Haematology and serum biochemistry of dogs naturally infected with canine parvovirus-2

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Abstract

Canine parvovirus is a deadly virus affecting the Canid family, causing virus-induced destruction of rapidly dividing haemopoietic precursor cells such as crypts of intestinal epithelial cells, thymus, lymph nodes, bone marrow precursor cells, blood cells and cardiac cells leading to multi-organ dysfunctions. The aim of this study was to determine the haematological, serum biochemical and electrolytic changes associated with canine parvovirus (CPV) -2 infection. An immunochromatographic test was used to differentiate the virus-positive and negative dogs using faecal samples. One hundred and sixty whole blood and serum samples were collected from apparently healthy and CPV-2-positive dogs in Plateau State, Nigeria. Haematological, serum biochemical and electrolytic analyses were done using standard methods. The data obtained were analyzed using descriptive statistics and a student *t*-test. Significance was accepted at probability values of p < 0.05. The haematological effect of CPV-2 showed a significant (P < 0.05) decrease in mean Packed Cell Volume (PCV), total red blood cell count, haemoglobin concentration, total white blood cell count, neutrophils, lymphocytes and platelet count. In addition, the CPV-2 significantly (P < 0.05) increased the mean aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, creatinine, triglyceride and malondialdehyde, while the mean total protein, sodium, potassium, chloride and cholesterol significantly (P < 0.05) decreased in the infected dogs. From the findings, CPV infection variably and significantly affected some haematological and serum biochemical parameters of infected dogs. Therefore, clinicians should endeavour to incorporate haematinics, haptatonics and immune-modulators during the management of canine parvoviral infection as supportive drugs with fluid therapy to improve the survivability of infected animals.

Introduction

Canine parvovirus is a small (about 25 nm in diameter), non-enveloped, single-stranded DNA virus. It is a significant and highly contagious canine pathogen belonging to the genus *Protoparvovirus* and the family *Parvoviridae* (Mira *et al*., 2018). Canine parvovirus (CPV) is widely distributed in the global canine population and remains a significant cause of morbidity and mortality in this species (Waner *et al*., 2004; Goddard & Leisewitz, 2010). The virus is a resistant and highly contagious agent capable of causing enteritis and myocarditis (Brunner & Swango, 1985), with severe leucopaenia in young dogs of up to 6 months of age. However, in recent years a number of cases have been reported in older dogs (Decaro *et al.,* 2008; Lamm & Rezabek, 2008; Ogbu *et al*., 2019). The principal pathogenetic factor in CPV-2 infection is the virus-induced destruction of rapidly dividing cells, including crypt intestinal epithelial cells, thymus, lymph nodes, and bone marrow precursor cells (Smith-Carr *et al*., 1997; Decaro *et al*., 2005; McCaw & Hoskins*,* 2006). As a result, intestinal mucosal barrier disruption, villous atrophy, and mal-absorption occur, leading to profuse diarrhoea and vomiting, severe dehydration or hypovolemia, metabolic acidosis (or alkalosis), bacterial translocation with subsequent coliform septicemia and endotoxemia, systemic inflammatory response syndrome (SIRS), hypercoagulability, multi-organ dysfunction, and death (Kalli *et al*., 2010; Goddard & Leisewitz, 2010; Sykes, 2014; Veir, 2014).

Canine parvoviral enteritis (PVE) has clinical similarities with other causes of acute gastrointestinal disturbances, including, though not limited to, canine distemper infection and other viral enteritides, haemorrhagic gastroenteritis, enteric bacterial infections such as salmonellosis, acute pancreatitis, hypoadrenocorticism, inflammatory bowel disease, intestinal intussusception, gastrointestinal foreign bodies, and various intoxications (Sykes, 2014). Therefore, clinical diagnosis of PVE necessitates the combination of compatible clinical and clinicopathologic abnormalities along with the detection of the viral antigen or the polymerase chain reaction (PCR)-based amplification of the viral DNA in the faeces (Mylonakis *et al*., 2016). The erythrocytic and leucocytic counts may be affected due to the effects of the infection on the bone marrow and other lymphoid tissues (Appel *et al*., 1979).

