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Molecular Detection of Canine Distemper Virus in Dogs by Polymerase Chain Reaction

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ABSTRACT: Canine distemper (CD) is a highly infectious and lethal disease that poses a significant threat to dogs and various other animal species. The disease, caused by the canine distemper virus (CDV), affects multiple body systems and can lead to severe complications. Its broad host range, including primates, cetaceans, and carnivores, underscores the virus's ability to cross species barriers and its potential impact on wildlife populations. The multi-systemic nature of CD manifests in a wide array of clinical signs, ranging from respiratory and gastrointestinal issues to neurological disturbances, making diagnosis challenging and treatment complex. A study conducted at the Veterinary Clinical Complex, Veterinary College and Research Institute in Orathanadu, Tamil Nadu, India, aimed to detect CDV in clinically suspected dogs using molecular techniques. The research focused on identifying the CDV nucleoprotein gene (N) in nasal swabs collected from 22 dogs exhibiting various clinical signs associated with distemper. These signs included respiratory symptoms, nasal discharge, excessive salivation, bronchitis, conjunctivitis, gastroenteritis, catarrhal pneumonia, and neurological abnormalities. Employing reverse transcription-PCR (RT-PCR) with primers targeting the N gene, the study successfully detected CDV in 6 out of 22 samples (27.27%). This finding not only confirms the presence of CDV in the studied population but also highlights the importance of molecular diagnostic techniques in accurately identifying the virus, potentially leading to improved disease management and control strategies.

Keywords: Canine distemper virus, RT-PCR, N gene, RNA.

INTRODUCTION

Canine distemper virus (CDV) is a highly contagious and potentially fatal pathogen that affects dogs and other carnivores (Osterhaus *et al.,* 1995; Visser *et al.,* 1993). The virus's structure and genomic composition contribute to its virulence and ability to spread rapidly among susceptible populations. The enveloped viral particle, ranging from 150 to 300 nm in diameter, contains a central core with helical structures. Its non-segmented, single-stranded negative-sense RNA genome encodes six structural proteins, each playing a crucial role in the virus's life cycle and pathogenicity. CDV's classification within the Paramyxoviridae family and Morbillivirus genus places it alongside other significant pathogens affecting various species (Griffin, 2001). The virus primarily targets the respiratory tract lymphatic tissues for replication, leading to characteristic symptoms such as lymphopenia and fever within days of infection (Peserico *et al*., 2019). This initial phase of viral replication and immune system suppression sets the stage for potentially severe clinical manifestations and complications associated with canine distemper. The virus's ability to rapidly replicate and spread throughout the host's body underscores the importance of early detection and intervention in managing CDV infections (Kalbermatter *et al*., 2023).

Currently, dogs exhibiting clinical symptoms of a chronically antibiotic-resistant respiratory disease, or a neurological disease are tentatively diagnosed with CD. In advance stages of infection, *i.e.,* when the central nervous system has already been infected with CDV, convulsions, lack of coordination, and myoclonus develop as more specific symptoms for CD diagnosis. Furthermore, CD is a complex disorder to be identified due to its wide range of clinical manifestations (Frisk *et al*., 1999; Bolívar *et al*., 2019; Karki *et al*., 2022). Therefore, early detection of infection is essential for isolating sick dogs and administering adequate care. For this reason, to identify a lesser amount of virus in early infection, a sensitive, rapid, and specific method is required. Different methods for detecting CDV antigen or RNA have been used, but the majority of them are time-consuming and complicated, and they may generate inaccurate results, making them inappropriate for antemortem diagnosis. New diagnostic methods are being developed as a result of recent advances in molecular biotechnological applications. The CDV

RNA is detected in blood samples and cerebrospinal fluid of dogs suspected of developing the disease using a reverse transcription polymerase chain reaction (RT-PCR) (Frisk *et al*., 1999; Bolívar *et al*., 2019).

Canine distemper (CD) is a severe and potentially fatal viral disease that affects dogs worldwide. Caused by the canine distemper virus (CDV), a member of the Morbillivirus genus within the Paramyxoviridae family, CD presents with varying clinical signs depending on factors such as the host's age and immune status, virus strain virulence, and environmental conditions (Carvalho *et al*., 2012). Despite widespread vaccination efforts over many decades, CD continues to pose a significant threat to canine populations globally (Rendon-Marin & Ruíz-Saenz 2024). The diagnosis of CD presents unique challenges, particularly due to the presence of high antibody titers resulting from previous vaccinations or subclinical/clinical infections, which limits the effectiveness of serological diagnostic methods. Given the infectious nature of CD and its high mortality rates, rapid and accurate diagnosis is crucial for implementing timely control strategies and appropriate medical interventions (Gilbert *et al*., 2020). Canine distemper is highly contagious, and viral shedding may follow infection for 60–90 days (Greene *et al*., 1990). Transplacental infection has been documented in domestic dogs (Krakowka *et al*., 1997). Dogs that recover can shed virus for 2-3 months (Williams, 2001).

