



SARS-CoV-2 Delta Variant Does Not Sustain Replication in Companion Dogs: Insights from an Experimental Infection Model

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Abstract | The potential role of companion animals in the transmission and pathogenesis of SARS-CoV-2 remains poorly understood. This study aimed to evaluate the susceptibility of domestic dogs to a human-derived SARS-CoV-2 Delta variant under experimental conditions. Seronegative, clinically healthy dogs were randomly assigned to control and experimental groups. The experimental group was intranasally inoculated with the strain SARS-CoV-2/Indian-Delta/KZ_Almaty/07.2021. Clinical signs were monitored, and samples were collected for hematology, biochemistry, serology (ELISA and microneutralization assay), RT-PCR, and histopathology over a 21-day period. Infected dogs exhibited mild, transient clinical signs including diarrhea, slight fever (up to 40.1°C), and mild weight loss. RT-PCR detected viral RNA only in rectal swabs on day 3 post-infection. Hematological changes included temporary reductions in leukocyte, erythrocyte, and lymphocyte counts. Antibody responses were detected by microneutralization assay and ELISA, with the former showing sustained increases through day 21. Histological examination revealed mild bronchial and alveolar inflammation, with no evidence of viral pneumonia. All control animals remained clinically and pathologically normal. While SARS-CoV-2 exposure in dogs elicited mild clinical signs and measurable antibody responses, there was no evidence of sustained viral replication or active shedding. These findings suggest that dogs are not likely to serve as reservoirs or vectors for SARS-CoV-2 transmission. Continued research is essential to monitor the interspecies dynamics of emerging coronaviruses within the One Health framework.

Keywords | SARS-CoV-2, Dogs, Susceptibility, Delta variant, Experimental infection, Serology, RT-PCR, Histopathology, Zoonosis

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The coronavirus infection caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), initially identified in late 2019, is believed to be a mutated variant of a previously circulating coronavirus, with bats considered the primary natural reservoir (Zhang and Holmes 2020; Mallapaty 2020; Zhou *et al.*, 2020; Andersen *et al.*, 2020). Upon zoonotic transmission to humans, viruses can undergo further mutations, often enabling them to evade host immune defenses and increasing the risk of severe disease. Such events can transform animal-origin pathogens into serious public health threats, as exemplified by the human immunodeficiency virus (HIV), which crossed the species barrier from non-human primates (Sharp and Hahn, 2011).

Although current evidence suggests a bat origin for SARS-CoV-2, the intermediate host(s) facilitating its spillover into the human population remain unidentified. The exact transmission pathway to humans has not been fully elucidated. Despite widespread implementation of public health measures, gaps remain in our understanding of the virus's transmission mechanisms and epidemiological dynamics. In this context, examining the potential role of animals, particularly companion and domestic species in the transmission and persistence of SARS-CoV-2 requires ongoing investigation (Mulugeta *et al.*, 2020).

Coronaviruses, members of the family *Coronaviridae*, are enveloped, positive-sense RNA viruses that infect a broad range of animal species, including humans (Dhama *et al.*, 2020). It is widely accepted that previous coronavirus outbreaks, such as SARS and MERS, resulted from interspecies transmission events, typically originating in animals (Dhama *et al.*, 2020; Sit *et al.*, 2020). However, the susceptibility of domestic and companion animals to SARS-CoV-2 and their potential to transmit the virus under natural conditions remain incompletely understood.

Improved knowledge of viral host range, interspecies transmission routes, and host-virus interactions is essential for assessing zoonotic risks and guiding future surveillance strategies. Insights gained from studies on SARS-CoV-2 in animals may also inform the development of control strategies, including diagnostics, therapeutics, and preventive measures. Therefore, the present study aimed to investigate the susceptibility of selected companion animals to SARS-CoV-2 through experimental infection, contributing to a better understanding of the virus's potential animal reservoirs and transmission dynamics.

VIRUS

The "SARS-CoV-2/Indian-Delta/KZ_Almaty/07.2021" strain was obtained from the original virus isolate by adaptation in Vero E6 cell culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum and antibiotics. The infectious viral activity was assessed by titration in 1–2-day-old Vero cell cultures maintained in 96-well microplates. The viral titer was calculated using the method of Reed and Muench (Reed and Muench 1938) and expressed as $\log_{10}$ TCID₅₀/mL (Zhugunissov *et al.*, 2022). The virus had a titer of $10^6 \log_{10}$ TCID₅₀.

ANIMALS

Prior to the experiment, all dogs were quarantined for a period of one month at the Experimental Animal Facility. During this time, the animals were monitored daily for clinical signs of illness. To ensure the absence of prior SARS-CoV-2 exposure, all dogs were screened for viral RNA using a RT-qPCR assay. Nasopharyngeal swabs were analyzed with the *AmpliSens® Cov-Bat-FL* diagnostic kit (AmpliSens®, Russia), following the manufacturer's protocol.