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (Halliwell & Gutteridge, 1996). Reactive oxygen species are produced by living organisms as a result of normal cellular metabolism (Mak, 2008). At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components such as lipids, proteins and DNA (Bagis *et al*., 2000; Valko *et al*., 2007).

The prognosis of CPV infection is poor; this is evident in the high mortality rate irrespective of vaccinations. CPV attacks a number of rapidly dividing cells such as crypt intestinal epithelial cells, thymus, lymph nodes, bone marrow precursor cells, blood cells and cardiac cells. These effects can lead to oxidative stress and haematological and serum biochemical changes (Schoeman *et al*., 2013; Shah *et al*., 2013). These complications have made its diagnosis and treatment difficult. Therefore, it is pertinent to study the haemato-biochemical and lipido-electrolytic changes associated with CPV-2 infection, especially with respect to age variations which is one of the intrinsic predisposing factors to parvovirus enteritis, hence the aim of the study.

Materials and Methods

Sampling technique

Two major Veterinary Clinics (Veterinary Clinics, Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom and Veterinary Clinic, University of Jos Veterinary Teaching Hospital, Jos); and four Kennels (Pam Kennel, Dog Villa, Gedeon Kennel and Shamby Kennel) from Plateau State were purposively selected for this study. Bi-monthly scheduled visits were made to the Clinics, while the Kennels were visited once throughout the period of study, and sampling was regularly done in the morning.

Forty samples comprising whole blood (20) and sera samples (20) from both apparently healthy dogs (10) and CPV-2 positive dogs (10) in Plateau State, North Central Nigeria, were collected from twenty dogs. From each dog, whole blood samples and sera samples were collected for haematological and serum biochemical analyses, respectively. These samples were collected from different breeds of dogs whose ages was categorized into two namely; 0 – 6 months and 6 months and above.

Sample collections

Rectal swab sampling for CPV-2 detection: Rectal swab samples were collected from dogs with clinical signs of gastroenteritis, such as foul, smelly and

haemorrhagic diarrhoea, vomiting, dehydration and weakness; suspected of CPV infection and also apparently healthy dogs. Rectal swabs were collected using swab sticks. The samples were tested using an immunochromatographic test kit for detection of CPV antigen (SensPERT® Canine Parvovirus Test Kit, VetAll Laboratories, Gyeonggi-do, Korea), according to the manufacturer's instructions.

Blood and serum sample collection
Blood samples for this study were collected from cephalic vein of the dogs. Blood samples (2mls) for haematology were collected into vacutainer tubes containing 2mg of Ethylene Diamine Tetracetic Acid (EDTA) as anti-coagulant. The sample bottles were rocked gently to mix the blood with the EDTA to prevent clotting. For biochemical analysis, 5mLs of blood was collected in a test tube without anticoagulant and the test tube left undisturbed in a standing position for 30 minutes to allow clotting. The clotted blood was centrifuged for 3 minutes at 30,000 revolutions per minute (rpm) using a micro centrifuge (Diehl *et al*., 2001). The serum supernatant was immediately aspirated into labelled sample bottles and refrigerated until use.

Ethical statement
The authors confirm that the experimental protocol adhered to the standard animal welfare guideline with approval number: UNN/eTC/16/81380 obtained from the Animal Ethics Committee of the University of Nigeria.

Diagnostic techniques
Immunochromatographic (IC) Test: Rectal swabs were tested using an in-clinic assay for detection of CPV antigen (SensPERT® Canine Parvovirus Test Kit, VetAll Laboratories, Gyeonggi-do, Korea), according to the manufacturer's instructions. This kit is a chromatographic immunoassay for the qualitative detection of parvovirus antigen in canine faeces. It can detect the pathogenic CPV subtypes CPV2a or CPV2b. Firstly, a faecal swab was collected per rectum or from the freshly voided stool and mixed with the assay diluents. The mixture was stirred evenly, and four (4) drops of supernatant from the extracted sample were added into the sample hole. As the test began to read, purple colouration moved across the result window in the center of the test device. The test result was interpreted within 5-10 minutes (Esfandiari & Klingeborn, 2000).

