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Seroprevalence of Coronaviruses FCoV and SARS-CoV-2 in domestic cats in Kerala

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Abstract

Seroprevalence of Feline coronavirus (FCoV) and Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) were analysed in a set of serum samples collected from domestic cats presented to tertiary care veterinary clinics during the first COVID pandemic wave in Thiruvananthapuram in 2021. In 156 samples analysed, virus-specific indirect ELISA detected the presence of FCoV antibodies in 72 samples (46.2 per cent) and SARS-CoV-2 antibodies in 12 samples (7.7per cent). Among the samples showing seropositivity for FCoV, 3.84 per cent had a significantly high antibody titre exceeding 1:6400 indicating the possible prevalence of Feline infectious peritonitis virus (FIPV), a serious and often fatal disease arising from mutations in FCoV. Among the 12 SARS-CoV-2 positive samples, six exhibited ability to neutralise SARS-CoV-2 pseudovirion infectivity, further confirming the specificity of the detection. Our data did not exhibit significant cross-reactivity between SARS-CoV-2 and FCoV antibodies. Even though there is no confirmed SARS-CoV-2 transmission from cats to humans, domestic cats may act as reservoirs, facilitate reverse zoonotic events and possibly promote viral mutations. Our observations reiterate the need of one health approach to prepare better for zoonotic threats and future pandemics.

Keywords: *FCoV, SARS-CoV-2, cats, seroprevalence*

Coronaviruses (CoVs) are positive-sense single stranded RNA viruses within the family *Coronaviridae*, subfamily Orthocoronavirinae classified into four genera: *alphacoronavirus*, *betacorona*virus, *gammacoronavirus* and *deltacoronavirus*. These viruses cause respiratory, enteric or systemic infections in mammals and are notable for their ability to cross species barriers, leading to outbreaks such as Severe acute respiratory syndrome (SARS) in 2003, Middle-East respiratory syndrome (MERS) in 2012 and COVID-19 in 2019. Their high mutation rates facilitate adaptation and host expansion (Dhama *et al*., 2020).

Feline coronavirus (FCoV), an *Alphacoronavirus,* commonly found in cats exists as two biotypes: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV). FECV causes mild enteric illness, while FIPV results in severe systemic granulomatous supportive disease, feline infectious peritonitis (FIP), which is sporadic, but fatal (Tekes

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and Thiel, 2016). Being serologically, morphologically and antigenically identical, FIPV and FCoV present a diagnostic challenge (Pedersen *et al*., 1981). Serological diagnosis is more valuable, as virus detection in FIP is challenging even with quantitative polymerase chain reaction (qPCR).

SARS-CoV-2, the *Betacoronavirus* shares 96.2 per cent sequence similarity with bat coronaviruses, suggesting an ancestral link (Ji *et al*., 2020). The virus utilises the conserved angiotensin converting enzyme-2 (ACE-2) receptor, allowing infection across multiple species (Liu *et al*., 2020). This has resulted in documented cases of transmission from humans to domestic and wild animals including dogs, mink, lion, tiger and white-tailed deer raising concerns about the risk of emergence of more infectious strains (Palermo *et al*., 2022; Cui *et al.*, 2022). In this study, we conducted analyses to assess the seroprevalence of FCoV and SARS-CoV-2 among domestic cats in Thiruvananthapuram district, Kerala, India.

Materials and methods

Sample collection

The study employed a randomised, sequential sampling approach. A total of 156 serum samples were collected from cats presented at the Multi-Specialty Veterinary Hospital, and District Veterinary Centre, Thiruvananthapuram, over nine-months from February to October 2021. The period coincided with the first COVID-19 wave in the district (Fig. 1). Animal owners were informed of the study objectives and verbal consent was obtained to use leftover blood samples for research.

