Isolation and Molecular Characterization of Canine and Feline Parvovirus Strains - An Updated Review

Abstract
Canine parvovirus is the most important and significant contagious viral cause of enteritis in pups, adult dogs and wild carnivores characterized by acute haemorrhagic enteritis, myocarditis and leucopenia. These viruses target the rapidly dividing cells. Phylogenetic analysis showed that all CPV isolates descended from a single ancestor which emerged during mid-1970s and were which was closely related to the long-known feline panleukopenia virus (FPV) that infected cats, mink, and raccoons but not dogs or cultured dog cells. FPV and CPV differ only by 0.5% in their DNA sequences. Inspite of their similarity, CPV-2 was not able to replicate in cat cells. FPV is not the only parvovirus species which infects cats, in addition to MEV, the new variants of canine parvovirus, CPV-2a, 2b and 2c have also penetrated the feline host-range, and they are able to infect and replicate in cats, causing diseases indistinguishable from feline panleukopenia. There are five to six amino acid (87, 101, 300, 305, 426 & 555) differences between CPV-2, CPV-2a and its variants. Amplification of full length VP2 gene followed by sequencing of the PCR products can give ample information about the antigenic and genetic differences between the original prototype CPV-2 and its variants.

Keywords: Canine parvovirus; Canine parvovirus variants; VP2 gene; Sequencing

Introduction
Canine parvovirus is the most important and significant contagious viral cause of enteritis in pups, adult dogs and wild carnivores characterized by acute haemorrhagic enteritis, myocarditis and leucopenia [1,2]. Now CPV infection is considered to be most threatening especially to the puppies between the time of weaning and six months of age. These viruses target the rapidly dividing cells.

Molecular characteristic of canine parvovirus type 2
Canine parvovirus belongs to the genus Protoparvovirus, family Parvoviridae. The virion is non-enveloped having icosahedral symmetry of 26nm diameter. It has a single stranded negative sense genome of 5.2 kb in length. This single-stranded virus has a DNA genome and use cellular replication machinery; their rate of nucleotide substitution is closer to that of RNA viruses than to that of double-stranded DNA viruses. Phylogenetic analysis showed that all CPV isolates descended from a single ancestor which emerged during mid-1970s and were which was closely related to the long-known feline panleukopenia virus (FPV) that infected cats, mink, and raccoons but not dogs or cultured dog cells [3]. FPV and CPV differ only by 0.5% in their DNA sequences. Inspite of their similarity, CPV-2 was not able to replicate in cat cells. FPV is not the only parvovirus species which infects cats, in addition to MEV, the new variants of canine parvovirus, CPV-2a, 2b and 2c have also penetrated the feline host-range, and they are able to infect and replicate in cats, causing diseases indistinguishable from feline panleukopenia [4]. CPV comprises of two open reading frames, one codes for structural proteins VP1 and VP2 while the other codes for non-structural proteins NS, and NS VP, is a capsid protein which is formed by proteolytic cleavage of VP2 capsid protein. The VP2 is the major capsid protein and plays an important role in determining the antigenicity and the host range of CPV. In 1978, CPV-2 emerged as a cause of a new disease in dogs throughout the world. The first documentation of the virus as CPV-2 was done in Canada [1]. Canine parvovirus (CPV) and feline panleukopenia virus (FPV) capsids bind to the transferrin receptors (TRs) of their hosts and use these receptors to infect cells. The binding is partially host specific, as FPV binds only to the feline TR, while CPV binds to both the canine and feline TRs [5].

Variants of canine parvovirus
After the emergence of canine parvovirus in late 1970s [1,6] due to rapid evolution, new antigenic types were evolved as CPV-2a, CPV-2b and CPV-2c (CPV2a with Asn426Glu) which have completely replaced the original CPV-2 [7,8]. Currently the original CPV-2 was not found in dog population but present only in vaccine formulations [8,9] (Figure 1).

