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Original Article

Detection of Canine Parvovirus from Suspected Clinical Samples in Pups

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Abstract

Canine parvovirus is one of the most devastating viral disease of dogs causing high morbidity and mortality. Parvovirus proliferates mainly in the gastrointestinal tract of adults and myocardium in case of pups. Two clinical cases were presented to the teaching veterinary clinical complex with a history of dogs of age group 3 months, having a temperature of 101^0F, diarrhea with foul odors and the dogs were not yet vaccinated. The clinical cases suspected of parvoviral infection were preliminarily screened by haemagglutination test and later confirmation was done by employing Polymerase Chain Reaction for the suspected fecal samples.

Keywords: Canine Parvovirus; HA; PCR Assay.

Introduction

Parvovirus a DNA virus belonging to the Parvoviridae family affects felines, canines and porcines. It is a non–enveloped, icosahedral virus, negative sense linear single stranded DNA virus with genome size of 5.2Kbp. It has two structural VP1 & VP2 and two non- structural proteins NS1 & NS2 of which VP2 protein is highly immunogenic and is responsible for the disease [1]. Canine parvovirus also named as CPV-2 is responsible for causing the disease in canines.

CPV is a highly mutant virus and undergone various changes in single nucleotide position, forming new CPV variants, namely CPV-2a, CPV-2b, new CPV-2a, new CPV-2b, and CPV-2c depending on the geographical locations [2,3].

The CPV in faecal samples can be detected by several methods like haemagglutination test, latex agglutination test, immunochromatographic test (IC), PCR based methods, Enzyme Linked Immunosorbent Assay and DNA hybridization.

Hence the present study was taken up to characterize the clinical cases both phenotypically and genotypically.

Material and Methods

The two fecal samples were collected in 0.2M Sorenson's PBS (pH 7.0) from the dogs presented to the Teaching Veterinary Clinical Complex, NTR CVSc., Gannavaram with a history of dogs of age group 3 months both were females having a temperature of 101°F, vomition and diarrhea with foul odors and the dogs were not yet vaccinated.

Methodology

Phenotypic Characterization

The fecal samples collected in 0.2M Sorenson's PBS (pH 7.0) were clarified by centrifuging at 3000 rpm for 10 minutes at 4°C and the supernatant was screened for CPV infection by haemagglutination (HA) test using 0.8% swine RBC. Ninety microlitres of processed faecal sample was treated with 10µl of chloroform and mixed well. The mixture was kept at 4°C for 10 min and later centrifuged at 10000 rpm at 4°C for 10 min. The supernatant was collected and used for haemagglutination test.

Two fold serial dilutions of 50µl amounts of the chloroform treated samples were made in 0.2M Sorenson's PBS of pH 7.0 in a 96 well 'U' bottom microtitre plates. To each well 50µl of 0.8 percent pig erythrocytes were added, mixed gently and allowed to settle at 4°C for 4 hrs. One well, added with 50µl of 0.2 M Sorenson's PBS of pH 7.0 and 50ìl of 0.8 percent pig erythrocytes, served as cell control. The highest dilution of sample showing complete haemagglutination was considered as the haemagglutination titre.

Genotypic Characterization

Genotypic characterization of the fecal samples was done by using PCR assay with CPV 2ab type specific primers [4]. The processed samples were screened by primer pair CPV- 2ab (F)/2ab (R) [5] that amplified at 681bp fragment of the gene encoding capsid protein VP2 of both CPV-2a and CPV-2b types having the sequence as CPV-2ab [F] 5'-AAGAGTGGTTGTAAATAATT-3' and CPV-2ab [R] 5'CTATATAACCAAAGTTAGTAC-3'. The DNA was isolated from faecal samples by boiling method [6].

A total of 100µl of processed faecal sample was used for template DNA preparation. The samples were boiled at 96°C for 10 min and immediately chilled in crushed ice. Then samples were centrifuged at 12,000×*g* for 10 min at 4°C. The supernatants were diluted 1:5 in distilled water to reduce residual inhibitors of DNA polymerase activity [7] and used as sample for PCR.

