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Repeated detection of SARS-CoV-2 in pet dogs in Ibadan, Oyo State, Nigeria: a cause for vigilance

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Abstract

Background The COVID-19 pandemic of 2020 was unprecedented in its devastating impact on the global economy, public health, travel and tourism, education, sports, religion, and social lives. Studies conducted thereafter on the disease and its causative agent, SARS-CoV-2, have highlighted the need for effective and sustainable public health interventions.

Methods This study investigated the prevalence and endemicity of SARS-CoV-2 infection in pet dogs using immunochromatography assay (IC) and quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) of their blood, rectal swabs, and nasal swabs in Ibadan, Oyo State, Nigeria between 2022 and 2024.

Key findings For the IC, positivity rates of 11.7% (23/197), 85.7% (6/7), and 100% (3/3) were recorded for 2022, 2023 and 2024 while for the RT-qPCR, positivity rates of 37.9% (11/29), 33.3% (2/6) and 100% (3/3) were recorded for 2022, 2023 and 2024. This repeated detection of SARS-CoV-2 in three of the dogs tested over the three-year period suggests continuous shedding of the virus by these animals and indicates endemicity of the virus in the study area. Findings highlight the urgent need for optimized SARS-CoV-2 rapid diagnostic tools tailored for veterinary applications to ensure rapid and reliable detection of the virus, especially in resource-constrained settings.

Conclusion Considering the zoonotic nature of SARS-CoV-2 and its potential for mutation into more virulent strains that can be transmissible to humans, the findings of this study have significant implications for public health and implementation of One Health strategies by policymakers, and highlight the need for robust SARS-CoV-2 surveillance in domestic animals to mitigate potential zoonotic risks.

Keywords SARS-CoV-2, Endemicity, Rapid diagnostic test, RT-qPCR, Dogs, Nigeria

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Introduction

Over the years, humans have been faced with the menace of emerging and re-emerging infectious diseases that constitute enormous threats and burdens to global health [1, 2], resulting in epidemics with attendant high morbidity and mortality in human and animal populations. In the last four decades, outbreaks of several newly emerging and re-emerging infectious diseases have occurred despite the implementation of prevention and control strategies. In December 2019, SARS coronavirus type 2 (SARS-CoV-2) emerged in Wuhan, China, likely originating in bats and spreading to humans through yet unidentified intermediary animals [3, 4]. This triggered a global pandemic of Coronavirus Disease 2019 (COVID-19), which has affected 777,315,739 persons globally and caused 7,083,869 deaths as of January 12, 2025 (https://data.who.int/dashboards/covid19/). Genomic studies revealed that SARS-CoV-2 may have initially been transmitted from bats to humans before escalating via human-to-human transmission [5].

Previous surveillance efforts in various parts of the world [6–20] have shown that dogs and cats are susceptible to SARS-CoV-2, especially in households where humans are infected or during quarantine periods, thus establishing cross-species (i.e., human-animal) transmission. The seroprevalence ranges from 0.2% in Dutch dogs without documented contact with COVID-19-positive people to 53% in dogs living in COVID-19-positive households in France [21].

Happi et al. [5]. detected SARS-CoV-2 RNA in dogs, cats, chickens, ducks, pigs, domestic ruminants, and lizards in Nigeria, thus demonstrating the high transmissibility of the virus across vertebrate hosts in the country. Agusi et al. [22]. also demonstrated presence of SARS-CoV-2 ELISA-reactive antibodies in dogs, rabbits and pangolins in Nigeria though most of the ELISA-reactive samples failed the virus neutralization and indirect immunofluorescence confirmation tests. These findings underscore the need for continuous surveillance of the virus within the country as well as prompt and reliable diagnostic tools, especially rapid diagnostic tests, which are crucial for the success of public health interventions.