Detection of antibodies to FCoV and SARS-CoV-2

Serum samples were tested for FCoV antibodies using Ingezim Corona Felino ELISA Kit (Cat No: 16.FCV. K1, Ingenasa, Spain) following the manufacturer's protocol. For the assay, 100 µl of the cat serum samples diluted 1:200 in sample buffer were used. High reactivity samples (OD_{450}> 1.25) were serially diluted up to 1: 12,800 to confirm the presence of FIPV specific antibodies. As more than 80 per cent of the FIPV-infected cats show an antibody titre greater than 1:6400, any sample surpassing this threshold was considered as FIPV-positive as per kit instructions. Antibodies specific to spike (S) proteins of SARS-CoV-2 in cat serum samples were tested further using multispecies IngezimCovid 19 S VET kit (Cat No. R.10.CoS. K1, Ingenasa, Spain). The kit detects specific IgG antibodies against the SARS-CoV-2 S protein in minks, ferrets, cats and dogs, with optical density cut-off values designated for each species. Notably, the assay demonstrated no cross-reactivity with antibodies to other coronaviruses. Each serum sample was diluted 1: 100 in the sample buffer provided and 100 µl was used and the assay was carried out following the manufacturer's instructions.

Production of SARS-CoV-2 pseudotyped virus

To produce secreted-alkaline phosphatase (SEAP) expressing vesicular stomatitis virus (VSV)-pseudotyped with SARS-CoV-2 spike protein, HEK293T cells at 80 per cent confluency were transfected with pCMV14-3X-Flag-SARS-CoV-2-S plasmid (Addgene plasmid # 145780) using polyethyleneimine (PEI) and simultaneously infected with G*VSV ΔG SEAP pseudovirus (Kerafast, # EH1035-PM). After 6 hours of incubation, uninfected virus was removed, and following washes, media with optiMEM supplemented with 1X

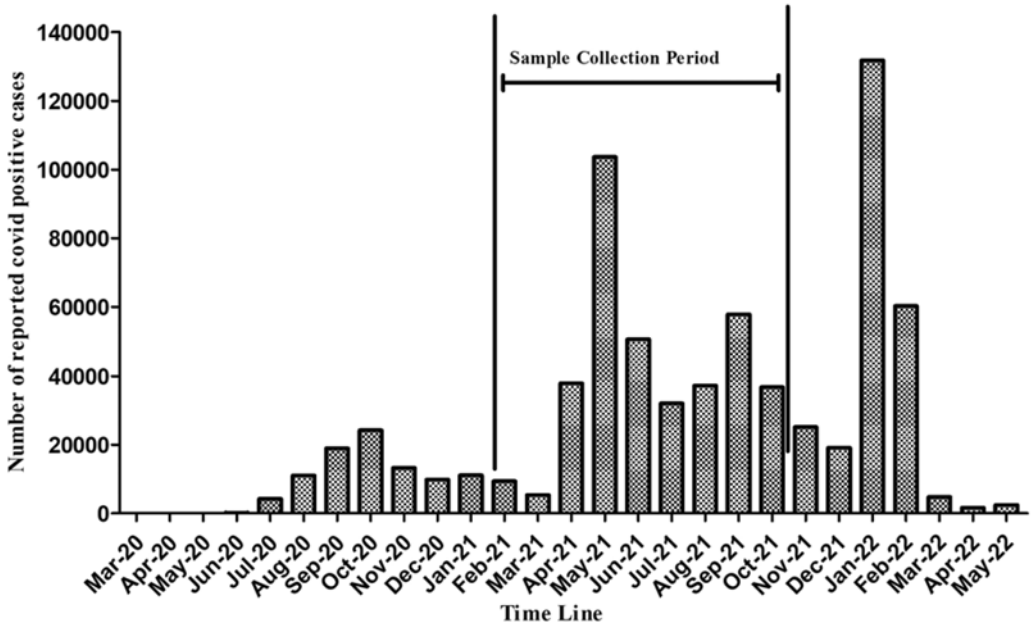

Fig. 1. Monthly COVID-19 positive cases in Thiruvananthapuram District (March 2020–May 2022) (Data: https://dashboard.kerala.gov.in/covid/dailyreporting-view-public-districtwise.php) and sample collection periods

