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Clinical manifestations and hemato biochemical alterations of dogs affected with parvo viral enteritis

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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 checkup that were apparently normal were taken as a control group. The disease was predominantly noticed in dogs between 0 to 6 months old. The hematological study that was conducted showed a reduction in number of thrombocytes of disease group (1.34±0.152) when compared to a control group (5.26±2.21) due to thrombocytopenia which is a good indicator for prognosis. There was a reduction in total protein and albumin in the disease group (4.74±0.68 and 2.86±1.67) compared to the control group (6.52±0.74 and 3.96±0.48) due to exudative type or permeability diarrhoea. Nine suspected samples were positive using H primer pair generated PCR product of 630 bp. 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) 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).

Keywords: PVE, CPV, Hemato biochemical, PCR.

Introduction
Parvovirus was first described as a clinical entity causing haemorrhagic gastroenteritis in dogs in 1977(Appel *et al.*, 1979) [1] and the aetiological 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 haemorrhagic 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 haemorrhagic) 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 diarrhoea, 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]. 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 parvoviral 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. They also reported that this primer could be great help in identifying the types/strains of canine parvovirus since it amplified the region where many of the important informative amino acid lied.

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, diarrhoea, 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]. Blood samples were collected in ethylene diamine tetra acetic acid vials from the cephalic vein of the animals under study. The estimation of RBCs, Hb, PCV and WBCs was conducted according to Sastry (1985) [21]. Blood was collected from the dogs and allowed to clot and the serum was harvested for the estimation of total protein and albumin calorimetrically with a commercially available diagnostic kit "agappi" using semi auto analyser as per (Doumas *et al.*, 1981) [9]. 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. 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 H rev	CAGGTGATGAATTTGCTACA CATTTGGATAAACTGGTGGT	All types	3556 – 3575 4166 – 4185	50ºC 630 bp

Results

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 checkup that were apparently normal were taken as a control group. Among the nine affected animals, 45 per cent were Non-descript dogs, 22 per cent Daschund, 11 per cent Boxer, 11 per cent Labrador and 11 per cent Dalmatian. 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 and among them 56 per cent were male and 44 per cent were female. The clinical manifestations were shown in Fig.1**.** On Faecal sample examination by direct smear method 11 per cent were positive for anchylostome ova. The hematological values of the control group and the disease group were showed in Table.2. The Serum biochemistry of the control group and the disease group were given in Table.3**.** Nine suspected samples were positive using H primer pair generated PCR product of 630 bp (Fig.2).

Table 2: Haematological changes in dogs with Parvo Viral Enteritis (PVE) compared to control group.

rameters	Disease group (N=9)	Control group (N=9)
Hb value	10.55±1.99	18.1±1.09
PCV	28.5±5.6	49.6±3.71
RBC	3.8±0.76	7.24±0.59
WBC	5.27±2.15	13.37±2.97
Neutrophils	3.87±1.59	6.99±2.16
Lymphocytes	1.33±0.6	2.61±0.62
Monocyte	0.037±0.05	0.23±0.09
Eosinophil	0.031±0.0032	0.84±0.68
Thrombocyte	1.34±0.152.	5.26±2.21

Table 3: Serum biochemical alterations in dogs with Parvo Viral Enteritis (PVE) compared to the control group.

Parameters	Disease group (N=9)	Control group (N=9)
Total protein	4.74±0.68	6.52±0.74
Albumin	2.86±1.67	3.96±0.48
Globulin	1.88±1.6	2.56±0.72
Albumin: Globulin ratio	2.82±2.44	1.55±0.67