Haematology: The Haematological determinations followed standard procedures. The packed cell volume (PCV) was determined by the micro-haematocrit method (Thrall & Weiser, 2002). The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008). The red blood cell (RBC) and total leukocyte counts (TLC) were done by the haemocytometer method. Differential leukocyte count was performed on thin blood smear made on clean grease-free glass slides stained following the Leishman technique and enumerated by the meander counting method (Thrall & Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Thrall & Weiser, 2002; Stockham & Scott, 2008). The erythrocyte sedimentation rate (ESR) was determined by the microhaematocrit Wintrobe method (Coles, 1986). The platelet count (PC) was done following the direct counting method of Rees & Ecker (1923).

Serum biochemistry
Serum biochemistry determinations were carried out using commercial test kits; Randox test kits (Randox, UK) for all serum biochemistry determinations. Serum ALT and AST activities were determined by the Reitman–Frankel method (Reitman & Frankel, 1957). Serum ALP activity was determined by the phenolphthalein monophosphate method (Babson *et al*., 1966), while a total serum protein was determined by the direct Biuret method (Lubran, 1978). Serum albumin was determined by the Bromocresol green method (Doumas *et al*., 1971). Serum globulin was calculated as the difference between serum total proteins and serum albumin (Colville, 2002), while serum total bilirubin was determined by the Jendrassik–Grof method (Doumas *et al*., 1973). Blood urea nitrogen was determined by the Berthelot–Searcy method (Fawcett & Scott, 1960), while serum creatinine was determined by the modified Jaffe method (Blass *et al*., 1974). Serum cholesterol and triglyceride were determined by the enzymatic colorimetric method (Allain *et al*., 1974). Serum electrolyte concentrations (Sodium, Potassium and Chloride) were analyzed using a spectrophotometric technique (Henry *et al*., 1974)
The method of Beutler *et al*. (1963) was followed in estimating the level of reduced glutathione. The reduced form of glutathione comprises in most instances the bulk of cellular non-protein sulfhydryl groups. This method is therefore based upon the development of a relatively stable yellow colour when 5', 5'- dithios − (2-nitrobenzoic acid) (Ellman's

reagent) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of Ellman reagent with the reduced GSH, 2-nitro 5-thiobenzoic acid possesses a molar absorption at 412nm.

GSH concentration in the samples - An aliquot of the serum was deprotonated by the addition of an equal volume of 4% sulfosalicyclic acid. This was centrifuged at 4,000 rpm for 5 minutes. Thereafter, 0.5 ml of the supernatant was added to 4.5 ml of Ellman reagent. A blank was prepared with 0.5 ml of the diluted precipitating agent and 4.5ml of Ellman reagent. Reduced GSH level is proportional to the absorbance at 412 nm (Figure 1).

Lipid peroxidation was determined by measuring the levels of Malondialdehyde (MDA) produced during lipid peroxidation according to the method of Varshney & Kale (1990). This method is based on the reaction between 2-thiobarbituric acid (TBA) and MDA: an end product of lipid peroxidation. On heating in acidic pH, the product is a pink complex which absorbs maximally at 532nm and which is extractable into organic solvents such as butanol. Malondialdehyde (MDA) is often used to calibrate this test and thus the results are expressed as the amount of the free MDA produced. An aliquot of 400μl of the sample was mixed with 1.6ml of tris-KCl buffer to which 500 μl of 30% trichloroacetic acid (TCA) was added. Then 500 μl of 0.75% TBA was added and placed in a waterbath for 45 minutes at 80^0C. This was then cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected and

absorbance measured against a reference blank of distilled water at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5 \, M^{-1} \, Cm^{-1}$

LPO (MDA formed/mg protein) = Absorbance x volume of mixture

E_{532nm} x volume of sample x mg protein

MDA FORMED = mmol/mg protein

Catalase activity was determined according to the method of Claiborne (1985). This method is based on the loss of absorbance observed at 240 nm as catalase splits hydrogen peroxide. Despite the fact that hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0041 $mM^{-1} \, cm^{-1}$ (Noble & Gibson, 1970) was used.

Hydrogen peroxide (2.95 ml of 19 mM solution) was pipette into a 1 cm quartz cuvette and 50 μl of sample added (Table 1). This was done to reduce the dilution of the samples (done according to the other protocols whereby H_2O_2 was prepared separately in distilled water (100mls) and the buffer was also prepared separately.

