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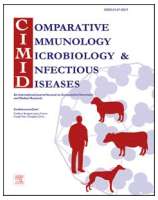

Review

Canine parvovirology – A brief updated review on structural biology, occurrence, pathogenesis, clinical diagnosis, treatment and prevention

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ABSTRACT

Canine parvovirus (CPV) is a major cause of hemorrhagic diarrhea and mortality in puppies worldwide. There are 2 types of Parvovirus which affects canines: Canine parvovirus 2 (CPV-2) and Canine parvovirus 1 (CPV-1) or the Minute Virus of Canine (MVC). CPV-2 originated from Feline panleukopenia virus and has undergone genetic variation to give rise to its three variants (CPV-2a, CPV-2b and CPV-2c). Amino acid substitutions in VP2 capsid protein have led virus to adapt new host range. The original CPV-2 was known to be dominant in Japan, Belgium, Australia as well as USA and later circulated throughout the world. Clinically, CPV-2 infection is characterized by anorexia, lethargy, depression, vomiting, leukopenia and severe hemorrhagic diarrhea. Several diagnostic tests have been developed to detect parvoviral infections which are categorized into immunological tests (latex agglutination test, SIT-SAT and ELISA etc.) and molecular based tests (PCR, mPCR and RT-PCR etc.). To control and manage the disease several treatments like fluid therapies, antibiotics, and adjunctive treatments are available and some are in various stages of development. Apart from this, many vaccines are also commercially available and some are in developmental stages. The present review contains detailed information regarding structural biology, occurrence, pathogenesis, clinical diagnosis, treatments and prevention in order to understand the need and the growing importance of CPV-2.

1. Introduction

Canine parvovirus-1 (CPV-1) commonly known as Minute Virus of Canine is genetically unrelated to Canine parvovirus-2 (CPV-2), whereas CPV-2 is one of the major entero-pathogen causing gastroenteritis in dogs/puppies, CPV-2 is non-enveloped virus with icosahedral capsid and contains single stranded DNA. Within the capsid, VP2 is the major capsid protein and determines the antigenicity of the virus. The virus is originated as host range variant from Feline panleukopenia virus (FPV). CPV-2 is highly fatal and contagious virus cause acute hemorrhagic diarrhea resulting in frequent mortality (>70% in puppies and <1% in adult dogs) and 100% morbidity throughout the world ([4,8]). The first case of parvoviral infection was reported in 1979 and from then the virus underwent genetic modifications at VP2 capsid protein to give rise to its 3 variants (CPV-2a, CPV-2b and CPV-2c). CPV-2 is one of the major gastroenteritis viruses present globally and predominant in countries like Japan, Belgium, Australia, Denmark, Brazil, France and United States of America (USA) [81]. The major clinical manifestations associated with CPV-2 are hemorrhagic diarrhea and in rare cases myocarditis [121]. CPV-2 infection is known to be present even in vaccinated kennels. The virus is highly contagious and can be transmitted from fecal-oral route as well as inanimate objects. The clinical symptoms associated with CPV-2 are often non-specific and often signs of depression, lethargy and fever are observed. Dog suffers from severe dehydration as a result of hemorrhagic diarrhea and vomiting that usually starts after 24 hrs of infection. Detection of CPV-2 is of utmost importance, especially in early stage of infection to further prevent the spread of disease. Detection of CPV-2 is mainly done by molecular and immunological based methods [90]. Several vaccines (heat killed and modified live virus) have been developed to control the disease [90]. The major reasons for the failure of vaccines are interference with maternally derived antibodies, incorrect timing of vaccination and more rarely the reversion of viral vaccines ([10] and [2]). Several post-exposure therapies have been developed to cure the disease, but they have their own limitations in terms of availabilities and cost [88]. However, further improvements are needed in the field of diagnostics, surveillance tools and pre as well as post-exposure treatment to manage the problems associated with CPV-2 infection. The present review is

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aimed to provide brief information regarding structure, epidemiology, pathogenesis, clinical diagnosis, treatments and prevention of CPV-2. This review will be helpful to explore futuristic research areas and generate awareness among scientific community, diagnosticians, practitioners, students, and teachers to manage and control the disease with highly scientific and efficient manner.

2. Taxonomy

CPV-2 is a highly infectious virus which causes acute enteritis in domestic and wild canids. CPV-2 belongs to the family of *Parvoviridae* that includes 2 subfamilies: *Parvovirinae* and *Densovirinae*. *Parvovirinae* is known to infect vertebrates whereas; *Densovirinae* infect invertebrates (Fig. 1). *Parvovirinae* includes Genus *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus, Bocavirus* and other unclassified vertebrate parvovirus (PARV4 and related viruses, chicken parvovirus and related viruses). On the other hand, subfamily *Densovirinae* includes Genus- *Iteravirus, Brevidensovirus, Densovirus* and *Pefudensovirus* (https://talk.ictvonline.org/ictv-reports/ictv_9th_report/ssdna-viruses2011/w/ssdna_viruses/151/parvoviridae [49] and [20]). Canine parvovirus along with Mink enteritis virus (MEV), Feline panleukopenia virus (FPV), Raccoon parvovirus (RPV) and Blue fox parvovirus (BFPV) have been grouped together in one subgroup having a common ancestor. CPV-2 bears 98% phylogenetic homology with FPV and considered to be closely related to FPV [90]. Despite of these similarities, they have specific host range, antigenic and hemagglutination properties. CPV-1 commonly known as minute virus of canine and is genetically unrelated to CPV-2 [83]. CPV-1 causes acute enteritis in dogs and considered to be related to Bovine parvovirus (BPV). There are no variants of CPV-1 reported till date [114].

3. Structure

CPV-2 is a non-enveloped virus with spherical capsid (25 nm) enclosing a linear single stranded negative sense DNA ((-) ssDNA). The viral capsid is made up of 3 major proteins encoded by the genome. The genome of CPV-2 contains two open reading frames (ORFs) which encodes non-structural proteins (NS1, NS2) and structural proteins (VP1, VP2) [24]. The capsid of CPV-2 has icosahedron (T = 1) geometry and have 60 copies of two structural proteins VP1 (5–6 copies) and VP2 (54–55 copies) which are produced by alternative splicing of mRNA ([61] and [12]). The VP3 protein is typically cleaved from VP2 by the action of host protease enzyme [91]. These capsid proteins have highly conserved central core made up of eight stranded anti-parallel β-barrel domains. The surface of the capsid consists of four loops inserted between the strands that produces spike like protrusions around threefold axes of about 22 Å. Loop 3 and 4 are usually grouped as a long GH loop. The structural configuration of these loops determines the antigenicity of virus. The antigen neutralization site also called as epitope A which is formed from loop 1 and 2 of one VP2 and loop 4 of threefold related molecule ([126] and [57]). The capsid also contains deep depression (canyon) of about 15 Å at fivefold axes and a 15 Å deep depression (dimple) at the twofold axes. The genetic organization of CPV-2 is shown in Fig. 2.

4. Evolution

The capsid of CPV-2 consists of 90% of VP2 and it determines the antigenicity, tissue tropism and host range of the virus [91]. CPV-2 is known to be closely related to FPV however, they differ in 6 amino acid residues in VP2 capsid protein. Mutations at VP2 capsid residues at 93 (Lys to Asn), 103 (Val to Ala) and 323 (Asp to Asn) allowed CPV-2 to bind to canine transferrin receptor (TfR) and replicate in canines. The mutations at VP2 residues at position 80 (Lys to Arg), 564 (Asn to Ser) and 568 (Ala to Gly) are associated with the loss of ability of CPV-2 to replicate in feline hosts. Soon after in 1979, CPV-2a replaced CPV-2 as a parallel evolution due to the amino acid substitutions at 87 (Met to Leu), 300 (Ala to Gly) and 305 (Asp to Tyr). These amino acid substitutions allowed CPV-2a to acquire affinity for binding to the feline transferrin receptor (TfR) and replicate again in feline host ([123] and [125]). The most important substitution introduced in feline host range was at amino acid residue 87. Apart from these substitutions, further changes occurred in VP2 capsid protein including amino acid residues 101 (Met to Thr), 297 (Ser to Ala) and 555 (Val to Ile) [125]. In 1982, CPV-2a

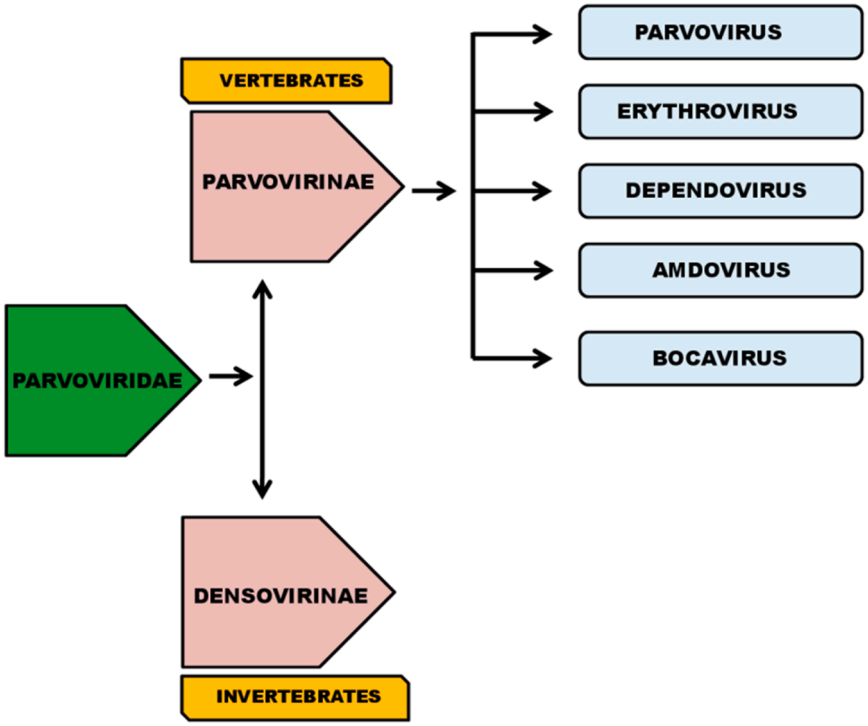

Fig. 1. Schematic representation of taxonomical relationship of *Parvoviridae*.

