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Deepika Kataria
Department of Veterinary Medicine, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Divya Agnihotri
Department of Veterinary Medicine, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Yogesh Chandrakant Bangar
Department of Animal Genetics and Breeding, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Tarun Kumar
Department of Veterinary Medicine, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Corresponding Author:
Deepika Kataria
Department of Veterinary Medicine, College of Veterinary Sciences, LUVAS, Hisar, Haryana, India

Comparative evaluation of haemato-biochemical alterations in dogs regarding CPV infection

Deepika Kataria, Divya Agnihotri, Yogesh Chandrakant Bangar and Tarun Kumar

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Abstract
Gastroenteritis in dogs comprises of multiple etiologies which includes infectious and non-infectious causes. Amongst all the factors viral gastroenteritis is the most common and major problem. Canine parvovirus (CPV) infection is the most pathogenic viral infection of dogs causing haemorrhagic gastroenteritis and myocarditis in pups. This study was planned to compare haemato-biochemical parameters in CPV infected and non CPV infected dogs which included a total of thirty six dogs which were diagnosed with polymerase chain reaction for the detection of CPV in faecal samples. CPV positive dogs showed decreased haemoglobin values with increased packed cell volume, leucocytosis, and neutrophilia with thrombocytopenia. CPV positive dogs also showed elevated levels of liver and kidney function parameters such as ALT, AST, BUN, total protein and serum creatinine. Serum levels of sodium, potassium and chloride were found to be decreased in the affected dogs. Five days of therapy lead to restoration of the clinical parameters in normal range.

Keywords: Gastroenteritis, CPV, haematological, biochemical, electrolytes

1. Introduction
Canine parvovirus (CPV) infection is considered as the most pathogenic viral infection of dogs causing haemorrhagic gastroenteritis and myocarditis mainly in young pups of less than six months of age. Acute CPV-2 enteritis can be seen in dogs of any breed, age or sex, but puppies between 6 weeks and 6 months of age are more susceptible (Panda *et al.*, 2009) [15] and the situation becomes more complicated due to the presence of different CPV variants i.e. CPV-2a, 2b and 2c. The CPV infection is considered as endemic in canine populations around the world which is characterized by anorexia, fever, lethargy, vomiting and hemorrhagic gastroenteritis in dogs of all age groups, but it is more fatal in pups. However widespread vaccination is practiced of domestic dogs but still there are several incidences of parvoviral enteritis in dogs, probably due to the emergence of new antigenic variants. Canine parvovirus is a single stranded, negative-sense DNA, 5.2 kb in length and has three structural (VP1, VP2 and VP3) and two non-structural (NS1 and NS2) proteins. Molecular diagnostic techniques like polymerase chain reaction had been the most reliable techniques having high degree of sensitivity and specificity in detecting CPV in faecal samples (Srinivas *et al.*, 2013) [19]. The highly contagious nature of canine parvoviral gastroenteritis requires earliest and rapid diagnosis so that effective and efficient management practices, control measures, and therapeutic interventions can be practiced against CPV infection.

2. Materials and Methods
The present study was conducted on thirty six dogs presented to VCC, COVS, LUVAS, and Hisar. Six apparently healthy dogs brought for routine health checkup and/or vaccination constituted the healthy control group. Complete history of the affected dogs regarding the duration of illness, appetite, frequency of vomition and diarrhoea, deworming and vaccination status was recorded. Blood was collected aseptically from each dog from cephalic/saphenous vein with 22/24 gauze scalp vein set. Two ml of blood was collected into a tube coated with K_3ethylenediamine-tetraacetic acid (K_3 EDTA) for hematological examination and three ml blood was poured in plain tube with clot activator for obtaining serum. The blood samples collected in plain tubes were kept undisturbed for one hour and then centrifuged at 3000 rpm