The virus has undergone mutations from the original CPV-2 to CPV-2a and CPV-2b. A third mutant, CPV-2c has been discovered in Italy [10]. The variants CPV-2a and CPV-2b differ from the original type CPV-2 by a few amino acid changes in the VP2 protein. The latter accounts for an extended host range in-vivo the reported rate of substitution refers only to the VP2 gene and for an increased affinity to canine transferrin receptors [3].
The difference between CPV types 2a and 2b is the presence of two single nucleotide polymorphisms (SNPs) in the capsid protein gene sequence, which determines amino acid changes in the major antigenic sites of the viral capsid [11].

Amino Acid Variations in the VP2 protein differentiating between FPV and CPV-2

Residue 426 had undergone two mutations since the emergence of CPV, first from asparagine to aspartate and more recently to glutamate, amino acid changes which constituted an important genetic marker for the new variants of CPV and the only coding change capable of distinguishing the CPV-2a strains from CPV-2b/2c strains [13]. A novel CPV mutant, CPV-2c is widely distributed and co-existing with other CPV types in Europe, North and South America countries. There was a single report of occurrence of CPV-2c in North India [14] (Table 2).

Epidemiology

Parvoviruses have been characterised by a rapidly changing epidemiology in dogs and cats. Feline panleukopaenia has been recognised as a disease of cats for over 100 years, and the virus (FPLV) was first isolated and associated with the disease in 1965 [15]. Twenty-three years later, in 1978, a variant of an FPLV-like virus called canine parvovirus CPV-2 emerged in the canine population, and appeared to spread rapidly worldwide with profound clinical consequences [16]. The precise origin of this virus remains the source of some speculation but possibilities include the evolution of the virus in a wildlife intermediate such as a fox [17].

In 1979 and 1980, an antigenic variant of CPV-2 was identified in several different countries using monoclonal antibodies and the variant was termed canine parvovirus type 2a [18]. By employing monoclonal antibodies (mAbs) and restriction enzymes (RE) for antigenic and genomic analysis of this virus, ample evidences were found that original CPV-2 was replaced in the field by viruses designated as CPV-2a [7] and CPV-2b [8] between 1979 and 1981. In the middle of 1980s, the virus underwent a further antigenic change, and the new variant was referred to as canine parvovirus type 2b, CPV-2b [19].
The isolation rates proved that CPV-2a and CPV-2b types increased yearly from 1979 to 1982 and in Japan all 50 isolates and in the United States 48 of 51 isolates collected after 1983 were CPV-2a and CPV-2b types [20]. Another new antigenic variant was detected in CPV-2 strains isolated from domestic dogs in Italy during 2001 [10] and was designated as CPV-2c. CPV-2c was broadly distributed in Italy, where it was co circulating with types 2a and 2b [21].

Presently the variants of CPV (CPV-2a and CPV-2b) were the predominant strains distributed worldwide and the original prototype CPV-2, no longer circulated in dog population [22-26].

CPV-2c had also been reported in Vietnam [12], Spain [23], Germany [27], United Kingdom [28] South America [24], United States of America [29], Central Portugal [30], Argentina [31], Greece [32], North India [14], Brazil [33] and Uruguay [34,35]. Recent reports revealed the predominant CPV strain in India as new CPV-2a (Ser 297 Ala) with one new CPV-2b (Ser 297 Ala) and another new CPV-2a (Ser 297 Gly) variant strain [36].

**Susceptible hosts/ reservoirs of CPV-2**

The main source of infection is faeces from the infected dogs. More than 10^6 virus particles per gram of faeces are shed during the acute phase in the enteric form. The faeces are the suitable clinical material for the detection of virus in the enteric form of disease [37]. The CPV spreads by faeco-oral route [11,38,39] and is shed for 8-12 days post infection via faeces [9,38].

CPV-2 can affect dogs of any age, however, severe clinical manifestation is seen in puppies between 6 weeks and 4 months old. All breeds of dogs are susceptible [3]. The crossbreeds are less susceptible in comparison to pure breeds like Rottweiler, Doberman Pincher, English Springer, Spaniels and German Shepherd, the exception to this being Toy Poodles and Cocker Spaniels [40]. Several species of wild carnivores, red foxes, wolves, raccoons and coyotes are susceptible to canine parvovirus infection [17]. Canine parvovirus infection has also been reported in the bat eared fox, honey badger, cheetah, African wild cat and Siberian tiger [41].