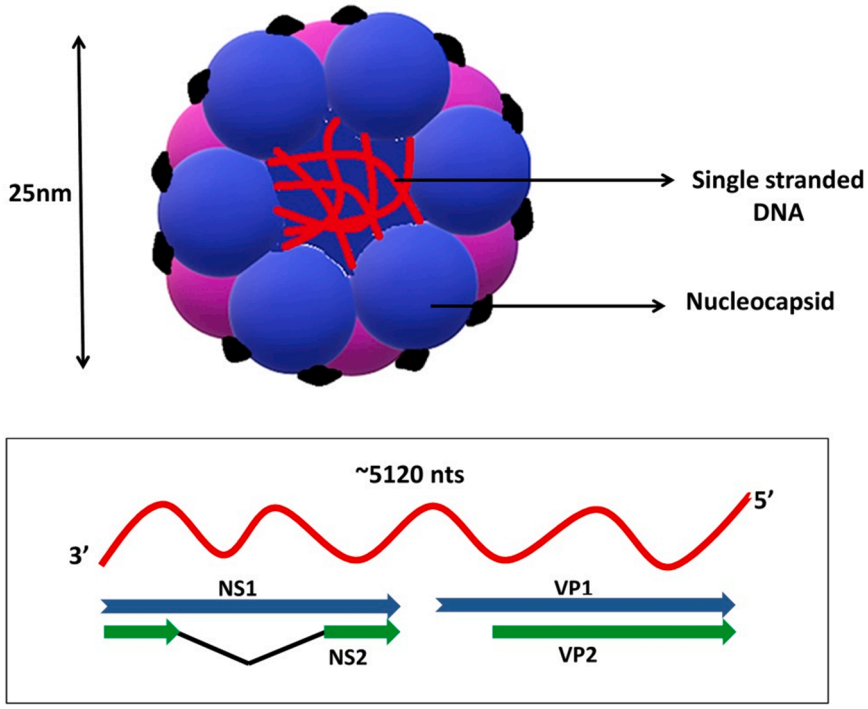

Fig. 2. Genetic organization of CPV-2 [42 and 50].
The figure is adopted and modified from http://online.anyflip.com/scbe/ubvw/files/basic-html/page3.html and https://viralzone.expasy.org/103?outline=all_by_species.

variant totally replaced the original strain CPV-2 worldwide. In 1984, CPV-2b emerged from CPV-2a which differed by single amino acid substitution at position 426 (Asn to Asp). CPV-2b was first detected in USA and was shown to be mutated at major antigenic region epitope A at threefold spike of VP2. Another variant, CPV-2c, was first detected in Germany, 1996 which had amino acid substitution at 426 (Asp to Glu) (Truyen et al.,). The genetic evolution of CPV-2 is shown in the Fig. 3. Other mutations were insignificant to give rise to epidemiologically relevant new variants. A mutation at 297 (Ser to Ala) was detected in both CPV-2a and CPV-2b. These mutations did not change the antigenicity of the variants as they were located on minor antigenic site (epitope B). Some variants have shown mutation at position 440 (Thr to Ala) that can influence the antigenic structure due to its presence in GH loop. Another important mutation was observed at 324 position of VP2 (Tyr to Ile) which is likely to affect CPV-2 host range. ([17] and [81]).

Some cases of co-infection of CPV-2 variants have been reported ([6] and [130]). In one of the cases, the dog was infected by CPV-2a and CPV-2c where the strains differed by 29 nucleotides. In other cases, the dog was infected by a recombinant strain which arose from recombination between CPV-2a and CPV-2c. These incidences indicate the generation of viral diversity and emergence of new genotypes of CPV-2 [81] and [102].

5. Worldwide distribution

CPV-2 and its variants are most common canine pathogens widely

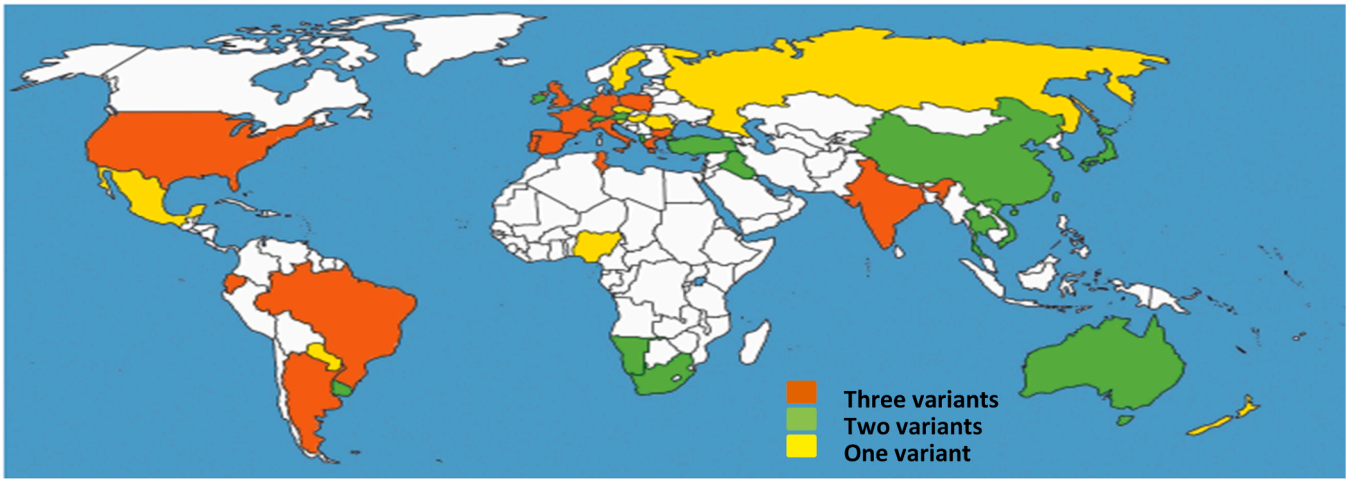

Fig. 3. Evolution of Canine Parvovirus. The figure depicts genetic variations and host ranges of CPV-2 and its related viruses. It also indicates the year of global spread of CPV-2 and its variants. [BFPV- Blue Fox Parvovirus, CPV- Canine Parvovirus, FPV- Feline Parvovirus, MEV- Mink Enteritis virus, RPV- Raccoon Parvovirus].
The figure is adopted and modified from Truyen [124] and [69].

distributed throughout the world. The amino acid substitutions have led the virus to evolve and adapt in different regions of the world. Initially, the original CPV-2 strain was identified in Japan, Belgium, Australia, USA, etc., and known to be dominant worldwide. After 1980, CPV-2 was replaced by new antigenic variant (CPV-2a) which further spread to France and Denmark (1979–1982). CPV-2a completely replaced original strain within 2–3 years that showed the epidemiological advantage and higher affinity to the canine cell receptors of CPV-2a variant over CPV-2. Further an antigenic variant CPV-2b was recognized in 1984 and in 2000 another mutant called CPV-2c was reported in dogs. However, the antigenic variation is also associated with new adaptation capability, phenotypical characteristics and epidemiological advantage among CPV-2 variants ([98] and [22] and [106]). CPV-2 infection is known to be present in 5 continents distributed worldwide. In India, the first case of CPV-2 infection was reported in 1982. The outbreaks of CPV-2 and its variants have been recorded in different parts of India which includes Assam, Tamil Nadu, Orissa, Pondicherry, Kerala, West Bengal, Haryana and Uttar Pradesh. The prevalence of CPV-2a has been documented in India, since 2001. CPV-2b is known to be more predominant in north India as compared to CPV-2a. CPV-2a infections are dominant in south and central India. CPV-2c was first documented in India in the year 2010 [90]. The global distribution of CPV-2 is shown in Fig. 4.

6. Pathogenesis

The most common route for infection of CPV-2 is fecal-oral route or contact with contaminated surfaces [120]. In kennel environment, the excretions from CPV-2 infected dogs can expose susceptible dogs for infection. CPV-2 has a tropism for rapidly dividing cells. First it invades pharynx and replicates in lymphocytes. After some days the virus is released into the bloodstream and eventually enters into bone marrow where it infects white blood cells causing profound leukopenia. After infecting lymphoid tissue, it causes viremia within 5 days of infection. Puppies born to unvaccinated parents who are infected with CPV-2, in first two weeks suffer from myocarditis as the virus replicates in heart muscles. Cardiac manifestation of virus is rarely observed as most of the puppies receive maternal derived antibodies (MDA) which protect the neonates. Once the virus is disseminated from infected leukocytes, the virus enters into the germinal epithelium of the crypts of the small intestine and causes diarrhea. The epithelial cells of intestine get matured in crypts of Lieberkuhn and migrate from germinal epithelium of crypts to villi. The major function of these cells is to aid absorption of nutrients. The virus infects the crypts of Lieberkuhn causing villous collapse. The turn-over of these rapidly dividing intestinal crypts gets impaired which results in characteristic lesions and thus disturbs the absorptive capacity of intestine. Both circulating and tissue associated lymphocytes gets infected resulting in lymphopenia and neutropenia. CPV-2 infected dogs are prone to secondary infections by bacteria. A villous collapse, inflammation and necrosis results in translocation of enteric microbial flora that is commonly associated with sepsis. Dog starts shedding virus particles at a detectable rate after 4–7 days of infection ([30,103,24,31] and [93]). The clinical manifestations of gastrointestinal tract due to CPV-2 infection are depicted in Fig. 5.