Despite the likely endemicity of SARS-CoV-2 infection in the post-pandemic era [23, 24], evidence of more severe disease upon reinfection [25, 26], and the identification of the southern region of Nigeria as a high-risk zone for SARS-CoV-2 transmission following analysis of transmission dynamics [27], longitudinal studies to investigate the endemicity of the disease in animals are lacking in Nigeria, hence this study. This may not be unconnected with the high cost of the RT-qPCR diagnostic test which limits its use in resource-limited settings such as sub-Saharan Africa where rapid and more cost-effective diagnostic tests are preferable. This study was therefore designed to investigate the prevalence and endemicity of SARS-CoV-2 infection in pet dogs presented at the Veterinary Teaching Hospital, University of Ibadan, Ibadan, Oyo State, Nigeria between 2022 and 2024.

Materials and methods

Study location and design

The study location was the Veterinary Teaching Hospital, University of Ibadan, Ibadan, Oyo State, Nigeria. It is a public tertiary hospital for animal care where clinical veterinary services are rendered to animals in the state as well as referral cases from neighboring States. The hospital is also a teaching hospital of the University of Ibadan for the clinical training of veterinary students.

The study used a longitudinal design where pet dogs (symptomatic and asymptomatic) of all ages whose owners routinely presented and verbally consented to the sampling were enrolled in 2022 and tested for SARS-CoV-2 antigen using immunochromatography assay (IC) and quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR). Subsequently, the IC-positive dogs whose owners further verbally consented to and presented for re-sampling in 2023 and 2024 were tested for evidence of active SARS-CoV-2 infection using IC and RT-qPCR.

Sample collection

In 2022 (May– October), blood (5 ml) was aseptically obtained once from 197 dogs through the cephalic vein (with minimal pain ensured) and dispensed into properly labelled EDTA and plain tubes. Most of the dogs were of the boerboel (29.4% of the dogs) and German Shepherd (23.9%) breeds. Caucasian (11.7%), Pit Bull (6.6%), and Rottweiler (6.1%) breeds of dogs were also among the dogs sampled. Breed distribution and other metadata are elucidated in the supplementary information of this paper.

In 2023 (March– April) and 2024 (March), blood and swabs (nasal and rectal) were obtained once each year from IC-positive dogs of 2022. In all cases, samples were transported to the laboratory within 24 h at 3 °C– 15 °C. Blood samples were centrifuged at 2500–3000 rpm for five minutes to separate the plasma/serum into cryovial and microcentrifuge tubes for storage at -20 °C until use. The swabs were collected in 1 ml virus transport medium (VTM) and also stored at -20 °C. Seven dogs were sampled in 2023 out of the population of IC-positive dogs of 2022 while three dogs were sampled in 2024 out of the population of dogs sampled in 2023 (Table 1).

SARS-CoV-2 detection by immunochromatography assay

The Standard Q COVID-19 Ag Test Kit (SD Biosensor, Korea), a rapid chromatographic immunoassay designed for qualitative detection of SARS-CoV-2 nucleocapsid

Table 1 Sampling distribution and tests conducted on samples

	2022	2023			2024		
	Plasma /Serum	Plasma /Serum	Nasal swab	Rectal swab	Plasma /Serum	Nasal swab	Rectal swab
Number of samples	197	7	5	6	3	3	3
Number of samples tested by IC	197 (23)	7 (5)	5 (2)	6 (4)	3 (3)	3 (3)	3 (1)
Number of samples tested by RT-qPCR	29[a] (11)	5[b] (1)	2[b] (0)	4[b] (1)	3 (3)	3 (3)	3 (3)

Key: IC– Immunochromatography Assay; RT-qPCR– Quantitative reverse transcriptase polymerase chain reaction; [a] All IC-positive plus 6 randomly selected IC-negative samples were tested; [b] Only IC-positive samples were tested; Number of positive samples are indicated in brackets

antigens in human nasopharyngeal samples was adapted for use in this study for SARS-CoV-2 detection in canine plasma, nasal swabs, and rectal swabs. Briefly, 50 µl of plasma or VTM was introduced into the specimen well of test device following which assay result appeared on the device result window within 10–20 min. The appearance of a test line and a control line whether bright or faint was interpreted as a positive assay result while a negative assay result was seen as the appearance of only a control line. The appearance of a test line only or the appearance of no line were interpreted as an invalid assay. An animal was considered positive by IC when any of its plasma, nasal swab or rectal swab was IC-positive.

