34	236.58
YES	11.73	3.58	12.90	5.81	1.90	3.72	0.56	5.62	260.83
p value	0.254	0.130	0,020	0.027	<0.0001	0.260	0.021	<0.0001	0.124
Grand Mean	11.19	3.99	13.69	6.12	2.15	3.81	0.62	5.96	249.47
Fever	WBC*	LYM*	HGB	RBC	ALB	GLB	ALB/GLB	TP	PLT*
NO	10.12	3.99	13.69	6.16	2.15	3.84	0.61	5.99	243.84
YES	20.30	3.98	13.69	5.79	2.15	3.57	0.69	5.71	297.53
p value	<0.0001	0.017	0.502	0.994	0.824	0.300	0.586	0.310	0.026
Grand Mean	11.19	3.99	13.69	6.12	2.15	3.81	0.62	5.96	249.47

*P<0.05 = Significance level (SL).

Table 5. Descriptive statistics of CCoV and CPV co-infection (n=41) by Fischer Exact and Chi-Square Tests according to the age, breed, gender, vital status, hemorrhagic enteritis and fever.

Variables	N=143	Count	Expected count	%Within CPVAg	p value
<1	83	23	23.8	56.1%	
Age					0.852
>1	60	18	17.2	43.9%	
Pure Breed	105	26	30.1	63.4%	
Breed					0.097
Mix Breed	38	15	10.9	36.6%	
Female	64	19	18.3	46.3%	
Gender					0.854
Male	79	22	22.7	53.7%	
YES	26	22	7.5	53.7%	
Dead*					<0.0001
NO	117	19	33.5	46.3%	
YES	76	20	21.8	48.8%	
H.Enteritis					0.580
NO	67	21	19.2	51.2%	
YES	21	4	6	9.8%	
Fever					0.434
NO	122	37	35	90.2%	

*P<0.05 = Significance level (SL).

Table 6. Descriptive statistics of CCoV and CPV co-infection (n=41) according to the biochemical and hematological results.

			95% CI		
Variables	N	Mean ± SD	Upper	Lower	p value
WBC* YES NO	41 102	5.9±2.3	9	5	<0.0001
LYM* YES NO	41 102	2.7±1.9	29	5	0.006
RBC* YES NO	41 102	5.3±1.9	31	11	<0.0001
HGB* YES NO	41 102	11.9±4.2	39	8	0.003
ALB YES NO	41 102	2.2±0.5	31	15	0.510
GLOB YES NO	41 102	3.69±1.0	22	6	0.394
Alb/Glob YES NO	41 102	0.6±0.19	15	11	0.726
TP YES NO	41 102	5.9±1.3	0.66	0.15	0.699
PLT* YES NO	41 102	203±63	97	31	<0.0001

*P<0.05 = Significance level (SL).

The chi-square test or, where appropriate, the appropriate Fisher's exact test (Kirwood and Sterne 2003) were used to compare the distributions of CCoV and CPV seropositive dogs according to explanatory variables which were associated with CCoV infected dogs at P<0.05 in univariate analysis; these were CPV serological status, age, vital status (alive/dead), diarrhea, fever, biochemical and hematological data. Biochemical and hematological results were categorized as being within (normal), above (high) or below (low) reference values (Villiers and Blackwood 2005).

Results

Descriptive statistics of breed, gender, age and CCoV-CPV co-infection are presented in Table 1 and clinical signs and laboratory findings are presented in Table 2. Statistical analyzes of hematological and biochemical results and comparative descriptive analyzes according to vital status (dead or alive), gender, enteritis and fever are presented in Table 3 and Table 4, respectively.

Seroepidemiology data of 143 dogs indicated that 79 (55%) were male and 64 (45%) were female, 38 (26.6%) were mixed and 105 (73.4%) were pure breed, 83 (58%) were 1 year old and younger, and 56 (39%) were 6 months old or younger and dogs of 31 breeds were affected by the disease (Table 1).

Hemorrhagic enteritis in 76 (53%) dogs was the most prominent clinical findings. Hematological and biochemical tests indicated, 47 (33%) of the dogs had low hemoglobin, 45 (31%) had erythropenia, 22 (15%), had leukopenia, 19 (13%) had leukocytosis, 26 (18%) had lymphopenia, 21 (15%) had lymphocytosis, 53 (37%) had hypoproteinemia, 122 (85%) had hypoalbuminemia, 22 (15%) had hypoglobulinemia and 53 (37%) had thrombocytopenia.

Significance levels were observed for WBC, HGB, RBC, GLB, TP and PLT values in dead dogs, HGB, RBC, ALB, ALB/GLB and TP values in dogs with hemorrhagic enteritis, and WBC, LYM and PLT values in dogs with high fever (P<0.05) (Table 4). No significance was observed (P>0.05) in the gender group.

CCoV Ag was detected in 143 dogs and CCoV and CPV Ag was detected together in 41 (28.7%) dogs from stool samples. Of these dogs, 26 died and CCoV and CPV co-infection were detected in 22, while CCoV was detected to be the sole agent in four dogs. Significance levels were observed in mortality and of WBC, LYM, RBC, HGB and PLT levels of the dogs with CCV and CPV co-infection. No significance (P>0.05) was observed in age, breed, gender, hemorrhagic enteritis and fever groups (Table 5-6).

