The Gastroenteritis Virus of Dogs: Canine Parvovirus and Its Diagnosis over the Years

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Dogs are among the most beloved companion animals. They are involved in number of activities of human being as pet, in various jobs. Domestic dogs are susceptible to the extremely contagious canine viral diseases, which pose a serious danger to canine health globally. The review involves the main viral etiology that causes gastroenteritis in dogs; the Canine Parvovirus which is highly prevalent causing heavy morbidity and mortality in affected dogs. Diagnosis of CPV infection in dogs involves various diagnostic assays. Lot of research has been conducted on various tests or techniques that can be used for diagnosis of CPV infection in dogs. This is important too as CPV is quite prevalent and of much concern. Therefore the information on diagnosis of CPV would help in early and quick diagnosis to prevent mortality by early treatment.

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1. INTRODUCTION: ABOUT THE CANINE PARVOVIRUS

Earlier in 1979 researchers examined numerous dogs that died from hemorrhagic diarrhoea and discovered mucosal lesions, particularly in the duodenum and upper section of the small bowel. Ultrathin sections of the these mucosa revealed particles that looked like parvoviruses in the nuclei of intestinal crypt cells. Negative staining electron microscopy of intestinal contents revealed the presence of numerous virus particles with a diameter of 24 nm and an icosahedral shape. These virions floated in cesium chloride at a density of 1.43 g/cm3 and agglutinate red blood cells from rhesus monkeys and pigs at 4 degrees celsius [1].

These viruses belonged to family Parvoviridae which comprises two subfamilies: the subfamily Parvovirinae, which contains viruses of vertebrates, and the subfamily Densovirinae, which contains viruses of insects. There are three genera in the subfamily Parvovirinae: the genus Parvovirus, Erythrovirus and Dependovirus. Member viruses of the genus Parvovirus cause important diseases in swine, cats, dogs, mink, mice, rats, hamsters, geese and ducks. The original canine virus, called Canine Parvovirus 1 or minute virus of canines, is taxonomically distinct. The other canine virus, called Canine Parvovirus 2, is related closely to feline and mink viruses.

Parvovirus virions are non-enveloped, 25nm in diameter having icosahedral symmetry. The genome consists of single molecule of linear single stranded DNA 5.2kb in size. The capsid is composed of 60 molecules of VP2 protein along with few molecules of VP1 formed by alternative splicing of same mRNA, and the entire sequence of VP2 is encoded within theVP1 gene. The virus also includes two non-structural proteins NS1 and NS2.

Canine Parvoviruses (CPVs) are non-enveloped DNA viruses that replicate in rapidly dividing cells. These viruses arose from the feline panleukopenia virus, and now have worldwide distribution. Canine parvovirus type 1 (CPV-1) is also referred to as minute viruses of canines and was initially reported in military dogs with diarrhea. This virus is antigenically distinct from CPV-2 and is more closely related genetically to bovine parvovirus.

2. EVOLUTION OF VARIANTS OF CANINE PARVOVIRUS

CPV-like viruses were first circulating in Europe in the mid to late 1970s, and early CPV type 2 isolates were essentially identical in nucleotide sequence, and were all replaced by the variant CPV type 2a strain which spread worldwide during 1979 and 1980. Other mutations have been seen worldwide in various countries since 1980, including a mutation changing VP2 residue 426 (referred to as CPV-2b) from Asn to Asp first seen around 1984 which spread worldwide. Further viruses with residue 426 as a Glu (referred to as CPV type 2c) have been identified in Europe [2,3], Vietnam [4] and in North and South America [5]. The reasons for the global spread of some mutations and the time of their emergence are hard to explain.

"CPV strains have undergone a series of evolutionary selections in nature, resulting in global distribution of new variants that have replaced the original CPV-2. Currently, the three major antigenic variants of CPV-2 which are known to be distributed among the dog population worldwide are i.e. 2a, 2b and 2c" [6]. "Isolation of CPV-2 was done for the first time in India by Ramadass and Khader in 1982 since then several occurrence of disease have been reported from different parts of the country involving different variants of CPV (2, 2a, 2b and 2c) both in vaccinated and unvaccinated animals" [7,8,9]. VP2 is the major capsid protein that plays an important role in the determination of antigenicity and host range of CPV.

"CPV-2 for the first time was isolated in India in 1982" [10]. "In 2000, another mutant CPV-2c was reported in dogs from Italy and it differs from CPV-2b by one amino acid at 426 position from Asp to Glu" [11]. "The prevalence of CPV-2a has been documented in 2001 in India [12]. It was also found that CPV-2b variants are more common in northern India compared to CPV-2a. Occurrence of CPV-2c was first reported in India in 2010" [13].