SARS-CoV-2 detection by multiplex real-time RT-qPCR

Viral RNA was extracted from plasma, rectal swabs and nasal swabs in aseptic conditions using the QIAamp Viral RNA Mini kit according to the manufacturer's instructions following which real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) for SARS-CoV-2 was carried out on all RNA extracts. RT-qPCR was done using the Allplex SARS-CoV-2 Assay kit (Seegene, Korea) which targets the nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase (RdRP)/spike (S) genes. ROX and CY5 were used as reporters for the N and RdRP/S genes, respectively without any corresponding quencher, while FAM and TAMRA were used as reporter and quencher for the E gene. The thermal cycling conditions using the QuantStudio 5 (Applied Biosystems, USA) were: 30 s at 60 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C, and lastly 30 s at 60 °C. The positive control sample used for the RT-qPCR validations was provided in the Allplex kit by the manufacturer. An animal was considered positive by RT-qPCR when any of its plasma, nasal swab or rectal swab was RT-qPCR-positive with a Ct value < 40.

Data analysis

Statistical analyses were performed using the IBM SPSS Statistics (ver. 23) and RStudio (2023.12.0 Build 369) software while data visualization was done using Microsoft PowerPoint and Microsoft Excel software (Office 2016, ver. 1808). Differences between the cycle threshold (Ct) values obtained were compared across the years using Mann-Whitney U test while Pearson's Chi-square test was conducted to test the strength of association between the test results and type of sample for the IC and RT-qPCR. Tests of association between test results (IC and RT-qPCR) and animal demographics (age groups, breed and sex) were conducted using Chi-squared or Fisher's Exact Test as appropriate. Statistical differences were considered significant at $p < 0.05$.

Results

Dogs tested in this study were mostly asymptomatic with IC positivity rates of 11.7% (23/197) and 100% (3/3) for the years 2022 and 2024 respectively while for the RT-qPCR, positivity rates of 37.9% (11/29) and 100% (3/3) were recorded for 2022 and 2024 (Fig. 1). 86.4% of RT-qPCR-positive samples were IC-positive (19 out of 22 samples) while 46.3% of IC-positive samples were RT-qPCR-positive (19 out of 41 samples) (Table 2). There were no significant associations ($p > 0.05$) between the IC results and each of the demographic groups as well as between the RT-qPCR results and each of the demographic groups.

In 2022, 12.1% (7/58) and 44.4% (4/9) of the boerboel breed of dogs tested were positive by IC and RT-qPCR respectively. Also, 10.6% (5/47) and 42.9% (3/7) of the German Shepherd breed of dogs tested were positive by IC and RT-qPCR respectively. 10.5% (10/95) and 33.3% (4/12) of female dogs tested in 2022 were positive by IC and RT-qPCR respectively. Similarly, 14.8% (13/88) and 43.8% (7/16) of male dogs tested in 2022 were positive by IC and RT-qPCR respectively. Also, 65.2% (15/23) of IC-positive dogs and 81.8% (9/11) of RT-qPCR-positive dogs were above a year old.