7. Replication

Like other viruses, the capsid of CPV plays an important role in host cell attachment. The virus undergoes certain activations to initiate the infection. These include receptor binding, exposure to low pH, rearrangement of capsid bonds and proteolytic cleavage of structural

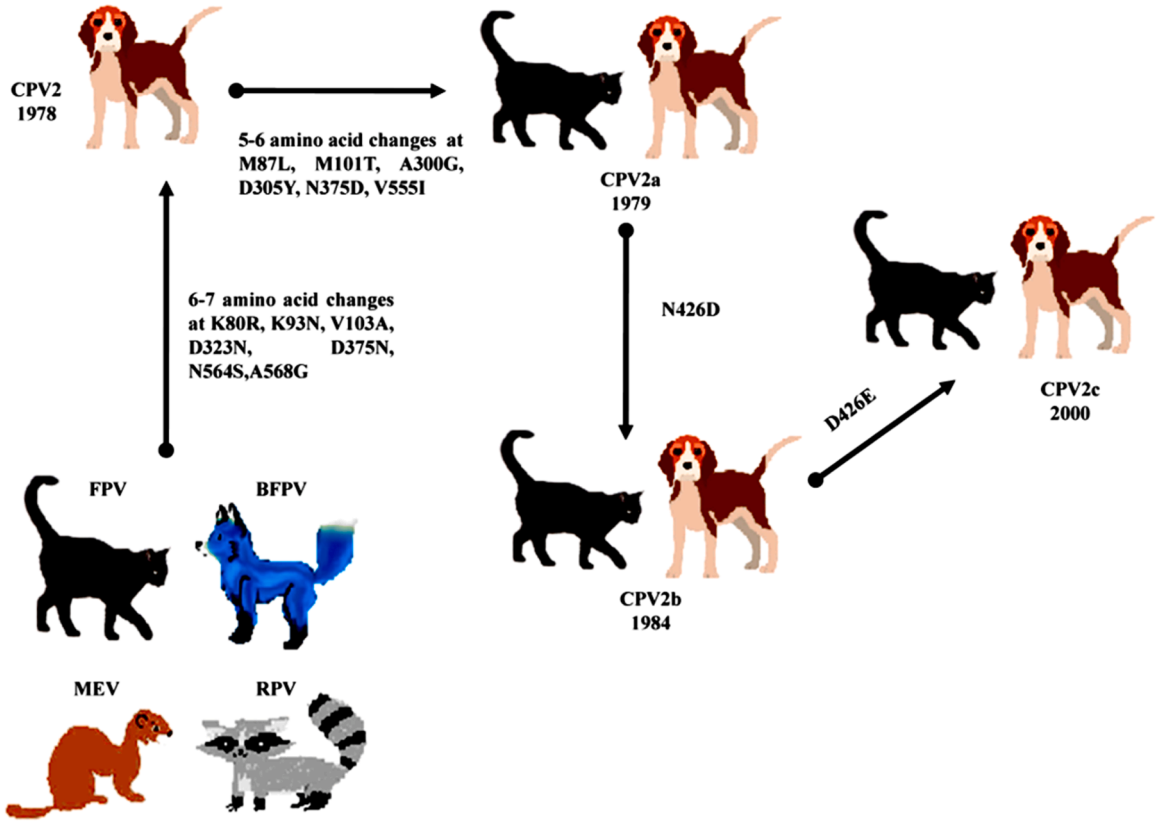

Fig. 4. Worldwide distribution of CPV-2 variants [(CPV-2a, CPV-2b between 1982 and 1985) and (CPV-2c during 1995–2009)] based on amino acid substitution at 426. Specific color code has been given which shows the distribution of particular variant in that region (orange indicates presence of all three variants, green indicates presence of 2 variants and yellow indicates presence of one variant).
The figure is adapted and modified from [81].

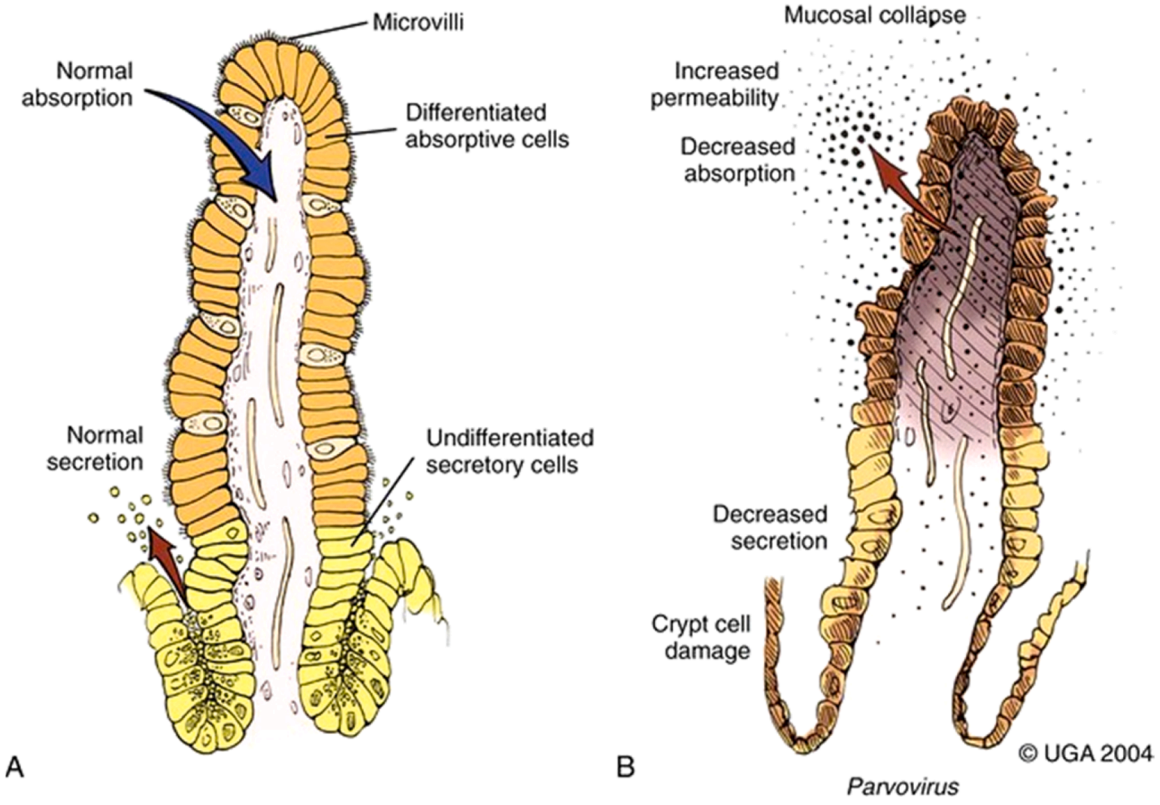

Fig. 5. A: Normal gastrointestinal tract showing differentiation along with villus. 5B: CPV-2 infected gastrointestinal tract showing villus collapse and necrosis of intestinal villus.
The picture adopted from [119].

proteins. These changes trigger virus attachment, endocytosis, membrane penetration and control trafficking in cytoplasm organelles as well as nucleus [91] and [109].

Transferrin receptor (TfR) is the principal receptor for both FPV and CPV-2. TfR is a homodimer and belongs to type II glycoprotein. Each monomer consists of an apical domain, helical domain and one protease like domain. TfR are variably glycosylated in different species. The TfR of canines have four glycosylations in VP2 residues at 93, 299, 300 and 323 [35]. The changes in amino acid at position 93 (Lys to Asn) and 323 (Asp to Asn) allows CPV-2 to attach to dog cells. The virus enters into the cell via clathrin mediated endocytosis and is further trafficked to early endosome. The low pH of early endosome causes dissociation of ligands from receptor. The virus moves to late endosome and finally ends up in lysosome. In lysosome, the release of phospholipase A2 (PLA2) enzyme domain of VP1 destroys the integrity of lysosomal membrane and the virus releases into the cytosol [12]. The virus is then transported from cytosol to nucleus with the help of VP1 nuclear localization signal for replication. The move of virus from early endosome to peripheral nucleus is mediated by microtubules and actin filaments. In nucleus ssDNA is converted into dsDNA, transcribed into mRNA and translated to produce viral proteins. Replication of virus occurs through rolling circle mechanism. The mature virion after capsid assembly is exported out from nucleus via CRM1 and NS2 mediated pathway. CRM1 is an exportin-1 present in virus, binds with the leucine rich nuclear export signals. It helps nuclear egression of assembled capsid and release of progeny after cell death and lysis ([127,28,29,97] and https://viralzone.expasy.org/103?outline=all_by_species).

Replication of CPV-2 is depicted in Fig. 6.

8. Clinical symptoms

CPV-2 infected dogs gradually develop fever at the early stage and the progression of infection results in vomiting and diarrhea. The consistency of stool varies and it appears yellow or may contain blood. The clinical symptoms usually develop from 3 to 5 days of infection and lasts for 5–7 days [90]. The clinical manifestations associated with infected dogs are anorexia, depression, lethargy, profuse diarrhea or hemorrhagic diarrhea, abdominal discomfort, pyrexia, dehydration and in worst cases death [93]. White blood cell count may drop below 2000–3000 cells/mL leading to leukopenia [24]. The duration of infection is influenced by the load of virus ingested by the dog. The mortality and morbidity rate in CPV-2 infected dogs are influenced by the severity of challenge, age of the animal and co-infection with other pathogens [90]. In co-infection scenario, the virus damages the intestinal tract and the rate of bacterial translocation increases in the blood stream resulting in septicemia, endotoxemia, systemic inflammation, coagulation disorders and septic shock. In some cases, *E. coli* had been recovered from the lungs and liver of infected puppies. Pulmonary infection often leads to respiratory distress [31]. Another rarely observed clinical manifestation in CPV-2 infected puppies below 3 months of age is myocarditis. During chronic infection, puppies suffer from agonal breathing. In case of mild infection, puppies are usually subjected to outpatient treatment [90]. In most of the cases outpatient is usually not recommended, as the pet owners often fail to provide oral treatment on time. Thus, the puppies use to deteriorate due to severe vomiting.

