RESEARCH ARTICLE

Molecular Detection and Phylogenetic Analysis of Canine bufavirus Associated with Canine parvovirus in Nagpur

Avinash Kanole, Shubhangi Warke*

Abstract

Viral enteritis is a major cause of mortality in dogs under six months of age. This study investigated the molecular characterisation of Canine bufavirus (CBuV) associated with Canine parvovirus (CPV) in faecal samples from dogs showing gastrointestinal illness. A total of 100 samples were screened, of which 46 were positive for CPV-2, indicating a prevalence of 46%. These CPV-positive samples were further tested for CBuV, and 3 samples were positive, showing a co-infection rate of 6.52%. Six representative samples (3 CPV-2 and 3 CBuV) were sequenced for phylogenetic analysis. The analysis showed a clear separation of CPV and CBuV into two major clades. All CPV sequences clustered within the CPV-2c lineage and showed 98.44%-99.03% homology with the Chinese strain MN119600. The CBuV isolates showed close genetic relationships with strains from Iran (99.93%), Thailand (98.72%), and China (99.12%), indicating genetic diversity among circulating CBuV strains.

Key words: Canine bufavirus, Canine parvovirus, PCR, Phylogenetic analysis.

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Introduction

A variety of microorganisms can lead to gastrointestinal infections in dogs, frequently causing mixed infections that worsen and complicate the clinical condition. *parvo viruses* belonging to the *Parvoviridae* family are small (25-30 nm in diameter), non-enveloped, and single-stranded DNA viruses of 3.9-6.3 kb in length (Cotmore *et al*., 2014; Penzes *et al*., 2019). Canine parvovirus-2 is a leading source of diarrhoea in dogs under 6 months of age (Schultz, 2006). The antigenic variants of the virus (CPV-2a, CPV-2b, and CPV-2c) have been found to cause acute haemorrhagic enteritis with vomiting and diarrhoea in dogs of all ages (Woods *et al*., 1980). In 2012, a novel *protoparvovirus,* later named Bufavirus (BuV), was identified through viral metagenomic analysis of faecal samples from diarrheic children in Burkina Faso (Phan *et al*., 2012). Since then, *protoparvoviruses* related to BuVs have been reported as sporadic cases in humans and non-human mammals (pigs, dogs, bats, rats, shrews, and non-human primates) (Kemenesi *et al.,* 2015; Sun *et al*., 2019). In 2018, a virus with a close genetic relationship to the human bufavirus (HuBuV) was detected in dogs with either gastroenteric or respiratory disease in Italy and Hungary, and it was named Canine bufavirus (Martella *et al*., 2018).

To date, Canine bufavirus (CBuV; species *Carnivore protoparvovirus 3*, genus *Protoparvovirus*, and subfamily *Parvovirinae*) has been reported in Italy, Hungary, China, Canada, India, Thailand, and Türkiye (Martella *et al.,* 2018; Li *et al.,* 2019; Sun *et al.,* 2019; Di Martino *et al.,* 2020; Ganji *et al.,* 2022; Charoenkul *et al*., 2024; Acar *et al*., 2025). Although the exact mechanism underlying CBuVs is still unknown, they are known to be linked to Canine enteritis (Martella *et al.,* 2018; Li *et al.,* 2019; Sun *et al.,* 2019). Additionally, CBuV has been detected in the serum of dogs with gastroenteritis (Li *et al.,* 2019). Positive correlations have been found between the presence of CBuV DNA and diarrhoea. Genetic studies suggest that the potential genetic diversity and recombination of CBuV may contribute to its evolution (Di Martino *et al.,* 2020). Multiple viral infections in dogs detected with CPV and CBuV were reported in a range of viral gastroenteritis cases. Co-infections are of particular concern as they may result in more severe clinical outcomes, including protracted or refractory diarrhoea, and may complicate the pathogenesis of the disease, thereby affecting the accuracy of diagnosis and the efficacy of treatment protocols (Charoenkul *et al.,* 2024; Ji *et al.,* 2024). Since the first discovery of CBuV in 2022, the virus has not yet been reported in India. Hence, the present study was undertaken to investigate the prevalence of Canine

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bufavirus infection along with Canine parvovirus in and around Nagpur (MS), India.

