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Review Article

Canine Circovirus: An Emerging Pathogen of Dogs

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Abstract

Canine Circovirus is a new pathogen affecting dogs and is gaining rapid clinical importance. Its relationship to Canine Parvovirus-2 (CPV-2) and virus associated with gastrointestinal issues such as vomiting, diarrhoea, and haemorrhagic enteritis/gastro-enteritis in dogs are still in nascent stage. Canine CV has been found in several internal organs of domestic dogs as well as wild canids (wolves, foxes, etc.), implying widespread nature of the virus Although pathogenic potential of the virus is still not fully known. Canine CV has been hypothesised to circulate and aggravate the clinical course of other canine viral disesaes like Canine Parvovirus, Canine Distemper Virus, Canine Retrovirus, etc. This review article seeks to out forward the most recent information on CanineCV including the morphology, replication, aetiology, epidemiology and various available diagnostic methods with preventive strategies including vaccines against Canine CV.

Keywords: Canine CV; ssDNA; Hemorrhagic Enteritis; Necrotizing Vasculitis; Rolling Circle Replication; RT PCR; Multiepitope Vaccine

Introduction

Canine Circovirus (CanineCV) is an icosahedral, circular, single-stranded DNA (ssDNA), non-enveloped virus with a diameter of between 15 and 25 nanometers with a circular genome that is around 2 kb in size [16]. It belongs to the Circoviridae family [23]. Canine Circovirus genotype 1 (CaCV-1) was first discovered in canine serum samples in 2012 [23]. The virus was then subsequently reported in dogs in many countries viz. Argentina, Brazil, China, Thailand, Taiwan, Germany, Italy, Argentina, and Colombia [12,22,24,26,51]. CanineCV has sparked significant interest due to its potential pathogenic role as recent data suggests that it may have a role in disease incidence, either as a direct cause or as a co-agent. Nonetheless, consistent evidence of its true pathogenic involvement in specific disease process remains elusive [16].

Epidemiology

Circoviruses have been identified in a variety of avian and mammalian species. PCV type 2 (PCV2) is one pathogen in the porcine respiratory disease complex (PRDC) that can cause respiratory diseases in pigs. Canine CV generally found in puppies and, to a lesser extent in adults and senior dogs [13]. Canine CV was discovered in both healthy and diarrheal canine cases (7.3% and 20.1%), with the prevalence of the virus being higher in the latter group [18]. It mainly affects the respiratory system Recently, an outbreak of deadly enteritis in puppies occurred in Italy, and a closely comparable version of CanineCV-1 was found in that outbreak [12]. Canine CV-1 strains found in the United States, Italy, Brazil, Germany, Colombia, and Argentina, as well as some strains found in China. The CanineCV-2 and CanineCV-3 genotypes contained the majority of the strains discovered in China [2,12,19,27].

