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Short Communication

EFFECT OF CANINE PARVOVIRUS ON CARDIAC TROPONIN T AND FAECAL CALPROTECTIN IN DOGS

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ABSTRACT: Parvovirus, regarded as one of the deadliest disease-causing agents in dogs has various detrimental effects in the body amongst which, cardiomyopathies and gastrointestinal aberrations are common causes for mortality. Electrocardiographic examinations along with the estimation of prominent cardiac and faecal biomarkers signify severity of the disease. The specificity of parvovirus to affect the myocardial cells, estimation of cardiac Troponin T biomarker for accessing myocardial damage is most necessary. Faecal calprotectin signifies the level of neutrophils lost in the faeces as the virus has a peculiar ability to cause sequestration of neutrophils and damage the intestinal epithelial cells.

Keywords: Parvovirus, Electrocardiography, Biomarkers.

Canine parvovirus is highly contagious disease, affecting the dogs globally. Two prominent clinical forms of the illness: enteritis, which causes vomition and diarrhoea in dogs of all ages and myocarditis, which causes heart failure in puppies younger than three months of age [1]. Hence, electrocardiographic examination along with estimation of cardiac and faecal biomarkers are utmost important for diagnosing parvovirus among dogs. The importance of study is highlighting the need for detecting the extent of cardiac and intestinal damage in dogs affected with parvovirus infection so as to have a better diagnostic and treatment strategies. The specificity of parvovirus to affect the myocardial cells, estimation of cardiac Troponin T biomarker for accessing myocardial damage is most necessary. Faecal calprotectin signifies the level of neutrophils lost in the faeces as the virus has a peculiar ability to cause sequestration of neutrophils and damage the intestinal epithelial cells. The Objective of study was to determine how parvovirus exels in causing severe destruction of myocardial and intestinal epithelial cells. The significance of study stands out in conducting a novel study in finding out how neutrophils remarkably indicate the level of damage to intestine by detecting feacal calprotectin in diarrheic faeces and how cardiac damage at the earliest be detected by using cardiac troponin T biomarker.

The study

Study design

The proposed work was conducted in the Department of Veterinary Medicine and Veterinary Clinical Complex (V.C.C), College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur, Madhya Pradesh. The proposed study was conducted for a period of six months (July – December, 2023). For this study, a total of 325 dogs presented at Veterinary Clinical Complex, College of Veterinary Science & A.H., Jabalpur (M.P.) were screened for the presence of clinical symptoms viz. dullness, vomition, anorexia, marked dehydration, watery faeces with red flakes of blood etc.

Out of 325 dogs with gastroenteritis, a total of 125 dogs with haemorrhagic gastroenteritis were found suspected of parvovirus. Out of suspected cases, 78 dogs were found to be confirmed for parvovirus

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infection. Out of which, 48 male dogs (71.64%) and 30 female dogs (51.72%) were found positive for parvovirus infection.

Collection of samples

Approximately 3 ml. blood samples were collected aseptically from cephalic or saphenous vein of dogs suspected for parvovirus. Out of which, 1 ml was collected in vial containing EDTA for routine haematology and 2 ml was collected in clot activator vacutainer vials. Serum was harvested after centrifugation, frozen and stored at -20˚C until further biochemical analysis.

Electrocardiographic examination

ECG was performed in confirmed cases of parvovirus. Electrocardiographic tracing was recorded with Cardiart 8408 View (BPL Limited).

The dogs were kept in right lateral recumbency with the legs perpendicular to the long axis of the body. After shaving and application of electrode jelly to the skin, electrodes were attached. The point just proximal to the olecranon on the lateral aspect was used for the forelimbs, whereas the point proximal to the patella on the lateral aspect will be used for hind limbs. Three bipolar limb leads (lead I, lead II and lead III) were used.

A paper speed of 50 mm/sec. was used. Before each recording, a one millivolt standardization pulse was made. Four or five beats were recorded on each lead. According to Tilley's (2008) guidelines, complexes and intervals were measured for interpretation of electrocardiograms.

P Wave: Amplitude (in mV) up to 0.4 mV (4 boxes tall) and duration (in second) up to 0.04 second (2 boxes wide) was measured and always positive in lead II and positive or isoelectric in lead I.

