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## Molecular detection of canine parvovirus from haemorrhagic enteric affections of dog in Orathanadu region, Tamil Nadu, South India

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**Abstract**

The present study deals with the rapid detection of Canine Parvo Viral (CPV) infection in various breed of dogs of Cauvery Delta Region, Tamilnadu by molecular means of diagnosis employing the polymerase chain reaction (PCR) method. A total of 168 haemorrhagic fecal samples were collected and out of those 112 were found positive by PCR. Among different age groups, 154/80,55/67 and 3/21 dogs were positive in pups aged 0-3 weeks,4-8 and 9-12 weeks respectively. All positive samples were from unvaccinated dogs. The breed of Labrador was found to be the most susceptible breed (n = 43) to Parvo viral infection. The positivity of confirmation (112/168: 66%) concludes PCR being a reliable one for early diagnosis and the study on variants of CPV-2b was ascertained by PCR and being the first report on molecular detection of CPV infection in dogs in this region. Type CPV-2c was not detected among the examined samples. On review of literature and on observance of recent reports the CPV Variant (CPV-2b) in India is gaining importance and mortality of pups due to CPV infection is high and precise and early diagnosis of CPV infection warrants early intervention of prior vaccination in all breeds of dogs against the deadly viral infection.

**Keywords:** canine parvovirus, CPV-2b, polymerase chain reaction, early detection

**1. Introduction**

Canine parvovirus 2 (CPV-2) causes acute haemorrhagic enteritis and myocarditis in canine, being contagious [9] and CPV-2 belongs to the family of Parvoviridae causing high morbidity and 10% mortality in canines in various parts of the world [1, 7]. Owing to the variants, CPV mutates and being highly contagious identification and characterisation is highly essential [6, 7]. Canine Parvo virus -2 (CPV-2) was first isolated in India in 1982 [11]. The disease is characterised by severe diarrhoea and vomiting later proceeding to fatal due to dehydration and more important if pups below 3 months of age are affected and die due to myocarditis [1]. CPV-2 is a non-enveloped single structured DNA virus of 5.2Kb size with icosahedral symmetry. The reports on Canine Parvo Virus (CPV) infection and its rapid detection by molecular means in this region are quite scanty and the present study deal with the occurrence pattern and molecular detection of CPV 2b from the clinically ill diarrhoeic dogs showing haemorrhagic affection during various seasons (Jan 2018 – Dec 2019) of Orathanadu region and part of CDZ of Tamilnadu, South India.

Comparing the traditional methods, molecular method of detection such as the PCR technique and its standardization for diagnosis of CPV-2 as a rapid diagnostic method has been widely accepted owing to high sensitivity and specificity [4, 9].

**2. Materials and Methods****2.1 Specimen collection**

Specimen collection Rectal swabs were collected from 168 dogs suspected for CPV showing haemorrhagic enteritis which were brought from nearby Government Veterinary Dispensaries and pet owners of Thanjavur District during the period from Jan 2018 to Dec 2019. The blood tinged faecal samples were aseptically collected by using sterile swabs in phosphate buffer saline (PBS, pH 7.2) (Hi-Media, Mumbai) and stored in -86 °C until further processing. The samples of infected dogs collected were homogenised [5]. In phosphate buffered saline (PBS, pH7.2) and homogenates (200µL) were frozen and thawed thrice, subsequently clarified by centrifuging at 10000 rpm for 15 minutes.

