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Alterations in haemato-biochemical and oxidative stress indices in dogs affected with parvoviral enteritis

IM Harizan, RK Khinchi, Manju, SK Sharma and VK Meena

Abstract
A total of 23 dogs, showing signs of diarrhoea and vomiting were confirmed positive for parvoviral enteritis by PetX Canine parvoviral antigen rapid test kit in Udaipur district. The positive dogs had significant lower mean of Hb, PCV, TEC and TLC with significant neutrophilia and lymphocytopenia. Mean value of serum ALT and AST was significantly increased while glucose, total protein, albumin and globulin were significantly decreased in parvoviral enteritis affected dogs. Serum Creatinine value was non-significantly differed in affected dogs than healthy dogs. Oxidative stress indices revealed significant increase in MDA and NOx values while significant decrease values of GST activity and catalase in parvoviral enteritis affected dogs.

Keywords: diarrhoea, vomiting, neutrophilia, lymphocytopenia. oxidative stress

Introduction
There are various gastrointestinal disturbances in dogs among which enteritis is the most common disease encountered in all breeds and age groups of canine population (Bhat *et al.*, 2013) [5]. Canine parvovirus infection is caused by parvovirus type-2, mostly seen in young puppies between 1-4 months of age and less prevalence is recorded in adult dogs (Kumar *et al.*, 2020) [24] due to inadequate derived maternal antibody to protect from parvo infection (Terzungwe, 2018) [37]. Canine parvovirus enteritis is a highly contagious and fatal disease affecting mainly intestinal tract. Affected dogs had clinical symptoms of anorexia, vomiting, diarrhoea and depression (Khinchi *et al.*, 2019) [22].
The reactive oxygen and nitrogen species play a complex role in many diseases and in metabolic regulation in a disease process, and oxidative stress has been implicated in several viral infections in man and animals. Reactive oxygen species (ROS) are produced as a defense mechanism by neutrophils and other cells during immune activation by viruses for amplification of signals (Peterhans *et al.*, 1987) [32]. Lipid peroxidation resulting from attacks of generated reactive oxygen species on double bonds in poly unsaturated fatty acids (PUFA) reduces the cellular elasticity and enhances cellular damage (Freeman and Crapo, 1982 [13]; McCord, 1983 [28] and Halliwell, 1987 [17]). The adverse health effects possibly due to oxidative damages in canine parvovirus diarrhoea can be prevented by ensuring adequate antioxidant defense (Evans and Halliwell, 2001) [11]. Role of oxidative stress in CPV-2 has been circulating with strong correlation about its role in the resultant anaemia (Panda *et al.*, 2009) [30]. There is no specific antiviral therapy to treat the dogs infected with canine parvo viral gastroenteritis. Symptomatic treatment is the only option to treat the patients that include intravenous fluid therapy to correct dehydration, hypoglycemia, electrolyte imbalance along with broad spectrum antibiotic, antacid, NSAID, multivitamins, antiallergic, antiemetic and antioxidants drugs.

Material and Method
During the study period of 6 months in Udaipur district, dogs with the symptom of vomiting and diarrhoea were confirmed for parvoviral enteritis by PetX Canine parvoviral antigen rapid test kit (Secure diagnostics Pvt. Ltd. Bhopal, M.P., India). The blood sample of healthy and parvoviral enteritis infected dogs were analysed for determination of Hb, PCV, TEC, TLC and DLC as per standard techniques by Feldman *et al.* (2000) [12]. Serum biochemical parameters *viz.*, Serum ALT, AST, creatinine, glucose, total protein, albumin and globulin were estimated by using automatic biochemistry analyzer (Mindray BC.2800 Vet.). The concentration of malondialdehyde (MDA) and catalase activity were estimated in 10% haemolysate by Placer

et al. (1966) [33] and Bergmayer (1983) [3], respectively. Plasma total nitric oxide (NOx) and glutathione S-transferase (GST) activity were assayed by using commercially available kits (EZAssay Nitric oxide Estimation Kit, Product Code: CCK061-20, Himedia, Mumbai and EZAssayTM GST Activity Estimation Kit, Product Code: CCK028-10, Himedia, Mumbai, India). The data were statistically analyzed and compared using standard formula given for mean, standard error and analysis of variance as per statistical methods described by Snedecor and Cochran (1994) [36].

