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Prognostication of haemato-biochemical, electrolyte and blood gas parameters in canine parvoviral enteritis#

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Abstract

Canine parvovirus (CPV) infection remains as a major threat to canine practitioners as well as pet owners as it requires intensive care for at least seven days and is associated with a high rate of fatality. This study conducted on 40 dogs with diarrhoea revealed a prevalence of 35 per cent (14/40) for CPV infection in Thissur, Kerala. Aetiology was confirmed by polymerase chain reaction (PCR) targeting the VP2 gene of CPV to get amplicon of approximately 583 bp length. Alterations in various blood parameters like blood gases, electrolytes, metabolites, haematological parameters and serum biochemical parameters are analysed and accessed as prognostic markers. Notable variations could be detected in the levels of pCO_2, pO_2, pSO_2, HCO_3^-, base deficit, per cent of granulocytes, total erythrocyte count (TEC), haemoglobin (Hb), volume of packed red cells (VPRC), total protein, albumin, blood urea nitrogen (BUN) and alkaline phosphate (ALP). Mortality was associated with major decrease in the levels of HCO_3^- and a greater base deficit as evidenced from the blood gas analysis values. No correlation could be obtained for hypokalaemia and elevated levels of lactate in relation with mortality.

Keywords: *Canine parvoviral enteritis, PCR, base deficit, mortality*

Canine parvoviral enteritis occurs among all breeds, irrespective of age and gender, but mortality is high among pups under three months of age. Anaemia and pancytopaenia are the expected major changes in CPV infection (Terzungwe, 2018). Even with prompt and aggressive therapy on conventional lines, mortality rate is high in infected dogs.

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This situation warrants better understanding of variations in haematology and blood gas electrolytes and their value as prognostic markers for designing better therapeutic protocols for the disease. Hence, in the present study various blood parameters are determined and correlated with mortality rate among CPV infected dogs.

Materials and methods

Sample collection for blood parameters

The study was performed from December 2020 to August 2021 in 40 dogs that were presented to University Veterinary Hospital, Kokkalai and Teaching Veterinary Clinical Complex, Mannuthy with dysentery, vomiting, anorexia and severe dehydration, which were untreated prior to presentation. Blood was collected from cephalic vein in three non vacuum vials for blood gas analysis, haematology and serum biochemical analysis. Four millilitres of blood collected in heparin coated blood collection vial was used immediately for blood gas and electrolyte analysis using Epoc ™ Blood Gas Analyser (Ottawa, Ontario, Canada). Two millilitres of blood collected in EDTA coated blood collection vial was used for haematological analysis using an Automatic Haematology Analyzer (Orphee, Mythic Vet 18, UK). Four millilitres of blood was collected in blood collection vial coated with clot activator, serum was separated out and used for biochemical analysis using a semi automatic analyzer (Erba Manheim, Chem-5 Plus v2, USA) by manufacturer's instruction. Values obtained from all the dogs were compared with normal reference values standardised at clinical laboratory in the College of Veterinary and Animal Sciences, Mannuthy (Table 1, 2, 3) and analysed statistically using independent t test.

Table 1. Reference range for various blood gases, electrolytes and metabolites in dogs

Sl. No.	Blood parameter	Normal values
1	pH	7.36 – 7.44
2	pCO_2	36 – 44 mmHg
3	pO_2	90 – 100 mmHg
4	cSO_2	94 – 98 %
5	HCO_3^-	24 – 26 mmol/ L
6	BE(ecf)	2 – 3 mmol/ L
7	Sodium	140 – 150 mmol/ L
8	Potassium	3.9 – 4.9 mmol/ L
9	Chloride	109 – 120 mmol/ L
10	Calcium	1.15 – 1.33 mmol/ L
11	Glucose	65 – 112 mg/ dL
12	Lactate	0.5 – 2 mmol/ L

Molecular identification

Approximately five gram of faecal sample was collected from all the animals and subjected to extraction of deoxyribonucleic acid (DNA) using QIAamp® DNA Stool Mini Kit (QIAGEN, Germany). The following primers were used to amplify a 583 bp region of VP2 gene of CPV-2: forward primer, 5′-CAG GAA

Table 2. Reference range for various haematological parameters in dogs

Sl. No.	Parameter	Normal value
1	Total leucocyte count (TLC)	6-17 $\times 10^3$/ µL
2	Lymphocyte count (LYM)	0.7-5.1 $\times 10^3$/ µL
3	Monocyte count (MONO)	0.2-1.7 $\times 10^3$/ µL
4	Granulocyte count (GRAN)	4.4-12.6 $\times 10^3$/ µL
5	Lymphocyte per cent (LYM %)	12-30 %
6	Monocyte per cent (MONO %)	3-10 %
7	Granulocyte per cent (GRAN %)	60-74 %
8	Total erythrocyte count (TEC)	5.5-8.5 $\times 10^6$/ µL
9	Haemoglobin (Hb)	12-18 g/ dL
10	Volume of packed red cells (VPRC)	37-55 %
11	Mean corpuscular volume (MCV)	60-77 μm^3
12	Mean corpuscular haemoglobin (MCH)	19-25 pg
13	Mean corpuscular haemoglobin concentration (MCHC)	32-36 g/ dL
14	Thrombocyte count (PLT)	160-525 $\times 10^3$/ µL