The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 minutes.

Calculation:

Catalase activity = (ΔOD/min x volume of assay system) = μmole H_2O_2/min/mg protein (0.0041 x Volume of Sample x mg protein)
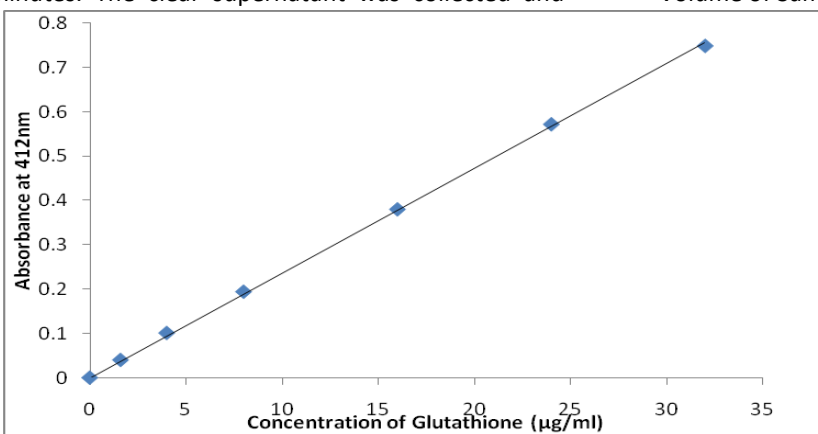
Figure 1: Standard curve for reduced glutathione

The level of SOD activity was determined by the method of Misra & Fridovich (1972). The ability of superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2 makes this reaction a basis for a simple assay for this dismutase. Superoxide ($O_2\bullet^-$) radical generated by the xanthine-oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per $O_2\bullet^-$ introduced increased with increasing pH (Valerino & McCormack, 1971) and also increased with increasing concentration of epinephrine. These results showed that autoxidation of epinephrine proceeds by at least two distinct pathways, only one of which is a free radical chain reaction involving superoxide ($O_2\bullet^-$) radical and

Table 1: Protocol for Catalase activity determination

Test	Blank	Sample
Phosphate buffer	3 mls	2.95mls
Sample	-	0.05mls
Total	3 mls	3mls

hence inhabitable by superoxide dismutase.

Statistical analysis
The data obtained were subjected to descriptive statistics using the statistical package for social sciences (SPSS) version 23. The effects of CPV-2 on haematological and serum biochemical parameters were analyzed using the student's t-test. Significance was accepted at probability values of $p < 0.05$. The results are presented in Tables.

Results
CPV-2 infection significantly ($P < 0.05$) affected the mean erythrocytic parameters of the infected dogs. The mean PCV, RBC counts and Hb concentrations were significantly lower in the CPV-2 infected than uninfected (Table 2).
Analysis of erythrocytic parameters of infected and uninfected dogs based on age showed that CPV-2 significantly ($P < 0.05$) decreased the mean PCV, RBC counts, and Hb concentration of infected puppies when compared with uninfected puppies (Table 2). Although there was a decrease in the mean PCV, RBC counts, Hb concentration of infected adult dogs, it did not differ significantly ($P > 0.05$) when compared with uninfected adult dogs (Table 2). CPV-2 infection significantly decreased mean MCHC ($P < 0.05$), whereas the decrease recorded in mean MCV and MCH, was not statistically significant ($P > 0.05$) when compared with the uninfected dogs (Table 2). Although there were decreases in the mean erythrocytic corpuscular values when analyzed based on age, it was not statistically significant ($P > 0.05$) when compared with the uninfected dogs of the same age group (Table 2). The mean leucocytic values and platelet counts were significantly ($P < 0.05$) decreased among CPV-2 infected dogs when compared with uninfected dogs (Table 2).
Analysis of the mean leucocytic values and platelet counts of infected and uninfected dogs based on age showed that CPV-2 significantly ($P < 0.05$) decreased mean WBC, neutrophils, lymphocytes and platelet counts among infected puppies (Table 2) and also decreased significantly ($P < 0.05$) WBC count, lymphocytes and platelet counts among the infected adults when compared with uninfected adult dogs (Table 2).
CPV-2 infection significantly ($P < 0.05$) increased the mean AST, ALT and ALP but significantly ($P < 0.05$) decreased TP and ALB (Table 3). Total bilirubin (TB) and direct bilirubin (DB) were not affected by CPV-2 infection. Among the puppies, CPV-2 significantly ($P < 0.05$) increased the mean AST, ALT and ALP but the