for 5 min and the separated serum were decanted in 2 ml eppendorf tubes and stored at-20 °C till further analysis. The blood samples stored in tubes coated with K_3 EDTA were analyzed in automated hematology cell counter (MS4s, Melet Schlosing Lab.). The haematological indices measured were haemoglobin (Hb) g/dl, packed cell volume (PCV) per cent, total leucocyte count (TLC) m/mm^3 and differential leucocyte count (DLC) (per cent) comprising of neutrophils (N) per cent, lymphocytes (L) per cent, monocytes (M) per cent, eosinophils (E) per cent and basophils (B) per cent along with total thrombocyte count (THR) m/mm^3. The serum samples were estimated using automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). The parameters measured were alanine amino transferase (ALT) (U/L), aspartate amino transferase (AST) (U/L), total protein (g/dl), urea (mg/dl) and creatinine (mg/dl). Electrolytes were measured in EasyLyte EXPAND analyzer which included sodium (mEq/L), potassium (mEq/L) and chloride (mEq/L). Faecal samples were collected from the rectum of thirty six gastroenteritic dogs for molecular study. These swabs were then mixed with 1ml of sterile PBS (pH 7.4), vortexed and stored at-20 °C. Genomic DNA was extracted using commercially available QIAamp DNA Mini Kit (Qiagen) following manufacturer's instructions. The faecal samples were screened for the presence of CPV viral DNA by amplifying the extracted DNA using conventional PCR. The cycling conditions and reaction mixture were used according to the method described by Kumar *et al.* (2011) [14]. Therapy was given for five days which included the antibiotics Ceftriaxone-Tazobactam, Ampicillin-Cloxacillin and Metronidazole were administered at the dosage of 25 mg/kg b.wt. i.m. o.d., 10 mg/kg b.wt. i.m. b.i.d. and 15 mg/kg b.wt. i.v. t.i.d. respectively. The antioxidant therapy included Vitamin C @20 mg/kg b.wt. i.v. o.d. and N-acetylcysteine (NAC) @70 mg/Kg b.wt. i.v. o.d. The therapy was supported with supportive and symptomatic treatment in accordance with the clinical conditions of affected dogs. The data obtained was analyzed by suitable statistical methods using statistical software package (SPSS 16). To compare various parameters obtained in diseased dogs with the healthy control dogs, the independent t-test was applied. For therapeutic efficacy, within and between the groups, two-way analysis of variance (ANOVA) with repeated measures was applied. The results are presented as Mean ± S.E. at 5 per cent level of significance (P<0.05). Therapeutic evaluation was estimated on the basis of remission of clinical signs and normalization hematological and biochemical values.

3. Results and discussion

A total of twenty one dogs were found positive for canine parvo virus infection out of thirty six gastroenteritic dogs, when diagnosed with PCR for CPV-2 variant which indicates that CPV infection is highly prevalent in causing gastroenteritis.

Alterations in hematological parameters of canine parvo viral gastroenteritis are represented in Table 1. Dogs found positive for CPV infection showed non-significant lower values of haemoglobin whereas non-significant higher mean levels of haemoglobin was observed in CPV negative dogs. Hemoglobinemia in gastroenteritis dogs were also reported in earlier studies (Biswas *et al*., 2005; Goddard and Leisweitz, 2010; Agnihotri *et al*., 2017; Bhargavi *et al*., 2017 and Bishnoi *et al*., 2016) [6, 11, 1, 3, 5]. On the other hand increased levels of haemoglobin were also stated in various studies on CPV gastroenteritis (Weiss and Tvedten, 2004 and Gaykwad *et al*., 2016) [21, 10] which indicates that it might be due to excessive fluid loss resulting in dehydration. In CPV positive dogs non-significant increased (P<0.05) mean values of hemoglobin were observed on day 3 and 5 of therapy while in CPV negative dogs a non-significant increase (P<0.05) was observed on day 3 of therapy. In CPV positive dogs, PCV was found non-significantly higher as compared to the control group. Increased PCV levels observed might be due to fluid losses and severe dehydration through vomition and diarrhoea as also suggested by Biswas *et al*. (2005) [6] and Bhargavi *et al*. (2017) [3]. The mean values of packed cell volume in CPV positive or CPV negative dogs found decreased non-significantly from day 0 to day 5 of the treatment. Dogs found CPV positive or found negative for the viral infection showed non- significant higher mean values of TLC than the control dogs on day 0. Leucocytosis during gastroenteritis observed in the present study could be due to secondary bacterial invasion in the damaged intestinal epithelium as also reported by Bhargavi *et al*. (2017) [3] and Bishnoi *et al*. (2016) [5]. Leucopenia in CPV enteritis is attributable to the destruction of haematopoetic progenitor cells of the various leucocyte types in the bone marrow and other lymphoproliferative organs such as thymus, lymph nodes and spleen which resulted in inadequate supply for the massive demand for leucocytes in the inflamed gastrointestinal tract (Goddard and Leisweitz, 2010) [11]. Dogs in both the groups showed normal values range of mean leucocyte count after five days of therapy. The mean values of neutrophil count were found to be significantly higher in CPV positive and CPV negative dogs as compared to the healthy control group on day 0. Neutrophilia in this study might be associated with secondary bacterial complications as also observed by Decaro and Buonavoglia (2012) [9], Agnihotri *et al*. (2017) [1] and Bhargavi *et al*. (2017) [3]. Dogs found positive or negative for CPV infection showed non-significant decrease in neutrophil count after treatment. Significantly low levels of mean lymphocyte count (P<0.05) were observed in CPV positive and CPV negative dogs than the healthy control group of dogs at day 0 in the present study. The lower levels of mean lymphocyte count might be due to the virus replication in the lymphoid organs resulting in lymphocytolysis as reported by Biswas *et al*. (2005) [6] and Dash *et al*. (2017) [8]. The changes in lymphocyte, monocyte and eosinophil count were found non-significant after treatment in both the groups and were found to be in normal limits as a result of therapy. Significant low levels of mean thrombocyte count was observed in CPV positive and CPV negative dogs as compared to the healthy control group of dogs on day 0. Thrombocytopenia in the dogs suffering from gastroenteritis might be due to the loss of blood through vomitus and faeces, increased destruction and/ or aggregation, decreased production and disseminating intravascular coagulation. It might also result from increased platelet utilization in the gastrointestinal tract combined with destruction of megakaryocyte bone marrow precursors (Rewerts and Cohn, 2000) [16]. Increase in the total platelet count was also observed in CPV positive and CPV negative groups after five days of therapy.