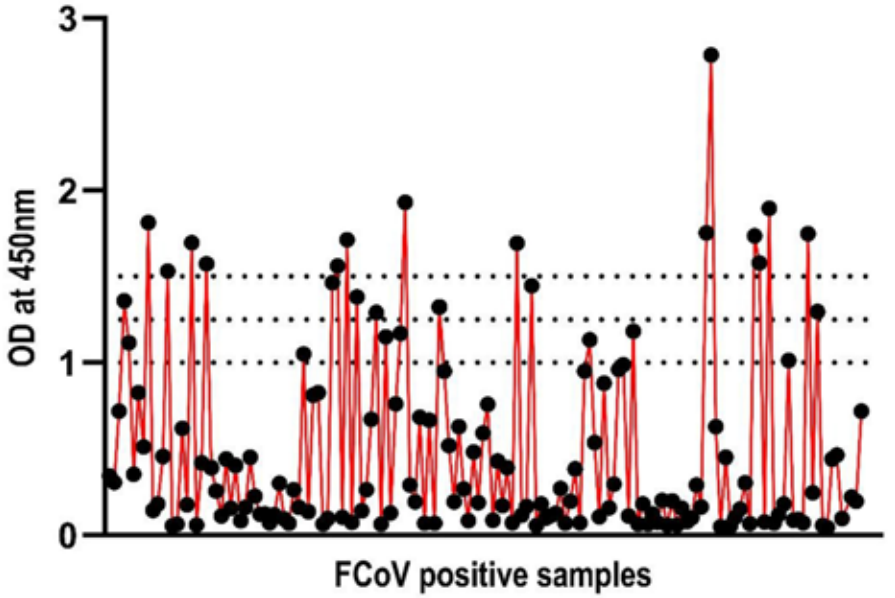

Fig. 2. Indirect ELISA reactivity of cat serum samples against FCoV. The OD_{450} values for 156 cat serum samples are plotted against FCoV antigen, with each dot representing an individual sample. Dotted lines indicate the cutoff values.

Non-Essential Aminoacids (NEAA), 1X Glutamax and 1X Penicillin /Streptomycin was added. Cells were incubated at 37°C in 5 per cent CO_2. Supernatants were collected at 48 h, adsorbed on HEK293T cells to remove residual G*VSV ΔG SEAP pseudovirus. The harvested supernatant containing VSV-SARS-CoV-2-S-SEAP pseudovirus was centrifuged to remove cell debris and stored at -80˚C.

Serum neutralisation test with SARS-CoV-2 pseudovirions

Cat serum samples were heat-inactivated at 56ºC for 30 min. Equal volume diluted test sera (1:40 dilution in DMEM) and VSV-SARS-CoV-2-S-SEAP pseudovirus (1:5 dilution in DMEM) were mixed and incubated at 37ºC for one hour. Anti SARS-CoV-2 spike antibody (Genetex # 1353565µg/µL) served as the positive control. Confluent HEK293T cells transfected with human ACE2 (hACE2) (Lupitha *et al*., 2022), in 96-well plates were incubated with 100 µL per well of the pseudovirus-serum mixture in triplicate at 37°C with 5 per cent CO_2 for 2 hours. The inoculum was replaced with DMEM with 2 per cent heat inactivated fetal bovine serum and plates were incubated at 37°C with 5 per cent CO_2 for 24 hours. Culture supernatant was collected, centrifuged at 1200 rpm for 10 min, heat inactivated at 65°C for 10 min to remove the endogenous alkaline phosphatase and 10 µL was used for the assay. A volume of 90 µL of Quanti-Blue SEAP reagent (InvivoGen, # rep-qbs) was added and incubated for 14 h at 37°C. SEAP activity was measured at 615nm using TECAN i-Control infinite 200 Pro. The neutralisation percentage was calculated from SEAP assay using the following formula