"CPV types 2a and 2b co-circulated throughout the world (although in different proportions) over the 20 years without either becoming fixed. CPV-2a and 2b co-circulated in Brazil as early as 1986 while CPV-2b was the predominant variant circulating there between 1995 and 2001" [14-15]. "The dominant CPV type circulating in Italy at that time was CPV-2a, while both CPV-2a and 2b circulated at similar frequencies in 1999/2000.

There appear to be Taiwanese-Japanese and Indian CPV populations which are phylogenetically distinct from American and Vietnamese populations" [16,17,18]. Additional mutations that have been observed include Ser297Ala and Glu300Asp, which may affect receptor binding or host range properties of the viruses.

3. CLINICAL SIGNS IN DOGS

3.1 Clinical Signs in CPV-1

CPV-1 is usually a subclinical infection in dogs but may cause enteritis, pneumonitis, myocarditis, and lymphadenitis in puppies aged between 5 and 21 days. Most pups have mild symptoms, but those that worsen may be classified as having fading puppy syndrome. Affected pups may show diarrhea, vomiting, and dyspnea and constantly cry out.

3.2 Clinical Signs in CPV-2

"CPV-2 causes hemorrhagic gastroenteritis in dogs and spreads rapidly in both domestic as well as wild population of canines. The virus has affinity for villi of the small intestine where they replicate in the rapidly dividing epithelial cells. The virus sheds in large numbers in the feces for four to seven days post infection and thus, feces are known to serve as a source of infection. Therefore, feces constitutes as the most suitable material for detection of CPV" [19,20].

"Feline Kidney Cells were used to study canine parvovirus strain in dogs. The virulent CPV strain developed small plaques in Feline Kidney Cells, whereas the attenuated strains formed big plaques. And it was observed that the dogs who were inoculated with attenuated strain of virus showed no clinical symptoms of illness or changes in leucocyte levels. Clinical indications of sickness (anorexia, vomiting, diarrhoea streaked with blood) and significant alterations in leucocyte levels were seen in dogs infected with the virulent CPV strain" [21].

4. TESTS EMPLOYED FOR DIAGNOSIS OF CPV OVER THE YEARS

Diagnosis of CPV infection in dogs involves various diagnostic assays. Lot of research has been conducted on various tests or techniques that can be used for diagnosis of CPV infection in dogs. Year-wise use of different techniques for diagnosis of CPV is explained.

4.1 ELISA

Drane et al. [22] used "ELISA detection kit as a quick field test for canine parvovirus antigen identification in canine faeces samples. To confirm infection, the CPV ELISA was compared to the haemagglutination assay (HA) test, electron microscopy (EM) and/or virus isolation (VI). The CPV ELISA demonstrated an 87 percent sensitivity and a 100 percent specificity, compared to 87 percent and 63 percent for the HA. Because of the HA's low specificity, it has a low positive predictive value of 51%, compared to 100% for the CPV ELISA". The CPV ELISA requires no special equipment, is easy to use, and yields a visible result. Kumar et al. [12] "developed a polyclonal antibody-based antigen-capture ELISA (AC-ELISA) for detecting Canine Parvovirus antigens in dog faeces. The capture antibody is a rabbit anti-CPV polyclonal antibody, the tracing antibody is a guinea pig anti-CPV polyclonal antibody, and the detection system is anti-guinea pig HRPO conjugate. The AC-ELISA had 88.4 percent relative sensitivity, 100.0 percent relative specificity, and 91.4 percent relative accuracy".

Markovich et al. [23] used an "ELISA and a PCR technique to examine faecal samples. On faeces with CPV-positive PCR assay results, genetic sequencing was performed, and 25 (73.5%) were found to contain CPV type-2c, while 9 (26.5%) were found to contain CPV type-2b. There was no link identified between the CPV strain and the severity of the disease or the clinical outcome".