The PCR reaction mix was prepared with 10µl of Gotaq master mix, forward and reverse primer 1µl each, 6µl of DNA template and 2µl of nuclease free water. Amplification was carried out with an initial denaturation of 95°C for 5 minutes, followed by 30 cycles of denaturation [94°C for 30 seconds], annealing [55°C for 2 minutes], extension [72°C for 2minutes] and then final extension for 4 minutes at 72°C. After completion of the cycles, the PCR product obtained was subjected to 1% agarose gel electrophoresis. The gel was visualized under UV transilluminator at medium wavelength in GENE FLASH.

Results and Discussion

The earlier studies on canine parvovirus infections revealed that the occurrence of parvovirus in dogs is higher in unvaccinated dogs. Pups of age group 8 – 10 weeks are more prone to infection and the mortality rate is 91% as the severity of symptoms is relatively high. On preliminary screening of the two fecal samples fromhaemagglutination test with 0.8% swine R.B.C., both samples were shown a clear agglutination reaction with a HA titre of 1:512 and 1:128 indicating that the concentration of virion particles voided in the affected dogfeces is high[Fig. 1].

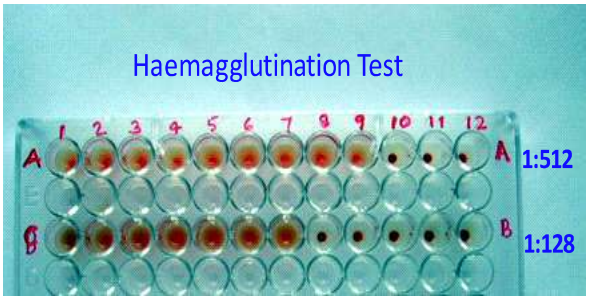

Fig. 1: Haemagglutination test for the two fecal samples showing titre 1:512 and 1:128

Parvoviral infected dogs excrete more than 10^9 virus particles per gram of feces and play a major role in transmission of infection among the dogs [8]. Further molecular confirmations were done by subjecting both the samples for PCR and were amplified with CPV2ab primers with a VP2 gene product size of 681 bp. The first sample produced a thick band as the concentration of the virion particles is high when compared to sample 2. The product size of 681 bpsuggests that the strain belongs to CPV-2a, further confirmation of the strain can be revealed by partial sequencing of the VP2 gene of the obtained PCR product.

Sequencing of the VP2 gene aids in analysing the substituted amino acid at the 426 bp position of the VP2 gene which determines the type of strain. In the present study the two samples revealed a product size of 681bp indicating that

Fig. 2: PCR Product [681bp] positive for CPV-2a strains of two fecal samples

the strain belongs to CPV-2a [Fig. 2]. The best reviews emphasizes that the most prevalent strain of CPV in India was CPV-2a.The detection of two CPV-2a strains in canines strongly correlates with theprevious reports [9,10]. Further, while co-relating the phenotypic and genotypic characterization in the first sample the HA titre is 1:512 indicating the virus voided in the faeces is relatively high when compared to the second sample which is having a lesser titre of 1:128. Based on the concentration of the virus, variations in the intensity of the PCR band emphasize that more virion particles are present in sample one when compared to sample 2.

Conclusion

The Parvoviral infection can be detected by both phenotypical and genotypical methods and are 100% accurate in confirming the presence of infection. Polymerase chain reaction is highly sensitive [11] and employing specific CPV-2ab primers aids in detecting the type of circulating strains. Further confirmation of the strain should be carried out by partial sequencing of the VP2 gene, which is highly sensitive in identifying the strain.

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Indian Journal of Anesthesia and Analgesia	Monthly	7500	7000	586	547
Indian Journal of Biology	Semiannual	5500	5000	430	391
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International Journal of Neurology and Neurosurgery	Quarterly	10500	10000	820	781
International Journal of Pediatric Nursing	Triannual	5500	5000	430	391
International Journal of Political Science	Semiannual	6000	5500	450	413
International Journal of Practical Nursing	Triannual	5500	5000	430	391
International Physiology	Triannual	7500	7000	586	547
Journal of Animal Feed Science and Technology	Semiannual	78500	78000	6133	6094
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