P-R interval: Duration (in second) was measured, 0.06-0.14 second (3 to 7 boxes).

QRS complex: Amplitude (in mV) and duration (in second) was measured. Maximum amplitude of R wave 3.0 mV (30 boxes) in large breeds and 2.5 mV (25 boxes) in small breeds and duration of QRS complex was maximum 0.05 second (2.5 boxes) in small breeds and 0.06 second (3 boxes) in large breeds in lead II and III. QRS Complex positive in leads II and III.

S-T segment: No depression-not more than 0.2 mV (2 boxes).

T Wave: It can be positive, negative or biphasic

Q-T interval: It was 0.15 to 0.25 second (7.5 to 12.5 boxes) at normal heart rate.

Cardiac biomarker – Troponin T

Cardiac troponin T was estimated in dogs having parvovirus by using commercially available canine specific ELISA kit (TnT ELSIA KIT) and ELISA instrument as per the standard methods described. Serum samples were stored at -20˚C and used for analysis of troponin T concentration. For the calculation, Multiskan FC ELISA reader was used.

Calculation

Relative $O.D._{450}$ = ($O.D._{450}$ of each well) – ($O.D._{450}$ of zero well)

The mean absorbance value (OD_{450}) for each set of reference standards, controls and samples was calculated. The standard curve was constructed by plotting the mean absorbance of each reference standard against its concentration in ng/mL on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. On the basis of mean absorbance value of each sample the concentration of troponin T (ng/mL) was determined from the standard curve.

Faecal biomarker – Calprotectin

Faecal calprotectin was estimated in dogs having parvovirus by using commercially available ELISA kit (BUHLMANN fCAL turbo) and ELISA instrument as per the standard methods described. Faecal samples were stored at -20˚C and used for analysis of calprotectin concentration. For the calculation, Multiskan FC ELISA reader was used.

Test procedure of calprotectin estimation

Calculation

Relative $O.D._{450}$ = ($O.D._{450}$ of each well) – ($O.D._{450}$ of zero well)

The mean absorbance value (OD_{450}) for each set of reference standards, controls and samples were calculated. The standard curve was constructed by plotting the mean absorbance of each reference standard against its concentration in µg/g on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis. On the basis of mean absorbance value of each sample the concentration of calprotectin (µg/g) was determined from the standard curve.

Results and discussion

Electrocardiographic findings in dogs affected with parvovirus infection

The ECG findings are shortlisted in Table 1, Fig. 1 and 2.

Table 1. Electrocardiographic findings in dogs affected with parvovirus infection.

S. No.	ECG tracings	No. affected (n=78)	Distribution (%)
1	Normal sinus rhythm	09	11.53
2	Normal sinus rhythm with biphasic T wave	06	7.69
3	Low voltage QRS complex	18	23.07
4	ST coving	15	19.23
5	Reduced QT duration	12	15.38
6	Sinus Tachycardia	10	12.82
7	Increased P wave amplitude	08	10.25

Table 2. Mean values of cardiac troponin T (ng/mL) in dogs affected with parvovirus infection.

Groups	Cardiac troponin T (ng/mL)
Parvovirus infected dogs (n=78)	0.15^{a}±0.05
Heathy control (n=6)	0.01^{b}±0.02

Table 3. Mean values of cardiac troponin T (ng/mL) in dogs of different age groups affected with parvovirus infection.

Parameter \ Groups	0-2 M	3 M	4 M	5 M	6 M
Cardiac troponin T (ng/mL) (Mean±SE)	0.18^{a}±0.02	0.15^{ab}±0.01	0.14^{b}±0.03	0.14±0.06	0.14±0.05

Table 4. Mean values of cardiac troponin T (ng/mL) level in dogs of different sex groups affected with parvovirus infection.

Parameter \ Sex group	Male	Female
Cardiac troponin T (ng/mL) (Mean±SE)	0.15±0.05	0.15±0.01

Table 5. Mean values of cardiac troponin T (ng/mL) in dogs of different breeds affected with parvovirus infection.

Parameter \ Breeds	Non-descript	Labrador Retriever	German Shepherd
Cardiac troponin T (ng/ml) (Mean±SE)	0.16^{a}±0.01	0.14^{b}±0.05	0.13^{b}±0.02

Table 6. Mean values of faecal calprotectin (µg/g) in dogs affected with parvovirus infection.