Materials and Methods

The present study was carried out on the dogs presented to the Teaching Veterinary Clinical Complex of the Nagpur Veterinary College, Nagpur (MS), India, and from the private clinics in and around Nagpur with signs like foul-smelling diarrhoea, gastroenteritis, vomiting, weakness, and dehydration during the year 2024-2025

Sample Collection and Processing

Detailed history, clinical signs, and epidemiological parameters were recorded. Faecal swabs were collected into a sterile Hiculture Collecting Device. A total of 100 samples were collected for this study, out of which 70 samples were from symptomatic dogs and 30 samples were from healthy ones. A sterile Hiculture Swab was inserted in to rectum and collected ~1 gm of faeces and emulsified in 1 mL of 0.1 M PBS of pH 7.2.

DNA Extraction and Amplification of the *VP2* Gene of Canine Parvovirus and Canine Bufavirus

Viral DNA was extracted from samples by using the Promega DNA isolation kit according to the manufacturer's protocol. The viral DNA extract was kept at a temperature of -20 °C. The conventional Polymerase chain reaction was performed for the detection of CPV as per the method described by Sheikh *et al.* (2017). The forward and reverse primers used for amplifying the 681-bp VP2 gene fragment of CPV were forward 5¢-GAAGAGTGGTT GTAAATAATT-3¢ and reverse 5¢-CCTATATAACCAAAGTTAGTAC-3¢. The PCR protocol was standardised using a 25 µL reaction mixture containing 12.5 µL of PCR Master mix, 2 µL each of forward and reverse primers, 2.0 µL of DNA template, and sterile nuclease-free water 6.5 µL. The cycling conditions for PCR included an initial denaturation of DNA at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 2 min, and extension at 72°C for 2 min, followed by the final extension of 72 °C for 10 min, and hold at 4 °C.

The CBuV was detected through conventional PCR targeting the VP2 gene of 202 bp using primers 5¢-GAAGAGTGGTTGTAAATAATT-3¢ and 5¢-CCTATATAACCAAAGTTA GTAC-3¢ as previously described by Martella *et al.* (2018). The PCR protocol was standardised using 25 µL of reaction mixture as above. The cycling conditions for PCR included an initial denaturation of DNA at 94 °C for 2 min, followed by 45 cycles of denaturation at 94 °C for 30s, annealing at 53 °C for 30s, extension at 72°C for 30 s, followed by the final extension of 72° C 10 min and hold at 4 °C. In each PCR run, both positive (reference standard DNA) and negative (no template DNA) controls were included to ensure specificity and absence of contamination. The final amplified product was analysed by agarose gel electrophoresis on 1% agarose gel and visualised under a gel documentation system (Syngene, UK).

Sequencing and Phylogenetic Tree of the *VP2* Gene of CPV and CBuV using Bioinformatics Tools

The representative positive PCR products from this investigation were subjected to sequencing using professional sequencing services. With the use of several bioinformatic tools like BLAST and others, the raw nucleotide sequences of the *VP2* gene of CPV and CBuV were edited, aligned, and used for phylogenetic analysis. The nucleotide sequences of the *VP2* gene were compared with the CPV and CBuV sequences available in the National Centre for Biotechnology Information, using the MEGA XI program (Kumar *et al.,* 2018) and the phylogenetic relationship was established utilizing Maximum likelihood method.

Results and Discussion

Out of the 100 samples subjected to conventional PCR amplifying *VP2* gene fragment, 46 (46%) were found to be positive for CPV, producing an amplicon of 681 bp (Fig. 1). Nearly similar findings were reported by Dorlikar (2018) by testing 91 faecal samples; among these, 41 samples were found positive by PCR with a product size of 681 bp. The incidence rate of Canine parvovirus was found to be 45.05%. Soma *et al.* (2013) analysed a total of 233 diarrhoeic Canine faecal samples collected from veterinary clinics in Japan between January 2009 and December 2011 and reported a PCR positivity rate of 45.9% (107/233) for CPV. In the current study, out of 100 samples, 5 (5%) samples were found to be positive for CBuV using conventional PCR with an amplicon size of 202 bp (Fig. 1). Of which 04 samples were associated with gastroenteritis, and 01 sample was found positive without clinical signs. The results of our study are nearly similar to those reported by Acar *et al.* (2025), who reported a positivity rate of 4.20% (5/119) for CBuV in rectal swab samples collected from dogs with gastroenteritis. Similarly, Di Martino *et al.* (2020) reported a prevalence rate of 8.8% (13/147) and 5.0 % (3/60) of CBuV in gastroenteritis-affected dogs and healthy dogs in Italy, respectively.

