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Review Article

Emergence of Novel Canine Distemper Virus Strains-A Real Threat to Terrestrial Domestic and Wild Animals

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Abstract

Canine distemper virus (CDV) is one of the most important pathogens of domestic and wild animals and widely distributed virus around the world. It belongs to thegenus Morbillivirus within the Paramyxoviridae family. At least six orders and over 20 families of Mammals are susceptible to this virus. CDV is highly contagious and have high morbidity andmortality in wild and domestic animal populations. Although there is only one serotype of the virus, it has widest host range with a constant threat to the conservation of the multiple endangered species worldwide. The lack of ecoepidemiological information of CDV transmission other than dogs has led to investigate the importance of the infection in a multihost scenario. The ability to jump the species barrier to infect a variety of mammals including primates has made apprehension that it can infect human being in near future. It is the need of the hour to elucidate the transmissionof CDV in different environments, and to have better understanding about the intricate epidemiological dynamics of CDV in multiple hosts. Among the genes of CDV, H gene is preferred for phylogenetic analysis of different genotypes owing it high mutation rate. However, the full genome sequencing would offer better insights about the substitution rates, glycosylations, and homologous recombination points that would explain the pathogenicity, species jump ability and vaccine failure of this virus as well as enable us to explain in detail its evolutionary informations and better understanding about the intricate epidemiological dynamics of CDV in its multiple host infections. This review is aimed to provide an overview about the recent emergence of CDV genotypes in different species of wild animals, pathogenicity and diagnosis so that the disease can be prevented and control in an efficient and effective manner and it impact on the conservation of a galaxy of wild animals and control can be minimized to a great extent.

Keywords: Canine distemper virus (CDV); Wild animal; Phylogenetic; Morbillivirus; Phocine distemper virus; dolphin morbillivirus; Porpoise morbillvirus; Geneotype; Genetic dversity; Pathogenicity; RT-PCR; Taqman Real time RT-PCR, SLAM; Nectin-4

Introduction

Canine distemper virus (CDV), a pantropic morbillivirus with worldwide distribution causes a fatal disease in dogs and other wild animals. The disease is highly contagious and readily transmitted between susceptible hosts. It was initially described as an infectious disease of domestic dogs but now it is recognized as a global multihost pathogen infecting and causing mass mortalities in a wide range of carnivore species. CD is generally controlled by attenuated vaccines; however, in recent decades, several outbreaks in properly vaccinated dogs and expansion of host range have been reported [1,2,3]. These outbreaks might be due to the emergence of new field strains able to avoid the immune response generated by the "old strains" currently used in the vaccines and/or because of the capacity of new field strains to infect other carnivore hosts [4,5]. The last decade has seen the numerous CDV outbreaks in various wildlife populations causing the potential threat to the conservation of a number of endangered species due to its ability to switch from one host to another [3,6]. In addition lethal infections have been described in non-carnivore species and non human primates demonstrating the remarkable ability of the pathogen to cross species barriers. Mutations affecting the surface protein H required for virus attachment to host cell receptors are associated with virulence and disease emergence in novel host species including primates that have raised several concerns of a potential zoonotic risk of CDV in humans. Further, the expanding the host range of CDV in a variety of wild animals is posing a conservation risk leading to extinction threat to several free ranging and captive non domestic carnivores. The ability of CDV to switch hosts has raised concerns about the threat it poses to several endangered wildlife species [5].

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Virus Properties

CDV is a large (100–250 nm) ssRNA virus belonging to the genus *Morbillivirus* of the family *Paramyxoviridae*. Other members of the genus include Measles virus of man, rinderpest (RP) virus of cattle, PPR virus of small ruminants and emerging viruses of aquatic mammals (phocine, dolphin, seal and porpoise distemper viruses) [5]. CDV is an enveloped, negative-sense, single-stranded RNA virus with a diameter between 150 and 300 nm and a genome length of 15,690 nucleotides, organized into six contiguous, non-overlapping, transcription units encoding for six structural proteins, known as nucleocapsid

