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Full Length Article

A Novel Approach to Diagnose and Treat Coronavirus in Dogs (*Canis lupus familaris*)

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Abstract

This study was conducted with an aim to diagnose canine coronavirus (CCoV) and to determine the antiviral effect of an antimalarial drug chloroquine (CQ). A total of thirty (n = 30) PCR-confirmed CCoV positive dogs (*Canis lupus familaris*) were randomly selected and included in the study for the drug trial. These 30 dogs were further divided into two groups; A (control) & B (treatment) containing 15 dogs each. Blood samples were collected from every dog to study the hemato-biochemical parameters, *i.e*., CBC, LFTs and RFTs during the course of the experiment. In group A, 5 out of 15 dogs recovered and remained alive while 10 died (mortality rate 66.7%). In group B, 10 out 15 dogs recovered and 5 out of 15 died during the course of this study (mortality rate 33.7%). CQ should be considered for treatment in CCoV as it has good antiviral activity against coronavirus in dogs.

Keywords: Canine coronavirus; Chloroquine; PCR; Clinical trial; Antiviral; *In-vitro* study; Dog

Introduction

Domesticated dogs (*Canis lupus familaris)* have become an essential part of people around the world. They influence people's daily lives as they impart delight, diminish friendlessness and psychological issues and give people emotional support (Deng *et al.* 2018). Pakistan has a huge population of livestock and other domestic animals such as dogs. Dogs possess peculiar attributes like guarding, sniffing, hunting and retrieving. These specialties of dogs are utilized by military, rangers, police, anti-narcotic forces, and other agencies. There are three million dogs in Pakistan (Towakal *et al.* 2010). Canine coronavirus (CCoV) was regarded as a pathogen of dogs in 1971 (Binn *et al.* 1974). Viruses of the coronaviridae are single stranded RNA viruses with a genomic length of 30 kbp. CCoV infection is very common in younger dogs, especially those kept in large groups, breeding facilities, shelters and kennels (Stavisky *et al.* 2012). Canine Corona Virus (CCoV) infects epithelial cells of intestinal villi causing mild to severe diarrhea (Saif and Heckert 1990). Pups are highly prone to develop severe and fatal disease. The infected dog is dull, lethargic, may or may not be febrile, anorectic, shows vomiting, bloody diarrhea and dehydration, Coronavirus infection is not diagnosed accurately as it mimics another viral infection caused by Canine Parvovirus (CPV). Thus, clinicians fail to educate the dog owners confidently about the prognosis of the disease and it causes great economic loss to dog breeders (Sulehria *et al.* 2020). CCoV can be diagnosed by cell-line culture method, PCR and immune-chromatography based test kits (Yoon *et al.* 2018). Chloroquine is known since 1934. Besides from its reputed antimalarial action, it has good antiviral effects especially against viruses like coronaviruses, retroviruses & flaviviruses and HIV (Savarino *et al.* 2003).

Materials and Methods

Experimental details and treatments

Experimental material: A total of thirty (n = 30) dogs (*C. lupus familaris)* positive for CCoV reported from different private and public veterinary clinics in Lahore were included in the study from January 2019 to December 2019. CCoV was identified from diarrheic dogs that were presented at different private and public veterinary clinics. For this purpose fecal samples were collected from the morbid dogs and were rendered for rapid detection (Fig. 1) using immuno-chromatography based rapid detection test kits (Sulehria *et al.* 2020) manufactured by Quicking Biotech China (Pvt. Ltd.). (Fig. 1).

Confirmation of CCoV by PCR

PCR assay was performed for the confirmation of CCoV. The CCoV RNA was extracted through RNA Fast Extraction stool Kit (Cat # RP8001, Bioteke Corporation China). To confirm the correct extraction and quality of the RNA, all samples were quantified by using a Nano drop 2000 spectrophotometer. To confirm the presence of CCoV, a 321 (bp) fragment of the M-gene of CCoV was targeted (Figs. 2, 3) by using protocols & PCR conditions as described by (Agnihotri *et al.* 2018; Sulehria *et al.* 2020).