mean TP and ALB were significantly ($P < 0.05$) decreased (Table 3). The infection also significantly ($P < 0.05$) increased the mean ALT and ALP among adult dogs, while the means of other parameters did not differ significantly ($P > 0.05$) between infected and uninfected dogs (Table 3).
The mean urea and creatinine concentrations were significantly ($P < 0.05$) increased, but the mean Na, K and Cl were significantly ($P < 0.05$) decreased in CPV-2 infected dogs (Table 4). The analysis based on age groups revealed that the mean urea and creatinine were significantly ($P < 0.05$) increased in the CPV-2 infected puppies, whereas the mean Na, K and Cl were significantly ($P < 0.05$) decreased (Table 4). Although there was an increase in the mean urea and creatinine concentrations and a decrease in mean Na, K and Cl in CPV-2 infected adults when compared with uninfected adult dogs, the changes were not statistically significant ($P > 0.05$) (Table 4).
CPV-2 infection significantly ($P < 0.05$) decreased the mean cholesterol but significantly ($P < 0.05$) increased the mean triglyceride in the infected dogs. The mean low-density lipoprotein and high-density lipoprotein were decreased among the infected dogs but did not differ significantly ($P > 0.05$) when compared with the uninfected dogs (Table 5). The lipid profiles of both CPV-2 infected and uninfected dogs did not differ significantly ($P > 0.05$) based on the age groups (Table 5).
The mean MDA was significantly ($P < 0.05$) higher in CPV-2 infected dogs, while the means of GSH, CAT and SOD did not differ significantly ($P > 0.05$) when compared with the uninfected dogs (Table 6). There was no significant difference ($P > 0.05$) in the mean of all the oxidative stress biomarkers analyzed between the CPV-2 infected and uninfected puppies (Table 6). Among the adult dogs examined, the mean MDA significantly ($P < 0.05$) increased in the infected dogs, while the changes in mean GSH, CAT and SOD were not significant ($P > 0.05$) when compared with the CPV-2 uninfected adult dogs (Table 6).

Discussion
The CPV-2 infection in dogs affected the erythrocytic (PCV, RBC, HB, MCHC), leukocytic (WBC, neutrophils, lymphocytes) parameters, and platelets manifested in anaemia thrombocytopaenia and leucopaenia associated with neutropaenia and lymphopaenia. This is similar to Castro *et al*. (2013), Shah *et al*. (2013), Dash *et al*. (2017) and Andrea *et al.* (2017), who reported decreased haematological parameters in CPV-2 affected dogs. Amaravathi *et al*. (2016), Bhargavi *et al*. (2017) and Arora *et al*. (2018) also

Table 2: Overall mean ± SEM of haematological parameters of CPV-2 infected dogs in North Central Nigeria