4.2 PCR and Nested PCR

The nested PCR was created by Hirasawa et al. [24] "using a double-nested primer pair. The PCR primer pairs' sequences were chosen from the conserved region of the CPV VP1/VP2 gene. On agarose gel electrophoresis, a single PCR with the outer or inner primer pair could identify 10 fg of viral DNA, whereas the nested PCR could detect as low as 100 ag of DNA, which was proven to be 100 times more sensitive than the single PCR. The nested PCR was also used to amplify samples from feline panleukopenia virus and mink enteritis virus, both of which have a very close antigenic similarity to CPV. Restriction enzyme analysis and Southern hybridization were used to establish the reaction's specificity. Two restriction sites (HaeIII and HindIII sites) in

the PCR product were conserved among the samples suspected of CPV infection. The single PCR assessed the number of genome copies in positive samples to be around 10^9-10^{11}/g, whereas the nested PCR estimated it to be around 10^{11}-10^{13}/g". Schunck et al. [25] "also used the PCR technique for detection of parvovirus in dog and cat faeces. After a quick and simple boiling pre-treatment, a touch-down approach was performed to enable the selective amplification of virion DNA from faeces. PCR had a sensitivity of ten infectious particles per response, which equates to a titer of roughly 10^3 infectious particles per gram of unprocessed faeces. This makes the PCR around ten to one hundred times more sensitive than electron microscopy. This PCR assay is recommended as a tool for routine parvovirus diagnosis due to its quick and simple sample preparation". Senda et al. [26] "employed polymerase chain reaction to discover wild-type canine parvovirus strains that contaminate dog vaccines. The detection limit was 10 copies of CPV-2 genomic DNA, according to sensitivity analyses". Uwatoko et al. [27] "developed a quick and specific technique for detecting the virus in faeces using polymerase chain reaction and primers based on the canine parvovirus VP2 gene sequence. A specific 226-bp sequence was amplified by the PCR using a pair of primers. These findings imply that the PCR technique can detect CPV in dogs early enough to prevent CPV secondary infection in veterinary facilities". Pereira et al. [14] "also developed a PCR technique to identify canine parvovirus strains in faeces obtained from sick dogs between 1980 and 1986 and 1990 and 1995. Their findings revealed that the CPV epizootic in Brazil followed the same pattern as the one seen in the United States, with CPV-2 emergence followed by replacement by CPV-2a and 2b variations. CPV-2a was the most common strain identified in 1980, but from 1990 to 1995, it was largely supplanted by CPV-2b".

Kaur et al. [28] "devised a differential PCR method. The antigenic types CPV-2a and CPV-2b were determined to be the most prevalent using differential PCR. It was also discovered that dogs who had been vaccinated had positive CPV results, implying the presence of additional CPV antigenic types. Thus, differential PCR primers can be utilised in a single PCR run to detect distinct antigenic kinds of CPV". Behera et al. [29] "too used PCR to detect canine parvovirus infection in 71 faecal samples from suspected diarrheic canines. The PCR assay identified 29 (40.85 percent) of the 71 samples to be positive. Infection rates were highest in Deshi/local breeds (34.48 percent), followed by German shepherd (17.24 percent), mixed and Labrador retrievers (10.34 percent), Rottweiler and German spitz (6.90 percent each), and finally Dalmatians, Neapolitan mastiff, Pug, and Great Dane (3.45 percent). The infection was shown to be more prevalent in the age group of 3-6 months (41.37 percent), followed by equal occurrences of 27.59 percent in the age groups of 1-3 months and 6-12 months, and a low incidence in the age categories above 12 months (3.45 percent). Males (86.21 percent) were more likely than females to be affected (13.79 percent)".

4.3 Restriction Enzyme Analysis

Greenwood et al. [30] "isolated canine parvovirus from clinical instances of enteritis and divided them into three groups using restriction enzyme analysis: CPV2, CPV2a, and CPV2b". In an another study, Kumar et al. [12] "investigated the prevalence of CPV-2 infection in diarrhoeic dogs in order to determine the role of various canine parvovirus variants circulating in India. Polymerase chain reaction was used to distinguish CPV-2a from CPV-2b. PCR and restriction endonuclease (RE) analysis using Mbo II were used to detect the CPV-2c variant in the samples. PCR testing revealed that 78 of the 129 faecal samples tested positive for canine parvovirus. Twenty seven of the 78 samples were positive for CPV-2a, 39 for CPV-2b, and 12 for CPV-2c. This study also discovered that a variant of CPV, CPV-2c, is circulating in India".

Nandi et al [13] "identified various mutants of CPV2 that are widespread in India and described them using polymerase chain reaction and restriction endonuclease mapping".

Castillo et al. [31] used PCR, "restriction fragment length polymorphism and partial sequencing of the structural viral protein VP2 coding area. This was the first study of CPV2 genetic characterisation in Chile, and it shows that CPV2c to be very common". Kumari et al. [32] "used multiplex PCR and restriction fragment length polymorphism PCR to determine that CPV-2a was the prevalent strain in Andhra Pradesh, with no CPV-2c found".