Table 1: Hematological alterations (Mean ± S.E.) in dogs suffering from canine parvo viral gastroenteritis

Parameters	Day	Healthy control	CPV Positive (n=21)	CPV Negative (n=15)
Hemoglobin (g/dl)	0	10.67±0.69	10.04±0.45	11.11±0.67
	3	10.67±0.69	10.73±0.40	11.44±0.52
	5	10.67±0.69	11.32±0.33	11.37±0.47
PCV (%)	0	34.83±2.71	36.03±1.37	35.69±2.14
	3	34.83±2.71	35.42±1.11	35.11±1.60
	5	34.83±2.71	34.94±0.77	34.93±1.10
TLC (m/mm3)	0	13.02±0.54	18.07±6.51	14.45±2.00
	3	13.02±0.54	16.26±3.12	12.42±1.16
	5	13.02±0.54	13.67±2.13	13.91±1.29
Neutrophil (%)	0	72.17 ± 2.95^{A}	84.23 ± 1.27^{Bb}	83.00 ± 0.20^{B}
	3	72.17 ± 2.95^{A}	81.84 ± 0.85^{Bb}	81.00 ± 1.28^{B}
	5	72.17 ± 2.95^{A}	78.32 ± 0.64^{Ba}	79.57 ± 1.04^{B}
Lymphocyte (%)	0	23.17 ± 3.71^{B}	14.71 ± 1.91^{A}	13.16 ± 1.17^{A}
	3	23.17 ± 3.71^{B}	13.81 ± 0.80^{A}	15.43 ± 1.36^{A}
	5	23.17 ± 3.71^{B}	17.84 ± 0.73^{A}	15.91 ± 1.05^{A}
Monocyte (%)	0	3.50±0.62	2.96±0.47	2.54 ± 0.58^{a}
	3	3.50±0.62	3.93±0.41	3.47 ± 0.68^{b}
	5	3.50±0.62	3.92±0.33	3.16 ± 0.31^{b}
Eosinophil (%)	0	2.33±0.33	2.10±0.38	2.33±0.33
	3	2.33±0.33	1.93±0.20	2.67±0.33
	5	2.33±0.33	2.25±0.22	2.67±0.21
Thrombocyte (m/mm3)	0	418.00 ± 59.03^{B}	212.19 ± 24.46^{A}	262.00 ± 49.66^{A}
	3	418.00 ± 59.03^{B}	234.02 ± 4.34^{A}	300.10 ± 41.64^{AB}
	5	418.00 ± 59.03^{B}	265.32 ± 21.60^{A}	278.62 ± 29.36^{A}

The means bearing different superscripts (a, b) differ significantly (P<0.05) within the groups.
The means bearing different superscripts (A, B) differ significantly (P<0.05) between the groups.