Parameters	CPV-2 Infected Dogs			CPV-2 Infected Puppies			CPV-2 Infected Adults dogs		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Pack cell volume (%)	21.20 ± 0.55^a	43.20 ± 1.39^b	0.027	19.33 ± 0.33	41.67 ± 1.12	0.040	21.50 ± 1.04	40.50 ± 0.87	0.750
Red blood cell count ($\times 10^{12}$/L)	4.38 ± 0.17^a	10.82 ± 0.68^b	0.002	4.37 ± 0.22^a	11.55 ± 0.92^b	0.006	4.40 ± 0.33	9.73 ± 0.83	0.123
Haemoglobin concentration. (g/dl)	6.90 ± 0.48^a	13.50 ± 1.11^b	0.029	7.17 ± 0.60^a	14.00 ± 1.81^b	0.012	6.50 ± 0.87	12.75 ± 0.85	1.000
Mean corpuscular volume (fl)	54.72 ± 1.57	62.83 ± 2.04	0.469	53.88 ± 2.49	60.34 ± 2.30	0.640	55.99 ± 1.45	66.57 ± 3.21	0.213
Mean corpuscular haemoglobin (Pg)	22.36 ± 0.77	26.37 ± 1.04	0.190	22.19 ± 1.23	24.62 ± 1.04	0.776	22.61 ± 0.82	28.99 ± 1.24	0.534
Mean corpuscular haemoglobin concentration (g/dl)	27.69 ± 0.81^a	33.56 ± 1.21^b	0.027	27.99 ± 1.30	31.94 ± 1.35	0.770	22.25 ± 0.79	35.98 ± 1.79	0.249
White blood cell count ($\times 10^9$/L)	2.75 ± 0.19^a	11.12 ± 0.40^b	0.026	2.39 ± 0.22^a	9.63 ± 0.56^b	0.005	2.66 ± 0.30^a	11.62 ± 0.67^b	0.004
Neutrophil (%)	23.66 ± 0.66^a	80.28 ± 1.98^b	0.018	20.90 ± 0.53^a	78.97 ± 3.03^b	0.038	23.79 ± 1.07	82.25 ± 2.03	0.106
Lymphocyte (%)	8.53 ± 0.47^a	40.50 ± 1.44^b	0.016	8.27 ± 0.71^a	43.10 ± 1.52^b	0.020	8.91 ± 0.57^a	36.59 ± 1.19^b	0.044
Platelets ($\times 10^3$/L)	2.30 ± 0.89^a	8.40 ± 0.45^b	0.010	2.50 ± 0.26^a	8.58 ± 0.54^b	0.032	2.00 ± 0.20^a	8.13 ± 0.88^b	0.029

Different superscripts in a row (a and b) indicate a significant difference between the group mean values of the compared group at ($P<0.05$) while lack of superscript indicates no significant difference

Table 3: Overall mean ± SEM of liver function markers of CPV-2 infected dogs in North Central Nigeria

Parameters	CPV-2 Infected Dogs			CPV-2 Infected Puppies			CPV-2 Infected Adults dogs		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Aspartate aminotransferase (AST) (u/l)	154.83 ± 3.10^b	29.14 ± 1.31^a	0.006	154.15 ± 4.30^b	27.20 ± 1.63^a	0.002	155.85 ± 5.02	32.05 ± 1.24	0.180
Alanine aminotransferase (ALT) (u/l)	156.57 ± 7.47^b	34.64 ± 1.66^a	0.006	161.62 ± 11.10	33.82 ± 2.65	0.051	148.99 ± 8.85^b	35.86 ± 1.48^a	0.000
Alkaline phosphatase (ALP) (u/l)	143.88 ± 7.81^b	42.36 ± 2.29^a	0.004	152.80 ± 9.90^b	45.18 ± 2.70^a	0.009	135.50 ± 6.84^b	35.63 ± 2.09^a	0.028
Total Bilirubin (mmol/l)	0.46 ± 0.08	1.09 ± 0.08	0.717	0.48 ± 0.10	1.04 ± 0.11	0.751	0.43 ± 0.16	1.17 ± 0.13	0.425
Direct Bilirubin (mmol/l)	1.52 ± 0.12	1.44 ± 0.11	0.942	1.56 ± 0.16	1.43 ± 0.15	0.931	1.47 ± 0.14	1.47 ± 0.18	0.744
Total Protein (g/l)	1.85 ± 0.15^a	7.58 ± 0.31^b	0.031	1.95 ± 0.19	7.38 ± 0.43	0.012	1.69 ± 0.26	7.87 ± 0.47	0.451
Albumin (g/l)	2.51 ± 0.31^a	4.18 ± 0.22^b	0.029	2.62 ± 0.51	3.86 ± 0.23	0.038	4.65 ± 0.34	2.34 ± 0.25	0.758

Different superscripts in a row (a and b) indicate a significant difference between the group mean values of the compared group at ($P<0.05$) while lack of superscript indicates no significant difference

Table 4: Overall mean ± SEM of kidney function markers and electrolytes of CPV-2 infected dogs in North Central Nigeria