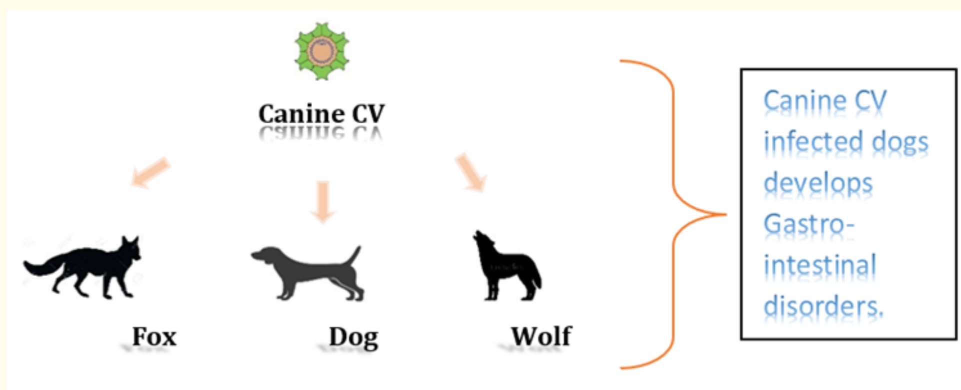


Figure a

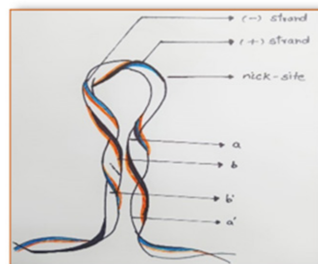
Specifically, several studies reported the coexistence of CPV-2 with Canine circovirus (Canine CV) and Canine adenovirus (CAdV) type 1 and 2 [10,14,23]. Additional mammalian circoviruses, such as PCV-3 in pigs and canine circovirus-1 (CanineCV-1) in dogs, have recently been discovered using next-generation sequencing (NGS) [23] has been associated with several disease entities accompanied by manifestations like vasculitis, haemorrhages, thrombocytopenia, neutropenia, and diarrhoea (2016). It's interesting to note that dogs with Canine CV infection frequently have other intestinal or respiratory diseases [2,19,45,52].

Genomic organization

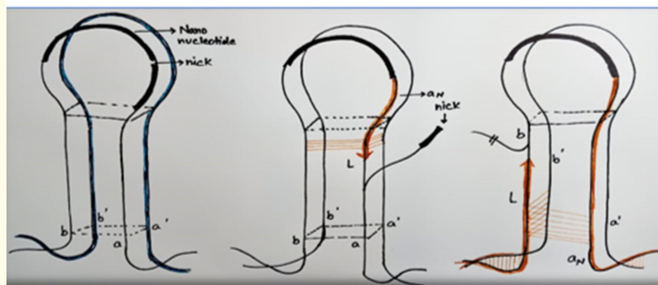
Similar to other animal circoviruses, Canine CV has an ambisense genomic organisation with the genome made up of a single circular DNA strand that is around 2063 nucleotides in length. Two inversely arranged (open reading frames) ORFs that encode for the rolling circle replication initiator protein gene replicase (Rep) and a capsid protein gene (Cap) respectively, similar to other animal circoviruses have been found in Canine circovirus [6,1216]. The viral *Rep* proteins are made up of 303 amino acids and the viral capsid protein of 270 amino acids. The capsid and replicate proteins of Canine circovirus shares <25% and <50% identities with other mammalian circoviruses [23,24]. It has been seen that one of the intergenic noncoding region of CanineCV shares 91% nucleotide identity over 150-out of a sequence of pine marten torque tenovirus of Anelloviridae family [48] thus providing the first documentary evidence of an evolutionary relationship between these two different virus families (Circoviridae and Anelloviridae [23].

Replication strategy of Canine CV

This virus replicates its genomes using a circular, ds replicative form (RF) DNA intermediate produced during the S phase of cell division by host cell DNA polymerases. The RF acts as a template for the production of viral ssDNA, most likely through the rolling circle replication (RCR) mechanism. Their genome consists of two coding and two noncoding parts [11,22]. Rep is an essential replication protein dimer that needs to be coded for the DNA. In order for viral or cellular polymerase to act, DNA must be cut at a specific location, exposing a free 3' OH [11]. Since the CV virus is a small virus, it can easily enter the cell nucleus where the viral DNA is replicated. A 12-nt pair (stem) and a 10-nt open loop (CATAGTATTA) make up the palindrome sequence in Canine CV at the origin of replication site [11].



3D Model of viral DNA



Rolling circle replication mechanism

Figure b

Viral pathogenesis

As circovirus is found in different species. Psittacine beak and feather disease is a result of circovirus infection in psittacine birds, which causes dystrophy in the epidermis of the feathers and beak, hyperplasia and necrosis of the integument, lymphoid depletion, and immunosuppression (PBFD). [34]. Although Canine CV has been isolated from cases of high positivity rate in cases of diarrhoea, its pathogenicity is still debatable [50]. Canine CV's pathogenic role in domestic and wild canids is still not fully established, and further research is required to understand this virus' pathophysiology. Similar to their role in PCV2 infections, viral and bacterial infections have the potential to greatly (Figure c).

