CASE REPORT
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FATAL CANINE PARVOVIRUS-2C INFECTION IN A PUP FROM KERALA, INDIA: A DEFINITIVE DIAGNOSIS USING ARMS-PCR

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ABSTRACT

Canine parvovirus (CPV) is a highly contagious viral disease in dogs, especially affecting puppies. A five month old, male Labrador Retriever pup with anorexia, pyrexia, lethargy and haemorrhagic gastroenteritis with haematemesis and haematochezia was presented to the University Veterinary Hospital, Mannuthy, Kerala Veterinary and Animal Sciences University. Haematological analysis showed severe leukopaenia, with granulopaenia and lymphopaenia. Lateral flow assay for CPV yielded a positive result. Molecular diagnosis using amplification refractory mutation system polymerase chain reaction (ARMS-PCR) confirmed the presence of CPV-2c. Despite the initiation of supportive therapy, the pup succumbed to death the next day. This the second report of CPV-2c from a dog in Kerala and represents the pioneering use of multiplex ARMS-PCR for detecting CPV variants in Kerala.

Keywords: Canine parvovirus- 2c, Amplification refractory mutation system polymerase chain reaction, Haematemesis, Haematochezia

INTRODUCTION

Canine parvovirus-2 (CPV-2) is a small, non-enveloped, single stranded DNA virus belonging to the family carnivore protoparvovirus 1, with a genome size of around 5000 base pairs (Cote *et al*., 2024). It was first recognized in the late summer of 1978 from USA (Appel *et al*., 1978) as the etiological agent of an epizootic severe gastroenteritis of dogs. By the end of 1980, CPV-2 was completely replaced globally in dogs by a genetic and antigenic variant termed CPV-2a. Subsequently, the VP2 residue 426 changed from Asn to Asp and then from Asp to Glu in the so-called CPV-2b and CPV-2c respectively.

Charoenkul *et al*. (2019) conducted a comprehensive surveillance study in Thailand, spanning a period from 2016 to 2018, which highlighted the emergence of CPV-2c in both domestic dogs and cats. Their findings highlighted the growing distribution of this variant in Southeast Asia, reflecting its adaptability and potential for causing widespread outbreaks. In India, the occurrence of CPV-2c has been documented in various regions, with studies providing critical insights into its distribution. For instance, Harikrishnan *et al*. (2023) focused on Tamil Nadu, utilising ARMS-PCR to detect CPV-2c among dogs. Their research confirmed the presence of this variant, indicating its significant impact on canine health in the region. Similarly, in Kerala, Krishna *et al*. (2024) reported the detection of CPV-2c through conventional PCR methods followed by sequencing.

Most assays for typing CPV face limitations such as inconclusive results, high costs, specialized lab requirements, labour intensity, and the need for skilled technicians. Therefore, a simple, cost-effective, specific, rapid, and robust assay was needed for routine use in diagnostic labs. Amplification Refractory Mutation System PCR meets these needs by detecting and typing haplotypes or single nucleotide polymorphisms through differential PCR product sizes on agarose gel electrophoresis (Newton *et al*., 1989).

CASE HISTORY AND OBSERVATIONS

A five-month-old unvaccinated male Labrador Retriever puppy was presented to the University Veterinary Hospital in Mannuthy, displaying symptoms of lethargy, anorexia, fever, and haematemesis (Fig.1A and 1B) for the past three days, along with the onset of haematochezia (Fig.2) from the morning of day of presentation. On clinical examination, the puppy exhibited high fever with a body temperature of 103.6°F, congested mucous membranes, lymphadenopathy, tachypnoea (44 per minute), tachycardia (154 per minute), and dehydration. Diagnostic tests included a faecal sample analysis to rule out parasitic ova, a blood smear examination to rule out haemoparasitic infection, a complete blood count (CBC), and a lateral flow assay to detect CPV infection. The faecal sample and blood smear were negative for ova of parasites and haemoparasitic infection, respectively. Complete blood count revealed leukopaenia with a white blood cell (WBC) count of $2.4 \times 10^{3}/\mu L$, granulopaenia ($1 \times 10^{3}/\mu L$) and lymphopaenia ($1.1 \times 10^{3}/\mu L$). The lateral flow assay kit yielded a positive result for CPV from the faecal sample (Fig.3). The faecal sample was collected in a sterile vial containing phosphate-buffered saline and subjected to ARMS-PCR analysis (Chander *et al*., 2016) for

