JOURNAL OF MICROBIOLOGY, IMMUNOLOGY AND BIOTECHNOLOGY
Journal homepage: www.jakraya.com/journal/jmib

REVIEW ARTICLE

Current Approaches in the Diagnosis of Canine Parvovirus: An Overview

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Received: 26/02/2015

Revised: 19/03/2015

Accepted: 22/03/2015

Abstract

CPV causes acute hemorrhagic gastroenteritis and myocarditis in dogs. CPV is prone to genetic evolution and has undergone several mutations that have led to various antigenic variants of CPV-2 that have replaced the original CPV-2. Currently, there are three main antigenic variants, i.e. 2a, 2b and 2c circulating in the dog population worldwide. The genome of CPV is about 5.3 Kb and VP2 plays an important role in the determination of antigenicity and host range of CPV. Mutations affecting VP2 are mainly responsible for the evolution of different antigenic variants. It spreads rapidly in the domestic as well as in the wild population of canines. The virus is shed in large quantity in the feces particularly 4-7 days post infection and infected feces serve as a source of infection. There are a number of methods that are used to diagnose CPV *viz*., virus isolation using cell culture, hemagglutination, hemagglutination inhibition, electron microscopy, enzyme linked immunosorbent assay but molecular diagnostic assays are very handy and can be used for its detection as these have high sensitivity and specificity.

Keywords: Canine Parvovirus, Antigenic types, VP2 gene, Diagnosis, PCR.

1. Introduction

Canine parvovirus (CPV) was first identified in 1978 in USA (Appel *et al.,* 1979) and was designated CPV type 2 (CPV-2). After its emergence, CPV-2 spread globally and today it is endemic in most populations of domestic and wild canids (Parrish *et al.,* 1988). CPV-2 is a variant of Feline panleukopenia virus (FPV) from which it differs in less than 1 per cent at the nucleotide sequence level and there are as few as six coding nucleotide differences in the VP1/VP2 protein viz. at positions 3025, 3065, 3094, 3753, 4477 and 4498 (Parrish, 1991; Truyen *et al.,* 1995). The biological effects of these few genomic changes were enormous, in that CPV-2 acquired the canine host range, but lost the ability to replicate in cats (Truyen *et al.,* 1994). After the emergence of CPV-2 two new antigenic types of CPV with altered antigenicity became wide spread viz. CPV type 2a (CPV-2a) and CPV type 2b (CPV-2b). Another antigenic variant CPV type 2c (CPV-2c) was reported in Italy (Buonavoglia *et al.,* 2001). This variant has an amino acid substitution, Asp-426 Glu in the capsid protein which is important for the antigenic properties of CPV-2.

CPV-2 is classified in the family *Parvoviridae* genus Parvovirus. It is a negative-sense, linear, single-stranded DNA virus (Parrish *et al.,* 1982). It is a non-enveloped icosahedral virus having a size of approximately 20nm (Reed *et al.,* 1988). The genome consists of 5323 bases (Reed *et al.,* 1988) of single-stranded DNA and encodes two structural proteins viz. VP-1 and VP-2 (Mochizuki *et al.,* 1993) and two non-structural proteins viz. NS-1 and NS-2 (Cotmore and Tattersall, 1987). VP-1 gene is located between 2285 and 4537 (2253 bases) and VP-2 gene between 2783 and 4537 (1755 bases) in genome of 5323 bases of CPV.

All sexes, ages and breeds of dogs have been found to be susceptible to CPV-2 infection (Castro *et al.,* 2007; Gombac *et al.,* 2008). The virus is shed in faeces (more than 10^9 virus particles/ gram of faeces) from infected dogs during acute phase of infection and infected faeces act as the main source of infection (Carmichael and Binn, 1981). Thus the transmission mainly takes place by the feco-oral route. The dogs can also become infected from the virus present on fomites such as shoes, clothing, hands of humans, food bowls and other utensils (Pollock and Carmichael, 1982; Carmichael, 1994; Decaro *et al.,* 2005). The virus is very stable in the environment, kennel and veterinary clinics and spread directly or indirectly among the dog population.

2. Diagnosis of Canine Parvovirus Infection

2.1 Electron Microscopy

Electron microscopy allows the identification and confirmation of CPV-2 viruses based on their size and morphology. Viruses are observed in groups or seen as single particles stained negatively with uranyl acetate, phosphotungstic acid or methylamine tungsten (Alicia *et al.,* 1999). The identification of the CPV-2 viruses in faeces can be carried out only during the elimination period of the virus which occurs between the 3rd and the 9th day of infection. However, the sensitivity of electron microscopy is believed to be relatively low due to the large quantities of viruses required for a positive test result (Esfandiari and Klingeborn, 2000).

2.2 Haemagglutination (HA) Assay

One of the important properties of CPV is haemagglutination. CPV agglutinates erythrocytes of rhesus monkey (Burtonboy *et al.,* 1979), African green monkey (Gagnon and Povey, 1979) and Porcine (Carmichael *et al.,* 1980). HA property of CPV is due to binding ability to the specific sialic acid receptor (Parrish *et al.,* 1988). HA and HI (haemagglutination-inhibition) tests are specific, rapid and inexpensive test for CPV diagnosis. Because of endemic nature of CPV and the wide spread administration of CPV vaccines, most of the dogs have significant antibody titres to CPV in the absence of active infection. Therefore, serology must be used with caution as a diagnostic tool. Paired serum samples are usually collected to detect a significant rise in CPV antibody titres in infected dogs.

