Clinical, hematological and biochemical assessment of dogs naturally infected with canine parvovirus before and after therapy

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ABSTRACT

To evaluate the effect of canine parvovirus infection on hematological and biochemical parameters in dogs, forty-two dogs aged 2 - 9 months admitted to the Veterinary Hospital at Faculty of Veterinary Medicine, Zagazig University and private clinics at Sharqia governorate between August 2018 and August 2019 were included in the present study. The canine parvovirus infected dogs (n=32) were positive for the rapid CPV Ag test kit with clinical signs of fever, diarrhea, vomiting, depression and anorexia. Hematological examination in canine parvovirus infected dogs showed decreased hemoglobin, RBCs, PCV% and leukocytes due to lymphopenia and neutropenia compared to healthy control dogs (n=10). The ALT, AST, globulin, alkaline phosphatase and serum creatinine significantly increased. After treatment of infected dog with intravenous fluid therapy, antibiotics, anti-emetics, hematological and biochemical parameters were improved after recovery. We concluded that, canine parvovirus infection has an effect on the hematological and biochemical findings dogs.

1. INTRODUCTION

Canine parvovirus infection is considered an important problem affecting dogs at an early stage of life and is an important cause of morbidity and mortality worldwide, it causes fever, diarrhea, vomiting and severe dehydration which predisposing to acute kidney injury (Nan et al., 2018). It is considered the main cause of diarrhea in dogs less than 6 months of age (Hackett and Lappin, 2003; Prittie, 2004; Yesilbas et al., 2007; Schulz et al., 2008). It usually affects dogs at any age, although puppies are more susceptible between 6 weeks and 6 months of age (Godsall et al., 2010; Iris et al., 2010). Adults with decreased or insufficient immunization program might be affected (Iris et al., 2010; Markovich et al., 2012). The pathogens spreads among dogs through fecal - oral route where the virus is shedded through feces 3 days after natural infection and continues for an incubation period of 3 - 4 weeks after the appearance of clinical disease (Decaro et al., 2005a; Godsall et al., 2010). Exposure of susceptible dogs to infected feces or vomits predispose to infection (Sykes, 2014). There are two types of canine parvovirus (CPV); CPV 1 and CPV 2. Most cases infected with CPV 1 are asymptomatic, while CPV 2 is mostly contagious (Nandi and Kumar, 2010a). Several studies were performed to describe the clinical features, hematological and biochemical parameters in dogs naturally (Azetaka et al., 1981; Carman and Povey, 1982) or experimentally (Hirasawa et al., 1987; Sutton et al., 2013; Parker et al., 2017) infected with canine parvovirus. Canine parvovirus is most commonly manifested as gastroenteritis. The initial clinical signs are nonspecific and characterized by loss of appetite, depression, and lethargy (Lamm and Rezabek, 2008). Typical clinical signs include vomiting, mucoid to hemorrhagic diarrhea, depression and leukopenia especially neutropenia and lymphopenia (Duffy et al., 2010; Hackett, 2011). However not all dogs infected with canine parvovirus are presented with bloody diarrhea. Fever may be observed in most cases (Decaro et al., 2005b). The intestinal mucosa is disrupted results in atrophy in the intestinal villi and malabsorption with profuse diarrhea and vomiting, the diarrhea and vomiting in some cases are hemorrhagic (Turk et al., 1990). In many small animal clinics, canine parvovirus is diagnosed based on the clinical signs and some can rely on the rapid CPV Test kits (Tinky et al., 2015). Although the most accurate method for diagnosis of canine parvovirus infection is the use of polymerase chain reaction on fecal samples which is highly recommended (Saasa et al., 2016). Canine parvovirus infection is usually accompanied by septicemia and toxemia which leads to systemic inflammatory response syndrome that progresses to multiple organ dysfunction and death (Turk et al., 1990). The dehydration status that follows parvovirus infection results in increased liver enzymes activity (van den Broek, 1990), which is predispose to the occurrence of acute kidney injury (Schmidt-Ott et al., 2007; Schoeman et al., 2013; van den Berg et al., 2018). Early kidney damage was observed in canine parvovirus infection accompanied by elevation of blood urea nitrogen and creatinine (van den Berg et al., 2018). Early detection of high-risk condition that put the kidneys and liver under acute
stress allows introducing the suitable intensive treatment and might improve the outcome of canine parvovirus infection (van den Berg et al., 2018). The aim of the present study was to investigate the effect of canine parvovirus infection on the hematological and biochemical parameters. Treatment trial was performed for diseased dogs to monitor the improvement in hematological and biochemical findings after therapy.