The N gene was amplified in all RT-qPCR-positive samples while the E and RdRP/S genes were amplified in 63.6% (14/22) and 9.1% (2/22) of the RT-qPCR-positive samples, respectively. There was no significant association ($p = 0.74$) between test result and type of sample for RT-qPCR but a significant association ($p = 0.00003$) between test result and type of sample for IC test was observed with plasma samples having the most negative IC results (85.0%) compared to rectal swabs (44.4%) and nasal swabs (37.5%). The 2024 mean Ct value of 28.06 ± 1.66 (mean ± standard deviation) was significantly lower than those of 2023 (34.20 ± 0.45; $p = 0.0015$) and

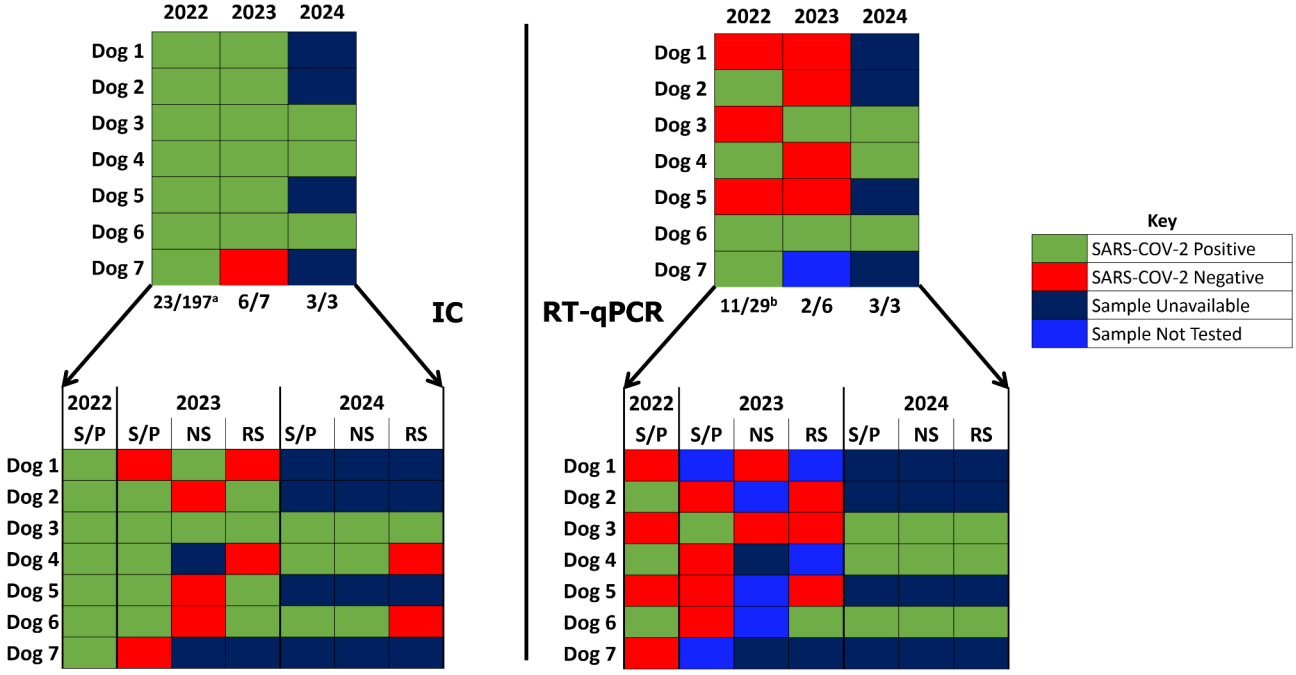

Fig. 1 Heatmap showing SARS-COV-2 detection results in seven dogs using IC and RT-qPCR. Virus was detected in at least two time points in dogs 1–6 using IC but only in 3 dogs using RT-qPCR. Dog 6 which consistently showed SARS-CoV-2 presence within the 3 years in both IC and RT-qPCR was reported to have later died in 2024. Virus detection in serum/plasma (S/P), nasal swab (NS) and rectal swab (RS) samples are shown in colour codes while positivity rates for each year are shown as number of positive samples/number of samples tested. Results of only 7 out of the 197 and 29 dogs tested by IC (**a**) and RT-qPCR (**b**) respectively are shown for the year 2022

Table 2 Contingency table showing overall distribution of SARS-CoV-2 detection results using real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) and immunochromatography assay (IC)

		RT-qPCR		TOTAL
		Positive	Negative	
IC	Positive	19	22	**41**
	Negative	3	5	**8**
TOTAL		**22**	**27**	**49**

2022 (32.64 ± 4.63; $p = 0.01$), both of which were not significantly different from each other ($p = 0.93$).