**CPV infection in cats**

In the late 1970s, CPVs evolved from FPV after crossing species barriers by acquiring five or six amino acid changes in the capsid protein gene [42]. Within 1 year, the first CPV (CPV-2) changed to the current subtypes, CPV-2a and CPV-2a derived strains. Whereas CPV-2 was not able to infect cats, the subtypes can cause clinical signs that cannot be distinguished from those caused by FPV [43-45].

CPV had been isolated from a variety of domestic cats, as well as other more-or-less related carnivores, including raccoons, Asian raccoon dogs, cheetahs, mountain lions, leopard cats and a red panda [41,46-49]. CPV-2a and CPV-2b were likely to act as newly emerged viruses in cats, some cat-specific positive selection(s), such as relative in vivo fitness and immune surveillance, could have operated as a driving force of CPV evolution [50]. CPV had presumably started a new process of readapting in feline hosts and confirmed the importance of viral host switching as a mechanism for the emergence of new viruses [4]. They also reported two CPV infections in 24 cat samples in Italy, one with CPV-2c and another with mixed infection of both CPV-2a and FPV.

This mixed infection pointed out the predominant epidemiological role of cats as

a) Carriers of carnivore parvovirus since it was susceptible to both the viruses and as

b) Sources of new variants of parvoviruses [50].

**Isolation of CPV/FPV in cell cultures**

The host ranges of FPV and CPV variants have been extensively studied in vitro by several authors. In general, CPV-type viruses replicate efficiently in feline and canine cell lines, while most FPLV and FPLV-like viruses can replicate efficiently only in feline cells [51,52]. The isolation of CPV from clinical samples by using various cell cultures has been used for the diagnosis of canine parvovirus.

**Isolation of FPV in CRFK cell line**: MDCK and CRFK was used for the isolation and cultivation of canine parvovirus [45]. Antigenic and genetic properties of isolates from pups were elucidated. Three paroviruses that were recently isolated from a domestic cat and 2 cheetahs in cell culture, detected by means of the polymerase chain reaction were shown to be typical feline paroviruses. Crandell feline cell cultures inoculated with material derived from the cat faeces showed mild cellular degeneration in comparison with the cell controls after 5 days.

**Isolation of CPV in CRFK cell line**: CRFK was used for the isolation and cultivation of canine parvovirus from the faeces of infected dogs and adapted the isolate to grow in CRFK cell lines. Rounding and degenerative changes were the cytopathic changes observed at third passage level 72 hours post infection. The harvesting was done by three alternate cycles of freezing and thawing. Presence of virus in the harvested cell culture supernatant was detected by PCR [53-56]. These studies show that Canine parvovirus variants obtained from dogs can be isolated from CRFK cell line which is of feline origin.

**Isolation of CPV in A-72 cell line**: The A-72 cell line was widely used for cultivation of CPV in cell culture and the first CPV-2c was isolated in A-72 cell line in Uruguay [10,34,57-59]. A-72 cell lines was used for isolation of CPV from samples found positive by PCR, CPV was observed in A-72 cell line from second passage onwards showing greater sensitivity of A-72 cell line over CRFK cell line [60]. Rounding, increased granularity and detached cells were the cytopathic changes observed from the second passage onwards [61]. The studies show that CPV could be efficiently isolated from A-72 cell line which of canine origin when compared to CRFK cell line (feline origin). 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.

**Molecular Characterisation of CPV/FPV by VP2 Gene Sequencing**

Amplification and subsequent sequencing of VP2 gene was useful in characterization of CPV by identifying the types of CPV currently present in the field [24-26,56].

22 CPV isolates from 27 faecal samples collected from various states of India [62]. The isolates were characterized by nucleotide sequencing of the VP2 gene and found that 18 were CPV-2a and 4 were CPV-2b. The genetic analysis of canine parvovirus was
done in a total of 13 CPV vaccines, which had been licensed in Korea since late 1980s, and a field isolate of CPV from a dog with CPV infection. The partial VP2 gene of CPV was amplified and sequenced from 13 vaccine strains and one field isolate. The results showed that of the 13 vaccine strains, 10 strains belong to the CPV-2, 2 strains to CPV-2b, the remaining one to isolate to CPV-2a type, respectively. Several mutations of amino acids were detected at residues of the critical region of the commercial vaccine strains [63]. This suggested that CPV-2 contained in most licensed vaccines had been replaced by antigenic variants designated CPV-2a or CPV-2b/c in the worldwide dog population.