Lesions of CDV infection are similar in nondomestic carnivores and in domestic dogs (Von Messling *et al*., 2000). The lung, liver, lymph nodes, brain, and spleen of any dead animal with suspected CDV infection should be collected for viral isolation and/or PCR. Immunohistocytochemistry on formalin-fixed tissues provides definitive evidence of CDV infection.

Early virus detection for canine distemper virus (CDV) demands a comprehensive strategy that integrates high sensitivity, precision, and rapid turnaround times, especially in cases where clinical manifestations are evident. The shortcomings of conventional serological approaches have driven the advancement of sophisticated diagnostic methodologies capable of delivering swift and dependable outcomes (Hao *et al*., 2019). Polymerase chain reaction (PCR) assays, particularly real-time PCR, have emerged as powerful tools for detecting viral nucleic acids with high sensitivity and specificity. These methods can detect CDV in various sample types, including blood, urine, and cerebrospinal fluid, even before antibodies are detectable. Additionally, rapid antigen detection tests, such as immunochromatographic assays, have been developed to provide point-of-care diagnostics (Bogdanchikova *et al*., 2016). These tests offer the advantage of quick results, often within minutes, and can be easily performed in clinical settings. However, ongoing research is focused on improving the accuracy and reliability of these rapid tests to match the gold standard of laboratory-based molecular diagnostics. Currently, several methods are employed for CDV diagnosis, including virus isolation, immunofluorescence assays (IFA), RT-PCR, and real-time PCR (Frisk *et al*., 1999).

Research has focused on optimizing these techniques for early detection and improved accuracy. Kim *et al*. (2006) utilized RT-PCR to compare various bodily secretions for early CDV detection in experimentally infected dogs. More recent studies, such as those conducted by Maneesh *et al*. (2023); Paidimuddala Charitha *et al*. (2023), have employed molecular detection methods and phylogenetic analysis to identify CDV in different geographical regions. The latter study reported a 26.6% detection rate of CDV from clinical cases using reverse transcriptase PCR. Additionally, DNA sequencing of the CDV's N gene has been used to confirm the presence of the virus in samples (Sarute *et al*., 2014; Shin *et al*., 2004), further enhancing the diagnostic capabilities and understanding of CDV variants. The aim of this study was to detect canine distemper virus in dogs from the Cauvery delta region of Tamil Nadu.

MATERIALS AND METHODS

The sample collection process for this study focused on domestic dogs exhibiting symptoms consistent with Canine Distemper Virus (CDV) infection. Twenty-two dogs were selected, with the majority being unvaccinated, representing a diverse range of ages and both sexes. These animals were brought to the Veterinary Clinical Complex for evaluation due to a variety of clinical signs characteristic of CDV, including respiratory issues (nasal discharge, bronchitis, catarrhal pneumonia), ocular problems (conjunctivitis), gastrointestinal disturbances (gastroenteritis), excessive salivation, and neurological abnormalities. The selection of these symptomatic dogs allowed researchers to target individuals with a high likelihood of active CDV infection.

The specific sampling method employed was the collection of deep nasal swabs. This technique was chosen due to its effectiveness in obtaining viral particles from the upper respiratory tract, a primary site of CDV replication. The depth of the swab collection is crucial for maximizing the chances of retrieving sufficient viral material for subsequent analysis. Following collection, the samples were immediately stored at an ultra-low temperature of -70°C. This rapid and extreme cooling is essential for preserving the integrity of viral RNA, which is highly susceptible to degradation at higher temperatures. The storage of samples at this temperature ensures that the viral genetic material remains stable and viable for the subsequent RNA extraction process, which is critical for accurate diagnostic testing and potential genomic analysis of the virus.

A. Extraction of RNA and conversion to cDNA

The RNA extraction process from nasal swabs using the BioBasic RNA extraction kit is a crucial step in preparing samples for further analysis. This method ensures the isolation of high-quality RNA from the collected specimens. Following the manufacturer's instructions meticulously is essential to maintain the

integrity of the extracted RNA and minimize contamination. The subsequent measurement of RNA concentration and purity using a NanoDropTM1000 spectrophotometer provides valuable information about the quality of the extracted RNA. The absorbance ratio of 260/280 nm at 1.8 indicates pure RNA, which is critical for downstream applications.

The conversion of RNA to complementary DNA (cDNA) using a commercial cDNA conversion kit from Bio-Rad is a vital step in the molecular biology workflow. This process, known as reverse transcription, allows for the stable storage of genetic information and facilitates various analytical techniques, such as PCR and gene expression studies. The storage of cDNA at -20°C ensures its preservation for future use, maintaining the integrity of the genetic material for subsequent experiments. This careful handling and storage of both RNA and cDNA samples are essential for obtaining reliable and reproducible results in molecular biology research.