Table 1. Tests employed in diagnosis of Canine Parvovirus

S. No	Authors	Name of Test employed	Name of the gene present	Nature of sample	Detection	Sensitivity and Specificity of test	Primer used
1	Drane et al	ELISA	-	Faecal sample	CPV antigen	87% sensitivity and 100% specificity	-
2	Kumar et al	AC-ELISA	-	Faecal sample	CPV antigen	88.4 percent relative sensitivity, 100.0 percent relative specificity, and 91.4 percent relative accuracy	-
3	Markovich et al	ELISA and PCR	CPV type-2c and 2b	Dog faeces	CPV antigen	-	-
4	Hirasawa et al	Nested PCR	-	-	CPV antigen	100 times more sensitive than the single PCR	conserved region of the CPV VP1/VP2 gene
5	Schunck et al	PCR	-	Dog and cat faeces	CPV antigen	sensitivity of ten infectious particles per response	-
6	Senda et al	PCR	-	-	CPV antigen	detection limit was 10 copies of CPV-2 genomic DNA	-
7	Uwatoko et al	PCR	-	-	CPV antigen	-	226bp VP2 gene sequence
8	Pereira et al	PCR	CPV-2 emergence followed by replacement by CPV-2a and 2b variations	Dog faeces	CPV antigen	-	-
9	Kaur et al	differential PCR	CPV2a, and CPV2b	-	CPV antigen	-	-
10	Behera et al.	PCR	CPV 2	Dog faeces	CPV antigen	-	-
11	Greenwood et al	RE analysis	Identified CPV2, CPV2a, and CPV2b	-	CPV antigen	-	-
12	Kumar et al	PCR and RE analysis	To detect CPV-2c	-	CPV antigen	-	RE analysis using Mbo II

S. No	Authors	Name of Test employed	Name of the gene present	Nature of sample	Detection	Sensitivity and Specificity of test	Primer used
13	Nandi et al	PCR and RE analysis	identified various mutants of CPV2	-	CPV antigen	-	-
14	Castillo et al	PCR and RE analysis	CPV 2c	-	CPV antigen	-	structural viral protein VP2 coding area
15	Kumari et al.	Multiplex PCR and RE analysis	CPV 2a	-	CPV antigen	-	-
16	Nho et al	*In situ* hybridization	A digoxigenin-labelled probe was used	-	CPV antigen	-	Full VP-1 capsid protein sequence and the middle half of the VP-2 capsid protein sequence
17	Esfandiari and Klingeborn	Immunochromatographic test	-	Dog faeces	-	Sensitivity was 95.8% and Specificity was 99.7%	-
18	Narayanan et al	PCR and Sequencing	CPV 2a and CPV 2b detected	Dog faeces	CPV antigen	-	Hfor/rev primers
19	Mohan Raj et al	PCR and Sequencing	New CPV-2a (CPV2a with 297-SerAla) was identified	Dog faeces	CPV antigen	-	-
20	Ntafis et al	PCR and Sequencing	CPV-2c	Dog faeces	CPV antigen	-	VP2 gene of CPV
21	Jobin et al	PCR and Sequencing	antigenic variation of CPV-2a	Dog faeces	CPV antigen	-	-
22	Gogone et al	PCR and Sequencing	CPV 2b and CPV 2c	Dog faeces	CPV antigen	-	partial VP2 gene
23	Costa et al	PCR and HA/HI	CPV-2b was the most common	Dog faeces	-	-	1. Primer pair P2ab, which identifies CPV-2a and CPV-2b 2. Primer pair P2b which identifies CPV-2b