In samples tested by RT-qPCR in 2022, none of the samples showed all three gene amplifications on RT-qPCR simultaneously. The N gene was the sole amplified gene in 6 samples, while the RdRP/S gene was not amplified in all samples except one. The Ct values of the N gene expressed by the animals ranged from 24 to 38.

In 2023, all 6 dogs sampled were IC-positive (100% positivity rate) with only one of the dogs being IC-positive in the plasma, nasal swab and rectal swab while three other dogs were IC-positive in only the plasma and rectal swab (Fig. 1). Only two dogs in 2023 were RT-qPCR-positive (33.3% positivity rate) with one dog expressing all three SARS-CoV-2 target genes in its plasma only with low viral loads (Ct values of 34 each), while the other dog expressed only the SARS-CoV-2 E and N genes in its rectal swab also with low viral loads comprising Ct values of 35 and 34 respectively (Fig. 2).

In 2024, all 3 dogs sampled were IC-positive and RT-qPCR-positive (100% positivity rate), expressing only the SARS-CoV-2 E and N genes in their plasma, nasal swabs and rectal swabs. The E gene amplification had a Ct range of 26–31 while that of the N gene was 26–30 (Fig. 2). The rectal swabs only of two of the dogs were however IC-negative (Fig. 1). Two of these dogs, a female adult Rottweiler (Dog 3) and a female adult Boerboel (Dog 6) living together were presented sick by the owner for the 2024 sampling with similar clinical presentations, including jaundice, pyrexia, and mild tick infestation without any obvious respiratory signs as at the time of presentation. They were both placed on antibiotics by the vet following a tentative diagnosis of canine ehrlichiosis, but Dog 6, which later showed melena, died a few days afterward.

Discussion

The detection of SARSCoV-2 infection in dogs in this study supports available increasing evidence t that several animal species can be naturally infected with SARS-CoV-2 including dogs, cats, tigers, lions and minks, whereas livestock like pigs, chickens and ducks are far less susceptible to infection with the virus [28]. According to Chomel and Sun [29], pets such as dogs and cats

Fig. 2 Heatmap showing SARS-COV-2 RT-qPCR results. Target SARS-COV-2 sites included nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase/spike RdRP/S genes in various samples obtained from seven of the dogs tested within three years. Repeated amplification of SARS-COV-2 genes in 2023 and 2024 was observed in Dog 3 while that of Dog 4 was observed in 2022 and 2024. Persistent amplification of SARS-COV-2 genes were however evident in Dog 6 for the three years. The Cycle threshold (Ct) values of the amplified genes are shown

are becoming an integral part of households sharing human lifestyles, bedrooms, and beds. These practices may predispose to potential public health risks, including increased emergence of zoonoses. Dog owners should be on the lookout for clinical signs associated with SARS-CoV-2 infection in dogs including mild and transitory nasal discharge, cough and diarrhoea [30], even though evidence suggests infected dogs are mostly asymptomatic [9, 31] as observed in this study, and may not even shed the virus [31]. Considering that dogs are increasingly becoming common household pets in Nigeria, having close cohabitation with their human owners, surveillance for SARS-CoV-2 in these species has become important from a One Health perspective especially since the infectious virus can persist in the environment outside the host for hours to days [32], thus enabling fomite transmission.

Dog 6 which was positive for the virus throughout the three years of the study but later died in 2024 may have been overwhelmed by the presence of the virus and other comorbidities which led to grave prognosis for the bitch. Veterinary practitioners may therefore consider asymptomatic canine SARS-CoV-2 infection as a comorbidity capable of worsening prognosis of illnesses in dogs.