(N), phosphoprotein (P), matrix (M), fusion protein (F), hemagglutinin (H), and polymerase protein (L), [7,8,9]. The nucleocapsid protein, viral polymerase, and phosphoprotein are associated with the genomic RNA to form the ribonucleoprotein complex required for viral transcription and replication. The hemagglutinin (H) and fusion (F) proteins present on the viral envelope are the antigenic determinants of the virus whereas the matrix protein is membrane associated [6]. Canine distemper virus has the widest host range among the morbilliviruses and has been recognized to cause potentially lethal disease in 8 families of the order carnivora. Out of the six proteins, only H and F proteins are present on the surface of the virus particles and H protein is the most variable in nature with substitution rates between $5.4X10^{-4}$ to $1.8X10^{-3}$ nucleotide substitutions per site, per year [10] and binds with the host cell receptors. The infection of CDV may be prevented by an adequate host immune response against the H protein [11]. Several comparative studies have revealed that the H gene is subjected to a higher genetic variability (approximately 10%) when compared to the other CDV genes and other Morbillivirus genus, and it has been widely employed to characterize field strains [12,13,14] which makes it suitable for lineage identification and genetic analysis. It has been shown that for this gene wild type and vaccine strains are highly divergent (7–10% for nucleotides and 8–11% for amino acids) with a nucleotide identity between 93–90% and amino acid identity between 92–89%.Phylogenetic studies based on the complete sequence of the H gene from several CDV strains isolated in distinct geographic locations around the world have revealed a genetic/antigenic drift explained by a geographic pattern. According to this pattern, there are 17 distinct lineages known as America 1 (that includes almost all of the commercially available vaccines), America 2, America 3, America 4, America 5, Artic-like, Rock born Like, Asia 1, Asia 2, Asia 3, Asia 4, Africa 1, European Wildlife, Europe/South America 1 and South America 2, South America 3 [2,4,5,12,13,15,16,17,18,19,20,21]. It is also a target for investigating the polymorphism of CDV isolates and for molecular epidemiological studies. There is only one serotype of CDV recognized with several cocirculating genotypes based on variation in the H protein. Based on this variability and in conjunction with a phylogenetic analysis CDV strains have been classified into different genetic lineages: two strains belong to the same lineage if they cluster together and share an amino acid divergence of < 3.5%; the strains belong to different lineages if they appear in separate clades and show values of divergence >4%. In South America there are two co-circulating lineages, the South America 2 (SA2) lineage exclusive to South America and the Europe 1/South America 1 (EU1/SA1) lineage, which is spread in Europe and South America. Genotypes are defined on the basis of strains falling within the same clade sharing >95% amino acid similarity in their H protein [4,22, 23,24,25,26,27,28 ,29,30,31,32,33].

Epidemiology

CDV was initially described as an infectious disease of domestic dogs. Now it has become known as a multi-host pathogen worldwide affecting animals of the order Carnivora namely Ailuridae, Canidae, Felidae, Hyaenidae, Procyonidae, Ursidae, Mustellidae and Viverridae [3]. Its ability to infect multiple species has led to mass mortalities in a range of carnivore species from wild canids to monkey and cynomolgus macaques) [34,35]. The infections of primates have raised a concern about its transmissibility to humans in future [36]. However, there is no report of CDV infecting humans. Although it is generally thought that canine distemper virus affects only canine, reports of CDV outbreaks in large felids such as lions (*Panthera leo*), leopards (*Panthera pardus*) and tigers (*Panthera tigris*) have disproved that concept [3,37]. However, domestic cats inoculated with highly virulent strains of CDV were seropositive to CDV with no signs of illness [38,39,40]. But when SPF cats were inoculated with the virulent CDV, it caused death of the animals. Similar is the case with cheetah. The last decade has seen numerous CDV outbreaks in various wildlife species worldwide [3]. Outbreaks were confirmed in critically endangered species such as the Ethiopian wolf and Amur tiger, giant panda in China [41,42,43,44,45,46,47]. In 1994, Serengeti National Park, Tanzania experienced a severe CDV epidemic which killed one third of the lions population and other species as well such as bat-eared fox, African wild dog, silver-backed jackal and spotted hyena [48,49,50,51,52,53,54]. More recently, CDV outbreaks occurred in several reserves within South Africa caused 95% mortality. This outbreak also infected and caused mortality in other carnivore species such as brown hyena and wild dogs. 'Silent' CDV epidemics in spotted hyenas and lions without obvious clinical signs, high mortality, or a reduction in population size was observed during the lethal in 1993–1994 in the Serengeti–Mara ecosystem. Spotted hyenas from the Maasai Mara National Reserve were more exposed to CDV than those near to human settlements indicating that domestic dogs may not be the only animals that transmit CDV to these wild species [4,46 ,55,56,57,58,59,60,61,62,63].