Alteration in biochemical parameters of CPV positive and CPV negative dogs is presented in Table 2. CPV affected dogs showed non-significant higher values of ALT and AST than control group before the start of therapy. These elevated levels of liver function parameters could be due to hepatic damage caused by viral infections which can lead to increased levels of enzyme activity in serum. Increase in ALT may occur as a result of hepatic hypoxia secondary to severe hypovolemia or the absorption of toxic substances due to loss of the gut barrier (Shah *et al*., 2013) [18]. Canine parvovirus positive and CPV negative group of dogs showed decreased mean values of total protein as compared to healthy control group on day 0. The decrease in the levels of total protein might be due to anorexia and decreased absorption through villi of intestines. Similar findings of decreased levels of total protein in the gastroenteritic dogs were also reported by Biswas *et al*. (2005) [6]; Baruah *et al*. (2007) [2]; Sagar *et al*. (2008) [17] and Bhargavi *et al*. (2017) [3]. While, non-significant higher values of total protein in CPV positive dogs than healthy control dogs was observed by Surendhar *et al*. (2018) [20] which could be because of dehydration caused by diarrhoeic losses in CPV infection. Significantly higher (P<0.05) mean levels of BUN were found in the dogs which were CPV positive and CPV negative as compared to the control group, while a non-significant increase in the mean levels of serum creatinine were observed in CPV positive and CPV negative dogs as compared to the healthy control group of dogs at day 0. Similar findings were also reported by Agnihotri *et al*. (2017) [1]; Bhargavi *et al*. (2017) [3] and Bishnoi *et al*. (2016) [5]. The increased values of BUN are suggestive of pre renal azotemia which might be because of reduced glomerular filtration rate (Biswas *et al*., 2005 and Bhat *et al*., 2015) [6]. Low mean values of serum sodium, potassium and chloride were also found in CPV positive and CPV negative groups as compared to the healthy control group. Hypokalaemia observed might be due to the loss of potassium in the diarrhoeic fluid along with sodium and bicarbonate. Haligur *et al*. (2009) [12] and Joshi *et al*. (2012) [13] also suggested that severe vomition, diarrhoea and dehydration in the dogs affected with gastroenteritis of varied etiologies might be responsible for hyponatremia. Hypochloremia might be due to the loss of chloride ions through vomition and diarrhoea and resulting intestinal villous atrophy, which is in agreement with Burchell *et al*. (2014) [7]. The values of ALT and AST were restored to normal in the gastroenteritic dogs in both the groups after five days of treatment. The restoration of total protein in all the affected dogs was because of hydration improvement with fluid therapy. The BUN and creatinine levels decreased to normal levels in CPV positive and negative dogs after five days of therapy indicating increased glomerular filtration rate. Fluid therapy (Ringer's lactate and DNS) helped in improving electrolyte imbalances by correcting metabolic acidosis.

Table 2: Biochemical and electrolyte alterations (Mean ± S.E.) in dogs suffering from canine parvo viral gastroenteritis

Parameters	Day	Healthy control	CPV Positive (n=21)	CPV Negative (n=15)
ALT (IU/L)	0	29.95±2.59	36.23±6.43	24.46±2.23
	3	29.95±2.59	29.95±2.59	27.51±2.37
	5	29.95±2.59	27.87±1.72	29.94±2.37
AST (IU/L)	0	39.27±3.74	67.43±19.86	34.14±2.95
	3	39.27±3.74	37.43±4.33	39.36±8.17
	5	39.27±3.74	35.60±2.39	37.44±3.44
Total Protein (g/dl)	0	6.38±0.29	6.09±0.43	5.98±0.40
	3	6.38±0.29	5.34±0.39	5.61±0.44

	5	6.38±0.29	5.00±0.40	5.23±0.46
BUN (mg/dl)	0	20.72 ± 2.17^{A}	62.67 ± 9.28^{B}	35.69 ± 5.59^{AB}
	3	20.72 ± 2.17^{A}	51.84 ± 7.03^{B}	31.61 ± 5.29^{AB}
	5	20.72±2.17	40.89±4.83	29.73±3.73
Creatinine (mg/dl)	0	0.82±0.09	1.08±0.12	1.25±0.16
	3	0.82±0.09	1.03±0.09	1.13±0.10
	5	0.82±0.09	0.96±0.06	0.99±0.06
Sodium (mEq/L)	0	143.15±2.10	137.27±1.64	135.91±2.32
	3	143.15±2.10	137.88±2.27	135.29±2.18
	5	143.15±2.10	137.29±1.90	138.38±1.54
Potassium (mEq/L)	0	5.20±0.22	4.25±0.15	4.24±0.16
	3	5.20±0.22	4.31±0.13	4.29±0.14
	5	5.20±0.22	4.42±0.17	4.45±0.16
Chloride (mEq/L)	0	107.8±01.05	103.11±1.67	101.78±2.58
	3	107.8±01.05	107.60±2.25	105.63±2.35
	5	107.8±01.05	108.70±2.02	112.14±1.07

The means bearing different superscripts (A, B) differ significantly (P<0.05) between the groups.

4. Conclusion

CPV positive dogs showed anaemia with increased packed cell volume, leucocytosis, neutrophilia with thrombocytopenia. Elevated liver and kidney function parameters along with decreased serum electrolytes were observed in all gastroenteritic dogs including those suffering from CPV infection. Although there was not much significant difference in the clinical recovery of CPV positive dogs in terms of haemato-biochemical parameters when compared to CPV negative dogs when targeted with combined antibiotic and antioxidants therapy along with supportive treatment.

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