S. No	Authors	Name of Test employed	Name of the gene present	Nature of sample	Detection	Sensitivity and Specificity of test	Primer used
24	Desario et al	Immunochromatography, haemagglutination, viral isolation and conventional and real-time PCR, RFLP	CPV-2a, CPV-2b, and CPV-2 Glu-426 mutant were detected	Dog faeces	-	-	-
25	Cho et al	LAMP	-	Dog faeces	CPV antigen	relative sensitivity of 100 percent	-
26	Mukhopadhyay et al	LAMP	-	Dog faeces	CPV antigen	-	two sets of outer and inner primers that target a total of six unique sites on the VP2 gene
27	Wilkes et al	iiPCR	CPV-2a, 2b, and 2c, as well as FPV were detected	Dog faeces	-	-	-
28	Decaro et al	TaqMan real-time PCR	Detecting type 2-based vaccinations and field strains (types 2a, 2b and 2c)	Dog faeces	CPV antigen	-	minor groove binder (MGB) probe assay
29	Elia et al	TaqMan real-time RT-PCR	RNA transcripts produced by CPV-2	Tissues of dogs	-	detection limit of $1x\ 10^2$ RNA copies	-
30	Kumar and Nandi	SYBR Green-based real-time PCR	-	Dog faeces	CPV antigen	analytical sensitivity of 10 copies	VP2 gene of CPV 2
31	Streck and colleagues	TaqMan PCR	-	blood and faeces samples	CPV antigen	-	-
32	Kaur et al	Multiplex real-time PCR	CPV 2a, CPV 2b, and CPV 2c were detected	Dog faeces	CPV antigen	-	VP2 gene of CPV
33	Hoang et al	Simple Probe real-time PCR	CPV-2a, 2b, and 2c	Dog faeces	CPV antigen	-	-
34	Puentes et al	Isolation of virus	CPV-2c-found in two isolated viruses	Dog faeces	CPV antigen	-	CRFK and MDCK cells used

S. No	Authors	Name of Test employed	Name of the gene present	Nature of sample	Detection	Sensitivity and Specificity of test	Primer used
35	Kaur et al	Isolation of virus	CPV-2b type	-	CPV antigen	-	MDCK cells used
36	Wang et al	RPA	CPV 2	-	-	Detection limit of 10 copies of CPV-2 genomic DNA	-
37	Geng et al	RPA (Real-time PCR)	CPV 2	-	-	analytical sensitivity of 10^1 copies/reaction	primers and an exo probe targeting the CPV-2 nucleocapsid protein gene
38	Meggiolaro et al	MT-PCR	vaccine-like CPV-2b subtypes, wild type CPV-2a subtypes, and vaccine-like CPV-2a subtypes	Dog faeces	CPV antigen	-	-
39	Xu et al	Polymerase cross-linking spiral reaction	CPV 2	Dog faeces	CPV antigen	Detection limit of this PCR was 3.9×10^1 copies	-
40	Dema et al	ARMS-PCR	CPV-2a, CPV-2b, and CPV-2c	Dog faeces	CPV antigen	-	Targeted the mutant 426 amino acid of VP2

4.4 *In situ* Hybridization

Using *in situ* hybridization in standard formalin-fixed, paraffin-embedded tissue sections; Nho et al. [33] "developed an improved approach for the identification of canine parvovirus. A digoxigenin-labelled probe was constructed to complement DNA sequences that code for the full VP-1 capsid protein sequence and the middle half of the VP-2 capsid protein sequence. The small intestine, tonsil, lymph node, thymus, spleen, heart, liver, and kidney of dogs diagnosed with canine parvovirus infection at necropsy showed specific histologic localisation of canine parvovirus-infected cells. *In situ* hybridization detected the exact locations of viral infection with pinpoint accuracy. The presence of canine parvovirus in the liver, kidney, and heart tissues of the same pups could indicate increased virulence of this strain of canine parvovirus, as well as a broader tissue tropism not previously found in Korean canine parvovirus strains".

4.5 Immunochromatographic Test

For the detection of canine parvovirus in dog faeces, Esfandiari and Klingeborn [34] "developed a one-step immunochromatographic test using monoclonal antibodies. Comparative testing was performed in Sweden, Denmark, and the Netherlands on samples using this one-step test and three different enzyme-linked immunosorbent assays. The outcome measure sensitivity and specificity had been 95.8% and 99.7%, respectively, according to the results of the evaluation. These findings demonstrate that the one-step test can detect parvovirus in faecal samples from dogs, cats, and mink in a quick, simple, repeatable, and sensitive manner".

4.6 PCR and Sequencing

A study was carried out by Narayanan et al. [35] "in order to determine canine parvovirus strains prevalent in the Namakkal region of Tamil Nadu. The viral DNA was isolated from 120 faeces samples from clinically afflicted household dogs. By employing polymerase chain reaction and DNA sequencing techniques, the template DNA was used to detect and molecularly characterise canine parvovirus. PCR was carried out with Hfor/rev primers, and all positive PCR results were DNA sequenced. PCR and sequencing techniques revealed that 58 of the 120 faecal samples collected were positive for CPV-2a and 14 for CPV-2b".