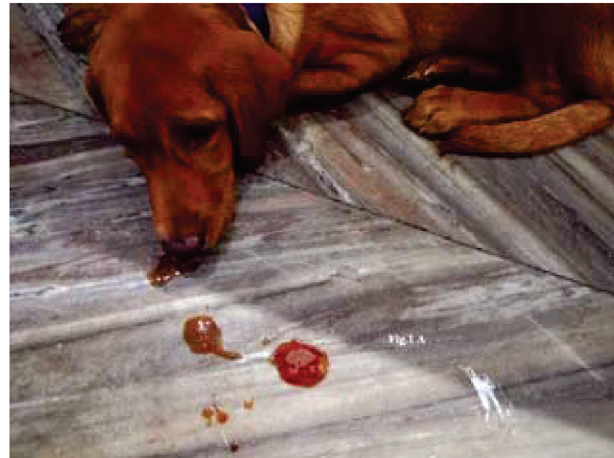

Fig. 1A and 1B: lethargic Labrador pup having haematemesis

Fig.2. Haematochezia

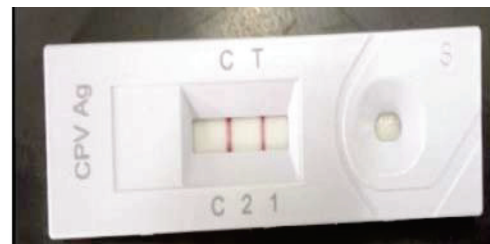

Fig. 3: Lateral flow kit: positive for

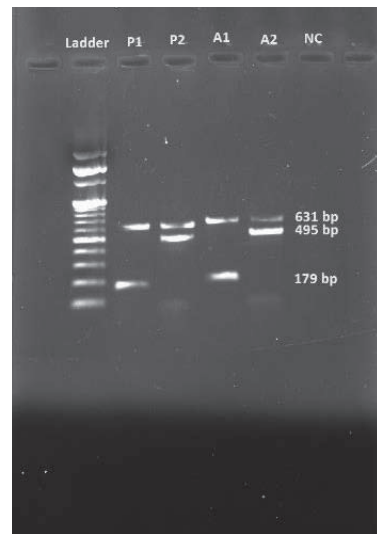

Fig. 4: Agarose gel (1.2%) showing PCR amplified product from positive CPV antigenic types. L: 100 bp DNA ladder, P1: PCR amplified product with CPV-2b/2c positive control (631 bp+179 bp), P2: PCR amplified product with CPV-2c positive control (631 bp+495 bp), A1: PCR amplified product with CPV2b/2c (631 bp+179 bp), A2: PCR amplified product with CPV-2c (631 bp + 495 bp), N1 and N2: Negative control.

identification of CPV-2 variant. Molecular diagnosis confirmed the presence of CPV with a positive result for CPV-2c, indicated by band sizes of 631 bp, 179 bp and 495 bp corresponding to CPV Genus, CPV-2b or 2c, and CPV-2c, respectively (Fig. 4). The positive sample were sequenced and submitted to NCBI Gen-bank and was assigned an accession number PQ037857.

TREATMENT AND DISCUSSION

Treatment was initiated prior to the receipt of PCR results, based on the preliminary diagnostic findings from CBC

and lateral flow assay. This approach was adopted to ensure that the patient received timely intervention, addressing the clinical signs of infection while awaiting the more specific molecular confirmation provided by the PCR test results. The treatment regimen commenced with the intravenous administration of Inj. amoxicillin-Sulbactam (12.5 mg/kg), Inj. Metronidazole (20 mg/kg), Inj. pantoprazole (1mg/kg) and Inj. Ondansetron at 0.5 mg/kg. Additionally, the patient received intravenous fluids to maintain hydration, alongside multivitamins delivered parenterally to ensure adequate nutritional support during the treatment period. Despite intensive care, the pup succumbed within one day due to severity of the infection.

While sequencing is not practical for routine use due to its cost and the technical expertise required, the ARMS-PCR method used in this study can efficiently differentiates between CPV-2a, 2b, and 2c, offering a significant advantage in diagnostic efficiency. Following ARMS-PCR, a positive reaction is confirmatory for the presence of the target allele (Little, 1995). Brown and Rogers (2001) suggested that severe neutropenia in cases of CPV enteritis can be attributed to a combination of factors, including endotoxemia, potential sepsis leading to the margination of neutrophils, a significant loss of neutrophils through the compromised intestinal wall, and the direct destruction of mitotically active myeloblasts by the virus. Goddard *et al*. (2008) found that leukopenia was a strong indicator of severe clinical CPV-2 infection, which aligns with our observations, as the affected animal ultimately succumbed to the disease. Viral-induced intestinal damage heightens the risk of coliform septicaemia. This can trigger a systemic inflammatory response, which may escalate to septic shock and result in mortality. Kalli *et al*. (2010) observed that puppies meeting the criteria for systemic inflammatory response syndrome (SIRS) characterized by a heart rate exceeding 140 beats per minute, a respiratory rate greater than 30 breaths per minute, and a body temperature either above 102.56°F (39.2 °C) or below 100.04°F (37.8 °C) experienced a higher mortality rate. These findings align with the observations in this case, where similar clinical manifestations were associated with a severe outcome.

Hernandez-Blanco and Catala-Lopez (2015) reported uncertainty about the cross-protection by the available CPV-2 vaccines which caused great concern to the veterinarians and pet owners. Sykes (2024) reported fatal myocarditis due to viral infection in pups infected *in utero* and pups without maternal immunity. The causes of death in acute CPV infections

include bacteraemia, endotoxaemia, and shock (Cote *et al*., 2024). In this case, death was likely attributable to severe diarrhoea and vomiting, which might have resulted in extreme fluid loss and dehydration, ultimately leading to shock and death. It is unlikely that myocarditis was the cause of death in this five-month-old pup.

SUMMARY

This case report describes a severe form of parvoviral infection due to CPV-2c in a young pup. A five-month-old male Labrador Retriever, with haemorrhagic gastroenteritis, was diagnosed with CPV-2c via ARMS-PCR. Despite supportive therapy, the pup's condition deteriorated rapidly, resulted in death the next day. This case points to the urgent necessity for advanced molecular diagnostic techniques, to ensure early and prompt diagnosis, facilitating timely and appropriate control measures.

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