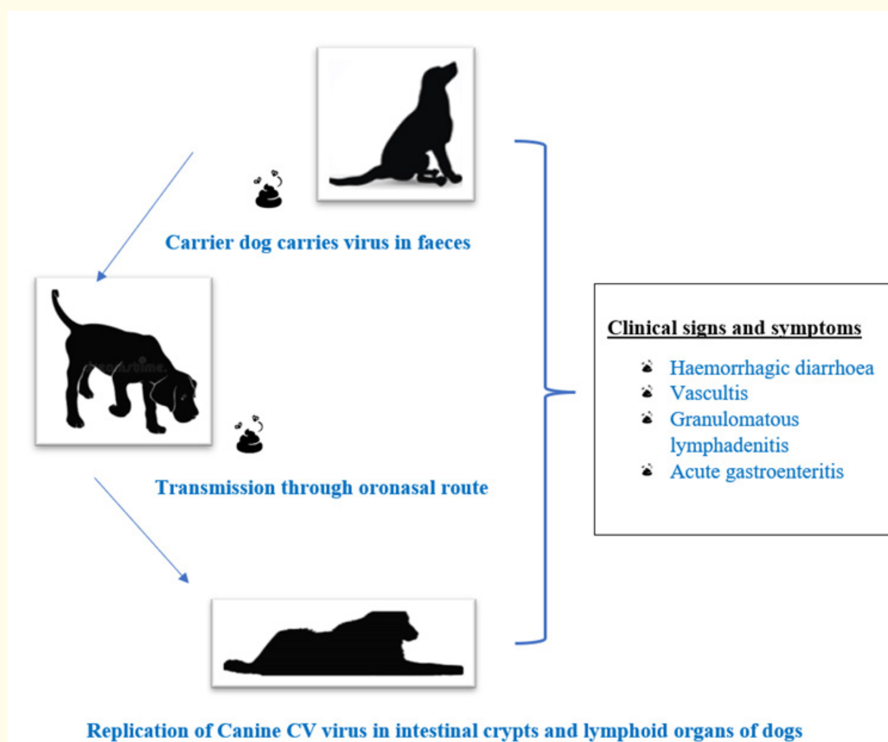


Figure c

Complicate the pathogenesis of Canine CV in domestic and wild canids [37]. Numerous studies have shown that Canine CV has a preference for certain tissues, such as lymphatic tissues [26,27], in the cases of dogs, wolves, and badgers. It was also found to have greater titers in some tissues, such as the spleen and lymph nodes tissues, than in others tissues thus compromising the immune status of the host [52]. Viral inclusions and multinucleated giant cell development are most prominent histopathological alterations seen in seen in dogs with Canine CV infection that are similar to PCV2 infections [42]. However, electron microscopy could identify macrophages in the lymph node that had cytoplasmic viral inclusions made of dense granular or paracrystalline organised virus [26,27]. Canine CV DNA was also discovered in a number of other lymphoid tissues, such as Peyer's patches, and in the adrenal cortex as well as in tiny, endothelial channels of the intestinal lamina propria in dogs that had neither a clinical nor histological enteric lesion [27]. This implies that the virus could infiltrate different body tissues with or without causing pathogenesis, which calls for further innovative research.

Signs and symptoms

Canine CV infection symptoms in domestic dogs which are similar to other canine enteric virus include vomiting, diarrhoea (which may or may not be bloody), lethargy, thrombocytopenia, neutropenia, and vasculitis. Canine CV most likely affects the intestinal endothelium cells and causes bleeding in a number of organs, including the liver, kidney, and lymph nodes. Studies have shown variations in organs, although necrotizing vasulitis and bleeding were prevalent and were common findings seen in all dogs. In-situ hybridization (ISH) of tissues revealed that the dogs were positive; the disease symptoms varied, and some of the dogs shared clinical, gross, and microscopic characteristics with symptoms related to PCV2 infec-

tion [26,27]. Animals with Canine CV illness are also susceptible to co-infection. CDV and CPV-2 infections along with Canine CV infection in domestic dogs and other wild canids, were the most typical co-infections [52]. Additionally, juvenile animals may be more likely than adult animals to experience life-threatening illnesses, but this theory has not yet been experimentally proven [52].

