

Status on the incidence of coronaviral infection (CCoV) in canine cases of haemorrhagic gastroenteritis by rapid lateral flow assay (LFA) in Namakkal

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Abstract

Canine coronavirus causes mild to severe gastroenteritis in dogs of all age groups, and co-infection with other enteric infections, especially with canine parvoviral enteritis caused by CPV-2, could result in mortality in puppies. Considering the need for clinical differentiation from the most fatal CPV-2 infection, risk of emergence of new pathogenic strains of coronavirus in dogs or other species and need for consideration of CCoV as core or non-core vaccine, an incidence study was undertaken in Namakkal region. Faecal samples were collected from the dogs presented with haemorrhagic gastroenteritis and tested by lateral flow assay test kits. All the cases were found to be negative for CCoV infection and no incidence could be recorded from this region.

Keywords: Canine coronavirus, Dogs, Haemorrhagic diarrhoea, Immunochromatography test, Incidence

Highlights

- Faecal samples from the dogs presented with haemorrhagic gastroenteritis were collected.
- Immunochromatography test kits were employed in the diagnosis of CCoV infection.
- No incidence of CCoV could be recorded in the dogs of different age groups and breeds from Namakkal region.

Canine coronavirus (CCoV) and canine parvovirus type 2 (CPV-2) are the most common etiological agents causing gastroenteritis in dogs worldwide. The CCoV is a single-stranded RNA virus causing mild to severe diarrhoea in dogs of all age groups and breeds with high morbidity (Appel, 1987). The virus destroys tips of mature epithelial cells on the intestinal villi, resulting in malabsorption. Most of the dogs recover within a week by appropriate therapeutic management. Since the disease is self-limiting and mortality is rare, the disease is rarely diagnosed in the laboratory (Desai *et al.*, 2020). However, CCoV can occasionally cause death in young puppies, especially when co-infected with canine parvovirus-2 (Hossain *et al.*, 2021).

In recent years, studies on coronaviruses have gained more importance due to the coronavirus outbreaks in humans. In addition, mutations in coronaviruses may result in serious diseases in new hosts like SARS-CoV-2 or cause changes in organ-tissue affinity (Timurkan *et al.*, 2021). Recently, apart from the classical enteric form, pantropic strains causing hypervirulent and multi-systemic infections are reported to be emerging (Decaro *et al.*, 2010).

However, data on CCoV infection in dogs have been limited, and most of the studies were based on serological studies (Yeşilbag *et al.*, 2004). A recent

study showed that puppies affected with CCoV exhibited haemorrhagic enteritis similar to that of CPV-2 infections (Pinto *et al.*, 2014). Hence, CCoV should always be considered in mind for the differential diagnosis of canine parvoviral enteritis caused by CPV-2 in dogs (Timurkan *et al.*, 2021).

At this point of time, an update has to be made in the vaccination regimen based on the CCoV prevalence as to whether the CCoV vaccine needs to be incorporated in the currently circulating core vaccines or can be used as a non-core vaccine in areas where the disease is prevalent. Published information on the incidence of CCoV in dogs from many states of India is still not available, including many districts of Tamil Nadu except Chennai. Hence, this paper reports the status of the incidence of canine coronavirus infection in dogs presented with haemorrhagic gastroenteritis from the Namakkal region of Tamil Nadu.

The dogs (n=50) presented with the signs of haemorrhagic gastroenteritis to the Infectious Unit of Clinics, Veterinary College and Research Institute, Namakkal, Tamil Nadu were randomly selected. The faecal samples were collected from dogs of different age groups, 0-3 months (n= 20), 3-6 months (n= 23) and 6-9 months (n= 7). The breeds included non-descript (n= 13), Labrador Retriever (n= 12), German

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Shepherd (n= 7), Chippiparai (n= 5), Dobermann Pinscher (n= 4) and other breeds (n= 9). All the cases had a history of no vaccination against the enteric diseases, CCoV and CPV.

The immunochromatography based lateral flow assay kits procured from Bionote, Inc. (Republic of Korea), Ubio Biotechnology systems Pvt. Ltd. (Quickvet®, Kochi) and J&G Biotech Ltd (PetX®, England) were used in this study. The faecal samples were collected by using the sterile swabs and dispensed immediately in the assay diluent tubes provided within the immunochromatography based lateral flow assay kits, which were stored at 2-8°C until further use. After allowing the tube for 1 minute to settle down the larger faecal particles, four drops of the supernatant were added into the sample hole(s) of the test kit. The results were interpreted within 10 minutes. The interpretation of the results is shown in Fig. 1. The positive result was indicated by the red lines both in control (C) and test (T) marks, whereas the negative result will be indicated by the red line only in C, not in T mark.

All the faecal samples from the above cases were negative by the lateral flow assay kits, and hence, no incidence of CCoV could be recorded in this region. Sakulwira *et al.* (2003) reported a prevalence of 12.8% for CCoV in dogs from Thailand by using semi-nested reverse transcriptase polymerase chain reaction (RT-PCR), and 77.7% of the CCoV cases were found to be co-infections with CPV-2. Worldwide, the emergence of pantropic CCoV from enteric strains could be witnessed by the previous studies in France and Belgium (Zicola *et al.*, 2012), Greece (Ntafis *et al.*, 2012), Brazil (Pinto *et al.*, 2014), Italy (Decaro *et al.*, 2012; Alfano *et al.*, 2019) and China (He *et al.*, 2020).

In India, serological and molecular-based detection of CCoV in dogs suggested a widespread distribution of CCoV in the canine population (Deka *et al.*, 2013; Agnihotri *et al.*, 2017). Deka *et al.* (2013) recorded a prevalence rate of 19.28% for CCoV infections in Assam, India by using sandwich ELISA. Agnihotri *et al.* (2017) reported an overall prevalence of 8.0% for CCoV infection in dogs by molecular detection (PCR) in Haryana, India. In Tamil Nadu, Sekar *et al.* (2003)

recorded a positivity of 16.8% for CCoV in dogs from Chennai city by using indirect immunofluorescence test (IFA). However, recently, Naveenkumar *et al.* (2020) recorded a negative prevalence of CCoV in dogs of gastroenteritis in Chennai by RT-PCR.

Desai *et al.* (2020) reported an overall prevalence of 4.58% for CCoV infection in dogs in Gujarat by immuno-chromatography (Bionote®) based lateral flow assay (LFA) tests kits. The prevalence for CCoV was recorded only in <3 months age group and no other age groups. The rapid CCoV antigen test kit was reported to show good diagnostic performance in a clinical setting, and no significant difference could be observed in comparison with polymerase chain reaction (PCR). With genomic mutation and recombination, coronaviruses have a huge potential for the emergence of new strains or novel virus as natural infection of SARS-CoV-2 was earlier reported in several domestic dogs (Hossain *et al.*, 2021).

Hence, periodical monitoring of CCoV infection in dogs with the signs of haemorrhagic or non-haemorrhagic gastroenteritis by molecular and serological epidemiology will help to determine the distribution of pantropic or enteric coronaviruses in the dog population and risk of possibility of emergence of novel strains in humans.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contributions: SS: Carried out the diagnosis of clinical cases by test kits and draft of the manuscript; GS: Involved in the revision of the manuscript.

Data availability statement: All data generated during this study are included in this article. The corresponding author is willing to provide the raw data upon reasonable request.

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Fig. 1. Lateral flow assay test kit showing negativity for the CCov in faecal samples collected from canine cases of haemorrhagic gastroenteritis

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