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D Sravanthi
Department of Veterinary Medicine, Rajiv Gandhi Institute of Veterinary Education and Research, Pondicherry, India

P Vijayalakshmi
Department of Veterinary Medicine, Rajiv Gandhi Institute of Veterinary Education and Research, Pondicherry, India

P Thiruselvame
Department of Veterinary Medicine, Rajiv Gandhi Institute of Veterinary Education and Research, Pondicherry, India

Correspondence
D Sravanthi
Department of Veterinary Medicine, Rajiv Gandhi Institute of Veterinary Education and Research, Pondicherry, India

Incidence of parvo viral enteritis and electro cardiographic alterations in the affected dogs

D Sravanthi, P Vijayalakshmi and P Thiruselvame

Abstract
A case control study was conducted at small animal medicine unit of Teaching Veterinary clinical complex, Rajiv Gandhi college of Veterinary and Animal Sciences, Puducherry for the period of 3 months. Total of nine dogs exhibiting signs of vomiting and diarrhoea that were brought to the hospital were utilized for the study. Nine age-matched dogs brought to the clinic for vaccination / routine check-up that were apparently normal were taken as a control group. The disease was predominantly noticed in dogs between 0 to 6 months old. The disease group showed the prevalence of infection about 45% in non-descript dogs and 56% male dogs which was due to over representation of this breed and sex to the hospital. The current study was conducted for the determination of electrocardiographic alterations in dogs with parvo viral enteritis (PVE) infection. The Electrocardiographic findings (Lead II, 50 mm/sec, 1 cm = 1 mv) were, the QRS complexes of the infected group were normal in width but showed a low R wave amplitude (<0.5 mv) that is low voltage QRS complexes indicates Pericardial effusion because of sepsis. There was a reduction in QT interval (< 0.16 sec) which indicates loss of chloride and potassium ions due to prolonged vomiting and diarrhoea and presence of elevated S-T segment indicates myocardial hypoxia and Pericarditis. Nine suspected samples were positive using H primer pair generated PCR product of 630 bp.

Keywords: PVE, ECG, electrocardiography, canine parvo virus

Introduction
Parvovirus was first described as a clinical entity causing hemorrhagic gastroenteritis in dogs in 1977(Appel *et al.*, 1979) [1] and the etiological agent was named canine parvovirus type 2 (CPV-2) to distinguish it from the antigenically unrelated virus canine parvovirus type 1(CPV-1), also known as minute virus of canines. CPV 2 is important pathogen in domestic dogs and wild carnivores causing acute hemorrhagic gastroenteritis, lymphopenia, nausea and fatal myocarditis in young puppies(Carmichael & Binn, 1981) [4]. The disease is highly contagious and is spread from dog to dog by direct or indirect contact with their feces. The dogs are infected through the oronasal route and after 3-10 days they develop an acute gastroenteritis characterized by loss of appetite, vomiting, fever, diarrhea (from mucoid to hemorrhagic) and leukopenia. CPVs are small, non-enveloped, DNA-containing viruses that require rapidly dividing cells for replication. It has two distinct presentations, a cardiac and intestinal form. The common signs of intestinal form are severe vomiting and dysentery and the cardiac form causes respiratory or cardiovascular failure in young puppies. Dogs infected with CPV-2 may develop acute bloody diarrhea, fever, and dehydration, which are sometimes followed by shock and sudden death (Nelson *et al*., 1979, Robinson *et al*., 1980, Lenghaus *et al*., 1984, Macartney *et al*., 1984 and Carman *et al.*, 1985) [17, 18, 19, 20, 3]. Breeds like Doberman Pinschers, Rottweilers, and English Springer Spaniels had significantly increased risk factor for CPV enteritis (Hoskins 1998)[11] while Rottweilers, American Pit Bull Terriers, Doberman Pinschers, and German shepherd had significantly higher risk factor for CPV corresponding to age and sex. High prevalence of CPV-2 infections was found in 1–2 month- and 3–6 month-old dogs, respectively (Sakulwira *et al*., 2003)[23]. Parvovirus is as one of the most common causes of infectious diarrhea in dogs younger than 6 months. Dogs between 6 weeks and 6 months of age were in increased risk(Hoskins 1998)[11]. CPV-2 induced disease is observed mainly in 6–12 week-old pups; whereas, younger dogs are generally protected from CPV-2 infection by maternally-derived immunity(Decaro *et al.*, 2004)[8]. In dogs, antibodies are transferred from the bitch to her pups mainly through colostrum, whereas only a small number (5-10%) of antibodies are transferred during pregnancy because of low permeability of canine placenta to immunoglobulins