Mohan Raj et al. [36] carried out research to "isolate and describe the virus by amplifying a portion of the VP2 gene and performing further sequence analysis, as well as to determine the phylogenetic connection of the field virus with reference strains. Fifty-one samples were determined to be positive by PCR out of 77, and all 51 samples were submitted to virus isolation in the CRFK cell line. Sixteen viruses were isolated, and 10 isolates were chosen at random for sequencing analysis, along with four clinical samples. New CPV-2a (CPV2a with 297-SerAla) was identified in all 10 isolates and four clinical samples. One of the field isolates was discovered to be phylogenetically related to New CPV-2a strains from Japan and India; another field isolate was discovered to have ancestral origins with New CPV-2a strains from Korea, the United States, Italy, Brazil, Germany, Taiwan, and Vietnam; and the remaining sequences had distinct lineages but shared molecular relationships with New CPV-2a reference strains".

Ntafis et al. [37] too used a "conventional polymerase chain reaction to detect canine parvovirus 2 and the VP2 gene of the two CPV-2c viruses shared up to 100 percent amino acid identity with the CPV-2c strains previously discovered in Europe, according to sequence analysis. The findings showed that, unlike other European countries, CPV-2a is still the most prevalent variation in Greece, and that the CPV-2c version seen elsewhere in Europe is also present".

Jobin et al [38] conducted research "to better understand the genetic epidemiology of circulating CPV-2 strains in India's northern area. Positive polymerase chain reaction samples were processed to oligonucleotide sequencing, and these isolates were discovered to be identical to CPV-2a except for amino acid residues 264, 297, and 440, and so categorised as an antigenic variation of CPV-2a. The mutation at location 264 has never been seen before in India. Furthermore, a worldwide phylogenetic study verified the new CPV-2a isolates' molecular link with Chinese genomes".

To establish CPV-2's potential as an environmental danger for nonimmunized susceptible hosts, Gogone et al. [39] examined 50 faecal samples obtained from public locations in a municipality in Paraná state, Brazil. PCR technique targeting the partial VP2 gene found seven samples positive for CPV, with three

strains validated as CPV-2b variants and one as CPV-2c variants by sequence analysis. Phylogenetic analysis validated these findings, and canine mitochondrial DNA amplification and sequencing confirmed the species identity of the faeces samples' source. Their findings show that CPV is present in canine faeces contaminating city streets and emphasise the significance of environmental control to limit the risk of exposure to susceptible hosts.

4.7 PCR and HA/HI

Costa et al. [15] "used the polymerase chain reaction assay to analyse the genomic types of canine parvovirus circulating in the state of Rio de Janeiro, Brazil, from 1995 to 2001. A total of 78 faecal samples from gastroenteritic puppies were tested for canine parvovirus after being proven positive by haemagglutination/ haemagglutination inhibition assays or viral isolation in cell culture. A mixture of phenol-chloroform and silica-guanidine thiocyanate techniques were used to extract viral DNA from faeces samples. To discriminate between the old (CPV-2) and new forms of virus, PCR was used with differing pairs of primers. Using the primer pair P2ab, which identifies CPV-2a and CPV-2b, specific amplicons were identified for all samples. Because of their response with the primer pair P2b, 76 out of 78 samples were identified as CPV-2b. Thirty of the 78 samples were from previously vaccinated puppies, with enteritis symptoms appearing 1 to 12 days following immunisation in 15 of them. In 5 of the 15 puppies that had received old-type immunizations, PCR revealed wild virus CPV-2b infection. From 1995 to 2001, CPV-2b was the most common variety circulating in the state of Rio de Janeiro, according to research findings".

For the diagnosis of canine parvovirus type 2 infection in samples taken from dogs with diarrhoea, Desario et al. [40] used five laboratory tests like Immunochromatography (IC), haemagglutination (HA), viral isolation (VI), and conventional and real-time PCR. In 41, 50, 54, 68, and 73 of the samples, IC, HA, VI, and conventional or real-time PCR were able to detect CPV-2 antigen or nucleic acid, respectively. With an overall agreement of 94.38 percent, the best correlation was obtained between conventional and real-time PCR. Antigenic and/or genetic analyses of the CPV-2 strains were performed using monoclonal antibodies, restriction fragment-length polymorphism (RFLP), and/or sequencing analysis on 68 samples that tested positive by HA, VI, or conventional PCR. In total, 26 of the 68 strains studied were recognised as CPV-2a, 18 as CPV-2b, and 24 as a CPV-2 Glu-426 mutant.