Transmission and stability: CD is highly contagious and is readily transmitted between susceptible hosts through contact or aerosolized oral, respiratory and ocular fluids and exudates containing the pathogen. During the acute phase of infection, other body excretions and secretions (e.g. urine, faeces, skin) can also contain the virus [5,7,11,18]. Viral shedding may follow infection for up to 90 days and occurs even if the animal was subclinically infected [7]. CDV is sensitive to oxidizing agents, detergents and lipid solvents as well as UV radiation, heat, desiccation. The transmission of CDV is largely dependent on the close association between affected and susceptible animals. To sustain an epidemic of CD, dense populations of susceptible individuals and the continued presence of a biological reservoir are required. Domestic dogs, from communities surrounding protected wildlife areas, are often unvaccinated and occur in high densities with a rapid population turnover. They usually come in contact with the wildlife and spread the disease [1,7,64,65].

Clinical signs: CDV infection has to go through two phases for the development of the clinical disease: first there is an acute infection of the lymphatic system and second, there is an invasion of epithelial cells followed by viral shedding which allows transmission to other susceptible individuals [66]. CDV can also invade the central nervous system probably mediated by

a putative third receptor called GliaR located in glial cells [67,68]. The clinical signs of CDV infections in wildlife species largely resemble those in domestic dogs. However, the severity and the outcome of the infection may vary greatly among species and depend on several factors, such as strain virulence, host age and host immune status. The CDV infection is often subtle and rarely observed initially [69]. If an animal develops a strong immune response, no clinical illness ensues. An estimated 50–70% of CDV infections in domestic dogs are thought to be subclinical [69]. A weak immune response results in non-specific signs such as listlessness, appetite loss and fever. The strong immune response allows the infected animal to recover from infection but CDV can remail in the neurons, uvea, urothelium and skin (causing hyperkeratosis) [3] for quite a long time. CDV infection before the eruption of permanent dentition infect tooth buds causing enamel hypoplasia [6,68,70]. There are 2 forms of the disease: an acute systemic form and a chronic nervous form. Acute systemic disease occurs 2–3 weeks post-infection. The virus continues to replicate and spread throughout the body causing severe clinical signs, which include fever, mucopurulent oculonasal discharge, coughing, dyspnoea, depression, anorexia, vomiting and diarrhoea (which may be bloody) [71,72,73]. During this stage of infection, the virus is found in every secretion and excretion of the body. Neurological signs may be concurrent or follow systemic disease within 2–3 weeks. Signs are progressive and varied depending on the area of the brain affected but commonly include abnormal behaviour, convulsions or seizures, chewing-gum movements of the mouth, blindness, cerebellar and vestibular signs, paresis or paralysis, incoordination and circling [74]. Infection in the central nervous system results in acute demyelinization, and most animals die 2–4 weeks after infection [54]. Due to the immune compromising nature of CDV, clinical signs are often exacerbated by secondary bacterial infections of the skin and respiratory tract [10, 55].