2. MATERIAL AND METHODS

2.1. Animals and study design
The present study was conducted on forty-two dogs aged between 2 and 9 months with different breeds (German shepherd, Golden, Griffon, Pit bull, Rottweiler, and Husky) and average body weight of 8.8 kg, admitted to Veterinary Hospital of Faculty of Veterinary Medicine, Zagazig University and to a private pets clinic in Sharkia governorate during the period between August 2018 and August 2019. The dogs were classified into two groups; control group (n = 10) and canine parvovirus group (n = 32). Diseased dogs were diagnosed based on the clinical signs presented on admission and positive fecal CPV by rapid CPV Ag test kit. Following examination, the dogs were treated according to the standards for emergency care for pets. This study not includes experimental work, so the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC) was not required. Owners of the dogs have approved to include their dogs in the current study.

2.2. Clinical examination and samples collection
The dogs under investigation were examined clinically and thoroughly investigated at the time of admission. Fecal swabs were collected from diseased dogs included in the present study and used directly for rapid CPV Ag test kit (Lot no. 201806112, Zhenuri Biotech Co., Ltd., Shenzhen, China) according to the manufacturer’s manual. Blood samples were collected from all dogs, the sampling procedures were performed on admission (T₀) and 2 weeks following treatment (T₁) in diseased dogs, while in control dogs the sampling were performed once on admission (T₀). Blood was obtained from the cephalic vein and divided into two parts. First part was collected on EDTA tubes for whole blood samples and the 2nd part in plain tubes for serum collection. EDTA samples were examined immediately for hematological findings, including RBCs, WBCs, and differential leucocytic count, PCV%, hemoglobin and erythrocytotic and anemia indices using CELL - DYN 3500 (Spectra Diagnostika, California, USA). Hepatic and renal functional parameters were analyzed using a blood chemistry analyzer (Konelab Prime 60i, Konelab, Finland) included serum creatinine, blood urea nitrogen; AST, ALT, Alkaline phosphatase, total protein, albumin, and globulin.

2.3. Therapeutic trials
The diseased dogs were received symptomatic treatment after admission including intravenous fluid therapy, antibiotics (ampicillin 20 mg/kg in combination with metronidazole at a rate of 10 mg/kg through i/v route 3 - 4 times daily), anti-emetics (Ondansetron 1 mg/kg i/v) according to Martin et al. (2002), de Mari et al. (2003), Sykes (2014) and Mylonakis et al. (2016). Emetics (Ondansetron 1 mg/kg i/v) were administered three times daily (0800, 1200, and 2000), anti-emetics (Ondansetron 1 mg/kg i/v) were administered thrice daily (0800, 1200, and 2000), and anti-diarrheal drugs (Atorvastatin 0.5 mg/kg i/m) were given for 2 doses. In addition, symptomatic treatment (emetics, fluid therapy, and anti-diarrheal drugs) was administered for all dogs during application of therapy due to severe dehydration and deterioration in general health condition. There was an improvement in the results of the hemoglobin, erythrocytes,

2.4. Statistical analysis
The results were statistically analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and were expressed as mean ± standard deviation (SD). The differences between the diseased and control dogs were analyzed using t-test. After therapy (T₁) results were compared to that obtained on admission (T₀) using t-test. The results were considered significant at a p<0.05.

3. RESULTS

3.1. Study population
3.2. Results of clinical examination
According to the clinical examination and the results of rapid CPV Ag test kits, the dogs were classified into control dogs (n=10) which were clinically healthy with no signs of the disease; there were no signs of diarrhea and negative fecal CPV Ag test. CPV infected dogs (n=32) showed signs of fever, diarrhea which were hemorrhagic in 12 dogs (37.5 %) and without blood in 20 dogs (62.5 %), vomiting, depression and anorexia. The fecal CPV Ag test was positive. The case history revealed that the CPV diseased dogs were unvaccinated against parvovirus according to the owner’s declaration. The dogs after treatment showed an improvement in the general health condition and disappearance of signs if diarrhea and dehydration.