Groups	Faecal calprotectin (µg/g)
Parvovirus infected dogs (n=78)	17.38^{a}±2.38
Healthy control (n=6)	0.11^{b}±0.01

The results of electrocardiographic abnormalities detected in dogs with parvovirus infection revealed similar findings with [2] who reported a significant increase in low voltage QRS complex along with an increase in Q-T interval in dogs affected with parvovirus infection. Similarly [3] observed prevalence rate of 53.33 per cent of decreased R amplitude and reduced Q-T duration in 20 per cent prevalence rate in dogs affected with parvovirus infection.

Alteration in ECG findings among dogs infected with parvovirus are related to altered or reduced conduction of myocardium signifying severe electrolyte disturbance in the body or due to sepsis caused by pericardial effusion [3]. Since, diarrhoea in parvovirus infected dogs causes severe loss of potassium and bicarbonate ions, biphasic T waves are suggestive of hypokalaemia.

ST coving highlights steep fall of the T wave pattern indicating ventricular tachycardia with delayed depolarization. Tachycardia encountered in cases of parvovirus infection might signify anaemic condition. Reduced Q-T duration is one of the indicators of chloride and potassium depletion, mostly due to vomition and diarrhoea [4]**.** Increase in P wave amplitude is evocative of right atrial enlargement.

Cardiac biomarker – Troponin T (cTnT)

The mean values of cTnT in dogs affected with parvovirus infection was significantly increased *i.e.*, 0.15±0.05, when compared with mean values of cTnT of control group with healthy dogs *i.e.*, 0.01±0.02. There was a significant increase in the mean cTnT value among dogs of 0-2 and 3 months of age i.e., 0.18±0.02 and 0.15±0.01 affected with parvovirus infection.

Table 7. Mean values of faecal calprotectin (µg/g) in dogs of different age groups affected with parvovirus infection.

Parameter / Groups	0-2 M	3 M	4 M	5 M	6 M
Faecal calprotectin (µg/g) (Mean±SE)	12.96±4.38	21.19±5.27	24.73±9.18	12.64±3.00	21.44±7.47

Table 8. Mean values of faecal calprotectin (µg/g) in dogs of different sex groups affected with parvovirus infection.

Parameter / Sex groups	Male	Female
Faecal calprotectin (µg/g) (Mean±SE)	17.34±2.96	20.45±5.15

Table 9. Mean values of faecal calprotectin (µg/g) in dogs of different age groups affected with parvovirus infection.

Parameter / Breeds	Non-descript	Labrador Retriever	German Shepherd
Faecal calprotectin (µg/g) (Mean±SE)	24.73±4.54	11.02±2.20	17.05±4.54

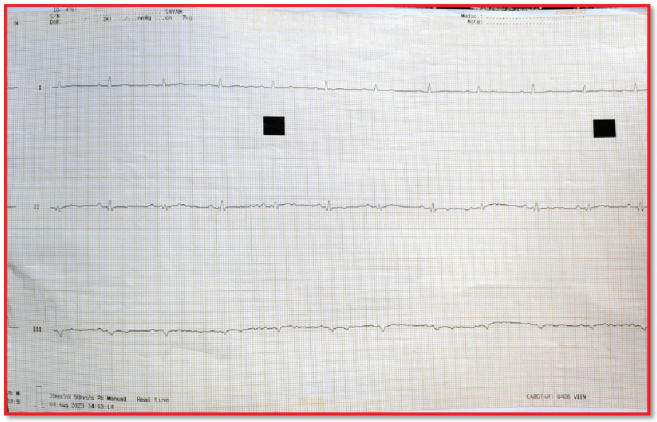

Fig. 1. Electrocardiogram showing low voltage QRS complex (QRS amplitude <0.5 mV).