## 2.2 Preparation and clarification of DNA Template

Being a DNA virus, CPV suspected samples after the storage were subjected to alternate freeze and thawing and the nucleic acids extracted from 200µL blood samples using Qiagen Viral Nucleic Acid Kit (Qiagen Inc. Germany) following the manufacturer's protocol with minor modifications as described [9]. The purified DNAs were stored at -86 °C until PCR. The PCR targeting partial VP2 gene (427bp) was performed. The amplified PCR products were visualized in

GelDoc apparatus (Bio-rad Inc, USA) via ethidium bromide staining after electrophoresis using in 1.2% agarose gel in TAE. Out of 168 rectal swabs collected, 32 were Labrador (80%), 35 Doberman pincher (68.5%), 31 were German shepherd (71%), 22 were Spitz (63.66%), 20 were Cocker spaniel (35%), 20 were nondescript (65%). The age group, gender, vaccination status were also recorded for the study purpose. (Table 1)

**Table 1:** Distribution of samples affected by CPV -2 infection (Jan- Dec 2019)

Age category	Breed category	Number of suspected samples for CPV	Number of samples confirmed by PCR for CPV-2b
0-3 weeks	Labrador	20	18
	Doberman pincher	18	13
	German Shepherd	16	12
	Spitz	8	6
	Cocker spaniel	9	
	Non-descript	9	5
4-8 weeks	Labrador	14	12
	Doberman pincher	13	10
	German Shepherd	11	10
	Spitz	11	8
	Cocker spaniel	9	7
	Non-descript	9	8
9-12 weeks	Labrador	6	2
	Doberman pincher	4	1
	German Shepherd	4	--
	Spitz	3	--
	Cocker spaniel	2	--
	Non-descript	2	--
	Total	168	112

## 2.3 Detection of CPV variant by PCR

The PCR primer sequence employed in their molecular typing of parvoviral DNA were published sequences of CPV-2b variant (Reed *et al.* 1988).

The primer sequence amplifying 427bp fragment of the gen5e

coding the capsid protein VP2 is as follows: (Pereira *et al.* 2000) (Table 2)

**CPV-2b** F-5'-CTTTAACCTTCCTGTAACAG3'  
R-5'-CATAGTTAAATTGGTTATCTA3'

**Table 2:** Thermocycling conditions for amplification of CPV-2 DNA extracted from clinical samples

Name of the virus variant	Forward Primer	Reverse Primer	PCR thermal condition
Canine Parvo virus-2b (CPV-2b)	F-5'-CTTTAACCTTCCTGTAACAG3'	R-5'-CATAGTTAAATTGGTTATCTA3'	Denaturation-94 °C/30s Annealing-52 °C/2mins Extension-72 °C/2mins

The samples were subjected to gradient PCR following the protocol as described above with the primer pair of CPV-2b variant. The above primer pair amplifies a 427 bp fragment of the original strain of CPV-2. The PCR program was as follows: 5 min at 94 °C, 30 cycles of 30 s at 94 °C, 2 min 55 °C, followed by 2 min at 72 °C and 5 min at 72 °C (Eppendorf Master cycler, Germany). To detect the new antigenic variants of CPV, a 427 bp fragment encoding capsid protein VP2 of antigenic type CPV-2b was amplified under similar PCR conditions. The PCR reaction was performed as described earlier. The PCR products were electrophoresed along with a 100 bp DNA ladder in 1% agarose gel containing 0.5 µg/ml ethidium bromide.

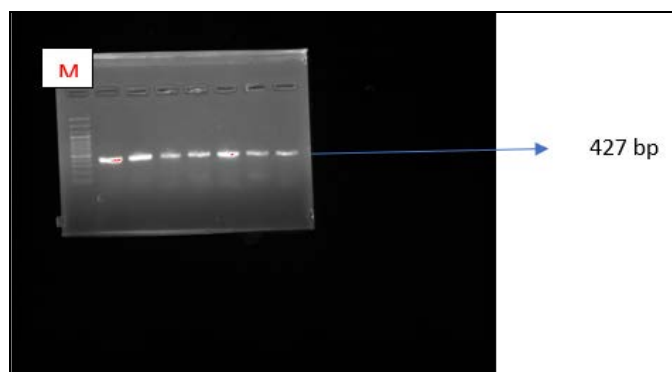
## 2.4 Purification of PCR products

The obtained PCR products were purified with PCR

purification kit (Gene JET, Thermo, USA) as per manufacturer's recommendations. The purified PCR products containing about 50 ng per µl DNA concentration and 260/280 ratio above 1.8 were used for further analysis using Bio-spectrophotometer (Eppendorf, Germany) The obtained PCR products were purified with PCR purification kit (Gene JET, Thermo, USA) as per manufacturer's recommendations. The purified PCR products containing about 50 ng per µl DNA concentration and 260/280 ratio above 1.8 were used for further analysis using Bio-spectrophotometer (Eppendorf, Germany)