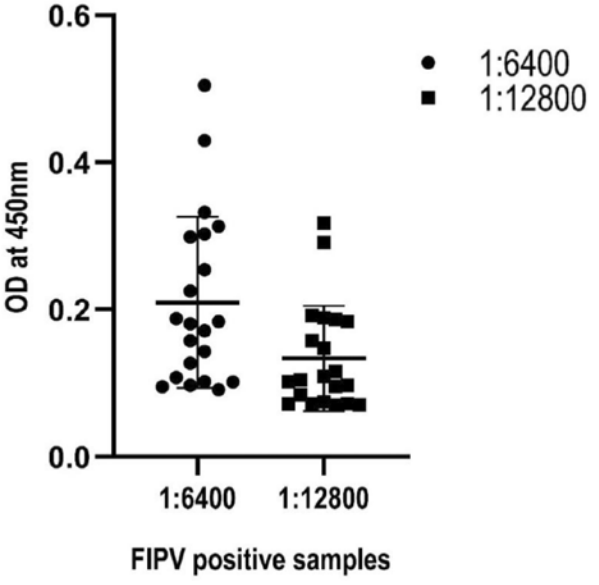

Fig.3. Antibodies to FIPV assessed by indirect ELISA. The 21 serum samples with a titre above 1.25 were serially diluted and the reactivity determined by iELISA.

$$\text{Percentage neutralisation} = \frac{\text{Absorbance of infection alone control} - \text{Absorbance of sample}}{\text{Absorbance of infection alone control}} \times 100$$

Samples with a neutralising percentage between 30-60 per cent were considered positive for SARS-CoV-2 neutralising antibodies in the serum.

Statistical analysis

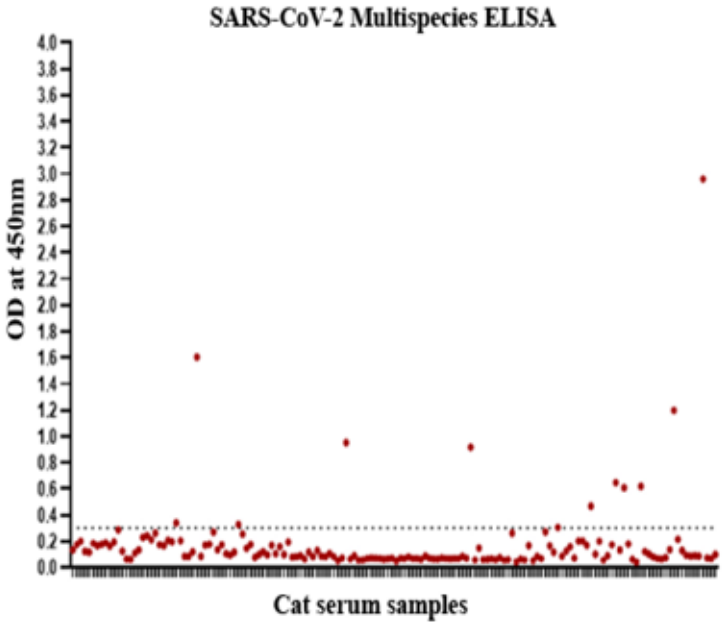

Fig.4. Indirect ELISA for anti-SARS-CoV-2 spike protein antibodies in cat serum. X-axis represents 156 samples, numbered sequentially from 1 to 156, Y-axis displays the OD values. Dotted line indicates the cutoff value of 0.30.