The detection of SARS-CoV-2 antigen and RNA in this study indicates natural infection with the virus and this may be attributable to the prolonged and close contact between the infected individuals and their pet dogs. Nishiura et al. [30]. noted that RT-qPCR and other laboratory tests can detect asymptomatic cases and confirm asymptomatic infection. However, the mechanisms by which asymptomatic carriers transmit SARS-CoV-2 and the extent of such transmission are still unclear [33]. In addition, the detection of 11.7% and 43.5% SARS-CoV-2 positivity by IC and RT-qPCR, respectively in dogs in this study, most of which were asymptomatic, suggests that they are a potential zoonotic risk for their owners and other humans with whom they have close interactions. Moreover, this finding is consistent with previous reports [12, 34–37] of SARS-CoV-2 detection in asymptomatic dogs. It is however unclear how these dogs contracted the virus although we suspect it could have been transmitted from their human owners. The inability to determine the source of infection for these dogs is a major limitation of our study as the owners did not give consent for their samples to be collected.

In this study, the repeated detection of SARS-CoV-2 by both IC and RT-qPCR in the blood, nasal swab, and rectal swab of these dogs over a course of three years may also be attributed to persistence of the virus after initial infection in tissues where it replicates without causing clinical disease. This has already been reported in humans [38] and has been identified as a risk factor for cancer development [39]. It is also an indication of active virus shedding to the environment which makes reinfection of a previously infected animal possible as previously described for most respiratory viruses including influenza viruses, rhinoviruses, and coronaviruses [23, 24]. This detection of the SARS-CoV-2 genetic material in dog samples after months of initial detection is consistent

with the findings of Hamer et al. [12]. who conducted a longitudinal study of 76 cats and dogs living with at least one SARS-CoV-2-infected human in Texas and found evidence of viral RNA persistence for at least 25 days after initial sampling in some of the animals. Machkovech et al. [40]. identified immunocompromising conditions as a risk factor for persistent SARS-CoV-2 infection, enabling viral evolution and transmission. They also underscored the possible role in persistent SARS-CoV-2 infection of viral replication in body tissues outside the upper respiratory tract especially since the ACE2 receptors, TMPRSS2 co-receptors and others required for viral infectivity are expressed in most cells of the body.

The significant association between test result and type of sample for IC in this study suggests the unsuitability of plasma or serum samples for SARS-CoV-2 detection using IC in dogs. This is consistent with the findings of Okwuraiwe et al. [42]. who reported that plasma was an inefficient sample type for COVID-19 diagnosis due to the low viral titers in the blood samples of asymptomatic COVID-19 cases. Nevertheless, the potential risk of COVID-19 transmission through transfusion of blood products as a result of viral shedding in plasma or serum following upper or lower respiratory tract infection is present. The use of RT-qPCR instead of IC in SARS-CoV-2 detection in blood should therefore be considered.

The IC kit (Standard Q COVID-19 Ag Test Kit) used in this study was validated and optimized for high specificity and sensitivity by the manufacturer (as stated in the kit leaflet) to detect SARS-CoV-2 nucleocapsid antigens in human nasopharyngeal samples only (Image in supplementary information) which makes it unsuitable for this study. Nevertheless, the kit was used for this study despite this limitation because it was the popular and readily available IC commercial kit for use in COVID-19 diagnosis and research as at the time this research project was conducted when there were no commercial kits validated for use with animal specimens. Similarly, the RT-qPCR kit (Allplex™ SARS-CoV-2 Assay kit) used in this study was also a popular and readily available RT-qPCR commercial kit for use in COVID-19 diagnosis and research at the time, and was validated and optimized by the manufacturer to detect the SARS-CoV-2 nucleocapsid (N), envelope (E), and RNA-dependent RNA polymerase (RdRP)/spike (S) genes in RNA samples extracted from human sputum and respiratory tract specimens using the Seegene NIMBUS and STARlet extraction kits. We however considered and used the readily available QIAamp Viral RNA Mini kit as a comparable alternative to the Seegene NIMBUS and STARlet extraction kits for RNA extraction. Though RT-qPCR is globally regarded as the gold standard in SARS-CoV-2 detection due to its high sensitivity and specificity [43], the RT-qPCR kit used in this study has been recently reported to be less suitable for SARS-CoV-2 detection in animals when compared to AviMol Dri Kit (AVICENNA, Dubai, United Arab Emirates) which was credited with the production of consistent results [5]. These aforementioned unsuitability of the IC and RT-qPCR kits could therefore have contributed to the discordance observed between the IC and qPCR results.