Parameters	CPV-2 Infected Dogs			CPV-2 Infected Puppies			CPV-2 Infected Adults dogs		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Urea (mmol/l)	38.78±3.40[b]	10.48±1.10[a]	0.007	28.36±3.28[b]	8.61±0.41[a]	0.000	51.91±3.22	12.03±1.07	0.131
Creatinine (mg/dL)	3.37±0.34[b]	0.63±0.11[a]	0.012	2.53±0.25[b]	0.44±0.23[a]	0.016	4.13±0.32	0.92±0.20	0.420
Sodium (mEg/l)	89.25±2.96[a]	158.39±4.94[b]	0.036	91.63±3.23[a]	159.71±5.88[b]	0.047	109.69±7.03	156.40±9.72	0.312
Potassium (mEg/l)	1.21±0.91[a]	6.03±0.28[b]	0.025	1.62±0.35[a]	4.26±0.11[b]	0.021	2.36±0.43	4.68±0.23	0.492
Chloride (mEg/l)	70.32±1.62[a]	141.87±3.46[b]	0.015	101.59±4.57[a]	123.87±1.85[b]	0.029	102.93±5.33	128.87±1.22	0.058

Different superscripts in a row (a and b) indicate a significant difference between the group mean values of the compared group at ($P<0.05$) while lack of superscript indicates no significant difference

Table 5: Mean lipid profile of CPV-2 naturally infected dogs in North Central Nigeria

Parameters	CPV-2 Infected Dogs			CPV-2 Infected Puppies			CPV-2 Infected Adults dogs		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Cholesterol (mg/dL)	92.65±2.48[a]	180.85±4.57[b]	0.011	93.98±3.55	186.75±5.85	0.230	90.65±3.53	171.98±5.25	0.567
Triglyceride (mg/dL)	307.78±6.93[b]	101.67±3.37[a]	0.001	291.44±2.87	94.45±2.32	0.974	332.30±2.23	112.52±2.50	0.969
Low DensityLipoprotein (mg/dL)	88.66±2.55	111.66±2.95	0.486	92.68±2.68	113.11±4.18	0.132	82.62±3.26	109.49±4.35	0.521
High Density Lipoprotein (mg/dL)	29.11±1.31	56.92±2.10	0.273	30.20±1.65	58.03±2.45	0.570	27.47±2.16	55.25±4.07	0.109

Different superscripts in a row (a and b) indicate a significant difference between the group mean values of the compared group at ($P<0.05$) while lack of superscript indicates no significant difference

Table 6: Overall mean ± SEM of oxidative stress markers of CPV-2 infected dogs in North Central Nigeria

Parameters	CPV-2 Infected Dogs			CPV-2 Infected Puppies			CPV-2 Infected Adults dogs		
	Positive	Negative	P-value	Positive	Negative	P-value	Positive	Negative	P-value
Malondialdehyde	151.23±14.91[b]	69.41±5.70[a]	0.016	123.72±8.07	61.77±7.24	0.568	162.49±6.67[b]	70.86±3.02[a]	0.042
Reduced Glutathione	6.02±0.51	8.84±0.39	0.135	6.00±0.61	9.23±0.41	0.056	6.05±1.01	8.25±0.70	0.557
Catalase	1.94±0.32	1.01±0.12	0.136	2.23±0.51	0.98±0.15	0.124	1.50±0.17	1.06±0.22	0.774
Superoxide Dismutase	1541.96±17.41	1008.95±13.21	0.151	1665.18±23.70	908.26±19.35	0.313	1357.13±25.48	1159.97±17.60	0.490

Different superscripts in a row (a and b) indicate significant difference between the group mean values of the compared group at ($P<0.05$) while lack of superscript indicates no significant difference

reported that CPV-2 caused a significant reduction in haematological parameters of infected dogs. These effects may be due to haemorrhagic gastroenteritis sequel to sloughing of intestinal epithelial and/or destruction of intestinal capillary villi (Hoskins, 1998; Mallela *et al.,* 2006; Mohan *et al.,* 2010; Sulthana, 2015), inadequate compensation for the lost blood cells and high demand for blood cells due to destruction of haematopoietic progenitor cells in the bone marrow and other lymphopoetic organs like thymus, spleen and lymph nodes (Goddard *et al.,* 2008). Greene (2012), Decaro & Buonavoglia (2012) and Judge (2015) also reported that leucopenia with attendant lymphopaenia and neutropaenia is a common condition in parvoviral enteritis due to the CPV effect on rapidly dividing leukocytic precursor cells of the bone marrow and lymphopoetic damage during the early stage of the infection. Meanwhile, some authors reported neutrophila, lymphocytosis and thrombocytosis in CPV infected dogs, which may be attributed to the stage of infection during the blood collection (Ramprabhu*et al* 2002; Chakrabarti, 2003; Kalli *et al.* 2010; Monteiro *et al.,* 2016; Kumar & Rajni, 2017; Agnihotri *et al.,* 2017; Kumar & Rajni, 2017; Mohanta *et al.,* 2018).

