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Molecular evidence and hematological alterations associated with the occurrence of coronavirus in domestic dogs in Pakistan

Sulehria, M.U.[1], Ahmad, S.S[1*], Ijaz, M.[1#], Mushtaq, M.H.[2], Khan, A.Y.[1] and Ghaffar, A.[1]
[1]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore, 54600, Pakistan
[2]Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, 54600, Pakistan
[*#]Corresponding author e-mails: vetsalim@uvas.edu.pk (S.S. Ahmad); mijaz@uvas.edu.pk (M. Ijaz)
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Abstract. Canine Enteric Coronavirus (CCoV) is one of the major enteric pathogen affecting dogs. This study aims to investigate the molecular prevalence, phylogenetic analysis, associated risk factors, and haemato-biochemical alterations in Canine Coronavirus in dogs in district Lahore, Pakistan. 450 fecal samples were collected from symptomatic dogs originating from various pet-clinics and kennels during 2018-2019. Samples were initially analyzed by sandwich lateral flow immunochromatographic assay and then further processed by RT-PCR (reverse transcriptase polymerase chain reaction) targeting the M gene followed by sequencing. RT-PCR based positive (n=20) and negative (n=20) dogs were samples for their blood for the haemato-biochemical analysis. A questionnaire was used to collect data from pet owners, in order to analyze the data for risk factors analysis by chi square test on SPSS. The prevalence of CCoV was 35.1%, and 23.8 % through Sandwich lateral flow immunochromatographic and RT-PCR respectively. Various risk factors like breed, age, sex, vomiting, diarrhea, sample source, body size, cohabitation with other animals, living environment, food, deworming history, contact with other animals or birds feces, and season were significantly associated with CCoV. The CCoV identified in Pakistan were 98% similar with the isolates from China (KT 192675, 1), South Korea (HM 130573, 1), Brazil (GU 300134, 1), Colombia (MH 717721, 1), United Kingdom (JX 082356, 1) and Tunisia (KX156806). Haemato-biochemical alterations in CCoV affected dogs revealed anaemia, leucopenia, lymphopenia, neutrophilia, and decreased packed cell volume, and a significant increase in alkaline phosphate and alanine transaminase. It is concluded that infection with canine coronavirus appears widespread among dog populations in district Lahore, Pakistan. This study is the first report regarding the molecular detection and sequence analysis of CCoV in Pakistan.

INTRODUCTION

Canine Enteric Coronavirus (CCoV) is one of the major etiological agents of gastro-enteritis in Dogs (Duijvestijn *et al*., 2016). It was first regarded as a pathogen of dogs in 1971 by Binn and coworkers from dogs with the signs of diarrhea (Bandai *et al*., 1999). Coronaviruses are enveloped, single stranded, positive-sense RNA viruses with a genome size of approximately 30 kb (De Groot *et al*., 2011). It belongs to the genus Alpha coronavirus and has a close genomic relation with Feline coronavirus (FCoV) and transmissible gastroenteritis virus of swine (TGEV) (Adam & Carsten, 2012). CCoV were distinguished into serotypes I and II based on the differences in their protein spike. Genetically, these serotypes looks similar but protein structure distinguishes them (Pratelli *et al*., 2003; Licetra *et al*., 2014). CCoV-2 has been further classified into two subtypes, CCoV-2a and CCoV-2b (Soma *et al*., 2010). Like other RNA viruses, CCoV can mutate, resulting in more virulent strains. These mutated strains can increase the severity

of enteric illness (Escutenaire *et al*., 2007). Coronavirus invades and destroys mature cells on the intestinal villi, resulting in a reduction of absorptive surface area and malabsorption; which can ultimately leads towards gastroenteritis (Decaro *et al*., 2008). CCoV is a highly contagious and often fatal disease, characterize by fever (39.5°– 40°C), lethargy, anorexia, vomiting, hemorrhagic diarrhea and neurologic signs (ataxia, seizures) (Buonavoglia *et al*., 2006). Mostly the affected dogs recover in 7 to 10 days and mortality rate is very low but CCoV infection is very common in young dogs, especially which are kept in large groups, like in breeding facilities, shelters and kennels (Stavisky *et al*., 2012). If untreated, affected animals may die due to dehydration or electrolyte abnormalities. Additionally, co-infection with adenovirus, parvovirus or distemper virus can increase the fatality rate (Licitra *et al*., 2014).