## 3. Result

Out of 143 PCR positive sample, 112 (66%) showed positive amplification of 427 bp indicating that it belongs to CPV-2b (Fig-1)



**Fig 1:** PCR products (427 bp) for the CPV-2b gene of Canine Parvo virus -2 in fecal samples Lane 1-7 represent positive samples and Lane M represents molecular weight marker from 100 to 1000 base.

As per Table 2, the pups between 4-8 weeks of age were found to be vulnerable to CPV infection and as rectal swabs received from non-vaccinated animals nearly (66%) indicate the positivity of CPV infection in dogs in this region. As per the breed disposition, the Labrador was found to be the most predisposed breed to this condition (40 cases) followed by Doberman pincher, 31 German Shepherd, 22 Spitz, 20 Cocker spaniel and 20 were nondescript breeds. All the fecal samples of the suspected dogs found positive by practitioners were simultaneously tested and detected to 66% of CPV -1 antigen by the Qiagen Rapid Ag Test Kit and the PCR investigations with the pCPV-2b with the above primer set revealed positive results in all tested faeces samples.

#### 4. Discussion

CPV-2 occurs worldwide in domestic dogs and has mainly increased rate of incidence due to improper maintenance of breeding kennels, animal shelter and intensive rearing of dogs in house dwelling areas without proper vaccination and incidence of CPV infection is more severe in young puppies of younger than 3 months of age<sup>[8]</sup>.

In the present study, prevalence of CPV-2 in haemorrhagic diarrhoeic dogs was found to be 66% and correlates with findings<sup>[2, 8]</sup> mainly in India. In breed wise occurrence of CPV-2 in this Orathanadu region, prevalence of CPV was highest in Labrador (80%), followed by Doberman pincher (68.5%), German Shepherd (71%), Spitz (63.66%), Cocker spaniel (35%) and Non-Descript (65%). The age group, gender, vaccination status were also recorded for the study purpose. (Table 1) The percentage of positivity was on an overall of 66% which correlates with the findings<sup>[13, 14]</sup>. The low prevalence of CPV in adult dogs may be due to subclinical infections and the adequate amount of antibody to protect adult dogs against CPV-2. PCR for molecular typing has proved to be the rapid method of diagnosis using the specific primers for the CPV mutant as reported<sup>[10]</sup>. The ideal material for molecular typing of CPV-2 is fecal sample from suspected dogs of CPV. This has been already proved earlier<sup>[13]</sup> owing to the shedding of 10<sup>9</sup> virus particles/gm of feces during the acute phase of the disease.

#### 6. Conclusion

A rapid method by molecular means of diagnosis was standardised in detection of clinically affected Canine Parvo Viral (CPV) infection in various breed of dogs of Cauvery Delta Region, Tamilnadu by polymerase chain reaction (PCR) method. Among 168 haemorrhagic fecal samples suspected for CPV-1 infection collected, 112 were found positive by PCR.(66%) Among different age groups, 154/80,55/67 and

3/21 dogs were positive in pups aged 0-3 weeks,4-8 and 9-12 weeks respectively. The Labrador breed of dog was found to be the most susceptible breed (n = 43) to Parvo viral infection. This is the first report on molecular detection of CPV infection in dogs in this region. Early and rapid diagnosis of CPV infection warrants early intervention of the disease and this research study proves that PCR based diagnosis can be employed in future for precise diagnosis of CPV-2 infection in future from clinically affected dogs of various breeds.

#### 7. Acknowledgement

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