Table 3. Reference range for various biochemical parameters in dogs

Sl. No.	Parameter	Normal value
1	Total protein	5.4-7.5 g/ dL
2	Albumin	2.3-3.1 g/ dL
3	Blood urea nitrogen (BUN)	8-28 mg/ dL
4	Creatinine	0.5-1.7 mg/ dL
5	Alanine transaminase (ALT)	10-109 IU/L
6	Alkaline phosphatase (ALP)	1-114 IU/L

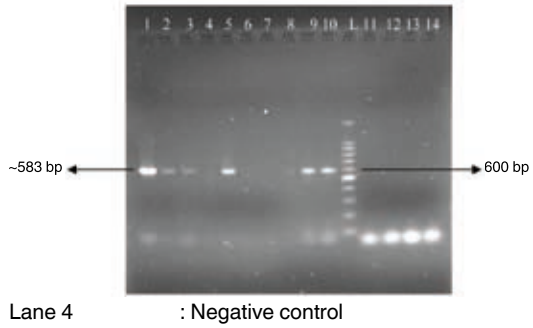
GAT ATC CAG AAG GA-3′, reverse primer 5′-GGT GCT AGT TGA TAT GTA ATA AAC A-3′ and the polymerase chain reaction was carried out for 30 cycles with denaturation at 94°C for 30 sec, annealing at 55°C for 2 min, extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min as done by Buonavoglia *et al.* (2001), using the S1000™ Thermal cycler (Bio-Rad, USA). Composition of PCR reaction mixture contained 12.5 µL of 2X- PCR master mix (Emerald Amp, Takara Bio, New Delhi), 4.5 µL of Nuclease-free water (Emerald Amp, Takara Bio, New Delhi), two microlitres each of forward and reverse primer, and four microlitres of DNA template. The commercial vaccine Megavac-P® (Indian Immunologicals, Hyderabad) was used as positive control for CPV-2.

Results and discussion

Genomic amplification product for CPV-2 was detected in 14 among 40 faecal samples of dogs with diarrhoea (Fig. 1).

Results of various blood gases, electrolytes, metabolites, haematological parameters and biochemical parameters were analysed and represented in Tables 4, 5 and 6.

Fig. 1. Agarose gel electrophoresis of PCR products specific for CPV-2



Lane 4 : Negative control
Lane 5 : Positive control
Lane 1,2,3,8,9,10 : Positive samples (CPV) (~583 bp)
Lane L : DNA marker 100 bp
Lane 11,12,13,14 : Negative samples

Table 4. Number of CPV infected dogs with various alterations in blood gases, electrolytes and metabolites

Parameters	Number of animals with		
	Decreased level	Normal level	Increased level
pH	2	8	4
pCO_2	9	1	4
pO_2	5	0	9
cSO_2	4	1	9
HCO_3^-	11	2	1
BE (ecf)	10	4	0
Sodium	6	7	1
Potassium	6	8	0
Chloride	5	8	1
Calcium	1	7	6
Glucose	1	6	7
Lactate	0	7	7

Table 5. Number CPV infected dogs with various alterations in different haematological parameters

Parameters	Number of animals with		
	Decreased level	Normal level	Increased level
TLC	2	9	3
LYM	0	11	3
MONO	0	12	2
GRAN	5	7	2
LYM %	0	8	6
MONO %	2	8	4
GRAN %	6	4	4
TEC	10	4	0
Hb	9	5	0
VPRC	9	5	0
MCV	0	11	3
MCH	0	13	1
MCHC	7	7	0
PLT	3	8	3

Table 6. Number CPV infected dogs with various alterations in different serum biochemical parameters

Parameters	Number of animals with		
	Decreased level	Normal level	Increased level
Total protein	6	5	3
Albumin	9	1	4
BUN	0	3	11
Creatinine	2	11	1
ALT	0	13	1
ALP	0	4	10

Parameters that showed significant variation at 5 per cent level included pO_2, sodium, chloride, per cent of monocytes, per cent of granulocytes, TEC, Hb, VPRC, MCH, MCHC, total protein and albumin (Tables 7, 8 and 9). Among the 14 animals with parvoviral enteritis, increased pO_2 was observed in 64.28 per cent of affected dogs. A decrease in chloride level could be noted in 35.71 per cent of dogs with infection. Decreased sodium level and granulocytopenia were observed in 42.85 per cent each. As CPV infection is characterised by haemorrhagic gastroenteritis that lead to loss of blood, a decreased TEC (among 71.43 per cent), Hb and VPRC (among 64.28 per cent, each), MCHC (50 per cent) and increased MCH (7.14 per cent) were also noticed. Serum biochemical analysis revealed decreased total protein, albumin among 42.86 per cent and 64.28 per cent, respectively.