Disease in different species of animals

Distemper in carnivore sp.: Within *Canidae* family, the domestic dog (*Canis familiaris)* is the species with more reports of occurrence of CDV and it is estimated that 70% of all infections are subclinical; however the acute generalized form produces high mortality rates [23,53,55]. Besides domestic dogs, CD has been reported in a large number of wild canids. They include Australian dingos, wild dogs, raccoon dogs, coyotes, black backed jackals, golden jackals and wolves. The Ethiopian wolf is recognized as the rarest canid species in the world and the most threatened carnivore in Africa. This species due to combined effects of rabies and CDV infections is almost extinct. On the other hand, there have been sporadic reports of CDV infection in large felids such as lions, panthers, leopards and tigers [3,7,37,44,52,74,75,76,77,78]. A serosurvey of CDV in jaguars, pumas and ocelots in the Brazilian Atlantic jungle reported for the first time the exposure of wild felids to CDV in this country. CDV infections have been reported in red foxes from various European countries including Germany, Italy, Spain and Portugal. Infected foxes exhibit abnormal behavior such as loss of fear for masters, disorientation, and/or respiratory distress. Morphologic findings comprise mainly conjunctivitis, pustular dermatitis, lymphohistiocytic polioencephalitis, and bronchointerstitial pneumonia with viral inclusion bodies and syncytia. Other animals susceptible to CDV infections are raccoons, red pandas, bears, ferrets, minks, polecats, badgers, otters, weasels, skunks, jaguars, lynx, bobcats, civets, hyenas etc [62,79,80,81,82,83,84].

***Distemper in noncarnivore* sp:** The remarkable ability of CDV to cross species barriers is exemplified by its infection of non-carnivore species such as peccaries and nonhuman primates. In 1989, a CDV epizootic with fatal encephalitis was observed in collared peccaries in the desert of southern Arizona (USA) [67]. In 1989, first cases of natural CDV infections in Japanese macaques was reported. In 2006, large CDV outbreaks occurred among rhesus monkeys in a breeding farm in Guangxi province (China) with death rates up to 30% (about 4000 fatalities) [35]. Animals displayed measles-like signs, such as respiratory distress, anorexia, fever, rash and conjunctivitis. In a subsequent CDV outbreak in Japan in 2008, similar fatality numbers and febrile systemic diseases were observed in colonies of long-tailed macaques. Post mortem examination revealed interstitial pneumonia, generalized lymphoid depletion and demyelination in the brain [34]. It has raised concerns about a potential risk of CDV infection in humans as primates are closely related to primates [35]. Two major host cell receptors namely signalling lymphocyte activation molecule (SLAM, CD150) and nectin-4 (polio receptor like 4) play a pivotal role in the CDV pathogenesis. Both receptors have Ig like variable domain (V) that binds with the morbillivirus [85,86]. SLAM as expressed on the surface of activated T and B cells, dendritic cells and macrophages [85]. Amino acid residues Y525, D526 and R529 of CDV-H binds with SLAM and 530 and 549 residues are important determinants of infectivity in carnivores [6]. Positive selection at site 549 of CDV-H with substitution of tyrosine by histidine (H) facilitate the spread of CDV from dog to non dog species [80]. Majority of CDV isolates from dogs have Y at site 549 whereas H in CDV isolated from other carnivore families [46]. Six to nine days after infection with CDV, the virus enters the epithelial cells of respiratory tracts, G.I. tracts, urinary tracts and endocrine system via nectin 4 receptor. Nectin 4 has been suggested to play a role in the neurovirulence [87]. In a host with weakened immune system, CDV will move to CNS producing neurological systems [88]. The monkey-adapted strain (CYN07-dV) has an intrinsic ability to use human nectin-4 for virus entry in vitro and easily become adapted to use the human CD150 following minor amino acid changes of the viral H protein. Thus, species jumps to human beings, especially in people with a lack of cross-protective measles immunity are proposed to happen in the future [3,89,90].