Treatments

A total of 30 dogs (CCoV positive) were randomly selected in the study (Table 2). These 30 dogs were further divided into two groups; A & B containing 15 dogs each. Group-A was taken as control group whereas Group-B was taken as treatment group. The dogs in both the groups A and B were given fluid therapy, anti-diarrheal medicine (Metronidazole @ 15 mg/kg q12h IV), anti-emetic (Metoclopramide @ 0.4 mg/kg q8h IV) medicine along with antibiotic (Ceftriaxone Sodium @ 50 mg/kg q12h). The treatment group (Group B) was given the same medicines and, additionally, Chloroquine @ 10 mg/Kg SQ q24h for consecutive 3 days.

Blood collection for hemato-biochemical analysis

Blood samples of the morbid dogs were collected aseptically from the cephalic or saphenous veins into EDTA coated (purple cap) and non-EDTA (yellow cap) coated vacutainers. 4 mL of blood was collected from each dog, 1 mL for Complete Blood Count (CBC) and 3 mL for Liver Function Tests (LFTs) & Renal Function Tests (RFTs). The samples were transported to the laboratory of Dairy Health Research Lab (DHRL), Department of Veterinary Medicine and Surgery (CMS), UVAS, Lahore, Pakistan by maintaining the cold chain 4ºC. The VET hematology analyzer (Model No. DW-3680/DW-36) was used for performing the CBC. While the serum samples were analyzed for estimation of biochemical parameters using a Semi-automated clinical chemistry analyzer machine (Model URIT-810).

Post treatment examination of dogs

The treated animals after the drug administration were re-examined after 14th day for rapid detection test using the kit. Simultaneously, the blood samples were also collected for hematological and serum biochemical analysis to check the hemato-biochemical changes.

Drug's efficacy formula

drug efficacy formula was taken from Asmaa *et al.* (2014);

$$\text{Efficacy} = \frac{\text{No. of animals cured}}{\text{Total no. of animals treated}} \times 100$$

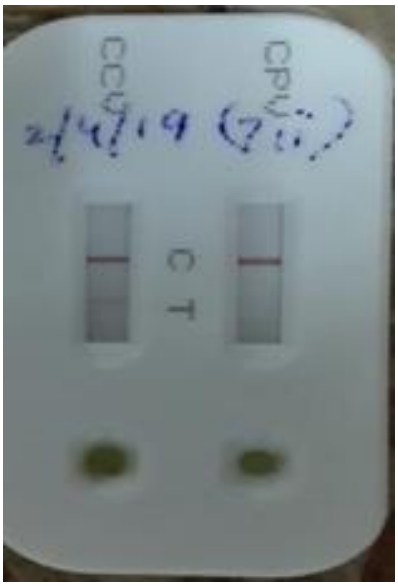

Fig. 1: A CCoV positive sample (Left Column with double bands) using a Rapid Detection Test Kit

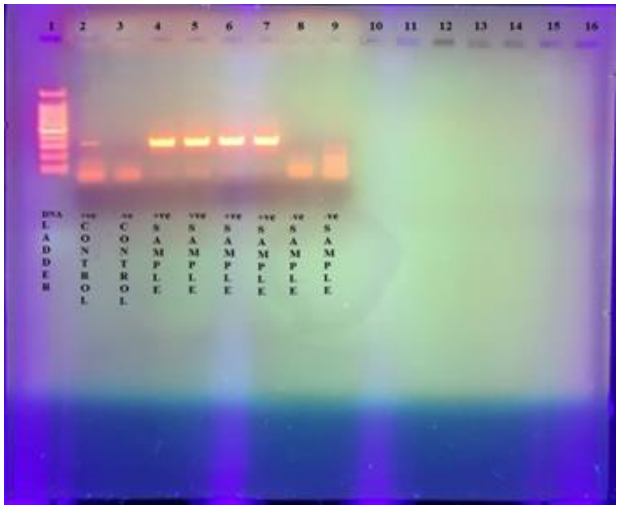

Fig. 2: PCR results for M-gene amplification of CCoV showing DNA ladder, positive and negative controls and positive and negative samples