Enzyme-linked immunosorbent assay, virus neutralization assay and western blotting can be used for the diagnose CCoV (Pratelli *et al*., 2002). CCoV is quite unstable in the environment so virus isolation is only succeeded, if samples contain high viral titers and are stored or transported in a cold chain. RT-PCR assays are documented as the best assay to detect CCoV infection even on a small amount of RNA availability (Bandai *et al*., 1999).

This is the first study for the molecular characterization of CCoV in domestic dogs in Pakistan. The study also focused the association of various risk factors accompanying the occurrence of CCoV and the effects on various hemato-biochemical parameters.

MATERIALS AND METHODS

Ethical consent

This study was duly approved by the advanced studies and research board, University of Veterinary and Animal Sciences, Lahore, Pakistan. Additionally, the author affirms the implementation of this work followed all technical, administrative, and scientific rules for animal research.

Study design and Sampling procedure

The current study was conducted in district Lahore from January 2018 to January 2019. A total of 450 dogs were included in this study irrespective of breeds, sex and age; which were sampled from private veterinary clinics and Dog kennels. The sampling inclusion criteria involved dogs presented with history of bloody diarrhea followed by progressive dehydration and anemia, pyrexia, restlessness and gastrointestinal dysfunction. Rectal swabs were obtained and stored at -40°C until further processing. A questionnaire was completed by each pet owner to obtain the information regarding the population characteristics in order to analyze these factors with the occurrence of infection.

Sandwich lateral flow immunochromatographic assay

All the fecal samples were initially processed by a rapid test kit (Quicking Biotech, China Cat No.W81066) with CPV+CCOV combined antigen. The kit was used according to the manufacturer instructions. Samples were thawed and further, they were diluted by using assay diluents until they were fully dissolved. For settlement of larger particles, the samples were incubated at room temperature for 1 min. Four drops of the supernatant from the diluent sample were added to the sample hole for each test. After 10 min, the test results were noted and the samples were declared positive when the test (T) line and control (C) line were present within the result window. The samples were considered negative when only the control (C) line appeared in the result window and is invalid if the control (C) line did not appear.

PCR Amplification

Samples were also subjected to RT-PCR assay to compare the efficacy of these two tests for the detection of Coronavirus Infection in Dogs.

The total RNA fast extraction stool kit (Bioteke Corporation China, Cat # RP8001) was used to extract viral RNA in stool samples according to the manufacturer instructions. The kit has previously proven to be highly efficient for the extraction of

RNA from fecal samples (Muhammed *et al*., 2016). Afterward the RNA extracts were further processed for cDNA synthesis by commercially available kits (GeneDirex, Las Vegas, Nevada, USA). All the extracted cDNA samples were processed by NanoDrop 2000 spectrophotometer in order to confirm the purity and concentration. To confirm the presence of CCoV, partial fragment of M Gene was amplified. The 2X PCR Taq Master Mix kit (BioShop, Canada) was used with the primers F-GTTATACAGAAGGACTAAGTCT and reverse R-GTTGAGTAATCACCAGC TTTAG which amplified a 321 bp fragment. For amplification, all the reactions were carried out in a volume of 25µl in 0.2 ml PCR tubes. RT-PCR was performed for amplification of the partial M gene as per Agnihotri *et al.* (2017) guideline for the confirmation of canine coronavirus infection in dogs presented with the history and signs of gastroenteritis. The reaction mixture contains PCR master mix (15 µl), forward primer (FP) (1.0 µl), reverse primer (RP) (1.0 µl), nuclease-free distilled water (6 µl) and template cDNA (2.0 µl). The PCR cyclic conditions were, initial denaturation 95°C for 5 min, denaturation 95°C for 30 sec, annealing 50°C for 30 sec, extension 72°C 1 min for 40 cycles, final extension was for 72°C for 15 min. Nucleus free water was used as negative controls. After amplification of the partial M Gene fragment, the corresponding electrophoresis was performed to visualize the expected band size. To this end, 1.5 % agarose gels were prepared in TAE (Tris, acetic acid, and EDTA) buffer. For each sample, 8 µl of the final solution was used with a molecular weight marker (100 bp Ladder Bioshop, Canada) and run at 100V for 40 mins. The gel was stained with ethidium bromide and view by trans-illumination. The bands at 311bp on 1.5% agarose gel were obtained by using the cutter and were sent for gel extraction followed by Gene Sequencing to Macrogen-USA.