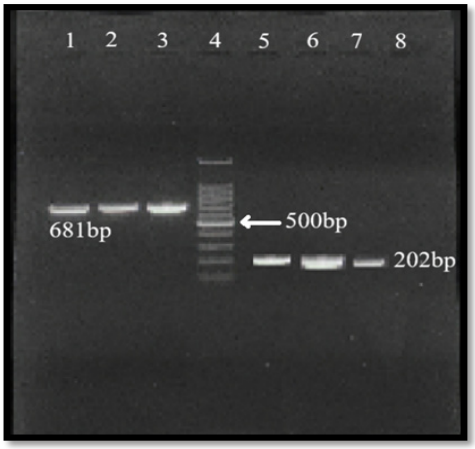

Fig. 1: Amplification of the *VP2* gene by conventional PCR of CPV and CBuV. Lane 1, 2, 3: CPV positive samples, Lane 5, 6, 7: CBuV positive samples, Lane 4: 100 bp ladder

Among the 46 CPV positive samples, 3 (6.52%) samples were also found to be positive for CBuV infection. Our results correlated with Ganji *et al*. (2022), who reported 7 of the 8 CBuV-positive samples co-infected with other canine enteric viruses, including CPV-2. Similarly, Abayli *et al*. (2023) from Turkey investigated 62 diarrheal dog samples previously tested for other viral pathogens (CPV-2, Canine coronavirus and Canine circovirus), CBuV was detected in two dogs (3.22%). One dog tested positive for 3 viruses, *i.e.*, Canine parvovirus, Canine bufavirus and Canine chaphamaparvovirus (CaChPV). Sun *et al.* (2019) from China investigated 540 samples from domestic dogs for Canine bufavirus, and found 10 (1.85%) samples positive for CBuV. They also found that all the CBuV-positive samples were co-infected with CPV. Their results were slightly different from our result where they found a low prevalence of CBuV (1.85%) compared to our study (6.52%).