Canine distemper virus and other morbilliviruses in marine mammals: Distemper in seals can be caused by CDV and the closely related but genetically different phocine distemper virus (PDV) [91]. The devastating PDV epidemic has been reported among harbor seals and gray seals in northwestern European waters in 1988. At the same time, epidemics with CDV strains of the Arctic group were observed among Baikal seals in Siberia. CDV was isolated also from Caspian seals during disease outbreaks with high mortality rates (60-80%) in 1997, 2000 and 2001 [92,93,94]. Similar to CDV, PDV infection

of seals leads to interstitial pneumonia and catarrhal enteritis, causing fever, diarrhoea, coughing, and dyspnoea. Other signs include nasal discharge, ocular discharge, anorexia, weight loss and abortion. Common neurological manifestations represent tremor, behavioural changes and lethargy. Brain lesions in PDV infected seals are similar to CDV induced acute polioencephalitis in dogs and measles virus inclusion body polioencephalitis in human beings, respectively. Distemper-like diseases in dolphins and harbor porpoises are caused by the dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV), respectively. Together with the pilot whale morbillivirus, isolated from a stranded long-finned pilot whale (Globicephalus melas), DMV and PMV are members of the cetacean morbillivirus group. Analysis revealed that these cetacean viruses are more closely related to rinderpest virus and peste-des-petits-ruminants (PPR) virus than to CDV. The isolated virus from Mediterranean monk seals during mass mortality closely resemble cetacean morbilliviruses, indicating cetaceans to pinnipeds transmission [95,96,97,98,99,100,101,102,103].

Genetic diversity of CDV: TheH protein of CDV determines the cellular tropism and host range when interacting with the SLAM receptor in host lymphoid tissues and with nectin-4 in epithelial tissues. The H gene has been widely used in genetic and phylogenetic analyses due to its highest variability [104,105,106,107]. Based on the nucleotide sequences of H gene a total of 17 lineages of CDV have been reported worldwide : America-1 (vaccine strains), America-2, America-3, America-4, America-5 Arctic-like, Rockborn-like, Asia-1, Asia-2, Asia-3, Asia-4, Africa-1, Africa-2, European Wildlife, Europe/South America-1, South America-2 and South America-3 [16,19,22,29,32,58,78,106,108,109,110,111,112]. Comparative genetic studies including several complete genome sequences of CDV revealed that certain residue located in the receptor binding region of the H protein are under positive selection [46,80]. The specific substitutions at sites 530 and 549 were systematically observed in non-dog species indicating their importance in host adaptation [46]. Domestic dogs have eight possible substitutions at position 530 (G/D/N/E/R/S/A/K) and two substitutions at 549 (H/Y) present in at least ten lineages; in non-canid wild animals there are seven substitutions at position 530 (G/D/N/E/R/C/V) and two substitutions at position 549 (H/Y) present in seven lineages. On the other hand, five lineages have three substitutions at position 530 (G/N/R) and two substitutions at position 549 (H/Y) in wild canids. Recent analyses of the CDV strain responsible for the outbreak in lions of Serengeti National Park in the 1990s demonstrated that all the species affected (including dogs and wild canids) had the 530D substitution [58]. It has been observe that CDV from dogs have a greater tendency to have 549Y, while substitution 549H occurs most commonly in wildlife, which indicates a possible association between this substitution and the affected species [80]. Further different CDV isolates in mink breeding sites (non-canid wild species), raccoon dogs and foxes (wild canid species) in China showed the trend 530G and 549H [112]. In monkeys (non-canid species), there was a trend of 530G and 549Y with an additional substitution at another site of the protein H (542F) that may play an important role in CDV adaptation to primates [112]. However, in an outbreak in urban raccoons (non-canid species) in Europe, researchers found the trend 530G and 549Y, and concluded that the Y549H substitution apparently is not essential to facilitate infection in wildlife other than canids. The explanation for this outbreak was that urban raccoons are in contact with foxes (wild canids), which are widespread species in urban areas thus having great contact with domestic dogs. Therefore, foxes act as a wild reservoir of domestic dog strains in which the substitution 549Y is predominant [59]. There was an 11.5% presence of the Y549H substitution in dogs from Brazil. In addition, all the dogs presented 530G and 580R, the latter representing 89% of the circulating strains. It is suggested “stray” dogs who are more likely to have contact with wildlife are acting like reservoirs in these ecosystems [109]. Regarding to wildlife, more sequences of the H gene are needed to determine the trend of the presence of substitutions at positions 530 and 549 because the 549H substitution has not been reported in the limited studies in the lineages of Asia, South America, and Africa [80]. With respect to CDV jump to humans, the literature reports that substitutions D540G and M548T in the protein H, besides Y267C in the protein V, C116Y in the protein F, and M267V in the phosphoprotein have allowed cell invasion in H358 cells. However, different authors suggest that there must be mutations in other genes allowing the intracellular adaptation of CDV [36,90]. The CDV complete genome sequences must be evaluated to identify other substitutions that can define its ability to jump between hosts other than H protein that may contribute to the inter-species jump and adaptation. There is only one serotype of CDV and recovered animals following single exposure showed lifelong immunity. However, there are different strains which differ in virulence and tropism leading to their differential pathogenic manifestations [18,37,113,114]. Epidemiological studies based on molecular techniques revealed that the H gene is subjected to a higher genetic variability (approximately 10%) compared to the other genes. This variability may affect some specific sites on the H protein which have an important role in immune neutralization leading to severe infections and clinical outcome. In recent years genetic divergent virus strains reported in CDV isolations around the world and several episodes of CDV were confirmed in vaccinated animals [113]. A lineage is characterized at the level of amino acid divergence within and between different phylogenetic clusters. Within group amino acid divergence is known to be less than 3.5%, whereas amino acid divergence between groups is greater than 4%. Phylogenetic studies based in the complete sequence of the H gene of several CDV strains isolated in distinct geographic sites around the world have revealed a genetic/antigenic drift explained by a geographical pattern. According to this, seven distinct lineages (clusters or genotypes) were reported until 2007. These are known as America 1 (includes almost all the commercially available vaccines), America 2, Artic-like, Asia 1, Asia 2, Europe, and European Wildlife [58,78,108].