Results and Discussion

Haematology

Mean±SE values of haematological parameters in healthy and parvoviral enteritis positive dogs are presented in table-1. The mean values of Hb, PCV, TEC and TLC were significantly (p<0.01) decreased in dogs affected with parvoviral enteritis as compared to healthy dogs. These findings were similar with Parthiban *et al.* (2016) [31], Andrea *et al.* (2017) [1] and Bhargavi *et al.* (2017) [4].

Table 1: Mean±SE values of haematological parameters in healthy and parvoviral enteritis affected dogs

S. No.	Parameters	Healthy control dogs (n=10)	Parvoviral enteritis affected dogs (n=23)
1	Haemoglobin (gm/dl)	11.79±0.398	8.74±0.346 **
2	Packed cell volume (%)	35.94±0.781	26.91±1.189 **
3	Total erythrocyte count (million/mm^3)	7.72±0.176	6.04±0.228 **
4	Total leucocyte count (10^3/mm^3)	10.24±0.251	6.78±0.235 **
5	Neutrophil (%)	68.2±0.533	72.39±0.359 **
6	Lymphocyte (%)	28.4±0.6	23.30±0.335 **
7	Monocyte (%)	1.5±0.167	1.91±0.197
8	Eosinophil (%)	1.9±0.233	2.39±0.264

* Means differ significantly (p<0.05) with control group
** Means differ highly significant (p<0.01) with control group

The decrease in mean values of Hb in dogs affected with parvoviral enteritis is might be due to suppression of erythropoiesis as a result of direct effect of parvovirus on the bone marrow (Boosinger *et al.*, 1982) [7], accumulation of toxic waste products during viremia and febrile phase (Jones and Hunt 1983) [18], further CPV damage also the capillary of the villi of intestine leading to loss of blood, which is responsible for the reduced Hb concentration (Yadav *et al.*, 2011) [39]. Lower values of PCV and TEC in canine parvovirus affected dogs might be due to damage of vascular epithelium of the intestine as recorded previously (Bhat *et al.*, 2013) [5] leading to haemorrhages and blood loss through the faeces and vomitus in the disease process (Behera *et al.*, 2014) [2]. Significant decreased TLC in parvoviral enteritis affected dogs was probably not only due to the destruction of hematopoietic progenitor cells of various leukocyte types primarily in the bone marrow but also in other lymphoproliferative organs such as the thymus, lymph nodes and spleen. This resulted in inadequate compensation for the massive demand for leukocytes in the inflamed gastrointestinal tract (Goddard *et al.*, 2008) [14].

The DLC (%) in parvoviral enteritis in dogs showed significant (P<0.01) neutrophilia % and significant lymphopenia % as compared to healthy group. A non-significant (P>0.05) difference of mean values of monocyte and eosinophils were recorded in parvoviral enteritis affected dogs as compared to healthy dogs. Similar findings were also recorded by Terzungwe (2018) [37] and Kataria *et al.* (2020) [20]. In the present study, neutrophilia is might be due to the involvement of bacterial complications in parvoviral enteritis and the lower value of lymphocyte counts might be due to the virus replication in the lymphoid organs resulting in lymphocytolysis (McCandlish, 1998 [27] and Chakrabarti, 2009 [8]).

Serum biochemistry

Mean ± SE values of biochemical parameters in healthy and parvoviral enteritis affected dogs are presented in table-2. The mean values of serum glucose were significantly lower (P<0.01) in dogs affected with parvoviral enteritis than healthy dogs. The observations recorded in present study were in agreement with the findings of Bhargavi *et al.* (2017) [4] and Kumar and Kumar 2017) [23]. Hypoglycemia in the parvoviral enteritis affected dogs may be due to inappetance/anorexia (Shinde *et al.*, 2000) [35] and it may also be due to decreased hepatic glucose production which was because of decreased blood supply to liver with portosystemic shunt which results in liver atrophy and liver cannot play its normal role in maintaining the blood glucose concentration (Joshi *et al.*, 2012) [19]. No significant difference was found in the mean values of serum creatinine in dogs affected with parvoviral enteritis as compared to healthy control dogs.