(Gillespie *et al*., 1958; Carmichael *et al*., 1962; Winters, 1981)[10, 5, 15].The IgG taken by suckling pups via colostrum reach the small intestine and are transported across the intestinal epithelium into the neonatal circulation by binding a surface membrane receptor of the enterocytes named Fcγ-R (Van de Perre, 2003) [22]. The highest permeability of the intestinal mucosa to IgG is reached immediately after birth, while a rapid decline is observed within 24h as a consequence of enterocyte maturation. Usually, the transfer of maternally-derived antibodies (MDA) is complete by 72 h after birth, so that the maximal immunity occurs at 36-48 h after birth (Winters, 1981; Chappuis, 1998) [15, 6].The level of MDA in pups is proportional both to the level of serum antibodies of dam and to the amount of colostrum taken by the pups. Thus, pups belonging to large litters usually receive a smaller amount of passive immunity than pups belonging to small litters, which are allowed to assume more colostrum(Pollock and Carmichael, 1982) [12]. MDA to CPV-2 can persist for 13-15 weeks (Pollock and Charmichael, 1982; Buonavogalia *et al*.,2001) [12, 16] thus protecting pups from natural infection but, conversely, preventing immunization after vaccination. It is a disease of un-vaccinated and under-vaccinated puppies and young dogs. It is extremely rare for older dogs with adequate vaccination status to acquire parvo viral enteritis unless their immune system is suppressed for some other reason. Buonavoglia *et al.*, (2001) [16]designed primer pair Hfor/ Hrev which can amplify 630 bp product of VP2 gene of canine parvovirus. They reported that this primer could be used for screening of faecal samples for the detection of Canine Parvovirus.

Materials and methods

This case control study was conducted at small animal medicine unit of Teaching Veterinary clinical complex, Rajiv Gandhi college of Veterinary and animal sciences, Puducherry for the period of 3 months. A total of nine dogs with naturally occurring CPV enteritis showing clinical signs such as anorexia, vomiting, diarrhea, dysentery, dehydration and pale mucosa were taken up for the study. Signalment of the animals that includes breed, age and sex were also recorded. Nine age-matched dogs that were clinically normal brought to the clinic for vaccination or routine checkup were taken as a control group. A general clinical examination followed by regional examination of the body system was conducted as per (Radostits *et al*., 2000)[14]. Faecal samples were examined by direct smear method that includes dilution of small quantity of faeces with few drops of water on the slide and spreading it evenly to get a translucent film. Then placing a coverslip over it and examined under the microscope. Three slides from different parts of the fecal samples were examined. The CPV suspected faecal samples were tested by PCR method using vaccine as a positive control. Electro cardio graphic studies were done on the control and the diseased animals (Tilley 1992)[24] with the parameters such as general inspection of heart rate and rhythm; P-wave morphology, uniformity and regularity; QRS complexes morphology, uniformity and regularity; P and QRS relationship by measuring P-R interval; S-T segment; T wave morphology, uniformity and regularity and Q-T interval. Hundred microliter of supernatant of the faecal sample was used for template DNA preparation. The supernatant was diluted to 1:10 in ultrapure water to reduce residual inhibitors of DNA polymerase activity(Decaro *et al.*, 2005)[7]. Template DNA was prepared by boiling at 96° for 10 minutes followed by immediate chilling in crushed ice. The processed faecal samples were screened by primer pair Hfor/ Hrev that amplifies a 630 bp fragment of the VP2 gene encoding capsid protein (Buonavoglia *et al.*, 2001)[16] and the amplified PCR product was analysed in 1.5% agarose gel electrophoresis for determination of their sizes (Table.1).