Diagnosis

No specific laboratory diagnostic method has been developed for the diagnosis of Canine CV infections; however, conventional polymerase chain reaction (PCR), electron microscopy, immunohistochemistry, *in situ* hybridization, and quantitative real-time PCR (qPCR) have been used to identify and confirm Canine CV infections

- **Polymerase chain reaction (PCR):** Canine Circovirus strain was used as a model. Using the expression vector pET-32a, the truncated cap gene was translated into a His-tagged protein and expressed in E. coli. The forward primer 5′-UGGATCCLINECTGACAGCTGATTG and the reverse primer 5′-UCTCGAGLINETTACAACTGGCG were used in PCR to amplify the gene. The PCR product was cloned into the pET-32a after being digested with BamHI and XhoI vector and transformed into E. coli Rosetta cells. rCap was found using a Western blot and mouse anti-His MAb that had been HRP-conjugated [50,51].
- **Electron microscopy:** Electron microscopy has been used to detect the presence of viruses in tissue sections/histological specimens, potentially avoiding the necessity for virus isolation or serology. Li., *et al*. (2013) re-fixed formalin-fixed afflicted tissues from a CaCV-infected sentinel dog in 2.0% glutaraldehyde and embedded them in epoxy resin for EM investigation. Toluidine blue was used to stain thick slices of the damaged tissues, and ultrathin sections from affected parts of the tissues were studied using a transmission electron microscope [26,27] Ultrastructural examination of the tissue revealed the presence of numerous intracytoplasmic inclusions in macrophages from affected dogs The majority of the inclusions were granular, electron-dense, and had a definite perimeter; some of them comprised paracrystalline arrays of 9-11m diameter icosahedral virions [26]. The inclusions discovered were spherical, irregular, and 0.5m in size, and they were generally concentrated (up to 25 per cell) within the cytoplasm.
- **Virus Isolation:** Canine fibroma (A-72, ATCC CRL-1542), Madin Darby canine kidney (MDCK, ATCC CCL-34), African green monkey kidney (VERO, ATCC CCL81), Walter Reed canine cells (WRCC), Crandell feline kidney (CRFK, ATCC CCL94), and Felis catus whole foetus (FCWF, ATCC CRL-2787) are some of the cell lines that have been exposed to positive samples from Canine CV cases (Li., *et al*., 2013). The newly trypsinized cells were also used in attempts to develop Canine CV (Li., *et a*l., 2013). After 5 days of incubation, the inoculation cells were tested for Canine CV using RT-PCR. The infected cells were observed for the emergence of cytopathic effects (CPE) [24,26,27]. Additionally, the cells were subcultured every 6 to 8 days for 5 successive passes [27]. However, all of these attempts at viral isolation using different cell lines failed [26,27]. More research on primary canine cells and/or other cells would help us comprehend the virus's cultural requirements. No experiment has been done using egg or experimental animals for virus isolation.