In 2007, a phylogenetic study of partial nucleotide sequences of the H gene of vaccinated and non-vaccinated dogs from Argentina found a new lineage denominated “Argentina” which forms a completely separate clade. In 2012, a new phylogenetic

study with full-length H gene sequences from South American dogs found that all Uruguayan, Brazilian and one Argentinean CDV strain belonged to the already known Europe lineage and called South America 2 (97). The antigenic profile of these new genetic variants are quite different when compared to vaccine strains [2,12,25,31,104,109,110,111].

Pathogenicity Predictions: Association predictions between morbidity and lethality related to the presence of specific substitutions at certain genomic positions have been presented by several authors [23,58,109]. It is important to note that these predictions are hypotheses that arise from the analysis of the sequences obtained by the authors and that due to the lack of large epidemiological studies no statistical association has been established. In the non-canid CDV strains from the 1993–1994 Serengeti outbreak a new substitution in the H protein (R519I) was associated with the presence of clinical signs in these wild species (African lion and spotted hyena). The presence of various combinations of substitutions at positions 519 and 549 of the protein H in strains from domestic dogs, wild canids, and non-canids wild species has been reported. A rare combination of substitutions in these positions (519I and 549H) was only observed in non-canid strains and was related to a fatal outcome only in non-canids (lion and hyena). The other combinations found (519R/549Y and 519R/549H) caused death only in domestic and wild canids [58]. Similarly, in a Brazilian study all the strains isolated from dogs harbored the substitution 549H causing the death of every animal [109]. In dogs from Medellin, Colombia, the presence of CDV of the South America-3 lineage and the combination of 549Y, 519R, and 530N; one dog presented 549H has been reported. All of these cases showed evidence of nervous symptomatology and respiratory disease with fatal outcomes [110]. The mutations association studies of the H gene are the first performed for CDV, and should be extended to the study of the complete genome to explain the pathogenesis in the various susceptible species. It has been reported that mutations in intergenic regions, and in the 5' and 3' UTRs can affect the virulence and pathogenicity of field strains [96]. Specifically, mutations in CDV M–F intergenic region were associated with a different viral phenotype, highlighting the importance of conducting association studies with complete genomes [115].