The RT-PCR products for the partial fragment of M-gene sequence of CCoV were subjected to sequencing. Blast queries of the resulted sequenced nucleotide indicated the sequence identity with the M-gene of Canine coronavirus in Dogs. For the comparative purpose, the nucleotide sequences of the M-gene from the NCBI database were aligned. The phylogenetic analysis was performed using Mega 7 by maximum likelihood algorithm with bootstrapping at 1000 replications.

Haemato-biochemical analysis

5 ml blood was collected from the cephalic/ saphenous vein of dogs aseptically for Haemato-biochemical analysis. 2 ml of blood in EDTA was used for hematological examination by using the VET hematology analyzer (Model No. DW-3680/DW-36), while 3ml blood was used for obtaining serum which was stored at -20°C till further analysis. The serum samples were analyzed for estimation of biochemical parameters using a Semi-automated clinical chemistry analyzer machine (Model URIT-810).

Statistical Analysis

Chi-square test was applied to various hypothesized risk factors, Odds ratio was determined to know the degree of association of risk factors, p-value less than 0.05 was considered significant (Ghaffar *et al*., 2020). Data regarding hemato-biochemical parameters was analyzed by independent sample t-test. Analysis was conducted on SPSS version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0.Armonk, NY: IBM Corp).

RESULTS

Epidemiology of Canine coronavirus in dogs

The Sandwich lateral flow immunochromatographic assay of 450 animals revealed 158 (35.1%) positive for coronavirus infection in Dogs, while RT-PCR has detected the 107 samples (23.8%).

A significant (p<0.05) association was recorded for prevalence of coronavirus among the breeds in dogs. The odd ratio suggested the highest potential risk factor for the disease dynamics from (OR=4.413; CI=1.451-13.422). Cross-breed dogs followed by Poddle and Labrador (OR=3.586;

CI=1.249-10.291), Rottweiler (OR=2.995 CI=1.089- 8.232), and Pitbull (OR=2.551; CI=0.959-6.785) respectively. While Pug dogs (OR=0.482; CI=0.207-1.124) have the lowest potential risk factors for the disease dynamic. Age at a different level of animals showed a significant (P=.007) association with prevalence of coronavirus in dogs, whereas the dogs with 1-3 years of age odd ratio 2.281 (CI=1.315-3.957) showed the highest potential risk factor. While the dogs >3 years of age OR=1.499; CI=0.898-2.501) were less susceptible as compared to the former one.

Sex was significantly associated (p=.006) with the prevalence of coronavirus in dogs while the (OR=0.536; CI=0.344-0.836) suggested that sex was not a potential risk factor for the prevalence of coronavirus in dogs. Vomiting condition was significantly associated (p=.000) with a prevalence of coronavirus in dogs and the odds ratio (OR= 4.329; CI= 2.700-6.943) also suggested that this condition is a high potential risk factor for the existence of the disease. The diarrhea status with blood or without blood showed a non-significant association (p=.184) but on the other hand, Odd ratio suggested that (OR=1.434; CI; 0.840-2.449) diarrhea status with blood or without blood is a potential risk factor for the prevalence of coronavirus in dogs population. Sampling source was also significantly associated (p=.011) with disease prevalence and the odd ration (OR=1.770; CI=1.137-2.754) also suggested it a potential risk factor for disease prevalence. The results declared that there was a significant association (p=.008) between the size of the dogs and prevalence of the disease, as the results of (OR=2.395; CI=1.373-4.178) also declared that dogs with large size were prone to coronavirus infection. As per the results of P-value (P= .005) and Odd ratio (OR=3.433; CI=1.388-8.488) it was concluded that Co-habitation with other animals is also one of the major factors for the disease dynamics of coronavirus infection in dogs. A non-significant (p=.08) association was observed between the Living environment of the animal and prevalence of Coronavirus infection in dogs while the odd ratio (OR=1.774; CI=0.944-3.333) results declared that this factor is a potential risk factor for the prevalence of coronavirus in dogs.