ELISA results of CCoV IgG titers were as follows; <10 ng/L in 3 (2%), 10-20 ng/L in 18 (13%), 20-30 ng/L in 16 (11%), 30- 40 ng/L in 14 (%10), 40-64 ng/L in 11 (8%) and >64 ng/L in 81 (81%) dogs, CCoV IgG antibody was detected. ELISA results calculation diagram and positive case distribution and percentages are presented in Figure 1 and Figure 2, respectively.

Discussion

Here the results of a study on the seroepidemiology and clinicopathology of canine coronavirus infection in dogs in Türkiye are reported. CCoV is one of a major pathogen of dogs, mostly causes mild and self-limited, high morbidity infections generally with gastroenteritis and low mortality. Coronaviruses have caused deadly diseases in humans in the last two decades and as a result, interest in coronavirus studies has increased greatly in recent years and continues to grow. Current scientific studies on coronaviruses were mainly focused on animal coronaviruses, diagnostic methods and antibody-virus interaction areas, as well as emerging diseases and novel anti-viral agents and treatment methods (Pourhatami et al. 2021). Coronavirus infection is common among dogs and its presence has been reported in previous studies by different researchers, both worldwide and in Türkiye (Aktutay et al. 2020; Gur et al. 2008; Haake et al. 2020; Pratelli et al. 2002; Tekelioglu et al. 2021; Willie at al., 2020; van Nguyen et al. 2017; Yesilbag et al. 2004).

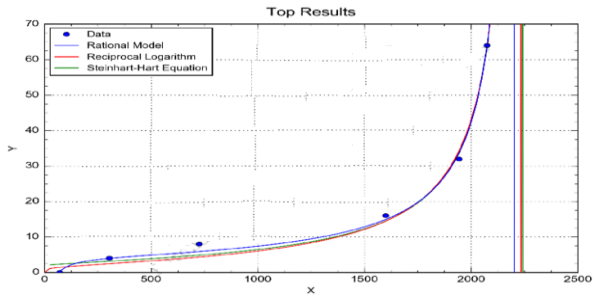

Figure 1. Calculations and result plot of quantitative measurements by comparing the optical densities of the samples with 2-fold dilutions of the standard solution.

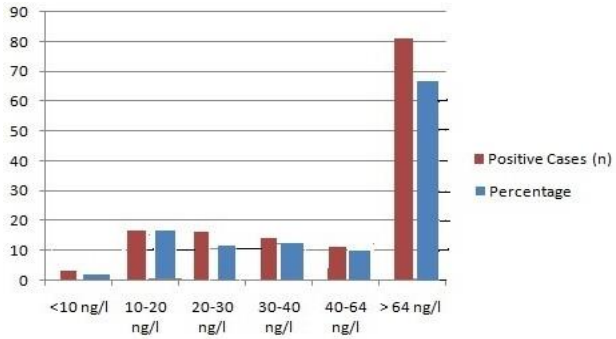

Figure 2. ELISA results diagram of positive cases and percentages.

In the current study, CCoV IgG antibody was detected by ELISA and the results are listed as follows; <10 ng/L at 3 (2%), 10-20 ng/L at 18 (13%), 20-30 ng/L at 16 (11%), 30-40 ng/L at 14 (10%) 40-64 ng/L in 11 (8%) dogs and >64 ng/L in 81 (81%) dogs. Pratelli et al. (2002 and 2004) and Rawland et al. (2021) reported the ELISA is a sensitive test method for detecting the rising antibody titers against CCoV and is significantly more sensitive than the virus neutralization test and stated that it can be used as an alternative technique to the virus neutralization test. In different studies Avcı et al. 24.4% (2015) and 14.87% (2016), Gür et al. (2008) 96.5% and Tekelioglu et al. (2021) 19.8% were reported seropositivity of CCoV in dogs in Türkiye. Furthermore, in molecular and bioinformatics Yesilbag et al. (2004) reported 15.5% CCoV positivity by RT-PCR and Aktutay et al. (2020) reported CCoV strains from different genogroups are circulating in Türkiye. Pratelli et al. (2002) in Italy, van-Nguyen et al. (2017) in Vietnam and He et al. ((2020) in China (P.R.C.) reported 73.4%. 43.3% and 23.94% serpositivities, respectively. Infection is also encountered in wild life and 28% seropositivity in wolves was reported (Watts and Benson. 2016). Rawland et al. (2020) reported increased CCoV IgG titers of zero to >1280 ng/L with both molecular evidence and seropositivity in bush dogs. Serological results determined that the CCoV IgG antibody levels in the dogs examined in the study ranged from 9.36 ng/L to >64 ng/L (Figure 2). The CCoV IgG seropositivity results obtained in this study are similar to the results of other studies as mentioned above. The differences were thought to be due to factors such as test methods, sampling animals and environment.