9. Risk factors

CPV-2 can infect dogs of all ages, sex and breed. Puppies become susceptible to infection mostly between the times of weaning to six months of age. The susceptibility depends upon the level of systemic protection acquired from their mothers. The level of protection depends upon the antibody titer of colostrum and also consumption of colostrum

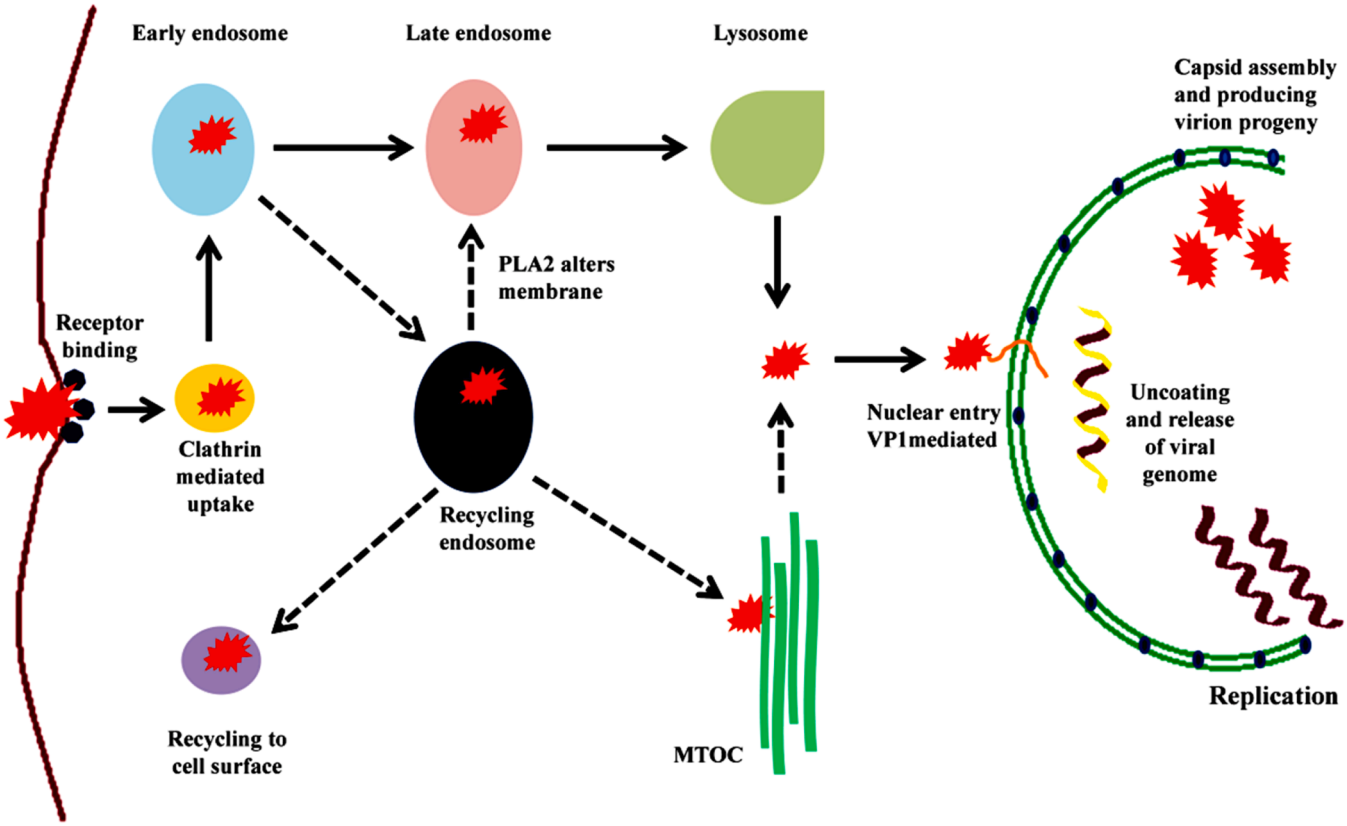

Fig. 6. Schematic representation of replication of CPV-2 PLA2- Phospholipase A2, MTOC-microtubule organizing center [46].
The figure is adopted and modified from [127] and https://basicmedicalkey.com/parvoviridae/.

by individual puppy. Moreover, maternal derived antibody significantly varies among puppies from a single litter. Puppies obtain vast majority of maternally derived antibodies during the first few hours of life. Systemic titer of MDA gradually decreases with age. If the serological titer of MDA falls below 1:80 (haemagglutination inhibition (HI)), it is no longer protective. Since colostrum and milk contains significant number of CPV-2 antibodies, they can potentially interfere with CPV-2 intestinal replication either by coating enterocytes or by trapping fecal CPV-2 particles, thereby preventing their replication in intestinal mucosa ([94] and [80]). A study was conducted in order to determine the role of maternal derived antibodies in protecting the puppies from infection. The experimental group contained 79 puppies from one breeding kennel and their MDA titer was determined once per week. Variability in MDA titer was observed (2 days of age) ranging from 1:10 (group A) to 1:1280 (group B). The variability in MDA level could be due to unequal colostrum ingestion from maternal or puppy's origin. The titer of absorbed maternal antibodies was observed to be associated with breed size and growth rate during the first 48 hrs of life [80]. Pure breeds of English springer, German shepherd, American Pit Bull Terriers, Doberman Pinchers and Rottweiler are more prone to CPV-2 infection than cross-breeds. A dog develops immunity for CPV-2 within a few days of infection and it remains for the rest of their life [32]. A comparative study conducted in India and Bangladesh showed that the rate of infection varied among the breeds: 30% in indigenous, 41.1% in Spitz, 22.2% in Lhasa and 21.4% in Doberman. The occurrence of CPV-2 infection was found to be 8.33% in vaccinated dogs and 60% in non-vaccinated dogs. Age wise distribution of CPV-2 infection was also observed which was found to be 57.4% for 1–3 months, 28.9% for 4–6 months, and 10% for above 6 months. Between males and females, the prevalence of CPV-2 infection was higher in females (49.4%) than males (33.7%) [38]. However, a study was conducted in Slovenia found that the patients suffering from CPV-2 was higher in males (83.3%) than females (16.7%) [33]. A retrospective study was conducted by Houston and co-workers to determine the susceptibility of sexually intact dogs and neutered dogs for CPV-2 enteritis. The results showed that sexually intact dogs (above 6-month age) were more susceptible to CPV-2 enteritis than neutered dogs [41]. Another study was conducted in Australia on CPV-2 infected dogs (1451 nos.) in order to analyze the risk factors associated with CPV-2 infection. About (89.5%) of infection were recorded in vaccinated dogs less than 12 months of age. One of the reasons contributing to higher incidence was interference of vaccine antigen with maternal antibodies. The increased severity and high fatality rate observed in CPV-2 infected dogs (under 12 month of age) might be due to the fact that humoral and cell mediated immunity does not develop fully until 1 year of age [70].

10. Diagnostics

A presumptive Diagnosis of CPV-2 is mostly carried out by considering the presence of clinical signs (depression, vomiting, diarrhoea, anorexia and fever) and the history of vaccination protocol. Veterinarians perform physical examinations for various parameters such as fluid deficit, mentation, abdominal examination, palpitation and rectal examination etc. Apart from these, other parameters like glucose level, electrolyte evaluation, WBC count, blood gas analysis, serum biochemistry and urine analysis are also performed [59]. However, clinical signs and physical examinations are considered as presumptive diagnosis of the disease and are not reliable in every case study. For confirmatory diagnosis, various detection methods have been employed which includes: Traditional methods, Immunological based methods and Molecular based methods, which are briefly described below [68].

11. White blood cell (WBC) count

One of the important clinical signs for parvovirus infection is reduction of immunologic cells (WBCs) production in blood. The WBC count depletes in high infection stages and increase in recovery stages. Hence white blood cell count is helpful parameter to monitor the infection stages and determining when the patient truly on the recovering. The WBC count is performed using blood smear after staining with Giemsa and cell count is performed by means of an automated cell counter (QBC Vet, USA).

12. Traditional methods

Traditional methods are old methods for isolation and identification of CPV-2 virus using mammalian cell lines. However, these methods are time consuming, laborious, expensive and require skilled professionals which limit their applications.

13. Virus isolation

Viral isolation by cell culture technique is the gold standard method for diagnosis. It is carried out by propagating the host cell lines in growth medium in order to form a monolayer. The virus stocks are checked for titer and added onto monolayer of cells [39]. CPV-2 has been observed to replicate in feline and canine cell lines. Several cell lines have been studied for culturing CPV-2 such as Crandall Feline Kidney cell line (CRFK), Walter Reed Canine Cell line (WRCC) and Madin Darby Canine Kidney cell line (MDCK). CPV-2 has been isolated from canine lungs and kidney cells. The virus has shown to produce characteristic cytopathic effects such as cell detachment, rounding of cells and formation of inclusion bodies in nucleus of host cells [68]. A-72 is known to be more prominent cell line for culturing CPV-2 as compared to other cell lines as it favors the growth of the virus. The cytopathic effects produced by CPV-2 on A-72 cell lines are negligible or poor even after 4 days of infection. The isolation and culturing of virus is considered to be highly specific, but these methods are laborious, expensive and time consuming [24].

14. Electron microscopy

Morphological identification of CPV-2 virus is carried out by electron microscopy. Burtonboy and co-workers demonstrated the morphological characteristics of CPV-2 using electron microscope by negative staining. Sample preparation is carried out by adding viral suspensions on 4% agar block (1 cm^2) and allowed it to spread evenly on the block. The negatively stained block with collodion solution of 2% phosphotungstic acid, with pH 7 is kept for 1 min. It is then placed in carbon coated grid and viewed under electron microscope at 80 kV [11]. Immune electron microscopy (IEM) is also performed using CPV-2 specific antibodies to increase the sensitivity ([112] and [68]). The disadvantage of using electron microscope is requirement of high viral load for detection. Moreover, it is highly expensive and requires skilled personals.

15. Immunological methods

Several immunological methods have been developed for direct virus identification that includes Latex agglutination tests, Slide inhibition test – Slide agglutination test (SIT-SAT), Hemagglutination assay, fluorescent antibody test, Enzyme linked immunosorbent assay (ELISA) and various immuno-sensor etc. Most of these laboratory methods are used for the detection of CPV specific antigen or antibody. ELISA is the most common test used by clinicians and practitioners as a confirmatory diagnosis of CPV-2 from feces and blood of infected dogs [84]. Apart from these, Slide Inhibition Test (SIT) and Slide Agglutination Test (SAT) are other tests used by clinicians for detection of CPV-2 by using porcine erythrocytes. Other immunological assays are also used in the research laboratories as a confirmatory diagnosis of CPV-2 from various sources. Although Latex agglutination test and SIT-SAT methods are cost-effective but are not commercially available. Also, hemagglutinin assay does not require expensive reagents but it requires laboratory environment and skills. ([9] and [132]).