A decrease in TLC was reported by Behera *et al*. (2014), Haligur *et al*. (2009), Mohanta *et al*. (2018) and Sagar *et al*. (2008). Leucopenia might be due to destruction of haemopoietic precursor cells in bone marrow, thymus, spleen and lymph nodes (Leisewitz, 2017), as happens in viral infections like CPV that targets fast multiplying cells of the body. Resulting immunosuppression may also render the animal susceptible for secondary bacterial infection and increase the severity of the condition (Mohanta *et al*., 2018). But only two dogs had leucopenia in the present study on the day of presentation, which was in accordance with De Laforcade *et al*. (2003), who stated that leucopenia that is characteristic in CPV infection will be present only in less than 50 per cent of animals on the day of presentation.

According to Zafar *et al.* (1999), damaged vascular epithelium of intestine and loss of blood through diarrhoeic faeces could cause anaemia as found in more than 60 per cent of animals in this study. Bhat *et al.* (2013) have reported that unaltered VPRC in dogs with haemorrhagic diarrhoea might be due to the masking of low VPRC by dehydration. Six among 14 dogs with CPV infection had normal VPRC. Thrombocytopenia and thrombocytosis could be noted in three animals each (21.43

Table 7. Comparison between blood gas and electrolyte values of CPV infected and control dogs

Blood electrolyte	Group				t value	p value
	Dogs with CPV infection		Non-diarrheic dogs			
	Total No.	Mean ± SE	Total No.	Mean ± SE		
pH	14	7.41221 ± 0.019613	10	7.46980 ± 0.021047	1.968^{ns}	0.854
pCO_2	14	30.386 ± 2.5121	10	28.270 ± 1.5870	0.646^{ns}	0.105
pO_2	14	121.079 ± 13.6594	10	167.244 ± 11.0712	2.626^{*}	0.031
cSO_2	14	95.171 ± 1.9829	10	99.422 ± 0.1899	2.134^{ns}	0.004
HCO_3^-	14	19.193 ± 1.3303	10	20.450 ± 0.9072	0.716^{ns}	0.080
BE (ecf)	14	5.400 ± 1.3972	10	3.230 ± 1.0459	1.154^{ns}	0.257
Sodium	14	141.786 ± 1.3391	10	148.000 ± 0.6325	5.707^{*}	0.000
Potassium	14	3.986 ± 0.1226	10	4.180 ± 0.1590	0.914^{ns}	0.365
Chloride	14	109.714 ± 2.2050	10	115.600 ± 0.8055	4.256^{*}	0.000
Calcium	14	1.3100 ± 0.03176	10	1.3140 ± 0.02156	0.104^{ns}	0.044
Glucose	14	100.500 ± 5.5586	10	103.300 ± 4.0608	0.407^{ns}	0.023
Lactate	14	2.5257 ± 0.38837	10	2.7200 ± 0.38206	0.338^{ns}	0.338

* - Means are significantly different at 5 per cent level
^{ns} - Non-significant

Table 8. Comparison between haematological values of CPV infected and control dogs

Haematological parameters	Group				t value	p value
	Dogs with CPV infection		Non-diarrheic dogs			
	Total No.	Mean ± SE	Total No.	Mean ± SE		
TLC	14	10.429 ± 1.5476	10	13.000 ± 1.1818	1.229^{ns}	0.064
LYM	14	3.193 ± 0.5792	10	2.700 ± 0.2867	0.763^{ns}	0.020
MONO	14	0.9286 ± 0.2066	10	0.5800 ± 0.06960	1.599^{ns}	0.02
GRAN	14	6.314 ± 1.1607	10	9.690 ± 1.1082	2.028^{ns}	0.331
LYM PER	14	32.1214 ± 4.76772		21.4100 ± 2.01282	2.070^{ns}	0.036
MONO PER	14	8.193 ± 1.094	10	4.530 ± 0.4261	3.009*	0.012
GRAN PER	14	59.686 ± 5.0897	10	74.060 ± 2.3901	2.556*	0.014
TEC	14	4.6507 ± 0.29348	10	5.8360 ± 0.50527	2.160*	0.100
Hb	14	10.700 ± 0.6000	10	14.420 ± 1.3186	2.603*	0.035
VPRC	14	32.886 ± 1.8831	10	40.100 ± 3.1714	2.075*	0.119
MCV	14	71.343 ± 1.4311	10	69.140 ± 1.1928	1.115*	0.175
MCH	14	22.636 ± 0.3957	10	24.630 ± 0.6660	2.731*	0.144
MCHC	14	31.857 ± 0.7147	10	35.590 ± 0.6248	3.736*	0.283
PLT	14	357.900 ± 30.8824	10	233.900 ± 45.2208	1.587^{ns}	0.340