3.3. Hematological analyses
Hematological examination table (1) showed that the hemoglobin concentration significantly decreased in canine parvovirus infected dogs compared to clinically healthy dogs. The erythrocytes were significantly decreased in canine parvovirus dogs compared to clinically healthy dogs. The packed cell volume in diseased dogs was significantly decreased compared to control dogs. The leucocytes count, neutrophils and lymphocytes were significantly reduced in canine parvovirus infected dogs compared to clinically healthy dogs. The platelets, monocytes, eosinophils and basophils did not differ significantly.

3.4. Biochemical analyses
Biochemical examination table (2) showed that, serum albumin significantly decreased in canine parvovirus infected dogs compared to clinically healthy dogs. The serum globulin was significantly increased in diseased dogs compared to clinically healthy dogs. Serum ALT, AST, alkaline phosphatase and serum creatinine were significantly increased in canine parvovirus infected dogs compared to clinically healthy dogs. The serum total protein and blood urea nitrogen did not differ significantly between the two groups.

3.4. Treatment trials
The diseased dogs were received an intensive care therapy included intravenous fluid therapy, antibiotic especially ampicillin at a dose rate of 20 mg/kg, anti-emetics. The protocol for treatment was given for at least 3 – 4 times daily until recovery. The hematological and biochemical analyses after therapy (T₁) were compared to that before therapy (T₀), the results are presented in table (1 and 2). Four dogs died during application of therapy due to severe dehydration and deterioration in general health condition. There was an improvement in the results of the hemoglobin, erythrocytes,
packed cell volume, leucocytes, neutrophils and lymphocytes in blood samples after therapy compared to that before therapy. The serum albumin, globulin, serum ALT, AST, alkaline phosphatase and creatinine values were significantly returned to normal values as in clinically healthy dogs.

Table 1 Hematological analysis of healthy control dogs and CPV infected dogs before (T₀) and 6 weeks after (T₁) treatment. Results are expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy control dogs</th>
<th>CPV infected dogs before treatment (T₀)</th>
<th>CPV infected dogs after treatment (T₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.4±0.45</td>
<td>9.6±0.6*</td>
<td>15.7±0.02</td>
</tr>
<tr>
<td>RBCs x10⁶/µl</td>
<td>6.8±0.98</td>
<td>4.2±0.63*</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>Packed cell volume %</td>
<td>48±0.3</td>
<td>34±0.1*</td>
<td>42±0.6</td>
</tr>
<tr>
<td>Platelets x10⁹/µl</td>
<td>300±1.2</td>
<td>280±2.1</td>
<td>310±0.8</td>
</tr>
<tr>
<td>WBCs x10⁶/µl</td>
<td>13.9±0.3</td>
<td>5.6±0.4*</td>
<td>10.9±0.8</td>
</tr>
<tr>
<td>Neutrophils x10⁶/µl</td>
<td>9±0.02</td>
<td>3.5±0.3*</td>
<td>7.8±0.9</td>
</tr>
<tr>
<td>Lymphocytes x10⁶/µl</td>
<td>3.6±0.05</td>
<td>1.8±0.02*</td>
<td>2.9±0.01</td>
</tr>
<tr>
<td>Monocytes x10⁶/µl</td>
<td>0.9±0.01</td>
<td>0.6±0.01</td>
<td>1±0.01</td>
</tr>
<tr>
<td>Eosinophils x10⁶/µl</td>
<td>0.2±0.01</td>
<td>0.2±0.01</td>
<td>0.2±0.01</td>
</tr>
<tr>
<td>Basophils x10⁶/µl</td>
<td>0.12±0.01</td>
<td>0.11±0.01</td>
<td>0.12±0.01</td>
</tr>
</tbody>
</table>

* Significant difference at p-value ≤0.05.