16. Latex agglutination test

Latex agglutination test is a clinical method to detect antigen or antibody from body fluids. It is semi-quantitative, cost-effective test which can be performed in short duration of time (https://vlab.amrita.edu/?sub=3&brch=69&sim=195&cnt=1) ([51]). Antibody coated latex particles like polystyrene beads can be agglutinated with CPV-2 antigen. The advantage of using polystyrene beads includes absorption capacity and stability. Rat monoclonal antibodies were generated against CPV-2 and designated as Rh antibodies. The assay was carried out by incubating suspected virus sample with Rh coated latex beads and incubated with GBS buffer (glycine-HCl 0.1 M buffer, pH 9.2, 0.17 M NaCl). The suspension was allowed to pass through flow cell auto-counter to detect non-agglutinated particles. The sensitivity of the assay for detection of CPV-2 antigen was 4 ng/mL [9].

17. SIT-SAT method

A twin test known as SIT-SAT method is developed for the detection of CPV-2. The test can be performed on a flat surface such as film rather than bottom shaped wells. Slide inhibition test (SIT) is developed for antibody typing and slide agglutination test (SAT) is used for antigen detection. SAT method can be performed by serially diluting the clinical sample followed by incubation with a fixed amount of RBC consisting of surface receptor for virus. The virus particles present in the sample binds with RBC and forms lattice that could be observed visually. Slide inhibition test is based upon the efficiency of viral antibodies to bind with the virus and prevent virus binding to RBC. The test is cost-effective, easy to use and does not require skilled professionals [62].

18. Hemagglutination assay (HA)

HA is an immunological method of tittering virus based on their ability to bind with the RBCs surface receptor and results in agglutination of the virus. The test is performed by serially diluting the virus suspension and tittered with a consistent amount of red blood cells (sh ttp://www.virapur.com/protocols/HA%20Protocol.pdf) [45]. The primary cell receptor for CPV-2 is transferrin receptor but it also has the capability to hemagglutinate the RBC (Fig. 7). It is shown that CPV-2 virus can interact and bind with the sialic acid receptor and agglutinate RBC. This hemagglutination is pH dependent and occurs at a pH 8, which is determined by VP2 of the capsid. Hemagglutination assay can be performed by using porcine, rhesus monkey, goat and canine RBC. The optimum temperature for hemagglutination was observed to be at 4 °C. The buffer system used for these assays are normal saline solution (0.9% NSS), phosphate buffer solution with BSA (15 mM PBS containing 0.1% BSA) and phosphate buffer saline solution (PBSS) (15 mM PBS containing 0.9% NSS) etc. The sensitivity of the assay can be compromised due to poor housing and management of red blood cells. Furthermore, an altered coefficient of erythrocyte sedimentation can also affect the HA ([68,75] and [87]).

19. Fluorescent antibody test (FAT)

In this test, CPV-2 specific antibodies are labelled with fluorescent dye and are used to detect the virus specific antigens. There are two types of fluorescent antibody tests based upon the tagging of fluorescent dye to primary or secondary antibody: direct fluorescent antibody test and indirect fluorescent antibody test. Direct fluorescence antibody test

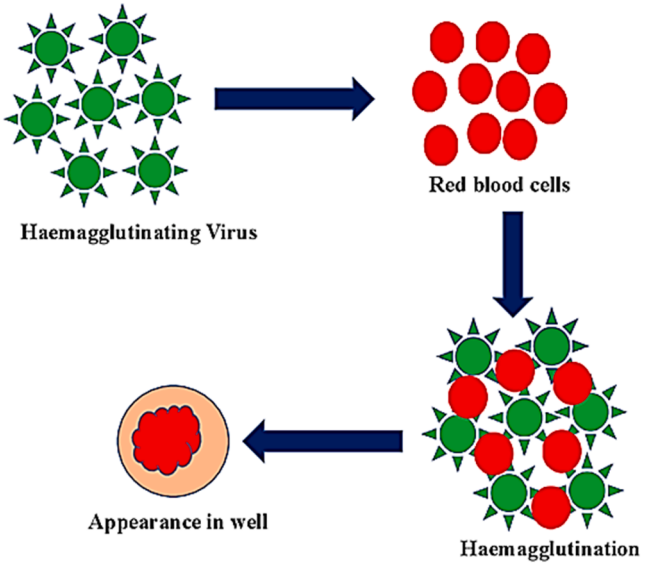

Fig. 7. Schematic representation of Hemagglutination assay. Red blood cells coagulation in well indicates positive for hemagglutination.

is performed using formalin fixed tissue and detecting the infection by using CPV-2 specific monoclonal antibodies. The test is highly sensitive and provides clear location of antibodies. However, the test is expensive, depends on availability of high-quality antibodies and requires skilled professionals. A magnetic bead aided indirect fluorescent method had been developed for CPV-2 detection. Although the level of detection is high but only few labs offer this test. In this assay, magnetic beads are first tagged with avidin and then allowed to react with biotin labelled antigens. A CPV-2 specific antibody present in sera is allowed to react with the virus which is detected by FITC labelled antibodies. The sensitivity of the assay found to be 0.3 ng/mL ([68,132] and [64]).

20. Enzyme-linked immunosorbent assay (ELISA)

ELISA is a commonly used plate-based assay performed in laboratories to detect and quantify antigen and target specific antibodies. It is a cage site detection method of CPV-2 antigen used worldwide by the veterinarians [90]. Several ELISA based assays have been developed to detect CPV-2 from clinical samples. One of the variants of ELISA, antibody-based ELISA (AC-ELISA) has been developed utilizing rabbit anti-CPV polyclonal antibody as a capture antibody and guinea pig anti-CPV polyclonal antibody as a tracking antibody. Horse radish peroxidase guinea pig anti-CPV polyclonal antibody is used as a detection antibody [66]. A double antibody ELISA (DAS-ELISA) is developed using two monoclonal antibodies (MoAbs) that recognize different epitopes on CPV-2 surface antigens (H1 and H2). The plate is coated with H1 and H2 specific monoclonal antibodies followed by addition of test sample. Then HRP conjugated H1 and H2 MoAbs are added and incubated. For chromogenic reaction TMB/H_2O_2 substrate was employed and optical density was measured at 490 nm in ELISA reader. The sensitivity of ELISA test for CPV-2 was found to be higher than hemagglutination test [108]. The Titer CHEK CDV/CPV ELISA is an antibody test developed for the sensitive, qualitative detection and differentiation of CPV-2 and CDV [71].

In another study, ImmunoComb test described as an enzyme labeled "dot assay", that detects antibody levels in serum, plasma or whole blood. (https://www.biogal.com/wp-content/uploads/2019/07/65CPD911–1.pdf) [43].

21. Immunochromatographic (IC) Strip Test

A gold nanoparticle-based IC-strip test is developed employing sandwich ELISA principle which utilizes anti-CPV monoclonal antibodies as detector molecule and rabbit anti-CPV truncated VP2 (CPV-tVP2) polyclonal antibody as a capture molecule. The use of polyclonal antibodies improves the efficiency of assay as it can detect multiple epitopes and its production is more economical. This strip-based assay is able to detect CPV-2, CPV-2a and CPV-2b with high specificity and sensitivity of about 6.6×10^5 $TCID_{50}$/mL [115].

22. Molecular methods

Molecular based assays developed for CPV-2 include PCR, Real-time PCR, mPCR, Peptide Nucleic Acid Assay (PNA), Insulated Isothermal PCR method (II-PCR), Loop-Mediated Isothermal Amplification (LAMP), Polymerase Spiral Reaction (PSR), Recombinase Polymerase Amplification Assay (RPA), and Fluorescence Melting Curve Analysis (FMCA). Most of these assays are capable of detecting CPV-2 from fecal and blood samples. These molecular tests are highly sensitive with specificity ranging from 66% to 100%. These molecular assays are rapid and are completed within 15–180 mins. In most of the cases suspected samples are sent by the clinician to practitioners in reference laboratories for conventional PCR and real-time PCR [26]. Other developed sensitive and specific molecular tests are also used as an alternative tool for confirmed diagnosis of CPV-2. However, most of these tests require expensive protocols, optimizing reaction conditions, highly skilled professionals, and sophisticated instruments and is time consuming which limits their applications in routine testing laboratories [15].

23. Polymerase chain reaction (PCR)

PCR is a widely used revolutionary in-vitro platform for detection and quantification of various pathogens from different sources. The PCR based assays are highly specific as they utilize specific pair of primers. PCR technique can also be applied to amplify unculturable pathogens. Various PCR based assays have been developed for the detection of CPV-2 and its variants from various sources. To identify the antigenic type of CPV-2, a differential PCR was developed which requires three sets of primers and the PCR was carried out by employing gradient of annealing temperatures [63]. Homogenous material like fecal matter often creates problems in conventional PCR by inhibiting the activity polymerase enzyme. A touch down PCR has been introduced to identify CPV-2 from fecal samples and can overcome this complication by introducing a pretreatment step such as sonication or boiling [113].

Amplification Refractory Mutation System PCR (ARMS-PCR) has been developed that can detect and differentiate between 3 variants of CPV-2. It utilizes one outer set of primers that recognizes conserved target region and allele specific inner primers (CPV-2a, CPV-2b and CPV-2c) which combines with specific outer primer product to give allele specific amplicons of different lengths [16].

Another PCR assay has been developed which combines with fluorescence lateral flow with magnetic bead purification. The purification of CPV-2 is carried out by using magnetic beads followed by PCR amplification and lateral flow immuno assay. Magnetic beads offer advantages such as functional groups for pathogen fixation and compatibility. Moreover, this method does not rely on gel electrophoresis and requires 80 mins for detection. The sensitivity of this assay is found to be 3×10^1 copies/μL [136].

Multiplex PCR (mPCR) is a molecular method which utilizes 2–3 sets of primers to carry out molecular typing in a single reaction tube. Several multiplex PCR have been developed for CPV-2 that can differentiate between three variants (CPV-2a, CPV-2b and CPV-2c). A mPCR is developed to differentiate between CPV-2a and CPV-2b by using 2 sets of primers [100]. Additionally, two more mPCR are developed which can differentiate Canine respiratory viruses (CRV) and Canine enteric

viruses using separate primer mixes. Out of these two, one mPCR is able to differentiate between canine respiratory viruses and Other mPCR can differentiate between canine enteric. The sensitivity of both multiplex PCR is found to be 1×10^4 copies/μL [37]. The disadvantages associated with PCR includes requirement of skilled professionals, expensive chemicals, matrix assisted inhibition and sophisticated instruments.