Repeated freeze-thaw cycles together with the – 20 °C storage facility used especially for the 2022 and 2023 samples which were stored for a long time before qPCR analysis in March 2024 could have negatively affected sample quality. Consequently, this could have contributed to the significantly lower 2024 mean Ct value observed for RT-qPCR analysis, the discordance observed between the IC and qPCR results, and the lack of significant association between the qPCR test result and type of samples analyzed for the study.

The accuracy of the results from the IC and RT-qPCR kits could be adversely affected by the high mutation rate of the SARS-CoV-2 [44, 45]. This was however mitigated in the RT-qPCR by the use of three target genes, and could be responsible for the poor amplification of the RdRP/S gene.

Conclusion

In this study, repeated detection of SARS-CoV-2 in the dogs in at least two time points over the course of three years was demonstrated. This highlights the endemicity of the virus in the region especially since the infected animals were actively shedding the virus as suggested by the presence of viral RNA and antigens in the nasal and rectal swabs. The animals also could potentially possess reservoir body sites for the continual propagation of the virus. This study has also revealed the need for SARS-CoV-2 commercial test kits optimized and validated for use with animal specimens to ensure reliable detection of the virus in animals. This however calls for continuous genomic sequence surveillance of the virus to detect variants that may evade detection by existing diagnostic kits. Longitudinal surveillance programs with intersectoral collaboration are particularly important for understanding transmission dynamics across populations which will be useful for policymaking especially in One Health interventions. The role of policymakers in this regard by ensuring sufficient funding for such research and development activities is paramount.

The RT-qPCR detection of SARS-CoV-2 infection in dogs in this study not only establishes the cross-species spread of the virus, but it also has significant implications for public health, as these dogs may become carriers/reservoirs with the potential for virus shedding into the environment. Intersectoral collaboration involving public health agencies, veterinary services, environmental scientists, and other stakeholders will enhance early

detection, response, and mitigation strategies to control the virus in humans, animals, and the environment. Further studies to unravel the role of other pets, domestic animals and wildlife in the epidemiology of the disease in Nigeria based on the One Health approach are advocated. In addition, as previously reported [46], we recommend that dogs in COVID-19-positive households be quarantined either at home or in holding facilities until proven to be PCR-negative.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04647-6.

Supplementary Material 1

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Author contributions
Conceptualization: OJO, ODO, HCT; Funding acquisition: OJO, HCT, BAS; Methodology: OJO, PIK, DOB, CCN, OOA, OO, HAN, OAB, KLZ; Investigation: OJO, PIK, CCN, OAB, KLZ; Formal analysis: PIK, DOB, CCN, OJO, HAN, KLZ; Data curation: PIK, CCN, DOB; Project administration: OJO, ODO, OO, HCT; Supervision: OJO, HCT, ODO, BAS; Writing– original draft: OJO, PIK; Review and editing: All authors.

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Data availability
Data is provided within the manuscript and also as supplementary.

Declarations

Ethics approval and consent to participate
Ethical approval for this study was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/093–0624/10). Also, informed consent of Dog owners was sought orally before enrollment of dogs into the study.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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