Table 2 Biochemical analysis of healthy control dogs and CPV infected dogs before (T₀) and 6 weeks after (T₁) treatment. Results are expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy control dogs</th>
<th>CPV infected dogs before treatment (T₀)</th>
<th>CPV infected dogs after treatment (T₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein (g/dl)</td>
<td>5.6±0.1</td>
<td>5.8±0.24*</td>
<td>5.6±0.32</td>
</tr>
<tr>
<td>Serum Albumin (g/dl)</td>
<td>3.9±0.32</td>
<td>3.5±0.64*</td>
<td>3.6±0.02</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>1.6±0.01</td>
<td>2.4±0.21*</td>
<td>1.6±0.04</td>
</tr>
<tr>
<td>Serum ALT (U/l)</td>
<td>20±6.62</td>
<td>53±0.89*</td>
<td>35±6.87</td>
</tr>
<tr>
<td>Serum AST (U/l)</td>
<td>17±6.66</td>
<td>45±0.74*</td>
<td>32±6.12</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>84.2±0.95</td>
<td>212±0.91*</td>
<td>156±2.65</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.9±0.01</td>
<td>3.7±0.24*</td>
<td>1.2±0.05</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dl)</td>
<td>12±4.56</td>
<td>13±6.85*</td>
<td>12±4.67*</td>
</tr>
</tbody>
</table>

* Significant difference at p-value ≤0.05.

4. DISCUSSION

The canine parvovirus infection is the most common cause of enteritis in dogs and most commonly seen in dogs at the age of 2 – 9 months. The morbidity and mortality rates were high especially in unvaccinated and/or untreated dogs (Prittie, 2004; Decaro et al., 2005a&b). Clinical signs on admission were nonspecific including: depression, anorexia, fever and lethargy while the typical signs include: vomiting, diarrhea which is developed to hemorrhagic later on, these signs was attributed to gastrointestinal inflammation process; similar clinical findings were reported by Thomson and Gagnon (1980), Sharp et al. (2010) and Mylonakis et al. (2016). Inflammatory mediators especially Interleukin-1 are released at the site of the gastrointestinal inflammation (Goddard and Leisewitz, 2010). The diseased dogs were diagnosed canine parvovirus infection by the use of rapid CPV Ag test kit which is considered animal side test, accurate, easy and cheap test for detection of the canine parvovirus antigen in the fecal samples of diseased dogs, it is recommended as an easy tool for the confirmation of parvovirus infection in dogs (AL-Hosary, 2018; Awad et al., 2019).

Canine parvovirus infection impacts the hematological parameters where the RBCs, PCV% and leucocytes count decreased significantly in canine parvovirus infected dogs compared to control dogs, which indicates signs of anemia manifested clinically by pale mucous membrane and general weakness. Leucopenia, neutropenia and lymphopenia was attributed to the cytotoxic effect of the virus on the hematopoietic cells and bone marrow during the acute phase of the disease, this was in agreement with a previous studies by Hankes et al. (1992) and Weiss et al. (1999). The Lowered PCV% in canine parvovirus infected dogs was due to the intestinal hemorrhage and bleeding. The biochemical analyses showed significant increase in serum ALT, AST, Alkaline phosphatase and creatinine values in canine parvovirus infected dogs compared to clinically healthy. The changes in the albumin and globulin were significant which is usually observed in enteritis and resulted from malabsorption, decrease dietary intake and protein losses (Craven et al., 2011). Blood urea nitrogen did not differ significantly between groups and this was disagreed to a previous results by Bhat et al. (2013), who observed significant increase in blood urea nitrogen due to pre-renal uremia which might reflect a serious problem in the kidneys during the course of the disease, although a previous study by van den Berg et al. (2018) stated that the serum creatinine and serum urea nitrogen are insensitive markers for assessment of renal injury. ALT and AST was significantly increased in canine parvovirus infected dogs compared to control dogs, this might be resulted from the hepatic disorders and the development of inflammatory bowel disease (Berghoff and Steiner, 2011). The canine parvovirus infection is more likely to occur at an early stage of life; therefore, it is recommended for owners of puppies less than 16 weeks to avoid contact with dogs at high risk for canine parvovirus infection or during recovery stage (Markovich et al., 2012).

This protocol agreed to that provided by Sykes (2014), Martin et al. (2002) and de Mari et al. (2003). During the treatment of the diseased dogs, there were 4 dogs died due to general systemic impairment, which might be related to...
severe dehydratation, anemia and kidney damage (Nandi and Kumar, 2010b).

5. CONCLUSIONS
The hematological and biochemical parameters were altered in dogs infected with canine parvovirus infection. Through clinical examination, rapid test kits are very important for diagnosis of canine parvovirus in dogs.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest

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5. REFERENCES