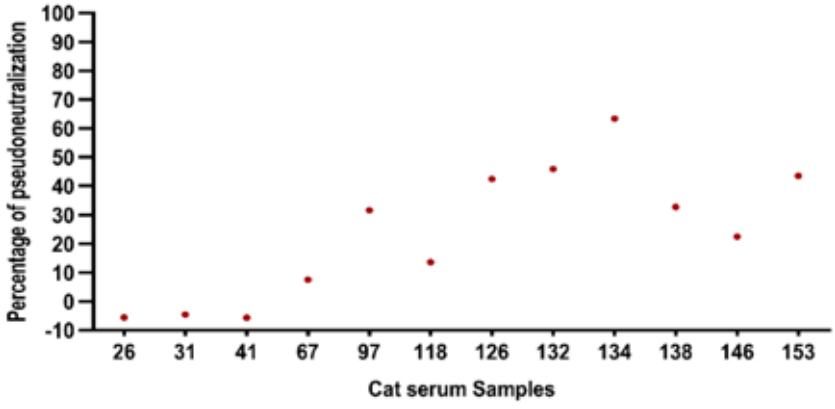

Fig.5. VSV-SARS-CoV-2-S-SEAP pseudovirus neut-ralisation test.

The GraphPad Prism (Version 8.0) software was used for all statistical analysis. Experiments were performed in three technical replicates and values were calculated based on Mean±SD.

Results and discussion

Detection of Feline Coronaviruses antibodies in cats' sera by indirect ELISA

A total of 156 serum samples collected from cats between February 2021 and October 2021 was screened for FCoV-specific antibodies using a commercial kit, with the cut-off threshold OD_{450} of 0.29 (Fig. 2). Results revealed that 46.15 per cent of samples were positive for FCoV specific antibodies. Among these, 17.9 per cent exhibited OD_{450} values over 1.00, 13.46 per cent surpassed 1.25 and 8.97 per cent exceeded 1.5. To confirm whether the samples were positive for FIPV, samples with OD_{450} values exceeding 1.25 underwent serial dilutions with the OD_{450} cut-off of 0.27. Six samples (3.84 per cent) exhibited antibody titres greater than 1:6400 with two exceeding 1:12800 (Fig 3).

Antibody response to SARS-CoV-2 by indirect ELISA

All 156 serum samples were screened for antibodies targeting the S domain of SARS-CoV-2 with the cut-off value set at an OD_{450} of 0.30. The results confirmed specific antibody response in 7.7 per cent samples (Fig. 4). Notably, the serum sample (#153) exhibited higher

absorbance than the positive control across all the replicates tested.

SARS-CoV-2 pseudovirus neutralisation efficiency by cat serum samples

To confirm the presence of SARS-CoV-2 neutralising antibodies, the ELISA-positive samples were tested using pseudovirus neutralisation assay based on the SEAP Reporter Gene (Condor Capcha *et al*., 2021). Among the 12 serum samples tested, six showed negligible neutralising activity, while the remaining six exhibited a reduction in SEAP expression yielding neutralisation percentage of 30 per cent to 60 per cent (Fig.5).

Coronaviruses are known for their ability to cross species barriers. Among companion animals, domestic cats seem to be particularly susceptible to viral infection from their human owners as seen during the SARS-CoV outbreak in 2003 (Martina *et al*., 2003). However, no confirmed cases of FCoV infection to humans have been documented. We observed a seroprevalence of 46.2 per cent FCoV antibodies in cat serum samples. This seroprevalence rate is higher than those reported in prior studies conducted in Australia (34 %) (Bell *et al*., 2006), Italy (39 %) (Spada *et al*., 2016), and Turkey (37%) (Tekelioglu *et al*., 2015). Detection of FCoV antibodies is instrumental in controlling the virus in cat populations enabling the effective separation of infected and non-infected cats (Addie *et al*., 1992).