The nature of the food is significantly associated (p=.000) with the prevalence of coronavirus infection in dogs but odd ratio results (OR=0.233, 0.390; CI=0.127-0.428, 0.208-0.730) didn't consider it as a potential threat for the prevalence of coronavirus infection in dogs. Deworming is significantly associated (p=.000) with the prevalence of coronavirus infection in dogs however the odds ratio 0.700, 0.230 (CI=0.373-1.314, 0.130-0.409) suggested that there is no co-relation between deworming history and dynamic of the disease. A significant (p=0.000) association was found between contact of Dog with other animals or birds feces but odd ratio 0.968, 0.303, 0.493, 0.078 (CI= 0.260-3.600, 0.116-0.791, 0.089-2.714, 0.032-0.189) is not agreed to consider it a potential risk factor for disease dynamics. The study showed a significant (p=.000) association between season and prevalence of coronavirus infection in dogs while odd ratio 4.821, 3.373, 1.974 (CI=2.452-9.477, 1.811-6.281, 1.119-3.482) also suggested that season has a greater effect on the prevalence of coronavirus infection in dogs (Table 1).

Phylogenetic Analysis

The current study samples clustered with the isolates from China (KT 192675, 1), South Korea (HM 130573, 1), Brazil (GU 300134, 1), Colombia (MH 717721, 1), United Kingdom (JX 082356, 1) and Tunisia (KX156806) (Fig. 1).

Hemato-biochemical parameters

There was a significant (P<0.05) decrease in Hb, RBCs, PCV, MCHC, Monocytes, and Platelets and significant increase in MCV in CCoV affected dogs as compared to healthy dogs (Table 2).

Biochemical analysis of this study declared that there was a significantly (P < 0.05) increase in AST, ALT, and urea while a significantly (P < 0.05) decrease was observed in Albumin in in CCoV affected dogs as compared to healthy dogs (Table 3).

Table 1. Analysis of different factors with the occurrence of CCoV in dogs

Category	Groups	Total	Positive (%)	Odd Ratio	95% CI for OR	P-Value
Breed	German Shepherd	45	16 (35.5)	Ref.	–	.000
	Labrador	45	06 (13.3)	3.586	1.249–10.291	
	Pug	45	24 (53.3)	0.482	0.207–1.124	
	Rottweiler	45	07 (15.5)	2.995	1.089–8.232	
	Mongeral	45	14 (31.1)	1.221	0.507–2.939	
	Crossbred	45	05 (11.1)	4.413	1.451–13.422	
	Pitbull	45	08 (17.7)	2.551	0.959–6.785	
	Shitzu	45	11 (24.4)	1.705	0.683–4.252	
	Poodle	45	06 (13.3)	3.586	1.249–10.291	
	Bully	45	10 (22.2)	1.931	0.761–4.898	
Age	< 1 Year	150	47 (31.3)	Ref.	–	.007
	1-3 Years	150	25 (16.6)	2.281	1.315–3.957	
	> 3 Years	150	35 (23.3)	1.499	0.898–2.501	
Sex	Male	225	41 (18.2)	0.536	0.344–0.836	.006
	Female	225	66 (29.3)			
Vomiting	Present	200	76 (38.0)	4.329	2.700–6.943	.000
	Absent	250	31 (12.4)			
Diarrhea	Present with blood	340	86 (25.2)	1.434	0.840–2.449	.184
	Present without blood	110	21(19.0)			
Sample source	Clinics	225	65 (28.8)	1.770	1.137–2.754	.11
	Kennels	225	42 (18.6)			
Body Size	Small	150	47(31.3)	Ref.	–	.008
	Medium	150	36 (24.0)	1.445	0.868–2.404	
	Large	150	24 (16.0)	2.395	1.373–4.178	
Cohabitation with other Animals	Not	430	97 (22.5)	Ref.	–	.005
	Yes	20	10 (50.0)	3.433	1.388–8.488	
Living area	Rural	50	17 (34.0)	Ref.	–	0.89
	Urban	400	90 (22.5)	1.774	0.944–3.333	
Food	Canned/Processed	150	17 (11.3)	Ref.	–	.000
	Raw meat/offal	150	53 (35.3)	0.233	0.127–0.428	
	Home cooked diet	150	37 (24.6)	0.390	0.208–0.730	
Deworming History	Dewormed in Last 3 months	150	20 (13.3)	Ref.	–	.000
	Dewormed in Last Year	150	27 (18.0)	0.700	0.373–1.314	
	Never Dewormed	150	60 (40.0)	0.230	0.130–0.409	
Contact with other animal feces	Cat Faeces	85	06 (7.00)	Ref.	–	.000
	Equines Faeces	55	04 (7.20)	0.968	0.260–3.600	
	Cattle/Buffaloe Faeces	105	21 (20.0)	0.303	0.116–0.791	
	Sheep/Goat Faeces	40	00 (0.00)	6.622	0.363–120.514	
	Birds Faeces	15	02 (13.3)	1.34	.78-5.54	
	Never contacted with any Faeces	150	74 (49.3)	0.493	0.089–2.714	
Season	Winter	111	45 (40.5)	Ref.	–	.000
	monsoon	113	29 (25.6)	1.974	1.119–3.482	
	Spring	113	19 (16.8)	3.373	1.811–6.281	
	summer	113	14 (12.3)	4.821	2.452–9.477	