The CCoV antigen was detected serologically from stool by lateral immunochromatography method from naturally infected sick dogs. Yoon et al. (2018) reported that CCoV tests have high sensitivity and specificity and do not cross-react with other CPV and CDV antigen tests. The relative speed and simplicity of such tests facilitate immediate treatment responses. These 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 of use. It has been reported that rapid viral infection diagnosis has a positive effect as it reduces the use of antibiotics and reduces the risk of antibiotic resistance and residue (Bullet et al. 2020). Similar to previous studies, detection of 9.39 ng/L to >64 ng/L CCoV IgG antibodies concurrently with CCoV antigen in dogs concluded that both test methods could be used in practice in canine health (Figure 2).

The significance was not observed in CCoV infection in dogs according to the gender and breed, but to the age (Table 1). Of the dogs 82 (52%) were 1 year old or younger and 56 (39%) were 6 months old and younger. Similar results, indicating that there is no relationship between breed and gender, and that young dogs are more sensitive than older dogs, were previously reported by Gür et al. (2008) and Yeşilbağ et al. (2004) in Türkiye, Jeoung et al. (2010) in Korea, Pratelli et al. (2002) in Italy and Takano et al. (2016) in Japan. On the contrary, Takano et al. (2016) declared that there is no relationship between age and infection. The difference was thought to be due to variables such as the sample size or the severity of the infection and its status in the population, and the habitus of the dogs.

The 41 (%28.7) dogs were presented co-infection with CCoV and CPV. Of the dogs 26 were died and 22 (53.7%) of these were co-infected. The four of the dogs were infected solely with CCoV. Mortality was observed to be higher in co-infected dogs. Jeoung et al. (2008) in Korea, Tekelioglu et al. (2021) in Türkiye, were reported co-infections with CCoV and CPV amongst dog population previously (Decaro and Buonavoglia 2011; El Wahed and Truyen 2021; Haake et al., 2020; Jeoung et al., 2008 and 2010; Tekelioglu et al., 2021; Zapulli et al., 2020).

There are few studies were observed in the literature containing detailed biochemical and hematological data associated with CCoV-infected dogs, until this date. In the current study, hematological and biochemical tests indicated, 47 (33%) of the dogs had low hemoglobin, 45 (31%) had erythropenia, 22 (15%), had leukopenia, 19 (13%) had leukocytosis, 26 (18%) had lymphopenia, 21 (15%) had lymphocytosis, 53 (37%) had hypoproteinemia, 122 (85%) had hypoalbuminemia, 22 (15%) had hypoglobulinemia and 53 (37%) had thrombocytopenia. Castro et al. (2013) reported similarly dehydration, lymphophenia, hypoproteinemia, hypoalbuminemia, mild anemia and trombocytophenia in CCoV infected dogs in Brazil. Sulehria et al. (2020) reported similarly erythropenia, thrombocytopenia, decreased hemoglobin and albumin levels. This is probably due to the degeneration and destruction of the mature enterocytes as a result of atrophy and/or cellular degeneration and/or necrosis of enterocytes resulting from infection and replication of CCoVs in the apical and lateral enterocytes of intestinal villi. All this pathogenesis causes villous atrophy and consequent indigestion, malabsorption and diarrhea (Licitra et al., 2014).

The CCoV vaccine is on the 'Vaccinations Not Recommended List' and not listed as a Core-Vaccine in the

WASAVA (2016) and KHVHD (2018) Vaccination Guidelines in contrast the CPV vaccine is in core vaccine list and routinely used in canine health. In general, the opinion is that the CCoV vaccines protect dogs from disease, but not from infection. It is accepted that protection against CCoV depends on the presence of local immunity and IgA in the intestine. Since dogs vaccinated parenterally do not produce an IgA response, they spread the virus in the stool (Jeoung et al., 2010; Pratelli et al., 2004, Tekelioglu et al., 2021; Tizard, 2021). For these reasons, their use is not common among dogs in Türkiye and worldwide.

It has been clearly demonstrated that a new canine-feline recombinant alphacoronavirus of CCoV origin, isolated and identified from pneumonia infections observed in humans in recent years, presenting high genetic similarity with each other (HuCCoV_Z19Haiti and CCoV-HuPn-2018), which can be transmitted from dogs to humans and can also cause human upper respiratory tract infections (Lednicky et al., 2021; Tortorici et al., 2021, Vlasova et al., 2021). To date, seven different pathogenic coronaviruses have been identified, four of which are endemic to humans and cause disease. This new zoonotic canine-feline recombinant alphacoronavirus has been recognized as the eighth pathogenic human coronavirus reported to cause human disease (Vlasova et al., 2021). It is concluded that studies on CCoV will attract attention and be a priority in the near future. Therefore, it is thought that the data of the present study may help to better understand the seroepidemiology and clinicopathology of the disease.

Conclusions

CCOV is circulating among dogs living in Türkiye. CCOV and CPV can cause co-infections. Although infection can be seen in dogs of all ages, it is more common in dogs younger than 1 year of age, and especially in dogs younger than 6 months, and causes diarrhea, hemaglitopenia, erythropenia, lymphopenia, leukopenia, thrombocytopenia, and hypoproteinemia.

Acknowledgements / Descriptions

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