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 paroviral infection as supportive drugs with fluid therapy to improve the survivability of infected animals.

Keywords: Age, Canine parvovirus, Dog, Haematology, Natural infection, Serum biochemical
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 malabsorption occur, leading to profuse diarrhoea and vomiting, severe dehydration or hypovolemia, metabolic acidosis (or alkalosis), bacterial translocation with subsequent coliform septicemia and endotoxaemia, 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 haematobiochemical and lipid-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 formula (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).

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′- dithio – (2-nitrobenzoic acid) (Ellman’s
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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 412 nm.

GSH concentration in the samples - An aliquot of the serum was deprotonated by the addition of an equal volume of 4% sulfosalicylic 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 4.5 ml 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 532 nm 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 400 µl of the sample was mixed with 1.6 ml 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°C. 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 x 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 pipetted 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 (100 mls) 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)

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•^-) radical generated by the xanthine-oxidase reaction caused the oxidation of epinephrine to adrenochrome and the yield of adrenochrome produced per O_2•^- 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•^-) radical and

Figure 1: Standard curve for reduced glutathione

Table 1: Protocol for Catalase activity determination

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer</td>
<td>3 mls</td>
<td>2.95 mls</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.05 mls</td>
</tr>
<tr>
<td>Total</td>
<td>3 mls</td>
<td>3 mls</td>
</tr>
</tbody>
</table>
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 thrombocytopenia 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Positive Mean ± SEM</th>
<th>Negative Mean ± SEM</th>
<th>P-value</th>
<th>Positive Mean ± SEM</th>
<th>Negative Mean ± SEM</th>
<th>P-value</th>
<th>Positive Mean ± SEM</th>
<th>Negative Mean ± SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pack cell volume (%)</td>
<td>21.20±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.20±1.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.027</td>
<td>19.33±0.33</td>
<td>41.67±1.12</td>
<td>0.040</td>
<td>21.50±1.04</td>
<td>40.50±0.87</td>
<td>0.750</td>
</tr>
<tr>
<td>Red blood cell count (× 10&lt;sup&gt;12&lt;/sup&gt;/L)</td>
<td>4.38±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.82±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
<td>4.37±0.22</td>
<td>11.55±0.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.006</td>
<td>4.40±0.33</td>
<td>9.73±0.83</td>
<td>0.123</td>
</tr>
<tr>
<td>Haemoglobin concentration (g/dl)</td>
<td>6.90±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.50±1.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.029</td>
<td>7.17±0.60</td>
<td>14.00±1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.012</td>
<td>6.50±0.87</td>
<td>12.75±0.85</td>
<td>1.000</td>
</tr>
<tr>
<td>Mean corpuscular volume (fl)</td>
<td>54.72±1.57</td>
<td>62.83±2.04</td>
<td>0.469</td>
<td>53.88±2.49</td>
<td>60.34±2.30</td>
<td>0.640</td>
<td>55.99±1.45</td>
<td>66.57±3.21</td>
<td>0.213</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin (Pg)</td>
<td>22.36±0.77</td>
<td>26.37±1.04</td>
<td>0.190</td>
<td>22.19±1.23</td>
<td>24.62±1.04</td>
<td>0.776</td>
<td>22.61±0.82</td>
<td>28.99±1.24</td>
<td>0.534</td>
</tr>
<tr>
<td>Mean corpuscular haemoglobin concentration (g/dl)</td>
<td>27.69±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.56±1.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.027</td>
<td>27.99±1.30</td>
<td>31.94±1.35</td>
<td>0.770</td>
<td>22.25±0.79</td>
<td>35.98±1.79</td>
<td>0.249</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CPV-2 Infected Dogs</th>
<th>CPV-2 Infected Puppies</th>
<th>CPV-2 Infected Adults dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>38.78±3.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.48±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.007</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>3.37±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.012</td>
</tr>
<tr>
<td>Sodium (mEq/l)</td>
<td>89.25±2.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.39±4.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.007</td>
</tr>
<tr>
<td>Potassium (mEq/l)</td>
<td>1.21±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.03±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.007</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CPV-2 Infected Dogs</th>
<th>CPV-2 Infected Puppies</th>
<th>CPV-2 Infected Adults dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>92.65±2.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.85±4.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.011</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>307.78±6.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>291.44±2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td>Low Density Lipoprotein (mg/dL)</td>
<td>88.66±2.55</td>
<td>111.66±2.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.015</td>
</tr>
<tr>
<td>High Density Lipoprotein (mg/dL)</td>
<td>29.11±1.31</td>
<td>56.92±2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.273</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CPV-2 Infected Dogs</th>
<th>CPV-2 Infected Puppies</th>
<th>CPV-2 Infected Adults dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>151.23±14.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.41±5.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.016</td>
</tr>
<tr>
<td>Reduced Glutathione</td>
<td>6.02±0.51</td>
<td>8.84±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.135</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.94±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.136</td>
</tr>
<tr>
<td>Superoxide Dismutase</td>
<td>1541.96±17.41</td>
<td>1008.95±13.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.151</td>
</tr>
</tbody>
</table>

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 lymphopoietic 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 lymphopoietic damage during the early stage of the infection. Meanwhile, some authors reported neutrophi, lymphocytosis and thrombocytosis in CPV infected dogs, which may be attributed to the stage of infection during the blood collection (Rampabhuuet 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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