Table 1: Screening of clinical samples by primer pair Hfor/ Hrev using PCR

Primers	Primer Sequence 5' – 3' Direction	CPV types Amplified	Position of the genome	Annealing temperature and product size
H for	CAGGTGATGAATTTGCTACA	All types	3556 – 3575	50ºC
H rev	CATTTGGATAAACTGGTGGT		4166 – 4185	630 bp

Results and Discussion

The dogs that were taken up for the study were subjected with the above examination and were summarized. Among the nine affected animals with the disease, 45 per cent were Non-descript dogs, 22 per cent Daschund, 11 per cent Boxer, 11 per cent Labrador and 11 per cent Dalmation (Fig.1). Hoskins (1998)[11] found that Doberman Pinschers, Rottweilers, English Springer Spaniels had significantly increased risk factor for CPV enteritis. Although it was common in Doberman Pinschers and Rottweilers the disease group showed the prevalence of infection about 45% in non-descript dogs and 56% male dogs (Fig.2) which was due to over representation of this breed and sex to the hospital. Among the nine affected animals with the disease, the animals under the category of 1-3 months were 33 per cent and the animals under the category of 4-6 months were 68 per cent. CPV infection was widely prevalent among canine population in Puducherry, Tamilnadu. Compared to sex prevalence among the nine affected animals with the disease, 56 per cent were males and 44 per cent were females. The disease was predominantly noticed in dogs between 0 to 6 months old as reported by other author (Parthiban, 2008)[13]. It was well known that increased intestinal epithelial turnover caused by changes in micro flora, diet and diminishing maternal antibody level were the predisposing factors to canine parvovirus infection in pups(Decaro *et al.*, 2004)[8]. The faecal samples examined by direct smear method from disease group showed 11 per cent positive for anchylostome ova whereas faecal samples from control group were negative for endoparasitic ova. The Electrocardiographic findings (Lead II, 50 mm/sec, 1 cm = 1 mv) showed the heart frequency values (b.p.m) of the infected group appeared decreased and they were significantly different in comparison to those of the control group (Table.2). The heart rhythms found in the infected group were regular. P waves are positive in lead II with a constant configuration. The QRS complexes of the infected group were normal in width but showed a low R wave amplitude (<0.5 mv) (Fig.4) that is low voltage QRS complexes indicates pericardial effusion because of sepsis. The P-QRS relationship is normal with a constant P-R interval. There was a reduction in QT interval (< 0.16 sec) which indicates loss of chloride and potassium ions due to

prolonged vomiting and diarrhea and presence of elevated S-T segment indicates myocardial hypoxia and Pericarditis. All the nine dogs in the study showed positive for the PVE (Parvo Viral Enteritis) (Fig.3). In this study PCR assay was found to be sensitive, specific and rapid technique for detecting CPV infection. The primer pair Hfor/Hrev (Buonavoglia *et al.*, 2001) [16] amplified 630bp product of VP2 gene. The higher sensitivity of PCR assay could be due to its ability to detect low level of virus (10^3 PFU / g of faeces).