Feline infectious peritonitis virus emerges by mutations in the avirulent FECV in a small percentage of infected cats, subsequently causing a fatal disease (Vennema *et al*., 1998; Pedersen, 2014). About 5-12 per cent of cats seropositive for FCoV eventually develop FIP, influenced by host and virus-related factors (Addie *et al*., 1995). Cats affected by FIP typically exhibit elevated antibody levels against FCoV and in our study, 3.84 per cent of samples showed ELISA titres greater than 1:6400, with two samples exceeding 1:12800. However, clinically less severe FIP can mimic FECV which shares antigenicity (Ishida *et al*., 1987) making serological diagnosis alone not reliable for confirming an FIP diagnosis. The outbreak of SARS-CoV-2 in December 2019 led to a global pandemic (Wu *et al*., 2020).

In this study, we detected SARS-CoV-2 antibodies in cat serum samples using ELISA and pseudovirus neutralisiation test (PVNT). The S1 domain of SARS CoV-2 spike protein contains the receptor binding domain (RBD) and harbor highly immunogenic epitopes that elicits robust neutralising antibody response (Yadav *et al*., 2021). Of the 156 serum samples analysed, 7.7 per cent were positive by ELISA with 3.9 per cent demonstrating neutralisation percentage of 30 to 60 in PVNT. This suggests that a subset of animals may have generated low-affinity antibodies insufficient to inhibit spike-ACE2 interaction. Comparable seropositivity rates in RBD-based ELISAs have been reported in other countries, including 14.7 per cent in China, 4.2 per cent in Germany and Italy, 3.3 per cent in the United Kingdom and 6.4 per cent in Spain (Schulz *et al*., 2021; Zhang *et al*., 2020).

The SARS-CoV-2-specific indirect ELISA showed a 4.5 per cent seropositivity rate among FCoV-positive samples. However, no significant correlation was found in OD_{450} values between SARS-CoV-2-positive and FCoV-positive samples, as assessed by the Wilcoxon Signed Rank test ($P > 0.05$). This result suggests that the ELISA kits used detect distinct antibodies, with negligible cross-reactivity. SARS-CoV-2 and FCoV belong to different virus genera, sharing only 44.5 per cent nucleotide identity and even lower amino acid homology (Sharun *et al*., 2020). Nevertheless, structural similarities between the RBD regions of FCoV and SARS-CoV-2 spike proteins, and the ACE-2 receptors in humans and cats, pose a chance for FCoV to cause human infection similar to SARS-CoV-2 causing infection in cats (Gao *et al*., 2022). Currently, there is no evidence of SARS-CoV-2 transmission from cats to humans. However, studies have shown that SARS-CoV-2 can spread among cats via respiratory droplets (Shi *et al*., 2020).

Unlike the COVID-19 disease surveillance system among humans (van Oosterhout *et al*., 2021), vigilance for SARS-CoV-2 infections among animal hosts, including domestic cats, remains limited (Cui *et al*., 2022). Our results reiterate the continued need for of pet animal surveillance to mitigate future pandemics considering the zoonotic sources emerging viruses.

Conclusion

This study highlights the significant prevalence of FCoV antibodies in domestic cats in this part of the country, which highlights the widespread exposure of the virus in feline populations. The detection of FCoV antibodies serves as an important tool for controlling the spread of the virus, particularly in differentiating infected from non-infected cats. While FCoV can lead to FIP, serological testing alone is not a definitive method for diagnosing FIP, due to antigenic similarities between FCoV and FECV. Additionally, the detection of SARS-CoV-2 antibodies in domestic cats suggests potential interspecies transmission, though no direct evidence of cat-to-human transmission has been found. Our findings emphasise the importance of continued surveillance of SARS-CoV-2 and other zoonotic viruses in pet animals to prevent future pandemics. Given the structural similarities between FCoV and SARS-CoV-2, further research is necessary to explore the potential risks of interspecies transmission and the implications for public health. In general, this study reinforces the need for vigilance in monitoring zoonotic diseases in domestic animals, particularly in the context of the ongoing global challenges posed by emerging infectious diseases.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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