4.8 LAMP

Cho et al [41] used the LAMP method to identify "canine parvovirus genomic DNA, with a relative sensitivity of 100 percent, indicating that this test has potential for application as a CPV diagnostic assay in a clinical environment. By developing two sets of outer and inner primers that target a total of six unique sites on the VP2 gene of CPV". Mukhopadhyay et al. [42] also developed a "highly sensitive loop mediated isothermal amplification approach. A simple boiling and chilling procedure was used to make the template DNA. One hundred four samples were found positive by LAMP, whereas 81 samples were found positive by PCR, out of 140 faecal samples examined by the developed LAMP and traditional PCR methods. Cross examination of common dog pathogens was used to test the specificity of the LAMP assay, which was then validated by sequencing".

Wilkes et al. [43] developed "a nucleic acid detection system that uses insulated isothermal PCR (iiPCR) technology and has high sensitivity and specificity. CPV-2a, 2b, and 2c, as well as FPV, were detected by iiPCR. The iiPCR technology has the potential to be a valuable tool for CPV-2 molecular detection that is both quick and accurate.

4.9 Real-time PCR

Decaro et al. [6] developed "TaqMan-based diagnostic tests for detecting canine parvovirus type 2 strains in the faeces of dogs suffering from diarrhoea, including a minor groove binder (MGB) probe assay for detecting type 2-based vaccinations and field strains (types 2a, 2b and 2c). These assays have been proven to be highly sensitive, specific, and repeatable, enabling for the detection of both type 2b vaccinal and field strains in the same specimens". Elia et al. [44] "designed a TaqMan real-time RT-PCR technique to detect RNA transcripts produced by CPV-2 replication. The assay had a detection limit of $1x\ 10^2$ RNA copies, and the standard curve had a linear range of $1x10^2$ to $1x10^9$ copies with high repeatability. The technique was subsequently used to measure the levels of mRNA in the tissues of dogs who were infected

with CPV-2". Decaro et al. [6] "used minor groove binder (MGB) probe assays for discriminating between CPV vaccine and field strains and the current investigation indicated that the majority of episodes of parvovirus-like sickness in dogs that arise soon after vaccination are caused by infection with field strains of CPV-2 rather than pathogenicity reversion of the modified live virus in the vaccine".

Cavalli et al [45] "investigated the antigenic relationships among the original CPV-2 and the variants CPV-2a, -2b, and -2c by using serum neutralization assay".

Kumar and Nandi [46] "developed a SYBR Green-based real-time polymerase chain reaction (real-time PCR) for identifying and analyzing canine parvovirus type 2 in dog faeces. The primers for this assay were developed and produced using the nucleotide sequence of the VP2 gene of CPV 2. The SYBR Green-based real-time PCR was proven to have an analytical sensitivity of 10 copies. When the findings of three different assays were compared, it was discovered that real-time PCR is more sensitive than HA and conventional PCR, allowing for the detection of low CPV 2 titers in infected dogs".

Streck and colleagues [47] "developed a quantitative TaqMan PCR for detecting and quantifying canine and feline parvoviruses in blood and faeces samples. Validation experiments were done utilising 10-fold serial dilutions of CPV-2 virus in CPV/FPLV-negative faeces and CPV/FPLV-negative serum samples, as well as a standard curve. All forms of canine parvoviruses, as well as FPLV, were found. Therefore they summarised real-time PCR is an improved and useful method for detecting and quantifying canine and feline parvoviruses in various sample matrices".

Kaur et al. [48] conducted a study using a de novo multiplex real time PCR to identify and antigenic type CPV. The study found that the multiplex real time PCR test described in the study can be employed for quick detection of CPV as well as typing of its three antigenic kinds, CPV 2a, CPV 2b, and CPV 2c.

Using the Simple Probe real-time PCR assay, Hoang et al. [49] developed a novel method for genotyping CPV-2 strains. Amplifying the DNAs prepared from clinical specimens revealed three distinct melting curve peaks in the SimpleProbe® assay. The melting peak of CPV-2b was 57.8°C

(CI 95 percent: 57.7-58.5°C), followed by CPV-2c at 52.3°C (CI 95 percent: 52.2-53.2°C) and CPV-2a at 50.2°C (CI 95 percent: 50.1-50.5°C). This technique can be utilised for molecular CPV-2 epidemiology investigations and is a reliable and sensitive tool for discriminating between CPV-2a, 2b, and 2c.

