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Research Article

PREVALENCE OF CANINE PARVO VIRUS INFECTION IN DOGS IN AND AROUND CHENNAI FROM MARCH, 2021 TO FEBRUARY, 2022

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ABSTRACT

Canine parvovirus (CPV) infection is contagious viral disease of canine. Dogs infected by three variants of CPV type. A total of 300 samples screened for canine parvo virus infection from March 2021 to February 2022 reported with the history of vomition and watery blood mixed foul smell diarrhoea presented in TeachingVeterinary Clinical Complex Hospital, Madras veterinary college. Out of 300 faecal samples, 150 samples were shown positivity by Amplification refractory mutation system-Polymerase Chain reaction for CPV infection. Non-descript breeds showed high prevalence (64%), highest positivity between 3-6 month of age group (42%), male shown highest positivity (55.33%) than female (44.66%). Unvaccinated dogs had the highest positive prevalence of (68.66%). This study concluded that through proper vaccination we will control the parvovirus infection among the dogs. Epidemiological factors like age, breed, sex play an important role in the prevalence of canine parvo virus infection.

Keywords: Canine parvovirus-2 variant, Prevalence, ARMS-PCR, Haemorrhagic bloody diarrhoea

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Canine parvovirus type 2 (CPV2) is one of the most important global pandemic contagious viral diseases affecting canine domestic population, especially young puppies causing acute haemorrhagic enteritis, myocarditis, vomition, and immunosuppression (Ahmed *et al.*, 2018). The virus causes major outbreaks of haemorrhagic enteritis leading to high morbidity and mortality rates in young dogs. The genomic substitution rate of CPV2 is very high (Decaro *et al.*, 2009). CPV is classified within the family Parvoviridae subfamily Parvovirinae, genus protoparvovirus and species carnivore protoparvo virus I (Cotmore *et al.*, 2019). Dogs with canine parvovirus enteritis usually exhibit high fever, depression, loss of appetite, lethargy, vomiting, and severe mucoid or bloody diarrhoea (Khare *et al.*, 2019). The incubation period following natural or experimental exposure ranges from four to fourteen days, and virus shedding starts a few days before the onset of clinical signs and progressively declining 3 to 4 weeks post-exposure (McCaw and Hoskins, 2006). CPV-2 can survive more than one year in the environment and infect the susceptible dogs through infected faeces, or vomitus by faecal-oral route (Albaz *et al.*, 2015). The viral genome is a single-stranded, linear, negative-sense DNA comprising about 5200 nucleotides. The genome encodes four proteins; two non-structural proteins called NS1 (involved in viral replication) and NS2 (has a role in capsid assembly) and two structural proteins termed VP1 (involved in cell infection) and VP2 (forms the viral capsid and is the main protective antigen) (Chinchkar *et al.*, 2006). CPV replicate in the intestinal crypts and the lymphoid organs, but the virus can spread to all body tissues (Pollock, 1982), including the brain. Once the virus enters in to the body it penetrates through the oro-nasal route. The virus replicates in gastro enteric associated lymphoid tissues and is disseminated by infected leukocytes to the germinal epithelium of the crypts of the small intestine and initiate (Pollock, 1982). In 2-3 week-old seronegative pups, CPV can also able to replicate in cardiac cells inducing a fatal myocarditis. This myocardial form is not always seen in all young pups are protected by maternally derived antibodies (MDA). The aim of this study was to find out the positive prevalence of canine parvovirus infection in and around Chennai from March 2021 to February 2022 based on the age, breed, sex and vaccination status of infected dogs and confirmed by Amplification Refractory Mutation System-Polymerase Chain Reaction.

MATERIALS AND METHODS

For one year, prevalence study was carried out at Infectious disease unit of Veterinary teaching hospital, Madras Veterinary College, Chennai from March 2021 to February 2022. A total of 300 dogs of different breeds of the age group ranging from 30 days to up to greater than 1 year (upto 3 yrs.) presented format different localities in and around Chennai was considered in the present study. Canine exhibiting clinical signs including fever, diarrhoea with foul smell and vomiting, tachycardia, dehydration and weakness were included in the study. Similarly

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different age groups, breed and gender. The details recorded for parvovirus suspected dogs were of different age groups, breed and gender with vaccination history were also recorded for parvo virus suspected dogs. Faecal samples were collected from suspected dogs by using Sterile cotton swab (Manufacturer, Hi-media). Later, the samples were stored at -20°C until further analysis.

DNA Extraction

Directly from commercial DNA extraction kit from faecal samples as per the instructions of manufacturer (Quiagen).