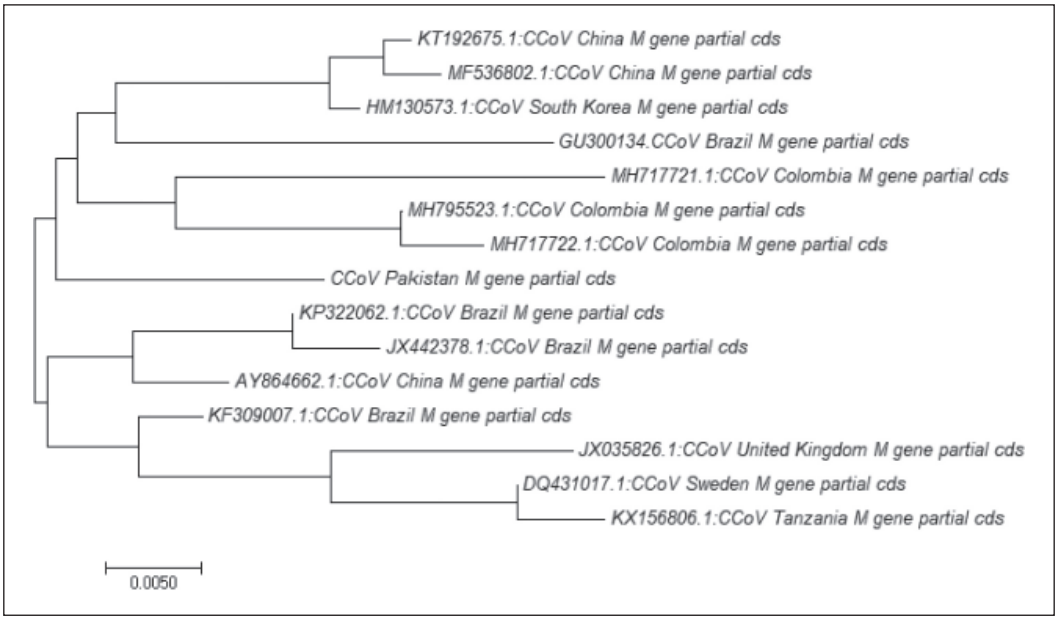

Figure 1. Phylogenetic analysis of local CCoV isolates.