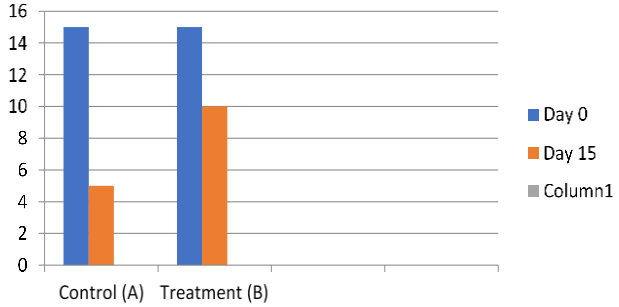

Fig. 3: No. of dogs alive at the start and completion of the trial

Statistical analysis

For statistical analysis, Chi-square ($\chi 2$) Test and Sampled paired *t*-test was applied to various hemato-biochemical parameters to determine the significant ($P < 0.05$) difference of mean and standard deviation before and after the treatment. All the statistical analyses were carried out using IBM® SPSS (statistical product and service solutions) Statistics® version 21.0.

Results

Clinical condition and scoring

The current study showed that corona virus infected dogs showed different clinical signs (Table 1). Prior to treatment, clinical examination revealed that all the dogs (100%, 30/30) were anorectic. Among the dogs (33.3%, 10/30) showed low body temperature *i.e*., less than 101ºF, fever (temperature more than 102.5ºF) was observed in 16 out 30 dogs (53.3%) whereas 4 out of 30 dogs (13.3%) had normal body temperature, but harbored the canine corona virus infection. Among the dogs, 21 out of 30 (70%) had been confirmed to have been vomiting. All the canine corona virus infected dogs (100%) showed diarrhea. The dogs showed pale mucous membrane were 26 out of 30 (86.7%), while 4 out of 30 dogs (13.3%) showed pink mucous membrane. Dogs appeared dehydrated with varying degrees. Out of 30 infected dogs, 24 (80%) were 4–5% dehydrated, 2 dogs (6.7%) were 6–7% dehydrated while 4 out of 30 (13.3%) were more than 7% dehydrated. It was observed that all the 30 dogs (100%) dogs had a poor body condition.

Survival rate

The clinical trial of chloroquine showed significant results. The Table 2 shows the comparison of the results between Group A (control group) and Group B (treatment group). In group A, 5 out of 15 dogs recovered and remained alive while 10 died, the mortality rate 66.7% in this group. On the other hand, in the group B, 10 out 15 dogs recovered and 5 out of 15 died during the course of this study. The mortality rate was observed to be 33.7% in this group suggesting that the mortality rate was higher in the control group where there was no administration of chloroquine.

Dynamics of hemato-biochemical parameters

There was significant improvement ($P < 0.05$) in RBCs, neutrophils, monocytes, eosinophil, lymphocytes, MCH and platelets count on 14th day post-treatment in Group B compared to the dogs in Group A, indicating a significant increase (Table 3). Table 4 clearly shows that in treatment group (B), the values of Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), bilirubin, alkaline phosphatase, total protein, globulin and urea were significantly lower as compared to the control group (A).

Table 1: Clinical Scoring of the Patients

S. No.	Clinical sign		No. of dogs	Percentage (%)
1	Anorexia	Yes	30	100
		No	0	0
2	Temperature	Below 101ºF	10	33.3
		101ºF to 102.5ºF	4	13.3
		Above 102.5ºF	16	53.3
3	Vomiting	Yes	21	70
		No	9	30
4	Diarrhea	Yes	30	100
		No	0	0
5	Mucous	Reddish Pink	4	13.3
	Membrane	Pale	26	86.7
6	Dehydration	4-5%	24	80
		6-7%	2	6.7
		More than 7%	4	13.3