24. Real-time PCR

Real-time PCR (RT-PCR) is one of the most revolutionary molecular platforms for detection of various pathogens. The RT-PCR based assay combines PCR with a fluorescent probe for detection of target gene within an hour or less and considerably faster than conventional PCR. The test can be used for qualitative and quantitative analysis and gives real time results. However, it is rather expensive and requires professional laboratories to perform the test. The advantage of RT-PCR is that it eliminates the need of gel electrophoresis-based analysis [34]. A combinatorial approach by using RT-PCR and mini-sequencing is developed to detect and differentiate three variants of CPV-2. The assay employed SYBR green based RT-PCR followed by mini-sequencing to differentiate the variants. The mini-sequencing method is carried out by using sets of primers each of which consists of one baseless of known single nucleotide polymorphisms (SNP) at 3′ end of primer. After primer annealing, the fluorescence labelled dNTPs were added and followed by strand extension by polymerase. The added nucleotide was then identified which determines the SNP present at that position ([89] and [34]). A Real-Time based minor groove binding assay (MGB) had been developed for differentiation among the variants of CPV-2. The probes employed in this assay were highly specific for each antigenic type and can bind with the specific target for amplifications. Further, the resultant fluorescence was monitored by RT-PCR [27]. Differentiation between CPV-2a and CPV-2b is also carried out by Real-Time recombinase polymerase amplification (RPA) assay. The assay is performed at 38 °C and requires 10^5-10^1 copies/μL molecules of template DNA [131]. CPV-2 variants were also detected by using SimpleProbe® RT-PCR assay consisting labelled sensor probe. The specificity and sensitivity of the assay obtained was 100% with 10^4 copies/μL of DNA [40].

25. Peptide nucleic acid assay (PNA)

PNA is an oligonucleotide amplification method that utilizes nucleic acid analogue consisting of pseudo peptide skeleton instead of sugar phosphate backbone. PNA oligonucleotides have higher hybridization properties and are chemically and biologically more stable. The electrically neutral nature of PNA makes them as a suitable probe for detection. A peptide nucleic acid array was developed which can differentiate between CPV-2a, CPV-2b and CPV-2c. The specificity of this assay is found to be 90.4% from various sources. The demerits associated with this test are requirement of expensive reagents; matrix assisted inhibition and required skilled professionals [3].

26. Insulated isothermal PCR method (II-PCR)

It is an isothermal PCR which works on the principle of Rayleigh-Benard convection method. It is capable of amplifying nucleic acid within 30 mins in a heating device. The PCR is carried out in a polycarbonate capillary R-tube™ (GeneReach, USA) and heated isothermally. The DNA is amplified due to thermal convection in the tube. This assay can further be made more advanced by integrating the fluorescent hydrolysis probe [18]. A field deployable device has been developed that employs II-PCR for on-site identification of CPV-2 infection. The reaction cycles were carried out in POCKIT™ Nucleic Acid Analyzer (POCKIT™) employing a hydrolysis probe. Optimal signals generated due to hydrolysis of probe were converted to S/N ratios (signal $_{after}$/-signal $_{before}$) and reported as positive/negative on display screen. The sensitivity and specificity of the assay was found to be 98.41% and 100% in feces [134].

27. Loop-mediated isothermal amplification (LAMP)

LAMP assay is based on the generation of artificial stem-loop DNA structure under isothermal condition at (60 −65)°C using DNA polymerase with high strand displacing activity. A sensitive LAMP assay is developed for detection of CPV-2 infection targeting VP2 gene. The assay employs two sets of outer and inner primers which amplify six distant regions of VP2 and the reaction can be completed within 60 mins. The limitations associated with LAMP assay are requirement of more than one set of primers, highly sensitive may lead to false interpretation and technical expertise to perform the assay. The assay is highly sensitive and specific with the detection limit of 0.0001 $TCID_{50}$/mL as the virus can be diluted up to 10^{10} [86]. Another LAMP assay in combination with enzyme linked immuno sorbent assay (ELISA) and lateral flow dipstick were developed for detection of CPV-2 with high sensitivity and specificity [117]. The limits of detection of LAMP-ELISA and LAMP-LFD are 10^{-1} and 10^{-1} $TCID_{50}$/mL, respectively. Fig. 8 depicts the schematic representation of LAMP-ELISA. A study was conducted by Parthiban and co-workers where the results of LAMP assay were compared with one step PCR and nested PCR. The detection limit of LAMP assay is found to be 100 fg of DNA which is much higher than one-step PCR or nested PCR with a detection limit of 10 ng of DNA [99]. An Immunocapture LAMP (IC-LAMP) is developed for CPV-2 which has reduced the time of assay and increased the sensitivity of the assay [118].

28. Polymerase spiral reaction (PSR)

PSR technique is based on a generation of spiral DNA structure using DNA polymerase with strand displacing activity. The amplification method is highly specific, rapid and can be performed under isothermal condition. Primers for PSR consist of exogenous sequence which helps to make a spiral like amplicon. After amplification signal intensity can be monitored employing reducing dye or DNA intercalating molecule like SYBR green [73]. Fig. 9 depicts the schematic representation of polymerase spiral reaction. Gupta and co-workers developed a PSR assay for sensitive detection of CPV-2 from clinical samples. The assay employs one set of primer and can be completed within 75 mins at 65 °C in a

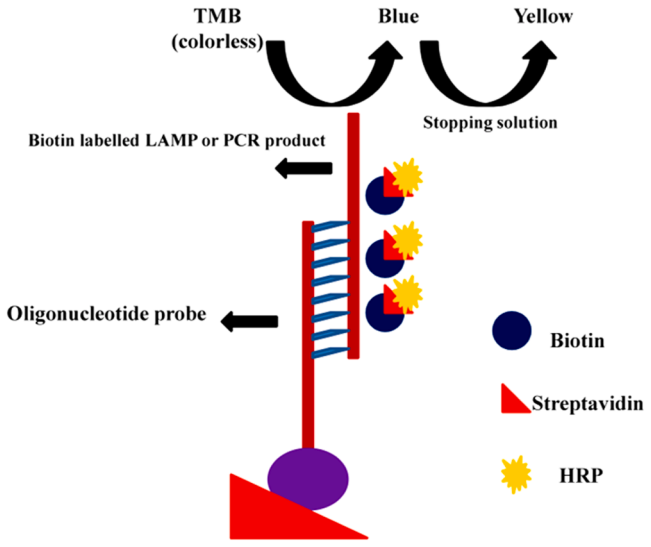

Fig. 8. Representation of the biotin-labelled specific oligonucleotide probes applied onto streptavidin-coated well and hybridize with biotin-labelled LAMP amplicons or PCR products. After washing the conjugates of streptavidin-horseradish peroxidase (HRP) were added to perform the ELISA detection. The picture is adopted and modified from [117].

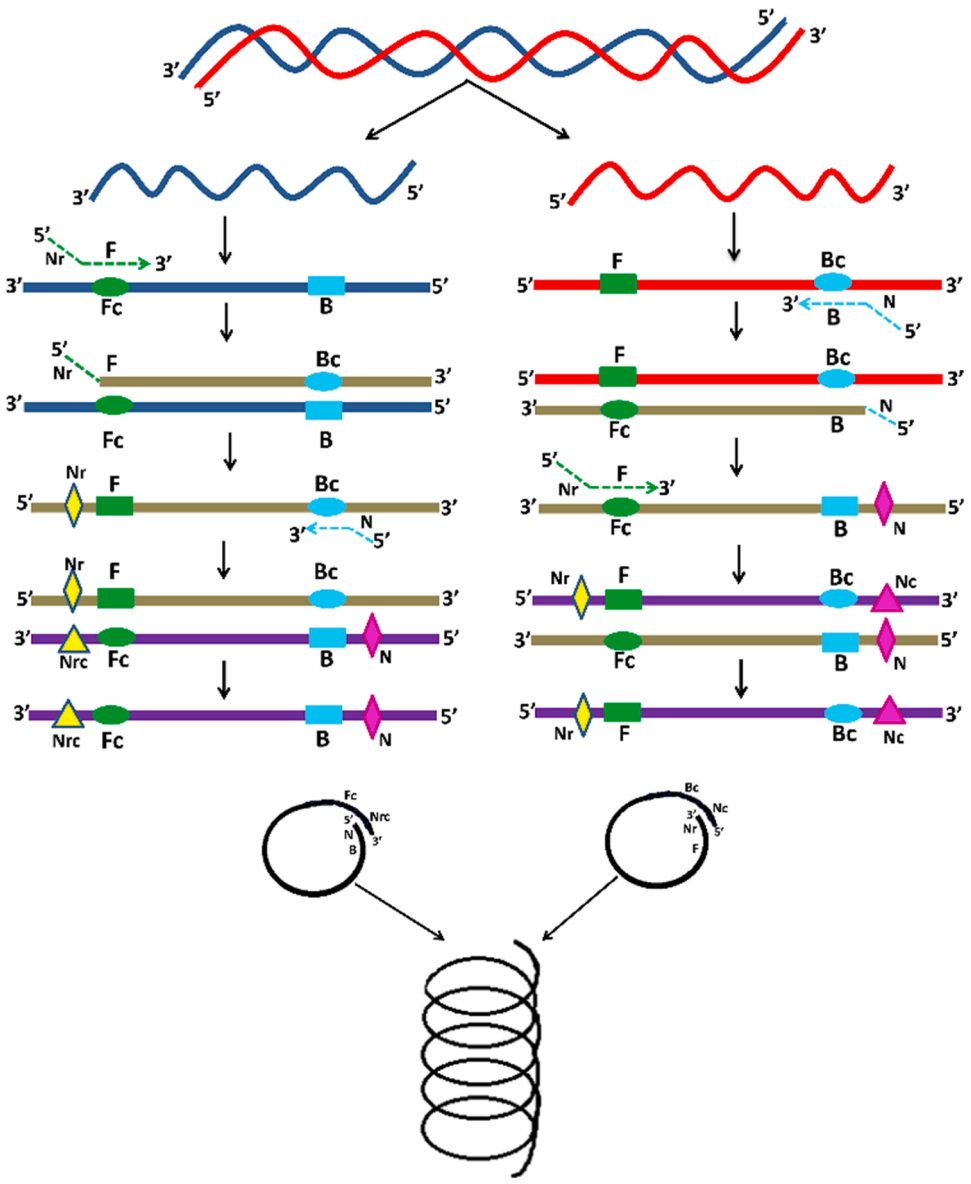

Fig. 9. Schematic representation of polymerase spiral reaction (PSR). The figure indicates the mechanism of primer annealing and extension to form spiral like product.
The figure is adopted and modified from [73].

water bath. The sensitivity of the assay is found to be 5×10^{-6} ng of parvoviral DNA (Gupta et al., 2017).