PCR amplification with sequence-specific primers was used to detect canine parvovirus DNA in 38 rectal swabs from Argentine dogs with canine parvoviral disease symptoms. Twenty-seven out of 38 samples analyzed were CPV positive. The classical CPV-2 was not detected in any of the samples, but nine samples were identified as CPV-2a variant and 18 samples as CPV-2b variant. Further sequence analysis revealed a mutation at amino acid 426 of the VP2 gene (Asp426Glu), characteristic of the CPV-2c variant, in 14 out of 18 of the samples identified initially by PCR as CPV-2b.

The primers CPV2655-F and CPV3511-R; CPV3381-F and CPV4116-R [64] and 555(F)/555(R) [10] were used for both amplification and sequencing of full length VP2 gene in 27 samples. The findings revealed that 100 CPVs belong to type 2b strains and 6 as type 2a strain which shows CPV-2b predominates in South Africa whereas all the 30 Nigerian samples were CPV-2a strains [65].

Molecular characterisation of CPV/FPV by partial VP2 gene sequencing

Canine parvovirus strains were detected and characterized in Brazil by screening 144 dog faecal samples by PCR for CPV-2, and 29.2% (42/144) of them were positive. Sequencing of the 583 bp fragment of the VP2 gene from the 42 positive strains revealed 78.6% (33/42) as type 2c, 19% (8/42) as type 2b and 2.4% (1/42) as type 2a [66].

The predominant CPV strain in India as new CPV- 2a (Ser 297Ala) with one new CPV-2b (Ser 297Ala) and another new CPV -2a (Ser 297Gly) variant strain by partially sequencing the VP2 gene of CPV which was reported by Mukhopadhyay et al. [36].

The full-length VP2 coding sequences from 11 CPV-2c strains, 11 CPV-2a strains and 2 CPV-2b strains revealed remarkable genetic diversity that included the circulation of all three variants (2a, 2b and 2c) and the existence of divergent evolutionary groups. CPV-2c was the most prevalent variant (54.47%), confirming the spread of this variant in America [67].

By sequence and phylogenetic analysis of VP2 gene reveals that out of 28 Taiwanese CPV 2 isolates, 15 were identified as new CPV 2a, and 13 were identified as new CPV 2b [68]. Many researchers carried out molecular characterization by sequencing the full length VP2 gene of CPV/FPV which is more reliable. The molecular characterization also reveals that the predominantly circulating strain which is causing infection in dog is CPV-2a then followed by CPV-2b. Recently CPV-2c strain has also been isolated from India. The full length sequencing of VP2 gene gives ample information about the biological properties of the viruses which helps in characterization.

Conclusion

Canine parvovirus (CPV) is one of the most important infectious agents of domestic dogs and wild carnivores worldwide which causes severe gastroenteritis with a high case fatality rate, particularly in puppies less than 3 months of age. Early and differential diagnosis of CPV is very essential for the effective clinical management of the affected dogs. Surveillance and genotyping of CPV from various geographical locations help in detecting the prevalent CPV types/strains. The CPV is now known to infect both wild and domesticated feline species. Since the cats are susceptible to both CPV and FPV viruses, superinfection and co-infection with multiple parvovirus strains occur facilitating recombination and high genetic heterogeneity leading to evolution of new virulent strains/virus. The cats also act as carrier and a source of infection to dogs. Considering the contradictory reports about vaccine efficacy, there is also an urgent need to consider developing vaccines incorporating the strains/types currently circulating in a particular geographical region. Moreover isolation of CPV in the susceptible cell line is very much essential for antigenic, molecular and phylogenetic analysis.

The main disadvantage of viral isolation in cell line 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. The strain currently circulating in the dog population causing disease is New CPV-2a then followed by New CPV-2b. Recently CPV-2c strain was isolated from dog in India. Amplification of full length VP2 gene followed by sequencing of the PCR products gives ample information about the antigenic and genetic differences between the original prototype CPV-2 and its variants which helps in molecular characterization.

References

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