Diagnosis: Ante-mortem diagnosis of CDV is preferred due to the disease's high infectious potential, combined with a high mortality rate and fast progression. Initial diagnosis of CDV based on the clinical signs is difficult due to the many varied clinical presentations of the disease and overlapping symptoms with other diseases. Differentiation from other diseases with respiratory, neurological and/or gastrointestinal signs, such as rabies, feline panleukopenia, coronavirus, toxoplasmosis, bacterial enteritis and parvovirus, should be conducted. There are a number of serological and immunological diagnostic tests have been developed for the detection of CDV in domestic animals. Diagnosis of CDV infection in wildlife is more difficult due to the challenges associated with acquiring and cold storage of samples in the field for further testing in the laboratory.

Molecular assays

The advent of molecular techniques has revolutionized the arena of diagnostic platforms of the infectious diseases with high sensitivity and specificity [26,116]. The test which has been widely used for the detection of CDV is the reverse-transcription (RT) PCR assay predominantly targeting the highly conserved N gene. While RT-PCR methods are more sensitive, specific and rapid compared to conventional culturing methods, they are still technically demanding and require several hours with additional post-PCR analyses. Sensitivity also varies depending on the sample source, extraction method and choice of primers. A more rapid diagnostic technique for the detection of CDV is real-time RT-PCR employing the TaqMan technology and targeting P gene to detect and quantify CDV in clinical samples and cell cultures. Additionally, nested PCR techniques have been developed for the detection of CDV as well as nested real time PCR. The technique was performed on several clinical samples and proved to be two orders of magnitude more sensitive than RT-PCR [14,89,104,117,118, 119,120,121,122].

***Serological assays*:** The tests which are used for the detection of antibodies to CDV are IFAT and ELISA. Both the IFAT and ELISA are used to detect IgM and IgG antibodies against CDV in domestic dogs and various non-dog hosts. The presence of IgM not only confirms current acute distemper infection but is used to retrospectively diagnose distemper by detecting seroconversion in paired serum samples collected during the acute and recovering phase of the disease [30,123]. The highly specific and sensitive serum-neutralization test is considered as gold standard for detecting antibodies to CDV (5). Serology as a diagnostic test is, however, not reliable in distinguishing between naturally acquired CDV infection (wild-type CDV strain), infection with attenuated virus vaccine strain [as used in the modified live vaccine (MLV)] or immune response to recombinant, virus-vectored vaccine and should thus if possible be combined with other techniques, such as RT-PCR and viral antigen ELISA [53,117,120].

Virus isolation: Virus isolation is typically conducted in pulmonary alveolar macrophages or by co-cultivation of infected tissues with mitogen stimulated lymphocytes derived from healthy dogs or ferrets [123,124]. These methods are demanding and time consuming, taking several days to weeks. Virus can also be isolated in MDCK and Vero cells with more intense CPE in Vero cells. Vero cells expressing the canine SLAM are highly sensitive for virus isolation, with cytopathic effects evident within 24 h of inoculation [107,124].

Treatment and control: The treatment and control of infectious viral diseases is often difficult, especially in wildlife populations. Treatment of CDV infection is commonly based on symptomatic and supportive therapy as there is no specific antiviral drug available for therapeutic use against CDV infection in any species, including domestic dogs. The orally administered pan morbillivirus inhibitor that targets viral polymerase showed some promising results [125]. Other compounds such as fucoidan, a sulfated polysaccharide found in brown algae, have also been evaluated for their ability to act as antiviral drugs against CDV [126]. In vitro results showed that fucoidan was able to inhibit initial steps of the viral replication cycle, strongly suppressing the formation of syncytia in infected cells. The antiviral activity

of several flavonoids (quercetin, morin, rutin and hesperidin) and phenolic acids (cinnamic, trans-cinnamic and ferulic acids), concentrating on their in vitro ability to inhibit stages of the CDV replication cycle. All flavonoids and phenolic acids demonstrated antiviral action against CDV infection [127,128]. Other methods of treating CDV infection that have been explored include mesenchymal stem cell therapy and the use of a veterinary pharmaceutical preparation of silver nanoparticles [129].