29. Recombinase polymerase amplification assay (RPA)

RPA is an isothermal PCR assay which employs recombinase enzyme to form a complex with oligonucleotide primer and to bind with a homologous sequence in target DNA. The single stranded binding (SSB) protein attaches with the displaced DNA and stabilizes the loop and initiates DNA amplification [116]. RPA based lateral flow strip (LFS-RPA) is developed for sensitive detection of CPV-2 employing *nfo* probe (46–52 nucleotides long) containing internal basic nucleotide analog (a tetrahydrofuran residue, or THF). The probe is labelled with FAM at 5′ and at 3′ it contains polymerase extension blocking group (such as a C3-spacer). The amplicons are detected by the naked eye in a lateral flow strip (LFS), utilizing anti-FAM gold conjugates and biotin-ligand molecules. The load of detection was found to be 1.0×10^2 copies/reaction [72].

30. PCR-restriction fragment length polymorphism (PCR-RFLP)

The technique utilizes restriction enzymes which recognizes and cleaves the DNA at a particular restriction site producing different fragments of DNA and examined by gel electrophoresis. The target DNA is first amplified by PCR and then subjected to RFLP. A RFLP was carried out in order to distinguish CPV-2b and CPV-2c by using *Pabs/Pabas* and *Mbo*II restriction enzyme [85]. In another study, PCR followed by RFLP was carried out by using *Rsa*I and *Hph*I in order to differentiate between CPV-2a and CPV-2b [110].

31. Fluorescence melting curve analysis (FMCA)

It is a probe-based assay to detect and differentiate between variants of CPV-2 based upon the melting curve analysis. The assay consists of two TaqMan probes (FAM labelled and HEX labelled). FAM labelled probe sequence is perfectly complementary with CPV-2a and bears 1 bp mismatch with CPV-2b and 2 bp mismatch with CPV-2c. The HEX labelled probe has complete complementarity with original CPV-2 and

bears 1 bp mismatch with other variants. The method is also capable of detecting samples containing more than one variant without sequencing. The assay is also able to detect 1–10 copies/reaction. The drawbacks associated with this assay are requirement of highly skilled labour, expensive chemicals and complicated protocols [74].

32. Biosensor

Quartz crystal microbalance (QCM) is a novel biosensor developed for the detection of CPV-2 based on antigen antibody reaction. The biosensor contains ProLinker™ B which enables the antibodies to bind with gold coated quartz surface. QCM is also regarded as label free technology as antigen-antibody interactions are studied without labelling. Gold electrodes are used as a carrier in QCM increasing the efficiency of detection as they generate stable frequencies. The sensitivity of detection is found to be 10^2 $TCID_{50}$/mL and specificity was 98% [65].

33. Commercially available kits

There are several commercially available kits in the market based on detection of antigen or antibody in CPV-2 infected dogs. The commercially available kits for CPV-2 are mostly based on antigen-antibody reaction such as ELISA, dot ELISA and immunochromatographic strip-based assay [115].

List of commercially available kits are given in the Table 1.

34. Treatment of CPV-2 infection in canine

The canines suffering from Parvovirus infection shows high morbidity (100%) and mortality ((>70% in puppies and <1% in adult dogs) ([90]). The treatments available for Parvovirus infection in dogs are supportive therapies which include fluid therapies, antibiotic treatments, antiemetic treatments, antiviral treatments, adjunctive treatments and dietary management.

35. Fluid therapy

CPV-2 infected patients often suffer from fluid losses due to severe diarrhea that can be balanced by taking fluid therapies. Balanced electrolyte solutions like lactated ringers' solution or normosol-R are usually recommended for initial restoration of intravascular volume and rehydration [59]. The rate and route of administration of electrolyte solution differs with patient condition [104]. In case of severe enteritis, subcutaneous absorption is mostly affected and hence venous access is commonly preferred. Electrolyte doses of about 7–12 mL/kg IV are recommended within the interval of 10 mins. This procedure is repeated until the parameters like heart rate, capillary refill time and mentation are improved. However, if the parameters do not reach the satisfactory levels, colloids such as hydroxy-ethyl starch (Voluven) or Pentaspan are recommended at a rate of 3–5 mL/kg intravenously [59]. The patients suffering from hypovolemic shock, fluids should be given through catheter placed in the cephalic or lateral saphenous vein. The rate of fluids to be administrated can be determined by physiologic endpoints. In some cases, venous access is prevented due to circulatory collapse, in that condition fluids can be given through intraosseous route. If the patient does not suffer from hypovolemic shock but experience dehydration should be rehydrated within 6–24 hrs. Once the perfusion parameters are restored the rate of administration of fluids can be decreased. Usually, the therapy depends upon the dog's systemic acid-base status and serum electrolyte concentrations [104].

Patients suffering from CPV-2 infection often suffer from protein losing enteropathy that may result in hypoalbuminemia (<2 g/dL) or hypoproteinemia (<4 g/dL). Synthetic colloids such as 6% hetastarch should be given to maintain intravascular oncotic pressure. A large amount of blood loss occurs due to hemorrhagic diarrhea. Therefore, blood transfusion is recommended for patients suffering from anemia. Usually, blood is administered at a rate of 10 mL/kg intravenously over 4–6 hrs [104]. The decision to transfuse blood should be based on clinical signs of anemia. Antibodies and serum protease present in transfused blood helps to neutralize the virus [88].

The serum potassium level in CPV-2 infected dogs gradually decreases and causes hypokalemia resulting in polyuria, cardiac arrhythmias, gastrointestinal ileus and malaise. To combat this condition fluid containing potassium chloride should be given at a rate of 0.5 mEq/kg/hr.2.5–5%. Dextrose could be added to the electrolyte solution for patients suffering from hypoglycemia [88].

36. Antibiotic treatments

CPV-2 infected patients often suffer from bacterial co-infections due to the destruction of epithelial cells of the gastrointestinal tract which leads to constant translocation of bacteria, resulting in endotoxemia and sepsis. To combat with the circumstance, β-lactam antibiotics like ampicillin (22 mg/kg q 8 hrs) or cefazolin (22 mg/kg q 8 hrs) are recommended along with aminoglycoside like gentamycin (2.2 mg/kg q 8 hrs) [59]. High dosages of aminoglycosides are not preferable for dehydrated patients. Enrofloxacin is another fluoroquinolone effective against Gram negative bacteria and can be given at a rate of (5 mg/kg q 12 hrs). Metronidazole works against anaerobic bacteria and given at 20 mg/kg IV q 24 hrs. Dogs with mild infections should not be recommended for antibiotic therapy [77].

37. Antiemetic treatment

One of the most common symptoms of CPV-2 enteritis is vomiting and can be managed by administrating antiemetic drugs such as metoclopramide and chlorpromazine. Metoclopramide is a dopamine antagonist that blocks the trigger zone present in the brain for nausea and also increases gastrointestinal motility. It is recommended at the rate of 1.0–2.0 mg/kg/24 hrs but is not considered safe for dogs which are at a risk of intussusceptions. In some cases, it may lead to muscle fasciculation and tremors. Chlorpromazine is a phenothiazine derivative

Table 1
Some of the commercially available detection kits for CPV.

Sl. No	Test	Company	Principle	Reference
1	SNAP parvo antigen test	IDEXX, United States	ELISA	[105]
2	Witness Parvo Test Kit	Zoetis, United states	Rapid Immuno Migration (RIM™) technology. [47]	https://diagnostics.zoetis.com/species/canine/parvovirus/witness-parvo-and-witness-cpv.aspx
3	FASTest parvo card	Vet lab, UK	Lateral flow	[112]
4	CPV Antigen Rapid Test Kit	Ubio Biotechnology systems Pvt. Ltd. [44]	Lateral flow	http://www.ubio.in/pdf/2015_06_13%2005_29_09.pdf
5	ImmunoRun CPV antigen detection kit	Biogal- Galed labs	Immunochromatographic assay	http://www.biogal.com/wp-content/uploads/2019/07/IR-CPV-255–27.06.11-.pdf
6.	Canine Parvovirus & Distemper IgMAntibody Test Kit	Biogal Galed Laboratories Acs Ltd.	Immunocomb	https://www.biogal.com/wp-content/uploads/2019/07/65CPD911–1.pdf ([52,43])

which also works by blocking dopamine receptor in brain, usually given at a dosage of 0.1 mg/kg IV q 4–6 hrs [77]. Other antiemetic drugs also recommended which includes maropitant, and ondansetron or dolasetron [59]. Ondansetron is considered as an effective anti-emetic drug than metoclopramide [107]. Maropitant is a neurokinin-1 inhibitor that blocks the chemoreceptor trigger zone of the brain. The recommended dosage of maropitant is 2 mg/kg ([78] and [7]).

38. Antiviral treatment

Antiviral treatment for CPV-2 is rarely implemented as no specific antiviral therapy has been approved [135]. Treatments with recombinant feline interferon-ω (r-FeIFN-ω) for CPV-2 infected dogs have been evaluated. The study was conducted on 32 breeds to evaluate the efficiency of feline interferon-ω. The dogs receiving feline interferon were designated as (group A) and those which received placebo were designated as (group B). Group A was given 2–5 MU/kg r-FeIFN-ω intravenously for three days. Along with this treatment, dogs were also given electrolyte solutions, antibiotics as well as antiemetic. After monitoring for 10 days no side effects were observed in group A. The mortality rate was 7% in group A and 29% for group B [23]. The antiviral activity of feline interferon is due to its structural similarity with canine interferon and it can bind with interferon receptor present on the surface of dog's cells [67].