Carmichael *et al.* (1980) defined conditions for HA and Haemagglutination-Inhibition (HI) reactions and demonstrated that CPV strongly agglutinate porcine RBC at pH 7.2. Mohan *et al.* (1991) reported 33 per cent prevalence of CPV using haemagglutination inhibition test on serum samples of dogs from different parts of Punjab.

2.3 Enzyme Linked Immunosorbent Assay

Indirect ELISA can detect IgM antiviral antibodies which are indicative of recent infection of CPV (Florent, 1986). Dot-ELISA has also been found more sensitive and specific in comparison to other serological tests such as HA test (Banja *et al.,* 2002). Double antibody sandwich ELISA test has been developed to detect CPV antigen in faecal samples (Rimmelzwaan *et al.,* 1991) having a sensitivity of 87% and a specificity of 100% compared to 63% and 87%, respectively of HA test (Drane *et al.,* 1994).

2.4 Viral Isolation Methods

The isolation of CPV from clinical samples by using various cell cultures has been used for the diagnosis of canine parvovirus. The various cell lines used for this purpose are Crandell Feline Kidney cell line (CRFK), Madin Darby canine Kidney cell line (MDCK), Walter Reed Canine Cell line (WRCC) etc. Primary cultures prepared from canine cells like kidney and lungs have also been used. The presence of virus is suspected by the occurrence of cytopathic effects (CPE) including rounding of cells or sometimes with the presence of intranuclear inclusion bodies which can be examined by giemsa staining and detachment of cells in cell culture system.

Isolation of CPV-2 requires cell culture capability, capable and skilled personnel and also the permissive cell lines to be used. Moreover, it is time consuming. The main disadvantage of viral isolation however is low sensitivity. It has been demonstrated in natural and experimental infections that CPV-2 is detectable by viral isolation only for a few days post-infection (Desario *et al.,* 2005). The isolation of CPV in cell culture cannot be applied to a spoiled sample in which viruses have been inactivated. The sensitivity of different cell lines may also be different.

2.5 Polymerase Chain Reaction and Nested PCR

PCR is a routine diagnostic method and a rapid and specific assay for identification of virus from faecal specimens and as compared to isolation of virus and HA test, PCR is found to be more sensitive (Mochizuki *et al.,* 1993). It is about 10 to 100 fold more sensitive than electron microscopy (Schunck *et al.,* 1995). In a study Uwatoko *et al.* (1995) concluded that PCR assay can detect fewer particles of CPV than conventional methods and thus able to detect CPV from faecal specimens in a rapid manner, provided that gel filtration of the samples through a spin column was done to remove inhibitory substances from the faecal specimens.

Hirasawa *et al.* (1996) used PCR to detect prevalence of CPV-2 in Japanese dogs between 1993 and 1995 and found the prevalence to be 54.1 per cent. Kim and Jang (1997) used different diagnostic assays for the diagnosis of CPV from faecal samples and found that 45% were positive by Haemagglutination test, 64 were positive in PCR and 87 were positive in nested PCR and concluded that nested PCR is a rapid, sensitive and specific method for detecting canine parvovirus.

Mochizuki *et al.* (2001) used PCR assay for detection of CPV-2 viral DNA in diarrhoeal specimens. Out of 84 diarrhoeal specimens, 21 (25%) were found to be positive by PCR assay. Sakulwira *et al.* (2001) detected and genotyped CPV by PCR using primer

sequence derived from conserved VP2 region of genome and thirty four out of 55 specimens (61.8 per cent) from dogs with enteritis were found to be positive for CPV.

Molecular typing of CPV in Pondicherry was done by Parthiban *et al.* (2010) using PCR based assays and CPV-2a and CPV-2b types were detected. Kumar and Nandi (2010b) analyzed 129 fecal samples and found 78 samples positive by PCR and out of these, 27 were CPV-2a, 39 were CPV-2b and 12 were CPV-2c type, respectively. This study also showed that CPV-2c is presently circulating in India.

2.6 Real Time PCR

Real time PCR is a newly developed variant of PCR and has been exploited for the diagnosis of Canine Parvovirus. Decaro *et al*. (2006) developed a minor groove binder (MGB) probe assay to discriminate between CPV type 2-based vaccines and field strains of canine parvovirus. Two MGB probes specific for CPV-2 and the antigenic variants (types 2a, 2b and 2c) respectively, were labelled with different fluorophores. The MGB probe assay was able to discriminate correctly between the old type and the variants, with a detection limit of 10^1 DNA copies with good reproducibility.

Li Ying Jun *et al.* (2010) also used TaqMan probe in Real-Time PCR for diagnosis of CPV. Kumar and Nandi (2010a) analyzed fecal samples (47) from dogs suspected of CPV- 2 infection by real-time PCR, hemagglutination (HA) assay and conventional PCR. It was observed that 24, 20 and 22 samples were found positive for CPV-2 by real time PCR, HA and PCR respectively indicating that real-time PCR is more sensitive than HA and conventional PCR and allow the detection of low titers of CPV-2 in infected dogs.

3. Conclusion

Therefore, from the above it could be concluded that though there are various methods for the diagnosis of CPV in dogs but, with the advent of molecular techniques the diagnosis of CPV has become quick, easy and reliable.

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