B. Reverse transcription-polymerase chain reaction

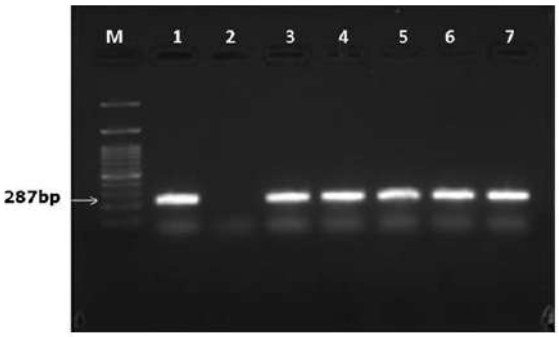
The primers targeting N gene of CDV were used for confirmation. The primer sequences CDF: ACAGGATTGCTGAGGACCTAT and CDR: CAA GAT AAC CAT GTA CGG TGC were used. Multicomponent vaccine NOBIVAC-6 was used as positive control.

Overall, a volume of 20 µL, including 10 µL of reverse transcription-PCR PreMix, 1 µL of gene specific primers (forward and reverse), 3 µL of nuclease free water and 5 µL of cDNA were added and mixed thoroughly inside an Eppendorf tube. PCR thermocycler conditions included pre-denaturation at 95°C for 5 min, denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, extension at 72°C for 1 min for 36 cycles, and final extension at 72°C for 5 min. The product of RT-PCR was visualized under UV light after staining the1% agarose gel with ethidium bromide.

RESULTS

The partial amplification of the viral N gene using specific primers in reverse transcription polymerase chain reaction (RT-PCR) provided valuable insights into the presence of Morbillivirus in the canine population studied. The detection of 6 positive cases out of 22 samples, representing a 27.27% prevalence, indicates a significant occurrence of this genus among the dogs examined. The N gene, which encodes the nucleocapsid protein, is a highly conserved region in morbilliviruses and is commonly targeted for diagnostic purposes due to its stability and abundance in infected cells.

The visualization of bands at approximately 287 base pairs (bp) on the gel electrophoresis, as shown in Fig. 1, confirms the successful amplification of the target region. This band size is consistent with the expected amplicon length for the specific primers used in the RT-PCR assay, providing further validation of the results. The presence of these bands in 6 samples suggests active viral infection or recent exposure to Morbillivirus in these dogs. This finding underscores the importance of continued surveillance and preventive measures, such as vaccination programs, to control the spread of morbillivirus infections in canine populations.



Lane M: 100bp ladder; Lane 1: Positive control; Lane 2: Negative control; Lane 3 – 7: Positive samples

Fig. 1. Agarose gel electrophoresis of PCR amplified products of CDV.

DISCUSSION

The N gene's high conservation makes it a reliable target for identifying Canine Distemper Virus (CDV). Reverse Transcriptase PCR (RT-PCR) has proven to be a highly sensitive and specific method for ante-mortem CDV detection. This finding aligns with previous research as reported (Paidimuddala Charitha *et al*., 2023), which reported a 26.6% CDV identification rate in clinical cases. The current study revealed a higher incidence of 270.27%, emphasizing the prevalence of CDV in the studied population. DNA sequencing of the CDV's N gene further confirmed the virus's presence in the samples, consistent with findings from other researchers (Mansour and Hasso 2021; Sarute *et al*., 2014; Shin *et al*., 2004; Krakowka *et al*., 1975).

The confirmation of CDV infection in domestic dogs within the Cauvery delta zone of Tamil Nadu raises concerns about potential disease spread to previously unaffected areas. To mitigate this risk, it is crucial to implement regular and consistent vaccination programs. These efforts should aim to reduce the introduction of contagious pathogens through comprehensive vaccination coverage. Additionally, authorities should develop and implement educational programs to inform dog owners about the importance of complete vaccine regimens for their pets. Such initiatives would contribute to better disease control and prevention, ultimately safeguarding the health of domestic dog populations in the region and beyond.

CONCLUSIONS

The diagnosis of distemper often necessitates laboratory examinations to validate disease in the absence of usual symptoms and clinical signs that are close to those of other illnesses. In the current research, CDV was detected by the N gene-based RT-PCR in 27.27% of the tested samples, indicating that the virus was circulating in the study region and because that, only animal with CDV clinical signs were taken a sample from it, which cannot be considered a reliable predictor of CDV

prevalence. The presence of CDV in the samples may also be confirmed by DNA sequencing of the CDV's N gene. Gene sequencing is mandatory to identify any nutation in the circulating virus and give the way for the development of specific vaccine for CDV.

FUTURE SCOPE

Canine distemper (CD) is the most important infectious disease of domestic dogs of any age. Incidence of clinical diseases is almost entirely in puppies between weaning and 6 months of age that has lost its maternal protection but acquired active immunity. The virus causes a high incidence of mortality and morbidity in some breeds of dogs, young aged and immune compromised animal. The absence of curative therapy and its genetic variation make the canine distemper infection the highest clinical diseases of the dog and other canids. Canine distemper infection has no curative treatment, therefore, to prevent dogs and other canids from this specific viral infection, vaccination using attenuated live virus vaccine should be advocated in endemic areas. Transmission is through aerosolization of respiratory exudate containing the virus, although other body excretions and secretions. Therefore, environmental hygiene where dogs kept should be strictly controlled. Emphasis should be given for susceptible groups of dogs during control and prevention of CDV infection in endemic areas.

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Conflict of Interest. None.

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