Discussion

This study was a novel attempt to diagnose canine coronavirus at molecular level and to treat this disease with chloroquine, an antimalarial drug. It was observed that CCoV infected dogs showed different clinical signs. The clinical examination revealed that all the dogs were anorectic, ten out of thirty dogs showed a decreased body temperature, sixteen out of thirty dogs showed fever, while the remaining four out of thirty dogs showed normal body temperature upon presentation but they harbored the canine corona virus infection. These same non-specific clinical signs have been observed by (El-Neshwy *et al.* 2019). Among the thirty dogs twenty-one dogs had been confirmed to have been vomiting. All the CCoV infected dogs showed bloody mucoid diarrhea and all of them had poor body condition. Dogs appeared dehydrated with varying degrees. Out of 30 dogs, 24 were 4–5% dehydrated, two were 6–7% dehydrated while four dogs were more than 7% dehydrated. Twenty-six out of 30 dogs showed pale mucous membrane whereas four dogs showed pink mucous membrane. The same has been observed by (Thomson and Gagnon 1980; Naylor *et al.* 2001; Godsall *et al.* 2010; Schultz *et al.* 2010; Kalli *et al.* 2010; Stavisky *et al.* 2012).

This study was a clinical trial, conducted to evaluate chloroquine as an antiviral drug against CCoV. The results suggested that in group A, 5 out of 15 dogs recovered and remained alive while 10 died, the mortality rate 66.7% in this group. On the other hand, in the group B, 10 out 15 dogs recovered and 5 out of 15 died during the course of this study. The mortality rate was observed to be 33.7% in this group suggesting that the mortality rate was higher in the control group where there was no administration of chloroquine. These results are in accordance with (Pardridge *et al.* 1998; Keyaerts *et al.* 2009; Kaptein and Neyts 2016) who proved the antiviral effects of chloroquine against human coronavirus, dengue virus and HIV respectively. As seen in Table 3, there was a significant improvement ($P <$

Table 2: Treatment Plan of Control and Treatment Groups

S. No.	Fluid Therapy + Antidiarrheal + Anti-emetic + Antibiotic		Fluid Therapy + Antidiarrheal + Anti-emetic + Antibiotic+ Chloroquine	
	Group (A) CCoV		Group (B) CCoV	
1	Died		Recovered	
2	Died		Died	
3	Recovered		Recovered	
4	Died		Recovered	
5	Recovered		Died	
6	Died		Recovered	
7	Died		Recovered	
8	Recovered		Recovered	
9	Recovered		Recovered	
10	Recovered		Recovered	
11	Died		Died	
12	Died		Recovered	
13	Died		Recovered	
14	Died		Died	
15	Died		Died	
Mortality Rate	10/15 (66.7 %)	5/15 (33.3%)	5/15 (33.3%)	10/15 (66.7%)
Drug Efficacy	33.3%		66.7%	

Table 3: Comparison of Hematological Parameters Before and After Trial

Parameters	Control Group (A)	Treatment Group (B)		*P-Value*
		Before (Day 0)	After (Day 14)	
Hb (G/dL)	9.19 ± 0.70	8.47 ± 0.92	9.87 ± 0.54	0.243
RBCs x10^6/μL	4.67 ± 0.31	4.27 ± 0.34	4.95 ± 0.14	0.000
PCV (%)	30.85 ± 2.00	28.44 ± 1.82	33.03 ± 2.82	0.921
MCV fl	74.25 ± 2.24	79.36 ± 1.65	71.48 ± 1.47	0.109
MCHC (G/dL)	29.06 ± 1.37	28.15 ± 1.27	31.18 ± 0.96	0.515
TLC (x103 /μL)	12.25 ± 0.41	11.53 ± 0.61	12.09 ± 0.52	0.548
Neutrophils %	70.51 ± 2.10	72.91 ± 2.68	68.53 ± 2.09	0.015
Monocytes %	4.32 ± 0.24	4.03 ± 0.18	5.11 ± 0.25	0.000
Eosinophils %	0.86 ± 0.14	0.49 ± 0.018	1.01 ± 0.18	0.017
Lymphocytes %	14.88 ± 0.44	13.99 ± 0.70	16.12 ± 0.76	0.000
MCH Pgs	21.65 ± 0.93	20.75 ± 1.50	22.69 ± 0.50	0.001
Platelets (x 105/μL)	386.24 ± 41.81	230.19 ± 14.93	419.23 ± 24.74	0.014