* - Means are significantly different at 5 per cent level
^{ns} - Non-significant

Table 9. Comparison between serum biochemical values of CPV infected and control dogs

Serum biochemical parameter	Group				t value	p value
	Dogs with CPV infection		Non-diarrheic dogs			
	Total No.	Mean ± SE	Total No.	Mean ± SE		
Total protein	14	5.9314 ± 0.46512	10	7.6950 ± 0.44596	2.640*	0.293
Albumin	14	2.2564 ± 0.23544	10	3.4970 ± 0.21485	3.724*	0.211
BUN	14	41.9271 ± 5.40638	10	30.3780 ± 2.80217	1.897^{ns}	0.050
Creatinine	14	0.9207 ± 0.17668	10	1.1790 ± 0.07204	1.180^{ns}	0.077
ALT	14	43.3493 7.17641	10	64.3710 7.60796	1.972^{ns}	0.505
ALP	14	188.5921 37.65129	10	152.2970 40.21433	0.647^{ns}	0.816

* - Means are significantly different at 5 per cent level
^{ns} - Non-significant

per cent) and the remaining eight dogs (57.14 per cent) had normal thrombocyte count. Thrombocytopenia in haemorrhagic enteritis has been attributed to blood loss in faeces (Decaro *et al.,* 2005) or due to the direct effect of viral particles on bone marrow (McCaw and Hoskins, 2006).

A decrease in Na^+, K^+, Cl^- and glucose reported by Bhat *et al*. (2013), but in this study, the number of animals with decrease in these electrolytes (42.85 per cent, 42.85 per cent, 35.71 per cent, respectively) was less than the number of animals with normal value (50 per cent, 57.14 per cent, 57.14 per cent, respectively) for these parameters. Hyponatraemia and hypochloraemia might be due to the loss through the vomitus (Bhat *et al*., 2013) and diarrhoeic faeces. Hypernatraemia could be due to dehydration, as suggested by Goldkamp and Schaer (2007).

Recovery rate among the affected animals with therapeutic management including fluid and electrolytes, antibiotics and supportive therapy was 10 (71.42 per cent) out of total 14 dogs (Fig. 2). Four animals (disease specific mortality rate -28.57 per cent) that died due to CPV infection were puppies less than three months of age. All the dogs that died were either partially vaccinated or not vaccinated at all.

The significance of variations in haemato-biochemical and electrolyte parameters as prognostic markers was investigated by statistical analysis using independent t-test. Hypokalaemia and increased lactate levels were reported to have correlation with survival rate (Nappert *et al.,* 2002), which was not evident in the present study. Even though decrease in bicarbonate level and increased base deficit were non-significant statistically, reduction in blood levels of these parameters were evident in all the cases (100 per cent) with fatality. This implicates metabolic acidosis probably complicated with hyponatraemia (50 per cent) or hypokalaemia (25 per cent) as proposed by Mair and Love (2012). Decreased pCO_2 reflects respiratory alkalosis. Combined occurrence of metabolic acidosis and respiratory alkalosis may have negated each other leading to normal pH (75 per cent), as reported by Kirby *et al.* (2015).

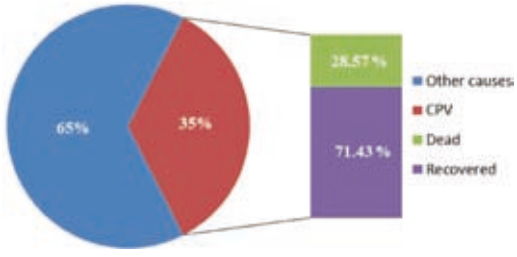


Fig. 2. Prevalence (35 per cent) and disease specific mortality rate (28.57 per cent) in dogs with CPV infection

Conclusion

The significant haemato-biochemical and electrolyte variations among CPV infected dogs warrant a better detection and correction of such variations for enhanced recovery rate among the affected animals. Mortality was found to be associated with decreased bicarbonate levels and greater base deficit. These findings suggest supplementation of intravenous sodium bicarbonate in the treatment of parvoviral enteritis. Hence assessment of blood gases, electrolytes, metabolites, haematological parameters and serum biochemical parameters are necessary to decide the proper line of treatment for CPV infections. It is always better to go for an individual analysis of these parameters rather than treating with the pattern recognition mode, as fluid and electrolyte loss varies with each and every case.

Conflict of interest

The authors report no conflict of interest.

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