Table 2: Mean ± S.E values of biochemical parameters in healthy and parvoviral enteritis affected dogs

S. No.	Parameter	Healthy control dogs (n=10)	Parvoviral enteritis affected dogs (n=23)
1	ALT (IU/L)	41.75±2.14	48.43±1.748 *
2	AST (IU/L)	32.67±1.7	39.68±1.780 **
3	Serum Creatinine (mg/dl)	0.8±0.03	0.99±0.057
4	Serum glucose (mg/dl)	102.59±0.95	86.49±0.587 **
5	Serum Total protein (g/dl)	6.59±0.14	5.11±0.12**
6	Albumin (g/dl)	3.67±0.13	2.64±0.112**
7	Globulin (g/dl)	2.92±0.1	2.47±0.05 **

* Means differ significantly (p<0.05) with control group
** Means differ highly significant (p<0.01) with control group

The mean value of serum total protein was significantly (P<0.01) lower in parvoviral affected dogs than healthy dogs. The observations recorded in present study were in agreement with findings of Bhargavi *et al.* (2017) [4], Khare *et al.* (2020)

[21] and Kumar *et al*. (2020) [24]. The mean value of albumin and globulin were significantly (P<0.01) lower in parvoviral enteritis affected dogs than healthy dogs. Similar findings were recorded by Salem *et al*. (2018) [34] and Kumar *et al*. (2020) [24]. The decreased values of total protein, albumin and globulin observed in parvoviral enteritis may be due to leakage of serum protein through the damaged capillaries of intestinal villi and may also be due to less absorption of protein through the damaged villi as opined by Biswas *et al.* (2005) [6].

The mean values of serum ALT and AST were significantly (P<0.05 and P<0.01, respectively) higher in dogs affected with parvoviral enteritis as compared to healthy dogs. The findings recorded in present study are in agreement with previous reports of Dash *et al*. (2019) [10] and Khare *et al*. (2020) [21]. Elevation in these enzymes may occur as a result of hepatic hypoxia secondary to severe hypovolemia or the absorption of the toxic substance of the gut barrier (Macintire and Smith, 1997) [26].

Oxidative stress indices

Mean±S.E values of oxidative stress indices in healthy dogs and parvoviral enteritis affected dogs are presented in Table 3. There were significant increase (p<0.01) in the mean values of MDA and NOx in parvoviral enteritis affected dogs than healthy dogs whereas the mean values of GST activity and catalase were significantly decreased (p<0.01) in dogs affected with parvoviral enteritis than healthy dogs. Similar findings were reported by Luo and Qiu (2013) [25]; Nykky *et al*. (2014) [29]; Khinchi *et al*. (2019) [22] and Ukwueze *et al*. (2020) [38].

Table 3: Mean ± S.E values of oxidative stress indices in healthy control and parvoviral enteritis affected dogs

S. No.	Parameter	Healthy control dogs (n=10)	Parvoviral enteritis affected dogs (n=23)
1	MDA (ηmol MDA /mg Hb)	1.14±0.073	2.58±0.093**
2	NOx (µM/ml)	3.4±0.14	8.28±0.169**
3	GST activity (µM ml-^{1} Min-^{1})	0.63±0.032	0.35±0.014**
4	Catalase (unit/mg Hb)	151.41±9.782	122.18±4.349**

* Means differ significantly (p<0.05) with control group

** Means differ highly significant (p<0.01) with control group

Virus-induced oxidative stress could be mediated by an early phase of liberation of pro-inflammatory cytokines. Participation of iron in Fenton reaction *in vivo* leads to production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 (Halliwell, 1994) [16] and results in increased lipid peroxidation. This might be one of the reasons for significant alteration in MDA and NOx in dogs suffering from parvoviral enteritis. GST is present in various tissues and can conjugate ROS with GSH. It plays an important role in protecting tissue from oxidative stress and its levels can reflect the antioxidant capacity of the body (Habig and Jakoby, 1981) [15]. Crnogaj *et al.* (2017) [9] reported that significant reduction in catalase found in diseased dogs could be attributed to the consumption of antioxidants that act as "scavengers" of free radicals during the oxidative processes.

Conclusion

In this study, the mean values of Hb, PCV, TEC and TLC were significantly decreased in dogs affected with parvoviral enteritis than healthy dogs. The DLC in parvoviral enteritis in dogs showed significant neutrophilia and lymphopenia as compared to healthy dogs. Among, biochemical parameters ALT and AST was significantly increased with significant hypoglycaemia, while serum total protein, albumin and globulin were significantly decreased in dogs affected with parvoviral enteritis than healthy dogs. The mean values of MDA and NOx were found significantly increased; whereas GST activity and catalase were found significantly decreased in parvoviral enteritis affected dogs than healthy dogs

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