Table 4: Comparison of Biochemical Parameters Before and After Trial

Parameters	Groups	Day 0	Day 14	*P-Value*
AST U/L	Group A	70.53 ± 4.11	57.40 ± 5.71	0.269
	Group B	71.86 ± 1.95	48.82 ± 5.06	0.000*
ALT U/L	Group A	141.51 ± 1.97	115.70 ± 4.69	0.182
	Group B	143.38 ± 4.95	106.62 ± 3.57	0.000*
Bilirubin Total mg/dL	Group A	0.61 ± 0.25	0.51 ± 0.034	0.533
	Group B	0.60 ± 0.26	0.42 ± 0.014	0.000*
Alkaline Phosphate U/L	Group A	279.08 ± 7.88	231.42 ± 18.25	0.559
	Group B	277.35 ± 8.09	193.15 ± 10.77	0.000*
Total Protein G/dL	Group A	8.57 ± 0.32	7.83 ± 0.37	0.050*
	Group B	8.42 ± 0.12	7.07 ± 0.16	0.002*
Albumin G/dL	Group A	2.97 ± 0.11	3.10 ± 0.07	0.667
	Group B	3.01 ± 0.13	3.08 ± 0.06	0.908
Globulin G/dL	Group A	6.16 ± 0.08	5.73 ± 0.24	0.005*
	Group B	6.18 ± 0.04	5.16 ± 0.11	0.017*
Urea mg/dL	Group A	55.36 ± 0.99	47.08 ± 1.11	0.731
	Group B	55.12 ± 1.01	34.42 ± 1.71	0.030*
Creatinine mg/dL	Group A	1.58 ± 0.57	1.48 ± 0.49	0.560
	Group B	1.54 ± 0.47	1.48 ± 0.47	0.714

0.05) in RBCs, neutrophils, monocytes, eosinophil, lymphocytes, MCH and Platelets count on 14th day post-treatment in Group B compared to the dogs in Group A indicating a significant increase. The same has been reported by (Sharma *et al.* 2008; Dongre *et al.* 2015; Agnihotri *et al.* 2017; Sulehria *et al.* 2020). It was uncovered in the current study that in the treatment group B, the values of AST, ALT, bilirubin, alkaline phosphatase, total protein, globulin and urea were significantly lower (better) as compared to the control group (A), (Shaker and Carey 1990; Berghoff and Steiner 2011; Bhat *et al.* 2013) were also of the same view.

Conclusion

The findings of our study conclude that canine coronavirus is circulating in the dog population of Pakistan. Chloroquine is a good and cost effective drug to treat canine coronavirus infection in dogs. The study has set a more authentic and reliable way to diagnose canine coronavirus infection in dogs. The advantage of using this line of diagnosis is that it will give more confidence to the practicing vets and will improve their clinical skills. Chloroquine may be referred to as a potential and novel drug for the treatment of canine coronavirus infection in dogs.

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Author Contributions

Conception, US, SS; methodological approach, US, SS, MI; critical examination, US, SS, MI and HM; writing initial draft preparation, US, SS; writing, assessment, and proofreading, US, SS and MI.

Conflict of Interest

All the authors have declared no conflicts of interest.

Data Availability

Data presented in this study will be available upon a fair request to the corresponding author.

Ethics Approval

The study design was approved by the Ethics Committee of the University of Veterinary and Animal Sciences Lahore, Pakistan under diary No. 502/dated 02.03.2018.

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References

Agnihotri D, Y Singh, K Batra, VK Jain, A Kumar, T Kumar, S Maan (2018). Molecular detection based epidemiology of canine parvovirus and canine coronavirus infection in diarrheic dogs in Haryana. *Ind J Anim Res* 8:367–374

Agnihotri D, S Yudhvir, M Sushila, V Jain, A Kumar (2017). Molecular detection and clinico-haematological study of viral gastroenteritis in dogs. *Haryana Vet* 56:72–76

Asmaa NM, MA El-Bably, KA Shokier (2014). Studies on prevalence, risk indicators and control options for tick infestation in ruminants. *Beni-Suef Univ J Basic Appl Sci* 3:68–73

Berghoff N, JM Steiner (2011). Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. *Vet Clin North Amer Small Anim Prac* 412:311–328