4.10 Isolation of Virus

In Uruguay, Puentes et al. [50] discovered CPV-2 in puppies with clinical illness. To isolate the virus, samples were processed and used to infect CRFK and MDCK cells. Two of the twelve samples tested positive for CPV-2. A 583-bp genomic area was amplified, and molecular characterisation was carried out using sequencing, phylogenetic analysis, and Restriction Fragment Length Polymorphism. CPV-2c-like viruses were found in two isolated viruses. The cytophatic effect of CPV-2 (vaccinal virus) and CPV-2c (isolated virus) on primary canine cell cultures and CRFK line cells was compared, and it was discovered that CPV-2c is less cytopathogenic in CRFK cells than in primary cultures. Kaur et al. [51] used the Madin Darby Canine Kidney cell line to isolate Canine Parvovirus from suspected canines and confirmed it using polymerase chain reaction and nested PCR. In addition, the VP2 gene of the CPV isolates was amplified and sequenced to establish the antigenic type that prevailed. The VP2 gene sequence analysis of the samples and vaccination strains revealed that the samples were of the CPV-2b type.

4.11 Recombinase Polymerase Amplification

Wang et al. [52] developed a recombinase polymerase amplification (RPA)-based approach for canine parvovirus type 2 (CPV-2) identification. RPA has a detection limit of 10 copies of CPV-2 genomic DNA, according to sensitivity analyses. RPA has the distinct benefit of being an isothermal reaction that can be carried out in a water bath, making it a viable alternative approach for detecting CPV-2 in resource-constrained environments. Using primers and an exo probe targeting the CPV-2 nucleocapsid protein gene, Geng et al. [53] developed a real-time RPA test for the detection of CPV-2. At 38 °C, the real-time RPA test was effectively completed, with findings acquired in 4–12 minutes. The assay exclusively detected CPV-2 and had no cross-reactivity with other viral infections, indicating a high level of

specificity. The real-time RPA had an analytical sensitivity of 10^1 copies/reaction of a typical DNA template, which was 10 times higher than the common RPA approach. The clinical sensitivity of the real-time RPA assay matched the real-time PCR results 100 percent. Meggiolaro et al. [54] used a multiplexed tandem PCR (MT-PCR) panel to identify the CPV-2 subtypes. There were vaccine-like CPV-2b subtypes, wild type CPV-2a subtypes, and vaccine-like CPV-2a subtypes found. In faecal samples, a high copy number indicated the presence of wild-type CPV-2a, but a fluctuating copy number indicated the presence of vaccine-like CPV-2b. A yardstick approach to a copy number or Ct-value to distinguish a vaccination strain from a wild type CPV-2 virus can be misleading in some situations. As a result, distinguishing the vaccination strain from a CPV-2 wild type subtype remains a difficult task. Xu et al. [55] developed an improved polymerase cross-linking spiral reaction method for detecting CPV-2 early and quickly. The amplification reaction was shown to be most effective when conducted at 62°C for 50 minutes, and it could be utilised to identify CPV-2 without causing cross-reactions with other canine infectious disease viruses. The positive amplification tube was exhibited as glowing yellow and the negative tube as bright purple, and the reaction outcomes were directly examined by the naked eye. Using gel electrophoresis or a visible dye, the detection limit of this PCR was 3.9 x 10^1 copies.

4.12 ARMS-PCR

Dema et al. [56] demonstrated a concise, single-step ARMS-PCR approach to discriminate CPV-2 antigenic classes by targeting the mutant 426 amino acid of VP2. A total of 150 faecal samples were tested using ARMS-PCR, with 18 of them being typed as CPV-2a, 79 as CPV-2b, and 6 as CPV-2c. The ARMS-PCR results were confirmed by sequencing incomplete VP2 genes from 14 samples at random. Phylogenetic study of partial VP2 gene sequencing of each of the CPV-2 variations revealed that CPV-2a and CPV-2b isolates belonged to a different clade of Indian lineage, while CPV-2c and Asian lineage had a common evolutionary origin. The created technique is a one-step, quick, sequencing-independent method for typing CPV-2 antigenic variations.

5. CONCLUSION

This review provides information on Canine Parvovirus causing gastroenteritis in dogs. This would help readers to have information on the virus and the various diagnostic techniques that have been used earlier and recently. Various diagnostic techniques have been developed and are being used routinely for diagnosis of CPV in dogs. This is important too as CPV is quite prevalent and of much concern among dog owners, breeders and veterinarians. Therefore the information on diagnosis of CPV helps in early and quick diagnosis to prevent mortality by early treatment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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