Amplification Refractory Mutation System- Polymerase Chain Reaction (ARMS-PCR) - partial amplification of canine parvovirus VP2 gene.

A reaction mixture volume of 20 µl was prepared and performed with the following PCR reaction was standardized for partial VP2 gene. The reaction mixture was prepared in 200 µl PCR tubes mentioned in Table 2. The amplification was performed in a thermo cycler (BioRad) witha reaction condition (Putty Kalyani *et al.*, 2020) comprised of an initial denaturation at 95 ºC for 5 minutes followed by 35 cycles of denaturation at 95 ºC for 30 seconds, annealing at 45 ºC for 30 seconds and extension at 72 ºC for 1 minute, with a final extension step at 72 ºC for 10 minutes with the specific primers mentioned in Table1. The amplified PCR products wereanalyzed (Fig. 1 and 1a) on 3% Agarose gel with the positive control and visualized under UV transilluminator (Biorad, India).

RESULTS AND DISCUSSION

Among 300 suspected faecal samples of dogs, a total of 150 (50%) samples showed positivity towards parvo virus infection by ARMS-PCR. Figure-1 and 1a showed the band size of 631 bp, 492 bp and 179 bp positive for Canine parvovirus using the earlier published primers (Putty Kalyani *et al.*, 2020). In this study the positive prevalence was calculated with respect to total positive (150) cases. The breed wise positive prevalence was found to be higher in non-descript breeds 64% (96/150) followed by Labrador 16% (24/150) followed by Spitz 4% (6/150), Chippparai 2.6% and lowest prevalence in Dalmatian, German shepherd, Rottweiler, Kanni, Golden retriever, Mongrel, Doberman, Lhasapso, Pug, Pitbull, Daschund (Table 3).

Age-wise prevalence of parvo virus in dogs among the positive cases revealed the infection being more in the age group of 3-6 months 42% (63/150) followed by 0-3 months 38.66% (58/150) followed by 6-9 months 8.6% (13/150) and a lowest positive prevalence in age groups above 9-12 months 4.6% (7/150) and greater than age of 1 year (1 to 3 yrs) was 6% (9/150) (Table 4). The prevalence over total suspected cases were found to be 55.33%

Table 1. Primer used for PCR (Chander *et al.*, 2016)

Primers	Sequence of Primers	Base pair
CPV Outer FP (OF)	TGATTGTAAACCATGTAGACTA	631 bp
CPV Outer RP (OR)	AAGTCAGTATCAAATTCTTTATC	631 bp
CPV Inner FP (IF)	ACTTTAACCTTCCTGTAAGAG	179 bp
CPV Inner RP (IR)	GTTGGTAGCAATACATTAGCA	492 bp

Table 2. ARMS-PCR Reaction mixture (20 µl)

Components	Volume (20 µl)
Master mix (Genei, Bangalore)	10 µl
Nuclease free water	2.0 µl
CPV –outer –forward primer –sigma	0.6 µl
CPV-outer –Reverse primer-sigma	0.6 µl
CPV-Inner –forward primer-sigma	0.6 µl
CPV- Inner –Reverse primer-sigma	0.6 µl
Template DNA	5.6 µl
Total reaction mixture volume	20 µl

Table 3. Breed-wise prevalence of parvovirus infection in dogs

Breed	Number of positive cases (N=150)	Positive Prevalence (%)
Non -descript	96	64
Labrador	24	16
Rottweiler	3	2
Golden retriever	2	1.3
Kanni	2	1.3
Great dane	1	0.6
Mongral	1	0.6
Chippiparai	4	2.6
Dalmation	3	2
Doberman	1	0.6
Spitz	6	4
GSD	3	2
Lashapso	1	0.6
Pug	1	0.6
Pitbull	1	0.6
Daschund	1	0.6

Table 4. Age-wise prevalence of parvovirus infection in dogs

Age of animals (month/yrs)	Numbers of positive cases (n= 150)	Positive Prevalence (%)
0-3	63	42
3-6	58	38.66
6-9	13	8.6
9-12	7	4.6
1to 3yrs	9	6

Table 5. Sex-wise prevalence of parvovirus infection in dogs

Sex	No. of positive cases (N=150)	Positive Prevalence (%)
Male	83	55.33
Female	67	44.66

Table 6. Vaccination details of positive cases

Vaccinated/ unvaccinated	No. of cases	Prevalence (%)
Unvaccinated	103	68.66
Vaccinated	47	31.33
Primary dose	31	20
II dose	5	3.33
III dose	11	7.33