Bhat AA, DR Wadhwa, SP Singh, I Singh (2013). Haematological and biochemical analysis in canine enteritis. *Vet World* 67:380–383

Binn LN, EC Lazar, KP Keenan, DL Huxsoll, RH Marchwicki, AJ Strano (1974). Recovery and characterization of a coronavirus from military dogs with diarrhea. *In*: *Proceedings, Annual Meeting of the United States Animal Health Association*, pp:359–366. Roanoke, Virginia, USA

Deng X, Zhang, J Su (2018) A multiplex PCR method for the simultaneous detection of three viruses associated with canine viral enteric infections. *Arch Virol* 163:2133–2138

Dongre J, HK Mehta, P Maheshwari (2015). Comparative evaluation of two treatment regimens against canine parvovirus infection. *Haryana Vet* 541:83–84

El-Neshwy W, H El-Zahar, A Morsi (2019). Clinical, hematological and biochemical assessment of dogs naturally infected with canine parvovirus before and after therapy. *Benha Vet Med J* 37:182–186

Godsall SA, SR Clegg, JH Stavisky, AD Radford, G Pinchbeck (2010). Epidemiology of canine parvovirus and coronavirus in dogs presented with severe diarrhoea to PDSA PetAid hospitals. *Vet Rec* 167:196–201

Kalli I, LS Leontides, ME Mylonakis, K Adamama-Moraitou, T Rallis, AF Koutinas (2010). Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. *Res Vet Sci* 89:174–178

Kaptein SJ, J Neyts (2016). Towards antiviral therapies for treating dengue virus infections. *Curr Opin Pharmacol* 30:1–7

Keyaerts ELIS, L Vijgen, E Rysman, J Verbeeck, MV Ranst, P Maes (2009). Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother* 53:3416–3421

Naylor MJ, RP Monckton, PR Lehrbach, EM Deane (2001). Canine coronavirus in Australian dogs. *Aust Vet J* 792:116–119

Pardridge WM, J Yang, A Diagne (1998). Chloroquine inhibits HIV-1 replication in human peripheral blood lymphocytes. *Immunol Lett* 64:45–47

Saif LJ, RA Heckert (1990). Enteropathogenic coronaviruses. *In: Enteropathogenic Coronavirus*, pp:185–252

Savarino A, JR Boelaert, A Cassone, G Majori, R Cauda (2003). Effects of chloroquine on viral infections: An old drug against today's diseases. *Lanc Infect Dis* 3:722–727

Schultz RD, B Thiel, E Mukhtar, P Sharp, LJ Larson (2010). Age and long-term protective immunity in dogs and cats. *J Compar Pathol* 142:102–108

Shaker M, WD Carey (1990). *Hepatic encephalopathy*. http://www.clevelandclinicmeded. com/medicalpubs/ diseasemanagement/hepatology/hepatic-encephalopathy/

Sharma R, R Kumar, P Goel, R Kumar (2008). Clinical haematology in canine haemorrhagic gastroenteritis. *Haryana Vet* 47:35–38

Stavisky J, G Pinchbeck, RM Gaskell, S Dawson, AJ German, AD Radford (2012). Cross sectional and longitudinal surveys of canine enteric coronavirus infection in kennelled dogs: A molecular marker for biosecurity. *Infect Genet Evol* 12:1419–1426

Sulehria MU, SS Ahmad, M Ijaz, MH Mushtaq, AY Khan, A Ghaffar (2020). Molecular evidence and hematological alterations associated with the occurrence of coronavirus in domestic dogs in Pakistan. *Trop Biomed* 37:963–972

Thomson GW, A Gagnon (1980). Canine parvoviral enteritis: A disease entity. *Can Vet J* 21:158

Towakal F, M Rabbani, K Muhammad, MS Khan, MZ Shabbir (2010). Major strains of canine parvovirus present in dog population of Pakistan. *Pak J Zool* 42:825–827

Yoon SJ, KW Seo, KH Song (2018). Clinical evaluation of a rapid diagnostic test kit for detection of canine corona virus. *Kor J Vet Res* 58:27–31