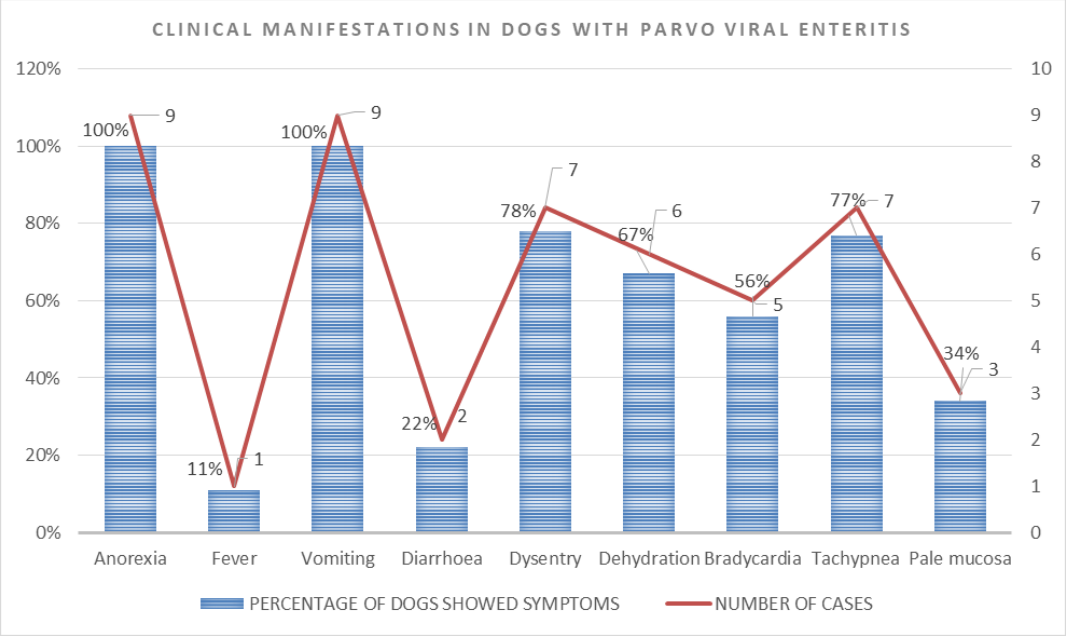

Fig 1: Clinical manifestations in dogs with PVE

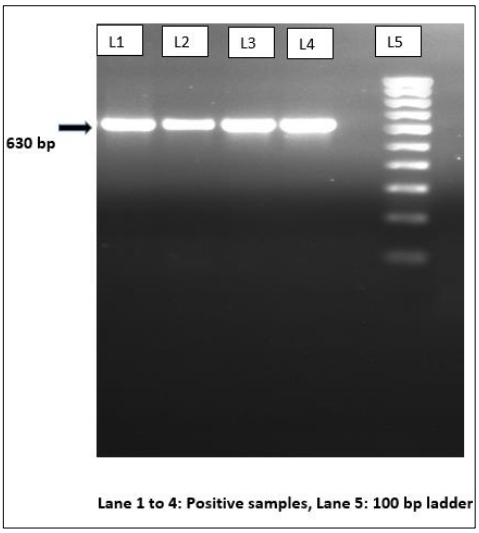

Fig: 2 Agarose Gel Electrophoresis of the amplified product: Lane 1 to 4: positive samples Lane 5: 100 bp ladder.

Discussion and Conclusion

CPV is a highly contagious and fatal infection of canine population characterized by vomiting, gastroenteritis in dogs. The study was undertaken for the clinical manifestations and haemato biochemical alterations in parvoviral enteritis of dogs. Hoskins (1998) [11] found that Doberman Pinschers, Rottweilers, English Springer Spaniels had significantly increased risk factor for CPV enteritis. In another work, Rottweilers, American Pit Bull Terriers, Doberman Pinschers, and German shepherd had significantly higher risk factor for CPV corresponding to age and sex. Although it was common in Doberman Pinschers and Rottweilers (Hoskins 1998) [11] 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. CPV infection was widely prevalent among canine population in Puducherry, Tamilnadu. 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]. All dogs of the disease group are within 6 months age indicates that the disease is common between 0 to 6 months old as reported by other author(Parthiban 2008) [13]. Thrombocytopenia was found to be mediated by virus-antibody immune complexes on platelet membranes. Decreased platelet production due to a direct viral effect on megakaryocytes was a likely contributor factor(Axthelm *et al.*, 1987) [2]. The haematological study that was conducted showed a reduction in number of thrombocytes of disease group when compared to a control group due to thrombocytopenia which is a good indicator for prognosis. There was a reduction in total protein and albumin in the disease group compared to the control group due to exudative type or permeability diarrhea. 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).

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