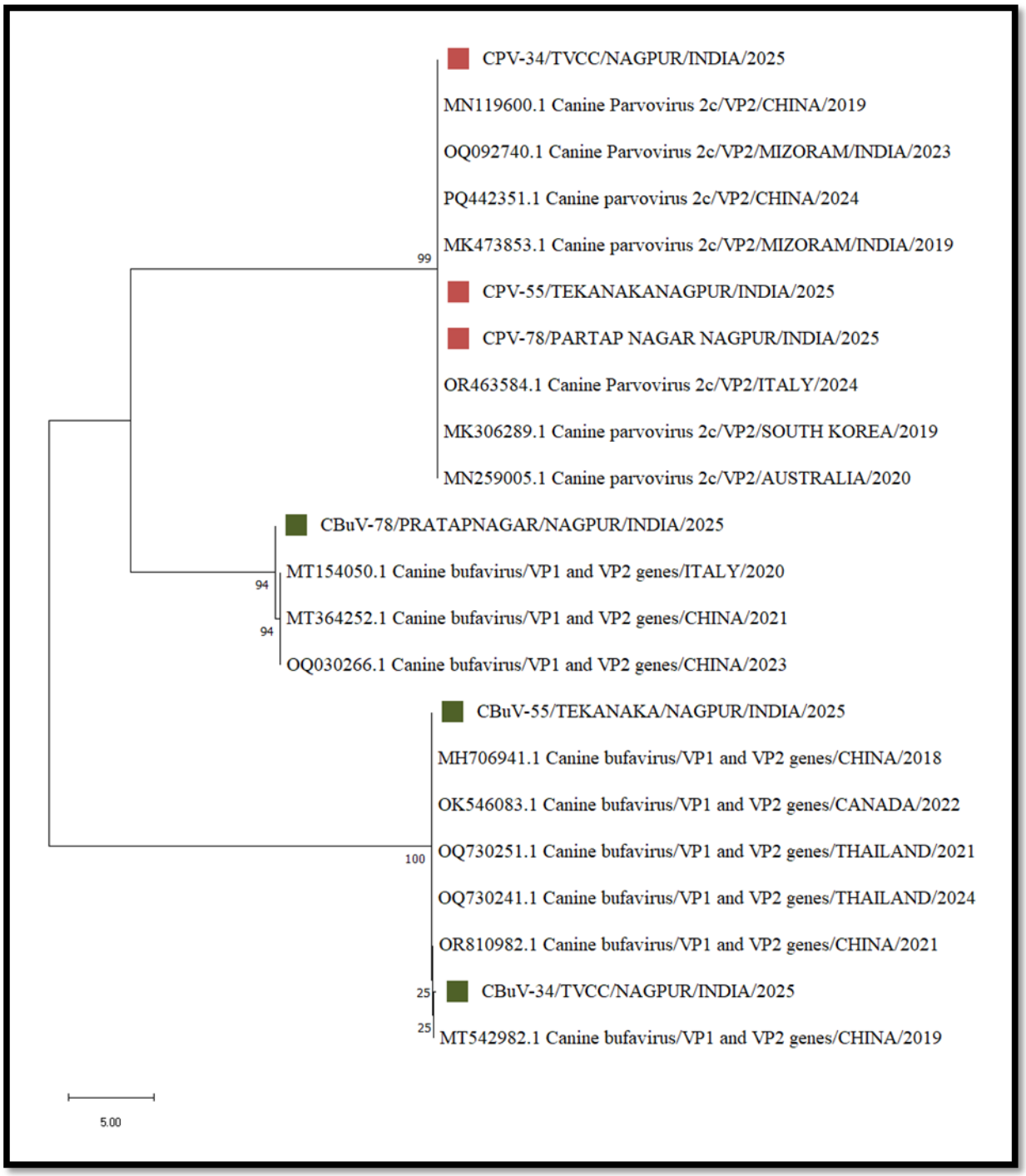

Fig. 2: Phylogenetic tree based on the partial VP2 gene sequences for CPV and CBuV.

Phylogenetic analysis comparing CPV-2 and CBuV sequences with global references showed that the two viruses formed distinct and separate clades, clearly differentiating them from each other (Fig. 2). The CPV-2 isolates (CPV-34, CPV-55, and CPV-78) exhibited 99.39% nucleotide homology with strains reported from Mizoram, India (Accession Nos. OQ097240.1 and MK473853.1), and showed similar homology with international CPV-2 sequences from China, South Korea, Italy, and Australia (MN119600.1, PQ442351.1, MK306289.1, OR463584.1, and MN259005.1). In contrast, the CBuV sequences (CBuV-34, CBuV-55, and CBuV-78) demonstrated 99.0-99.50% homology with global isolates from China, Italy, and Thailand (MH706941.1, OR810982.1, MT542982.1, MT364252.1, OQ030266.1, OK546083.1, MT154050.1, OQ730251.1, and OQ730241.1). The phylogenetic tree further indicated that, despite belonging to the genus *Protoparvovirus*, CBuV is genetically distant from CPV, as evidenced by less than 40% nucleotide identity between them. Similar observations were reported by Wang *et al*. (2020), suggesting that genomic recombination events may have contributed to the evolutionary divergence of CBuV from CPV.

Conclusion

The knowledge about new parvoviruses is still limited. Herein, we report for the first time the detection and comprehensive characterization of CBuV in Nagpur (MS), India. All CBuV strains in this study belonged to a novel CBuV-2 genotype. The current study forms a foundation for further studies on the epidemiology and genetic diversity of CBuV in India. The epidemiological data must be increased, and the pathogenic roles of both CBuV and CPV in the intestinal or extraintestinal systems still require elucidation.

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