Rapid, sensitive and cost effective method for isolation of viral DNA from feacal samples of dogs

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Abstract

A simple method for viral DNA extraction using chelex resin was developed. The method used was eco-friendly and cost effective compared to other methods such as phenol chloroform method which use health hazardous organic reagents. Further, a polymerase chain reaction (PCR) based detection of canine parvovirus (CPV) using primers from conserved region of VP2 gene was developed. To increase the sensitivity and specificity of reaction, nested PCR was designed. PCR reaction was optimized to amplify 747bp product of VP2 gene. The assay can be completed in few hours and doesn't need hazardous chemicals. Thus, the sample preparation using chelating resin along with nested PCR seems to be a sensitive, specific and practical method for the detection of CPV in diarrhoeal feacal samples.

Keywords: Chelating resin, CPV, Nested PCR, Virus, Sensitivity, Faecal sample.

Introduction

Canine Parvovirus belongs to the family *Parvoviridae* subfamily *Parvovirinae* and genus *parvovirus*. It is small, non enveloped virus comprised of linear, negative sense, single standard DNA of about 5.2 kb. It encodes two structural proteins (VP1 and VP2) and two non-structural proteins (NS1 and NS2).

It was first identified in 1978 in the USA by Appel et. al (1979) and was designated CPV type 2 (CPV-2) to distinguish it from previously recognized parvovirus of dogs known as minute virus of canines. The virus is shed in faeces (more than 109 virus particles/gm of faeces) from infected dogs during acute phase of infection and infected faeces acts as a main source of infection Carmichael and Binn (1981). CPV in feacal samples has been detected by several methods based on virus isolation in cell culture, hemagglutination (HA), electron microscopy, enzyme linked immunosorbent assay and DNA hybridization (Mochizuki et. al 1984; Teramoto et. al 1984). These methods are generally time consuming and need special equipment and materials (Green, 1984; Appel and Parrish, 1987).

In this study, we attempted to set up chelex resin based rapid, sensitive and cost effective method of viral DNA extraction from feacal samples of dogs. There is no need for phenol/chloroform extractions which are organic and health hazardous. Secondly, the time consuming steps such as precipitation with isopropanol/ethanol are eliminated. The extracted DNA is ready for applications such as polymerase chain reaction, Southern blotting and restriction digestion. Further, to increase the sensitivity and specificity of reaction, the nested PCR with a double nested primer pairs Sakulwira et. al (2001) was used.

Materials and Methods

Collection of faecal sample: Fifty canine faecal samples were collected over a period of two months duration between age group of 0-1 year with diarrhoea from various private clinics and veterinary hospitals located in Hisar district of Haryana.

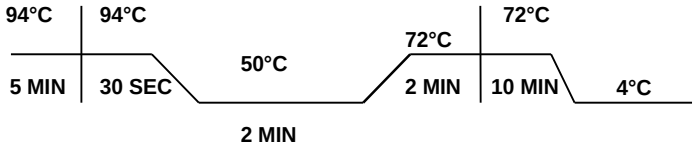
Extraction of viral DNA: The extraction of viral DNA from processed samples was done by using chelex resin (Minakshi, 2008, personal communication). In brief, 10% feacal sample was centrifuged at 3000 x g for 5 minutes. An aliquot of 25µl of the supernatant was taken and to this 200µl of 5% chelex resin solutions was added. The contents were mixed and 2 µl of Proteinase K (20 mg/ml) followed by 4 µl of 2M dithiotheriol (DTT) were added, mixed and incubated at 56°C for 45 min. After incubation the mixture was boiled for 8 minutes and vigorously vortexed for 10 seconds and centrifuged at 10,000 x g for 2 minutes (REMI Cooling Compfuge CPR 24). An aliquot of 10µl of supernatant was used for PCR assay.

Amplification of viral DNA

Vp2 gene specific semi nested PCR assay: The PCR assay was standardized using consensus sequence to obtain the maximum amplification of partial length of 747 bp product of VP2 gene.

Polymerase Chain Reaction: The PCR assay was

Fig 1: Cycling conditions used for amplification of partial lengthof VP2 gene (747 bp product).



standardized using VP2 gene specific primers Sakulwira et al. (2001). In brief, an aliquot of 10 ng-1µg of DNA template was added to mixture containing 25pmol of primer pair P1and P2 for the first amplification round and P1 and P3 for second amplification round in a 0.2 µl thin walled PCR tube. The PCR mixture containing 200µm dNTP mix, 1 x PCR buffer, 1.5 mM MgCl2 and 1.25 unit of Taq DNA polymerase was added to the same 0.2 µl tube on ice and final reaction volume was made 25µl by adding nuclease free water. The ingredients were mixed and spin for 10 sec. and put in thermocycler (Eppendorf Master Cycler Gradient TM, Germany) for 30 cycles of amplification (Fig.1).

Agarose gel electrophoresis: The PCR products were analyzed by agarose gel electrophoresis (AGE) using 1% agarose gel (Sigma) containing 0.5 µg ethidium bromide (Sigma) per ml in tris-acetate-EDTA (TAE) buffer along with 100 bp DNA ladder (MBI, Fermentas). The gel was visualized under UV transillu-minator (BIOVIS) and photographed on thermal paper (Glossy high density film, Sony) for record.

Results and Discussion

Out of 50 faecal sample extracted by chelex resin method a total of 33 samples (66.6%) were positive for CPV by PCR. The procedure took only one hour for extraction of viral DNA from faeces. The attraction of the method is that it produces high quality DNA for downstream applications such as PCR and DNA hybridization. The method is cost effective as it does not require expensive spin columns and instruments. Two primer pairs selected for the nested PCR effectively amplified the viral DNA. The feacal samples containing the virus produced single band of expected size i.e. 747bp (Fig.2) on the other hand no amplification was observed in the negative control.

In conclusion, the extraction of viral DNA using chelating resin along with nested PCR has been proved sensitive and specific test for the detection of CPV in diarrhoeic faecal samples of dogs.

Acknowledgement

The authors are thankful to CCS Haryana Agricultural University for providing infrastructural facilities, laboratory staff for technical assistance. Dr. Prem Singh Prof. (Surgery, COVS) and Dr. I. S. Chandna (Chandna Dog Clinic) generously supported in collection of samples from clinical cases. The studies reported in paper were supported by Department of Biotechnology, Government of India, New Delhi.

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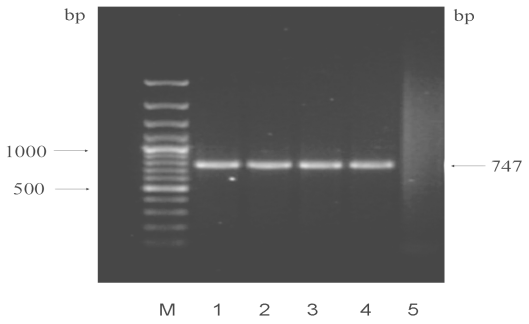

Figure 2: PCR products of VP2 gene of canine parvovirus yielding 747 bp product. Lane M: 100bp marker (MBI Fermentas), lane 2-4 showing amplified CPV DNA, lane1 is positive control using DNA from Nobivac DHPPi vaccine and lane 5 is the negative control