Another potential candidate for antiviral treatment of CPV-2 is Oseltamivir. It is a neuraminidase inhibitor antiviral drug originally used to treat Influenza virus. A small study was conducted where infected dogs were divided into two groups (treatment and control group). The treatment groups of dogs were given oseltamivir and rests were kept as a control group. Slight improvements in body weight as well as white blood cells were observed in antiviral treated dogs and drop in white blood cell count was observed in the control group. However, the role of Oseltamivir in treating CPV-2 infection remains speculative and needs further investigations ([79] and [111]). Efficiency of acyclovir was also studied in CPV-2 infected dogs. Dogs were divided into 3 groups (I, II, III). Group I received 20 mg/kg of acyclovir intravenously, group II was not given any treatment and group III was free from infection and treatment. Effect on group I showed successful prevention of CPV-2 replication in dogs [1].

39. Adjunctive treatment

Progression of CPV-2 infection gradually destroys intestinal barrier resulting in the release of Gram-negative bacteria in blood circulation and leads to sepsis and endotoxemia. The LPS remains stable for 2 weeks even if the bacterium dies, thus anti-LPS therapy has been recommended. Polyvalent equine origin antiserum against LPS endotoxin is effective for endotoxemia. This can be produced by injecting horses with appropriate doses of inactivated LPS. However, the anti-endotoxin therapy should be given before antibiotic treatment as LPS concentration can increase post-antibiotic therapy. A study was carried out where a group of dogs were experimentally infected and subjected to treatment with anti-CPV equine antibodies. The results showed that the puppies receiving equine antibodies on 1st and 2nd day before viral infection were found to be 100% protected from the infection. However, those puppies which were given antibodies on 4th day of infection resulted in providing 66% of protection ([133] and [5]).

The effect of exogenous granulocyte colony stimulating factor (G-CSF) was studied to combat neutropenia. The G-CSF helps in production, differentiation, maturation of granulocytes and also helps the bone marrow to produce new neutrophils. A study was conducted to determine the efficiency of recombinant granulocyte colony stimulating factor (r-hG-CSF) on infected dogs with leukocyte count of about $1.4 \times 10^9\ 1^{-1}$. The dogs were administered with the dosage of r-hG-CSF of about 5 μg/kg of body weight. The dose was given once in a day and results were compared with the control group containing untreated dogs with leukocyte count of about $2.2 \times 10^9\ 1^{-1}$. The leukocyte count in treated animals increased to $5.9 \times 10^{9}\ 1^{-1}$ whereas, the leukocyte count of untreated group decreased to $1.7 \times 10^9\ 1^{-1}$ ([92] and [82]).

Filgrastim (Colstim®) is a recombinant Methionyl Human Granulocyte Colony Stimulating Factor (r-metHuG-CSF) usually given to humans undergoing chemotherapy. In a study, CPV-2 infected rottweiler was administrated with filgrastim which gradually increased the leukocyte count from 1000 cells/μL to 5800 cells/μL [76].

40. Dietary management

CPV-2 patients suffering from osmotic diarrhea can be administrated with Nil pros (NPO) for six to twelve hours. Then doses of glucose, isotonic solutions and hydrolyzed protein can be given which substantially reduce diarrhea by improving intestinal mucosal health [59]. Early enteral nutrition limits the bacterial and endotoxin translocation, therefore reduces coliform septicaemia. One of the factors which affects enteral nutrition is gastric residual volume. Complex nutritional formulations can be given through nasogastric or oesophagostomy tube. Partial parenteral nutrition can be given through peripheral vein such as Procalamine at a rate of 60 mL/kg/day ([77] and [128]). Several oral recuperation fluids (ORF) can be given to infected dog to encourage voluntary appetite and increase caloric intake. The nutrients present in ORF include probiotics, omega 6/3 fatty acids and essential amino acids like glutamine, arginine and taurine. These nutrients aid in maintaining gastrointestinal integrity and promote healing [122]. CPV-2 infection often leads to an alternation of gut microbiota which can be restored by giving fecal microbial transplantations (FMT) in which the fecal matter from the healthy dog is fed to an infected dog. In a study conducted by Pereira and his co-workers showed higher clinical recovery rate for FMT treated CPV-2 infected dogs [101].

41. Prevention

CPV-2 is a highly communicable virus and therefore, to prevent the spread of disease several vaccines have been developed to control as well as manage the disease outbreaks. Some of the commercially available vaccines have been listed in Table 2. Vaccines developed against CPV-2 are mostly modified live virus vaccines (MLV) and more rarely inactivated vaccines [13]. Some vaccines are using CPV-2b strains, whereas some other still use the original CPV-2 strain (such as Nobivac) It has been studied that the administration of vaccine should start from 6 to 8 weeks of age and then every 2–4 weeks until 16 weeks of age or older. The dogs which are above 16 weeks or older are recommended 2 doses of vaccinations with an interval of 2–4 weeks but sometimes even a single dose of MLV is protective ([21] and [88]). Efforts have been made to develop new safe prophylaxis interventions which are in various stages of development. Virus like particle (VLP) based recombinant vaccine is under development. These vaccines come with the advantage of high immunogenicity and are highly safe [58]. DNA vaccines such as prokaryotic vector pTargeT containing VP2 are also under development stage. An experiment was carried out where dogs were divided into 2 groups and one group was given recombinant plasmid of about 100 μg and the other was given placebo. It was found that the dogs which were given recombinant plasmid showed to have serum neutralizing antibody dilution of about 1:256 which was significantly high to protect the dogs from infection [36]. Peptide vaccines containing major antigen neutralizing region N terminal of VP2 are also under development stage. The amino acid sequence of N terminal domain is highly conserved in Feline panleukopenia virus of cats, Mink enteritis virus, Minute virus of canines and Parvovirus. N terminal domain of VP2 has been utilized in peptide vaccines due to its importance in cell entry and it also displays epitope which can be recognized by CPV-2 [14].

The most common cause of failure of immunization is interference of materally derived antibody (MDA). Although MDA plays major role in

Table 2
Some Commercially available vaccines for Canine parvovirus are [48] ⟨http://www.biogal.com/wp-content/uploads/2019/07/IR-CPV-255–27.06.11-.pdf⟩ [53,54,56].

Sl. No	Product	Applicability
1	Nobivac Canine 1-DAPPV	Canine parvovirus, Canine distemper, Adenovirus type 1 (hepatitis), Adenovirus type 2 (respiratory disease) and Canine parainfluenza virus
2	Nobivac Canine 1-DAPPV+CV	Canine parvovirus, Canine distemper, Adenovirus type 1 (hepatitis), Adenovirus type 2 (respiratory disease), Canine parainfluenza and Coronavirus.
3	Nobivac Canine 1-DAPPVL2	Canine parvovirus, Canine distemper, Adenovirus type 1 (hepatitis) Adenovirus type2 (respiratory disease), Canine parainfluenza and against Leptospiral disease caused *L.canicola* or *L. icterohaemorrhagiae.*
4	Nobivac Canine 1-DAPPVL2 +CV	Canine parvovirus, Canine distemper, Canine adenovirus type 1 (hepatitis), Canine adenovirus type 2 (respiratory disease), Canine coronavirus, Canine Parainfluenza and against leptospiral disease caused *L. canicola* or *L. icterohaemorrhagiae.*
5	RECOMBITEK	CPV-2a, CPV2b, CPV2c
6	Virbac's CANIGEN® DHP	Canine Parvovirus enteritis, Canine Distemper, and canine Hepatitis
7	VANGUARD Plus 5	Canine parvovirus (CPV) Canine adenovirus type 1 (CAV-1), canine adenovirus type 2 (CAV-2) and canine parainfluenza.

Table adopted and modified: (https://www.merck-animal-health-usa.com/dp/8, https://www.zoetisus.com/products/dogs/vanguard-plus-5.aspx, https://in.virbac.com/home/dog/products/vaccine/canigen-dhp?preventiframecaching=1, https://www.boehringer-ingelheim.com/animal-health/companion-animals, products/recombitek#:~:text=RECOMBITEK%20%C2%AE%20offers%20a%20complet, e,disease%20and%20shedding%20against%20leptospirosis%3B*

protection against CPV-2 but they can also neutralize the vaccine. Inactivation of vaccine is fairly proportional to the amount of MDA present in the circulation. Puppies with high concentration of MDA titer exhibit low response to vaccine.

Apart from this other reason for immunization failure are poor vaccine storage, administration errors etc. Vaccine failure can also be due to host related factors such as age, gender, immune status etc [25].

42. Future prospective and conclusion

CPV-2 is of serious concern for unvaccinated puppies including breeds like English Springer, German Shepherd, American Pit Bull Terriers, Doberman Pinschers and Rottweiler. The future studies should be more focused on molecular pathogenesis; development of rapid detection systems and development of new vaccines that will be able to overcome MDA and therapeutics can substantially reduce the periodic occurrence of CPV-2 throughout the world. Further research on structural biology and molecular pathogenesis can help to understand the minute details about molecular pathogenesis and mechanism of action of the pathogen. Early detection can save many lives and can reduce the burden of the disease. Development of rapid, low-cost diagnostics can play a major role to prevent the disease in an outbreak and normal scenario. Miniaturized diagnostics like biosensors can be an attractive alternative over existing. New diagnostics like fluorescence biosensor, colorimetric biosensor; electrochemical biosensor and microfluidics-based biosensor can be developed employing aptamer/antibody as ligands. To reduce the morbidity and mortality of the disease, the development of affordable new generation vaccines and new adjuvant can play a major role to reduce the burden of the disease. Research should be focused on development of new modified live vaccines, DNA based vaccines, Subunit vaccines and knock out mutant vaccines to generate suitable prophylaxis. Post-exposure therapy will be an immediate choice to cure and save diseased canines. Extensive research is of pressing need for the development of a suitable therapeutic molecule based on antibody/aptamer that can be introduced with antibiotics therapy.

Conflict of interest

Authors declared no conflict of interest.

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