Table 2. Blood parameters of healthy and coronavirus affected dogs

Parameters	Healthy Animal Mean ± Standard Deviation	Coronavirus infected Mean ± Standard Deviation	P-value
Hb (G/dl)	13.23 ± 1.39	9.30 ± 1.69	.000
RBCs x 10^6/uL	6.41 ± 0.59	4.01 ± 0.60	.000
PCV (%)	42.62 ± 3.54	28.61 ± 4.42	.000
MCV	66.40 ± 5.73	79.30 ± 6.82	.000
MCHC (G/dl)	34.15 ± 1.87	26.27 ± 2.45	.000
TLC (x 10^3 /µL)	12.56 ± 0 .93	11.82 ± 0.58	0.41
Neutrophils %	65.84 ± 2.0	74.31 ± 1.40	.000
Monocytes %	6.65 ± 0.48	4.10 ± 0.55	.001
Eosinophils %	1.65 ± 0.67	0.50 ± 0.68	.000
Lymphocytes %	17.50 ± 1.70	14.05 ± 2.06	.003
MCH Pgs	23.10 ± 1.84	21.7 ± 1.98	0.3
Platelets (x 10^5/µL)	438.10 ± 61.20	243.8 ± 30.80	.000

* $P < 0.05$.

Table 3. Serum-Biochemical parameters of healthy and coronavirus affected dogs

Parameters	Healthy Animals Mean ± Standard Deviation	Coronavirus infected Mean ± Standard Deviation	P-value
AST U/L	35.75 ± 3.16	67.10 ± 4.78	.000
ALT U/L	96.10 ± 3.60	141.10 ± 12.10	.000
Bilirubin Total mg/dl	0.31 ± 0.12	0.61 ± 0.35	.09
Alkaline Phosphate U/L	130.35 ± 15.35	280.45 ± 41.04	.000
Total Protein G/dl	6.55 ± 1.01	8.51 ± 0.71	.004
Albumin G/dl	3.44 ± 0.43	2.94 ± 0.58	.04
Globulin G/dl	4.10 ± 0.47	6.28 ± 0.45	.002
Urea mg/dl	23.7 ± 3.77	55.20 ± 9.61	.000
Creatinine mg/dl	1.43 ± 0.22	1.59 ± 0.53	0.4

* $P < 0.05$.

DISCUSSION

The current study has documented 23.8% prevalence of CCoV by RT- PCR. The study findings are almost in line with the findings of Lu *et al.* (2016) and Duijvestijn *et al.* (2016), who reported 26% and 31.7% prevalence of CCoV from China and Netherlands respectively using RT-PCR. Divya *et al.* (2018) and Agnihotrie *et al.* (2017) showed discrepancies with our results as both studies reported 8% prevalence of CCoV in India. Curie *et al.* (2016) reported 0% prevalence of CCoV from southeast Brazil. Theses discrepancies might be due to the instability of RNA virus in the fecal material due to activities of endogenous RNases. Lack of accessibility of partially degraded RNA or intermittent shedding of the virus in feces may influence the sensitivity of PCR assay (Vermeulen *et al.*, 2011; Niamat *et al.*, 2019).

Furthermore, 53.3 % of the PCR positive dogs were of Pugs breeds, this breed was considered the most susceptible to CCoV, with the disease being more severe and having a worse prognosis if animals will be having other respiratory problems (Brandell & Olson, 2020). These findings disagree with findings of previous studies who have recorded the highest prevalence in German shepherds (Deka *et al.*, 2013). It might be due to the fact that Pakistani people keep pugs dogs usually as pet animals and this could be the major possible reason for the higher prevalence in this particular breed. This study revealed a significantly higher disease prevalence in age groups < 1 year (31.3 %), followed by > 3 years (23.3%) and (16.6%) in 1-3 years. Same trend was also observed with the advancement of the age by Deka *et al.* (2013). The main reason seems to be the lack of maternal immunity and poor efficiency of the immune system.

The current study findings of higher prevalence rate of CCoV infection in female as compared to male dogs was also supported by Stavisky *et al.* (2012). It might be attributed to the selective preference of keeping female as pets by owners. Deka *et al.* (2013) findings were not in accordance with current study findings. The preference of the male dogs as compared to female dogs was the justification by the authors.