Immunoprophylactic Agents: The most effective and economical way to control a viral disease is by using vaccines. In the 1960s, two MLVs against CDV were introduced. The first, the Onderstepoort vaccine, was developed from a natural isolate, passaged in ferrets (Mustela putorius furo) and then adapted to chicken embryos (these were later replaced with chicken cell culture) [130]. The second MLV was generated by adaptation of the CDV Rockborn strain to canine kidney cells. These modified live virus vaccines are sufficient for management of CDV in domestic dogs, but can on rare occasions cause post-vaccination encephalitis and lead to vaccine-induced illness [114]. The susceptibility of various species to vaccination with the MLV vaccine is largely unknown. Species differences in their response to vaccination have been observed, for example the avian cell adapted CDV vaccine can be fatal in European mink and ferrets [76,82], but was shown to give protection to the maned wolf, fennec fox and both red and grey foxes [82,131]. Concerns with differences in efficacy of MLV vaccines have led to the development of recombinant vaccines. Canarypox-vectored vaccines, developed for use in domestic canines, are incapable of replicating in the host cell, but can elicit an appropriate host immune response. Recent study on vaccine efficiency in tigers found that both the live attenuated and the recombinant canarypox-vectored vaccine appeared safe for use, although the live attenuated vaccine produced a significantly stronger and more consistent immune response in the tigers [61]. It is sometimes beneficial to immunize the domestic dog reservoir surrounding conserved areas to control CD in wild animals because most of the cases dogs transmit the disease to wild animals. While this approach benefits domestic dogs, vaccine coverage is rarely sufficient to reach the 95% target considered necessary to control CDV [132] and often fails to prevent infection in wildlife species that share their environment. Thus, the question whether endangered wildlife should specifically be targeted for vaccination is raised. Several challenges associated with wildlife vaccination need to be considered including (1) knowledge on the safety and efficacy of the vaccine in the specific species targeted; (2) mode of vaccine delivery either during opportunistic animal handling (when fitting tracking collars, translocation or medical examination), or by hypodermic dart (could cause injury and stress), or orally through laced bait (reduced efficacy if not eaten by target species) ; (3) the logistics of administering the required booster shots; and finally (4) the cost involved in initiating and implementing a vaccination programme in wildlife [73,133,134,135,136].

Conclusion

CD is a disease of a number of domestic and wild animals and worldwide in distribution. Although vaccines of high level of safety and efficacy are available for use in dogs, the vaccinated animals sometimes show mild form of the disease. Recently, with the emergence of a number of genotypes of CDV, it has become necessary to study in details the new strains and replacement of the old strains with the novel strains. CDV is also posing a serious threat to the conservation of several captive and free ranging wildlife populations [3]. Its ability to infect multiple hosts considerably hampers disease eradication. Until recently CDV had only been studied in domestic dog, with wildlife research greatly lacking. The monitoring CDV in different species of animals with or without symptoms, complex scenario of CDV transmission , the epidemiological importance of asymptomatic individuals and existence of dead end hosts which do not shed the virus such as cat and pig may be explored to have a clear insights about the epidemiological informations of CDV infections [38,137]. Multidisciplinary research is also needed to implement accurate strategies to mitigate CDV infection, particularly at the interface between wildlife and domestic animals. This includes limiting the contact between wild and domestic species or administering vaccines to domestic animals to reduce the impact of CDV on populations at risk of extinction. The proposal of vaccinating wild carnivore species against CDV has an adverse consequences as typical CDV clinical signs have appeared with fatal consequences in different wild carnivore species. Epidemiology of distemper in wildlife animals depends upon several factors such as virulence of virus strain, population density and herd immunity. Also the genetic diversity of CDV strains represents a possible cause for unpredictable disease emergence in domestic and wildlife populations. This is in contrast to the host specific pathogens such as measles virus and rinderpest virus, the broad and expanding host range of CDV considerably hampers disease eradication even by widespread mass vaccination [3,4,6].

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