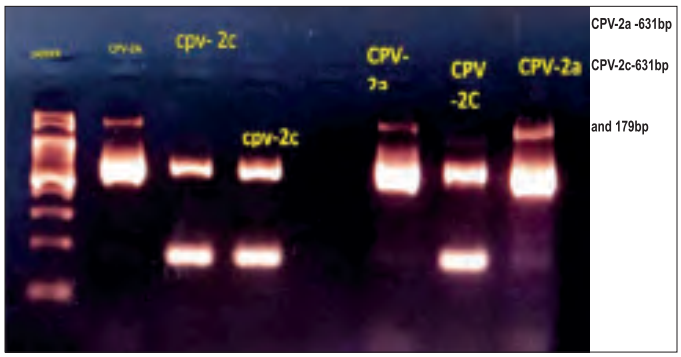

Fig. 1. Positive samples of CPV-2 variant
1-Ladder (100 bp), 2-Positive control, 3,4-Positive sample of CPV-2c (631 bp+179 bp), 5-Negative samples 6-positive sample of CPV-2a (631 bp+492bp), 7-Positive samples of CPV-2c, 8-positive sample of CPV-2a (631 bp+492 bp)

(83/150) in males and 44.66% (67/150) in females (Table 5). Among 150 positive cases unvaccinated was 68.66% (103/150) and 20% (31/150) was primary vaccinated dogs 3.3% (5/150) was secondary dose vaccinated dogs and tertiary vaccinated dogs was 7.33% (11/150) (Table 6).

In this study, overall positive prevalence of parvo virus enteritis was found in nondescript breeds (64%) followed by other breeds. The higher positivity in Nondescript might be due to over population, irregular vaccination protocol, (Behera *et al.*, 2015) roaming activity, increased chances of high exposure of infection. In overall male dogs showed higher positivity than female dogs.Gombac *et al.* (2008) in Slovenia and Srinivas *et al.* (2013) in Odisha found increased susceptibility of males for parvo enteritis. Males are predominantly available in field might be due to avoidance of female by the owners for breeding cycle issue, frequent exposure and behavioral pattern (Deka *et al.*, 2013). Highest positive prevalence as found in 0-3 months of age followed by three to six months, six to nine months, nine to twelve months, one to three years. Mainly due to the affinity of CPV to the mitotic intestinal cells, maternal antibody inter-reference and gut

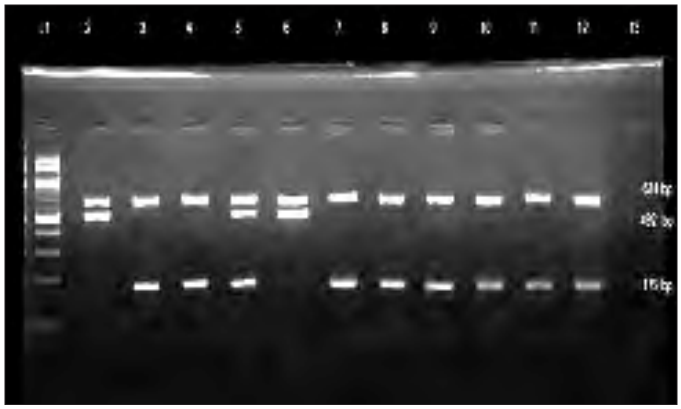

Fig.1a. ARMS-PCR Amplification of CPV-2 variants in dogs with tetra primers; 1-Ladder (100 bp), 2-Positive control, 3,4-Positive sample of CPV-2c (631 bp+179bp), 5-Positive samples of CPV-2b (631bp+492 bp+179 bp), 6-positive control of CPV-2a (631 bp+492bp), 7, 8,10,11,12-Positive samples of CPV-2c, 13-Negative control

Haemorrhagicdiarrhoea in dog

flora alteration through dietary change during weaning time increased the chances of CPV in less than three months of age.

Fall in maternal antibody after three months makes three to six months age group as vulnerable in endemic areas (Stepita *et al.*, 2013). Highest incidence in 6-9 month, 9-12 month followed by above one to three year reason due to improper vaccination schedule, lack of booster dose, improper cold chain and poor immunity (Carmichael *et al.*, 1983). Higherpositive prevalence in unvaccinated cases documented by many researchers (Houston *et al.*, 1996: Parrish, 1999 and Miranda and Thompson, 2016). Highest positive prevalence in unvaccinated is 68.66% and vaccinated is 31.33%. Occurrence of positivity in vaccinated cases may be due to improper vaccination protocol, improper cold chain maintenance and due to strain variation.

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