Detection by molecular methods

- An innovative SYBR Green real-time PCR assay (qPCR) that targets a fragment of 132 nucleotides in the intergenic region between the 3′ ends of the two main open reading frames was used to screen CanineCV suspected samples. the thermal cycling for the experiment involved 45 cycles of 95 C for 15 s and 60 C for 1 min each. After the final extension step, melting experiments were conducted by continuously increasing the temperature from 55 C to 98 C, with the target melting temperature falling between 93.2 C and 93.6 C. Positive samples were those with target DNA concentrations above or equal to the limit of detection and a particular melting peak in both replicates [3].
- **Real-time:** Canine CV samples were found positive using Real-time PCR (q-PCR) experiments with certain primers and a hydrolysis probe in DNA extracts from dogs' samples (Li., *et al*., 2013). Positive results were obtained from real-time PCR using the Light Cycler real-time PCR apparatus with thermocycling parameters of 10 min at 95°C, 45 cycles of 10 s each at 95°C, 10 s at 51°C, and 10 s at 72°C [3,27].
- **Immunohistochemistry analysis:** Intestinal samples from Canine CV infected dogs were found positive using PCR. Necrotic crypt epithelium contained significant amounts of CPV-2 antigen, according to immunohistochemistry. There was a significant amount of CanineCV-1 nucleic acid found in lymphoid tissues. For Canine CV-1 nucleic acid, medullary sinuses, marginal sinuses, and follicular centres were all strongly positive for CanineCV in the mesenteric lymph nodes. CanineCV-1 nucleic acid was found in the Kupffer cell nuclei of the liver. The presence of viral antigens/nucleic acids within the lesions was confirmed by IHC, and infection was confirmed by PCR [34].

- **In-situ Hybridization Analysis:** Tissues were obtained from necropsy cases of dogs whose clinical symptoms or histological abnormalities coincided with the sentinel animal afflicted ISH was carried out on these tissues and found the tissues to be infected with Canine CV (i.e., hemorrhagic diarrhoea and vasculitis) [27]. The infected dogs' macrophages, sub-capsular and medullary sinuses, and other lymphoid organs all showed significant cytoplasmic viral nucleic acid upon ISH analysis [3,16].

Detection of viral antigen/antibody

- **Serological Tests:** Serological tests provide diagnostic value in many infections and helpful in cases of infections with newly discovered or recently recognised viruses, for which the prevalence of infections, the age at which infections occur, or the spectrum of vulnerable host species is unknown [3,16,27]. The use of serological assays to measure virus-specific IgG or IgM is crucial for identifying infections, and serological epidemiology is a key method for understanding infections. The rising prevalence of Canine CV infection endanger the health of dogs. Because it is challenging to cultivate the virus, no quick and accurate serodiagnostic test has yet been created to identify antibodies for Canine CV infection. Canine CV hasn't been successfully identified by any cell culture method. Immunofluorescence and immunoperoxidase tests, as with other circoviral infections, can be used to detect the presence of virus antibodies and antigens [36]. Future sensitive and specific serological tests could be developed after a long-term study on Canine CV infection.
- **iELISA:** The Capsid protein (cap), produced in Escherichia coli, was used as a coating antigen in an indirect enzyme-linked immunosorbent test (iELISA). There was no cross-reactivity between the suggested iELISA and other relevant infections. By comparing the obtained results with those of a Western blot analysis, this assay's validity was established. According to the described protocol, iELISA was created using pure rCap [8,17,29,49]. Using canine serum samples, the relationship between rCap ELISA and Western blot was investigated [8].

Treatment

There is no specific treatment for Canine CV infection as it is seldom and diagnosis is not initiated and there is no known cure. When an infection is single or multifactorial, the sick animal may be saved by symptomatic treatment with steroids, broad-spectrum antibiotics, hydration, and electrolytes depending on the symptoms shown by the animal. Prevention and Management of Canine CV is currently not covered by any approved vaccines. Protecting dogs while maternal immunity is the greatest method of preventing Canine CV infection. Recently vaccines construct have been developed using immune-informatics tools that could be used further in future against Canine CV [22].

Vaccine against canine circovirus

A multi epitope vaccine construct against canine circovirus was successfully created using an in silico approach that targeted the replicase and capsid proteins using various immune-informatics tools First, highly antigenic epitopes were chosen. Molecular docking and molecular dynamics simulations were used for examination of epitopes interaction with molecules of dog leukocyte antigen (DLA). After that, GGS linkers were used to connect antigenic epitopes to RS09 and flagellin adjuvants, as well as PADRE sequence, to create a vaccine candidate. Various webservers were also used to predict the physiochemical characteristics, antigenicity, allergenic potential, secondary, and tertiary structures of the developed vaccine construct. The ability of the vaccine construct to be expressed and cloned was also examined using the insilico cloning method. It may be advantageous to protect the dogs from canine circovirus infections with this vaccine design [22].