The significant effect of CPV on the infected dogs based on age was supported by the report of Dash *et al*. (2017), who also reported that the haematological effect of CPV was more in puppies than in an adult. This could be due to the high susceptibility of puppies to CPV consequent upon the affinity for its rapidly dividing cells, inadequate/lack of immune response by puppies and deleterious effects of the virus on the maturing haematopoietic tissues.

CPV caused a significant increase in some liver function markers (AST, ALT, ALP, TP and albumin), kidney function markers (urea and creatinine) and triglyceride but decreased electrolytes (Na, K and Cl) and cholesterol. This is in consonance with Shah *et al*. (2013), Bastan *et al*. (2013), Amaravathi *et al*. (2016), Agnihotri *et al*. (2017), Salem *et al*. (2018) and Arora *et al*. (2018), who reported the same biochemical changes in CPV infected dogs. These biochemical effects could be a result of liver and kidney involvements, and intestinal villi destruction leading to protein-losing-enteropathy (Grigonis *et al.* 2002; Kumar and Rajni, 2017) and absorption of toxic substances sequel to depletion of gut barriers leading to hepatic hypoxia (Mohan *et al.,* 1993; Kahn & Line, 2005; Ettinger (2010); Shah *et al.,* 2013). Hypokalaemia, hyponatraemia and hypochloremia in CPV infected dogs could be attributed to excretion of potassium due to diarrhoea, loss of sodium and

chloride ions due to severe gastroenteritis and associated vomiting, respectively (Hoskins, 1998; Ettinger, 2010).

The effect of parvoviral enteritis on serum biochemistry based on age groups of the dog manifested in an increase in AST, ALP, urea and creatinine while TP, albumin, Na, K, and Cl, were decreased among the infected puppies. Among the adult dogs, the biochemical effect was evidenced only by increased ALT and ALP, while other parameters did not differ significantly. The severe effect on the puppies compared to adult dogs could be due to the naivety of the organs and preferential susceptibility of CPV in young ones due to the high rate of mitotic division. Also the adult dogs may have acquired a certain level of either humoral or cellular immunity due to previous vaccinations, recovery from the infection or subclinical infections.

The oxidative stress biomarker that was significantly affected by the infection was malondialdehyde (MDA). This aligns with Panda *et al*. (2009), who reported a significant increase in lipid peroxide levels. Russo & Bracarense (2016) stated that the measurement of oxidative stress biomarkers is used to determine the level of lipid peroxidation of the cell membrane. MDA is a product of lipid peroxidation of cell membranes and is one of the general biomarkers of oxidative damage (Sim *et al.,* 2003; Kadiiska *et al.,* 2005). According to Del Rio *et al*. (2005) and Russo & Bracarense (2016), MDA is both genotoxic and cytotoxic and has deleterious interaction with DNA and proteins of the host. The presence of many prognosticators has made detections of patients at high risk of death and their subsequent planned management more encouraging and successful (Schoeman *et al.,* 2013). The significant effect of diseases such as parvoviral enteritis on the levels of oxidative stress biomarkers may be due to a decrease in micronutrients that form an essential part of antioxidants due to bloody diarrhoea and anorexia (Panda *et al.,* 2009).

From the findings, it could be concluded that CPV infection variably and significantly affected the infected dogs' haematological, serum biochemical, and fatty acid levels. These could serve as prognosticators in determining the level of damage resulting from the multi-organ effects of CPV infection and aid in the clinical decisions during the management of parvoviral enteritis.

Management of CPV infection comprising haematinics, haptatonics and immune-modulators as supportive drugs with fluid therapy to improve the survivability of the infected animal is recommended.

Conflict of interest

The authors declare that there is no conflict of interest.

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