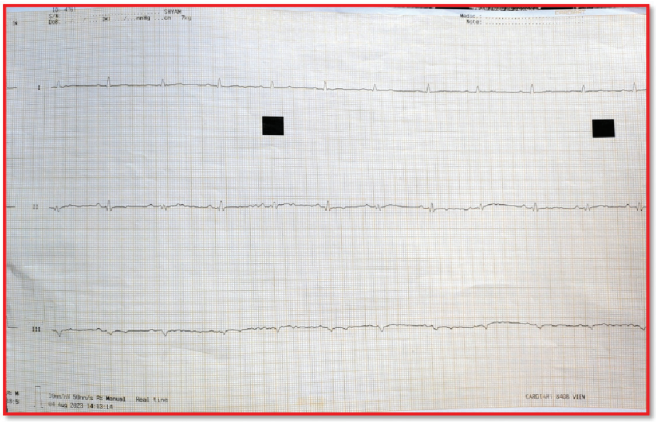

Fig. 2. Electrocardiogram showing ST coving suggestive of left ventricular enlargement.

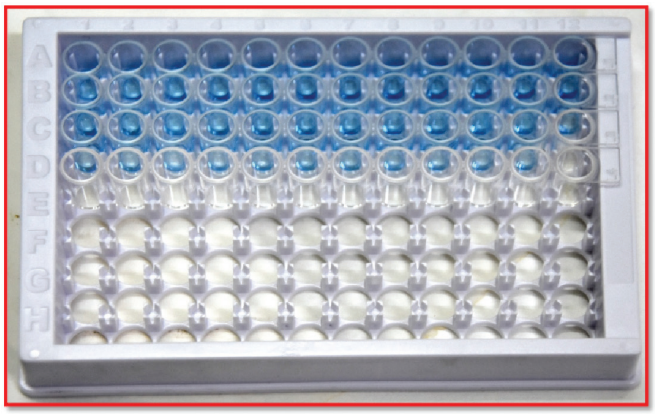

Fig. 3. Cardiac troponin T assay [A1 - 5: Standards, C 12: Control, Remaining wells: Test sample].

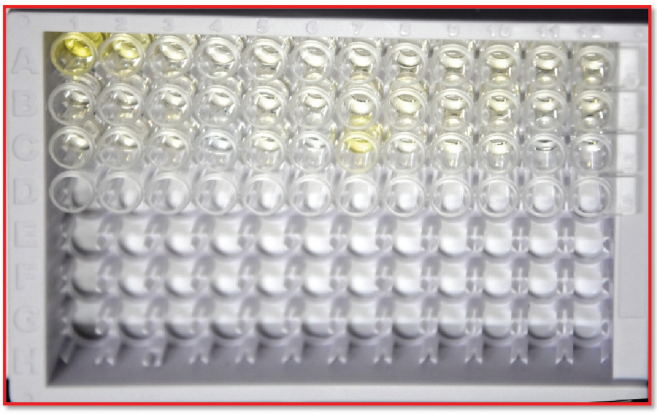

Fig. 4. Faecal calprotectin assay [(A1 - 5 : Calibrator), (C 12: Control). (Remaining wells: Test sample)].

The findings of present study are suggestive that parvovirus has more affinity to rapidly dividing myocardial cells of young dogs of 0 - 3 months of age. Non-descript breed of dogs with increased serum cardiac troponin T values might be due to over presentation of their population and lack of awareness among the pet owners for vaccinating the dogs which deprives them of immunity and uplifts the perpetuating nature of parvovirus in cases of reduced immunity leading to severe cardiac damage (Table 2, 3, 4, 5 and Fig. 3).

Faecal calprotectin

The mean values of faecal calprotectin in dogs affected with parvovirus infection was significantly

increased *i.e*., 17.38±2.38, when compared with mean values of faecal calprotectin of control group with healthy dogs *i.e*., 0.11±0.01. There was no statistically significant difference in age, breed and sex (Table 6, 7, 8, 9 and Fig. 4)

Calprotectin is a recognisable faecal biomarker used to assess intestinal inflammatory conditions and differentiate certain gastrointestinal abnormalities [5]. The results of present study are in accordance with the findings of [6] who reported significant increase in faecal calprotectin values in dogs with inflammatory bowel disease.

Conclusion

Parvovirus, acknowledged as one of the deadliest diseases of canines reflects multiple pathologies that are detrimental to life. Prevention from such disease emphasises a detailed investigation of the myocardial and intestinal biomarkers as both being peculiar sites of predilection. ELISA, being one of the sensitive tests helps us to use all the potential ways to combat these diseases by an early diagnosis. Electrocardiography, not only signifies the conduction defect but also aids in preliminary diagnosis of certain myocardial damages caused by parvovirus.

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