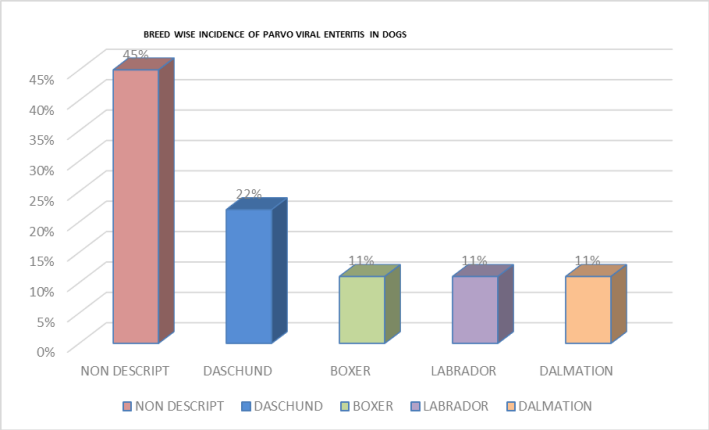

Fig 1: Breed wise incidence of PVE in dogs

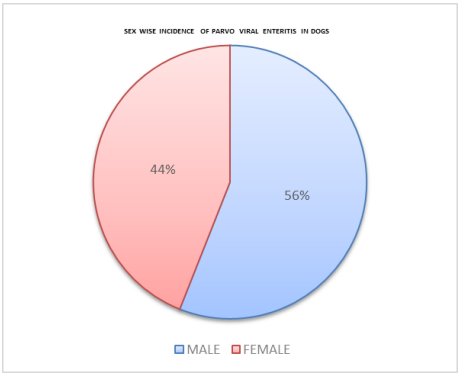

Fig 2: Sex wise incidence of PVE in dogs

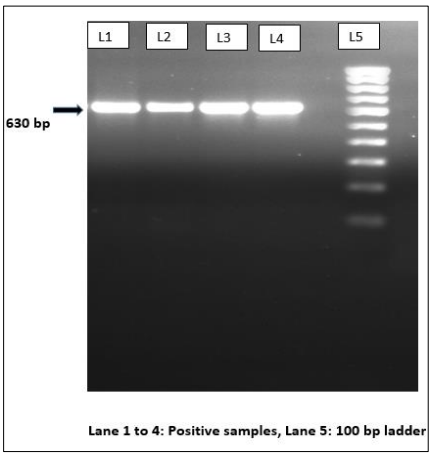

Fig 3: Agarose Gel Electrophoresis of the amplified product: Lane 1 to 4: positive samples; Lane 5: 100 bp ladder.

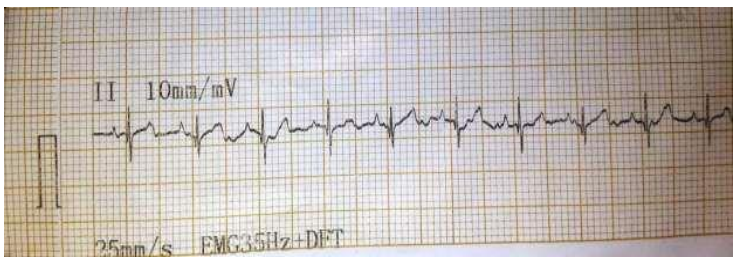

Fig 4: ECG of a 3.5 months old Labrador showing low R amplitude with deep S wave indicating low ventricular conduction.

Table 2: ECG findings of disease group of the dogs with PV and control group

Parameters	Disease group (N=9)	Control group (N=9)
P wave amplitude (mV)	0.23 ± 0.02	0.18 ± 0.01
P wave duration (sec)	0.03 ± 0.001	0.04 ± 0
QRS wave amplitude (mV)	1.32 ± 0.15	1.56 ± 0.13
QRS wave duration (sec)	0.03 ± 0.002	0.04 ± 0
PR interval (sec)	0.09 ± 0.006	0.11 ± 0.008
QT interval (sec)	0.13 ± 0.005	0.20 ± 0.006
T wave Amplitude (mV)	0.26 ± 0.03	0.35 ± 0.03