Vomiting and Diarrhea were the major clinical findings of CCoV in Dogs, the current study documented 38.0% and 25.2% of their prevalence respectively in CCoV infected Dogs. Various studies have documented these signs in their studies (Naylor *et al.*, 2001; Godsall *et al.*, 2010; Staviskyet *et al.*, 2012). Of the three size investigated in dogs, small size dogs had the highest prevalence (31.3%), followed by medium-size dogs (24%) and large size dogs (16%). This might be due to the more interest of pet owners for rearing of small breed of dogs because of their size and portable natures. Besides, our study reveals that dogs that have a history of living in rural environments were more likely (OD=1.774) to be infected with CCoV and had a higher prevalence (34%) as compare to dogs lives in the urban environment (22.5%). The current study investigated the dogs who had a history of Raw meat or offal found that (35.3%) tested positive for CCoV infection in dogs, followed by home cooked diet (24.6%) and a canned or processed diet (11.3%).

A trend has been observed in our study in the deworming status of CCoV positive dogs where 60 out of 150 dogs (40%) did not have any history of deworming. while 27 out of 150 dogs (27%) have a history of deworming in last year, and 20 out of 100 dogs (13.3%) had a history of deworming in the last three months. The results of the present study revealed that the dogs which were not having the history of contact with the feces were more susceptible (49.3%) to CCoV infection then those dogs which were having direct contact with Cattle/Buffaloes feces (20%), Equine feces (7.2 %), Birds feces (13.3), and small ruminants feces (0%).

It was observed that the prevalence of CCoV infection in dogs was higher during the winter season (40.5%) as compared to the monsoon, spring and summer seasons (25.6%, 16.8%, and 12.3%) respectively. Same trend is also documented by Deka *et al.* (2013). It might be due to the increased susceptible age groups of animals during the season following whelping and subsequent weaning pups along with the waning of maternal antibodies furthermore windy

weather in District Lahore might help in the rapid spread of both viruses within susceptible dogs during the period. In the present study, we find (0%) the prevalence relationship of CCoV infection from humans to dogs.

The values of haemoglobin and PCV were decreased in CCoV affected dogs. Previous studies have also reported such types of findings (Sharma *et al*., 2008; Dongre *et al*., 2015; Agnihotri *et al*., 2017). Anemia can be due to the fact that during the acute cases, the virus affects the bone marrow which ultimately results in myeloid and erythroid hypoplasia and severe form of haemorrhagic enteritis (Macintire & Smith, 1997; Sharma *et al*., 2005; Know *et al*., 2019). Diarrhea can cause dehydration and also leads toward the loss of body fluids (Bhat *et al*., 2015). Thrombocytopenia in this study in affected animals is also in line with the findings of Dongre *et al*., 2015 and Agnihotri *et al*. (2017). This might be due to the loss of blood with feces which ultimately results in the destruction or decrease in the production of platelets (Sharma *et al*., 2008). The study has revealed a little bit of leukopenia, acute lymphopenia, and monocytosis. The findings are strongly agreed with (Marinaro *et al*., 2010). Replication of virus in the lymphatic tissue and bone marrow can extinguish the lymphoid cells and actively mitotic myeloid precursors which can ultimately result in leucopenia (Haligur *et al*., 2009; Behera *et al*., 2014). Neutrophilia occurred in this study is justified by Bhat *et al*. (2015). It might be due to secondary bacterial infections, associated with the Coronavirus infections in dogs. Coronavirus infected dogs decrease in lymphocytes value and it might be due to virus replication in the lymphoid organ resulting in lymphocytosis.

Increase in the values of ALT and AST in this study is in line with the findings of Bhat *et al*. (2013). It might be due to reactive hepatopathy (Berghoff & Steiner 2011). This may occur as a result of hepatic hypoxia secondary hypovolemia or the absorption of toxic substances due to loss of the gut barrier (Shaker & Carey, 1990).

CONCLUSION

This study concludes that Coronavirus is prevalent in dogs of Pakistan. Various risk factors like breed, age, sex, vomiting, diarrhea, sample source, body size, cohabitation with other animals, living environment, food, deworming history, contact with other animals or birds feces, and season were significantly associated with CCoV. The hematological findings can help the clinician for the diagnosis of disease.

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Conflict of interest

The authors declare no conflict of interest.

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