Multiple strains of Canine CV were targeted by the multiepitope vaccine (MEV) construct. Each strain of Canine CV's capsid and replicase protein were used to predict a total of 545 MHCII-binding CD4+T cell epitope peptides. The vaccine was created using a variety of in silico identified antigenic, nontoxic, and conserved epitopes. Furthermore, stable interactions between the predicted MEV and the canine receptor TLR-5 were predicted by molecular docking and molecular dynamics simulations.. The chosen epitope's *in vivo* analysis clearly shows CD4+T-cell-dependent antibody production, which further suggests that the designed MEV construct has potential as a Canine CV vaccine candidate [22,25].

Conclusion

Canine CV is a newly identified virus that has been recently known to infect healthy dogs and dogs having diarrhoea. In addition to a variety of other non-specific signs and symptoms, the disease caused by Canine CV typically manifests in dogs as vomiting and diarrhoea as well as morbidity and mortality. However, necrotizing vasculitis and haemorrhages are the most common lesions seen in various organs in many clinical cases of Canine CV infection.

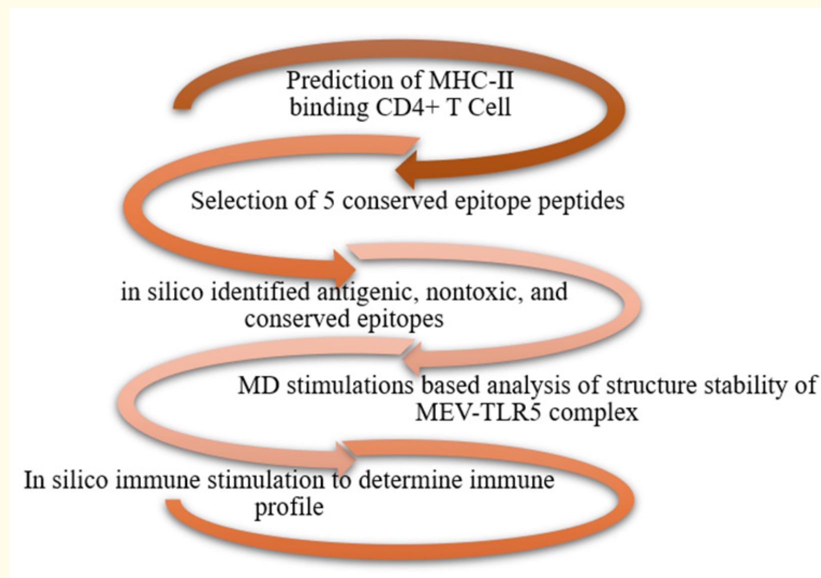


Figure d

The agent is frequently found to be associated with other canine enteric pathogens, which worsens the disease's clinical course. Canine CV has been found to be more prevalent in canine diarrhoea cases than in animals in good health. The use of molecular techniques, such as RT-PCR, to diagnose this new infection is still in its early stages, but they are reportedly very promising. Recently, it was discovered that an indirect ELISA can be successfully used to diagnose the virus. Since there are currently no available specific antivirals against this disease, the infection is managed symptomatically with standard treatment. Recently, A multi epitope vaccine construct against canine circovirus has been created using an in-silico approach that may have prophylactic potential. New studies are being conducted to determine the precise function of this virus as a primary etiological agent in canines and to speed up field diagnosis. However, as many cases have already been reported in many countries, veterinarians should be aware of the potential of Canine CV in causing canine enteric diseases and make suitable plans. So, as to clinically manage CanineCV infections either individually or as a co- infecting agent.

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