References

1. Appel MJG, Scott WF, Carmichael LE. Isolation and immunization studies of canine parvo-like virus from dogs with haemorrhagic enteritis. Vet. Rec. 1979; 105:156-159.
2. Axthelm MK, Krakowka S. Canine distemper virus-induced thrombocytopenia American Journal of Veterinary Research. 1987; 48(8):1269-1275.
3. Carman PS, Povey RC. Pathogenesis of canine parvovirus-2 in dogs: histopathology and antigen identification in tissues. Res. Vet. Sci. 1985; 38:141-150.
4. Carmichael LE, Binn LN. New enteric viruses in the dog. Adv. Vet. Sci. Comp. Med. 1981; 25:37.
5. Carmichael LE, Robson DS, Barnes ED. Transfer and decline of maternal infections hepatitis antibody in puppies. Proceedings of the Society for Experimental Biology and Medic. 1962; 109:677-681.
6. Chappuis G. Neonatal immunity and immunization in early stages: lessons from Veterinary medicine, Vaccine. 1998; 16:1468-1472.
7. Decaro N, Desario C, Campolo M, Cavalli A, Cirone F, Gabriella E *et al*. Canine parvovirus infection: which diagnostic test for the virus? J Virol. Methods. 2005; 126:179-185.
8. Decaro N, Desario C, Campolo C, Cavalli A, Ricci D, Martella V *et al*. Evaluation of the lactogenic immunity to canine parvovirus in pups. The New Microbiologica. 2004; 27:375-379.
9. Doumas BT, Bayse D, Bornerk, Carter RJ, Peters TJ, Schaffer R. A candidate reference method for determination of total protein in serum: Development and validation. Clin. Chem. 1981; 27:1642.
10. Gillespie JH, Baker JA, Burgher J, Robsoy Gilman B. The immune response to canine distemper virus. Cornell Veterinary. 1958; 65(1):125.
11. Hoskins DJ. Canine Viral Enteritis. In: Infectious Diseases of the Dogs and Cats. Greene, C.E. (2nd edn.).W.B. Saunders Co., Philadelphia, 1998, 40-48.
12. Pollock RV, Carmichael LE. Maternal derived immunity to canine parvovirus infection transfer, decline and interference with vaccination. Journal of the American Veterinary Medical association. 1982; 180:37-42.
13. Parthiban S. Isolation and characterization of canine parvovirus in Puducherry. MVSc thesis submitted to Pondicherry University, 2008.
14. Radostits Otto M, Mayhew IG, Doreen M, Houston. Veterinary Clinical Examination and diagnosis. Saunders Co. Ltd. Publisher and Distributors, West Bengal, India, 2001.
15. Winters WD. Time dependent decreases of maternal canine virus antibodies in new born pups. Veterinary record. 1981; 108:295-299.
16. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D *et al*. Evidence for evolution of canine parvovirus type 2 in Italy. Journal of General Virology. 2001; 82:3021-3025.
17. Nelson DT, Eutis JP, Mcadaragh JP, Stotz I. Lesions of spontaneous canine viral enteritis. Veterinary Pathology, 1979; 16:680-686.
18. Robinson WF, Huxtabee CR, Pass DA. Canine parvoviral myocarditis: a morphologic description of the natural disease. Veterinary Pathology. 1980; 17:282-293.
19. Lenghaus C, Studdert MJ. Acute and chronic viral myocarditis. Acute diffuse non-suppurative myocarditis and residual myocardial scarring following infection with canine parvovirus. Am J Pathol. 1984; 115:316-319.
20. Macartney L, McCandlish IAP, Thompson H, Cornwell HJC. Canine parvovirus enteritis 1: clinical, haematological and pathological features of experimental infection. Vet Rec. 1984; 115:210-210.
21. Sastry GA. Veterinary Clinical Pathology. 4th ed. New Delhi, India: CBS, 1985, 1-32.
22. Van de Perre P. Transfer of antibody via mother's milk. Vaccine. 2003; 21:3374-3376.
23. Sakulwira K, Oraveerakul K, Poovorawan Y. Detection and genotyping of canine parvovirus in enteritic dogs by PCR and RFLP. Science Asia. 2001; 27:143-147.
24. Tilley. Essentials of canine and feline electrocardiography. 3^{rd}ed. Lea and Febiger publisher and distributors, U.S.A, America, 1992.