To further confirm the seronegative status of the animals prior to experimental infection, serum samples were collected and tested for the presence of total antibodies against SARS-CoV-2. Serological screening was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit, *SARS-CoV-2-AT total ELISA-BEST-Vet* (Vector-Best, Russia). Only animals confirmed to be both RT-PCR-negative and seronegative were included in the study to ensure experimental integrity.

For the study, two groups of outbred dogs were randomly assigned into an experimental group and a control group. Each group consisting of five clinically healthy animals (n=5). All animals were between 1.5 and 2 months of age, with an average body weight ranging from 2.5 to 3.0 kg. To ensure uniformity, the maximum allowable intergroup weight difference was limited to ± 0.3 kg.

INFECTION PROCEDURE

Animals in the experimental group were intranasally inoculated with the SARS-CoV-2 strain "SARS-CoV-2/Indian-Delta/KZ_Almaty/07.2021" at a dose of 0.5 mL per animal, containing $10^6 \log_{10}$ TCID₅₀/0.1 mL. The inoculum was evenly distributed between the nostrils (0.25 mL per nostril). Control animals remained uninfected (Figure 1).

Following the inoculation, all animals were monitored daily for 21 days. Observations included assessments of general

health status and the development of clinical signs associated with SARS-CoV-2 infection.

Blood samples were collected from all animals on days 7, 14, and 21 post-infection to evaluate the humoral immune response. Antibody titers were measured using microneutralization assays (MNA) and enzyme-linked immunosorbent assay (ELISA).

On days 7, 14, and 21 of the experiment, one animal from each group (infected and control) was euthanized to collect lung tissues for histopathological examination. Euthanasia was performed by exsanguination via severance of the jugular vein, carotid artery, and trachea, to avoid potential cellular artifacts associated with chemical euthanasia agents (Figure 1).

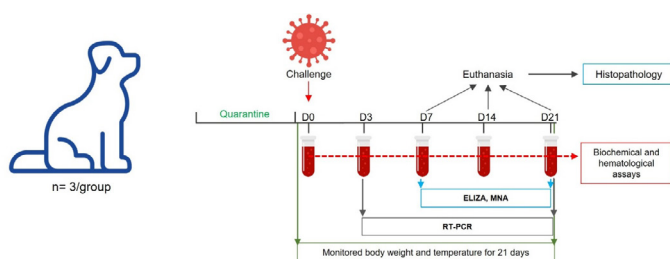


Figure 1: Study design and timeline of the SARS-CoV-2 Delta strain challenge study in dogs.

Viral RNA was extracted from 140 μ L of virus-containing material using the ALPREP magnetic bead-based extraction kit, following the manufacturer's instructions (Ali *et al.*, 2017). SARS-CoV-2 RNA was detected using the ALSENSE-SARS-CoV-2 RT-qPCR kit (Algimed, Belarus), which targets three loci of the *orf1ab* gene and the *S*, *E*, and *N* genes. Samples with cycle threshold (Ct) values ≤ 37 were considered positive for SARS-CoV-2 (Wu *et al.*, 2020).

ELISA

The SARS-CoV-2 antibody titer in dogs challenged with the Delta strain was assessed using ELISA. Briefly, 96-well plates were coated overnight with 0.1 μ g of purified S, M, and N proteins and then blocked with 2% BSA for 1 hour at room temperature. Diluted sera were added to each well, followed by incubation with goat anti-mouse antibodies conjugated with HRP for 1 hour at 37°C after washing three times with commercial saline. The reaction was stopped with 2M H₂SO₄, and the absorbance was read at 450/630 nm using an ELISA plate reader (Nurpeisova *et al.*, 2022).

MICRONEUTRALIZATION ASSAY (MNA)

Neutralizing antibody levels were determined by the inhibition of cytopathic effects (CPE). Briefly, Vero cells (3×10^5 cells/ml) were cultured in 48-well microtiter plates. A pretreated serum-virus mixture, containing an equal vol-

ume of diluted serum and SARS-CoV (200 TCID₅₀/ml), was inoculated into Vero cells and incubated for 6 days at 37°C in a 5% CO₂ environment. The CPE of each well was recorded daily. The highest serum dilution that completely prevented CPE in 50% of the wells was defined as the serum titer, with the virus control (no serum) showing complete CPE (Nurpeisova *et al.*, 2022).

HEMATOLOGICAL AND BIOCHEMICAL BLOOD ANALYSIS

Blood samples were collected from the caudal vein using sterile syringes and transferred into tubes with and without EDTA. Hematological analysis was performed using a Sysmex XN-1000 automatic hematology analyzer (Sysmex, Landskrona, Sweden) to measure hemoglobin, hematocrit, red blood cells (RBCs), eosinophils, monocytes, and lymphocytes.

Biochemical parameters were analyzed in serum samples using an A25 BioSystems autoanalyzer (BioSystems, Barcelona, Spain) and included: total protein, urea, creatinine, glucose, total cholesterol, total bilirubin, conjugated bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). All reagents were supplied by BioSystems (Spain).

HISTOPATHOLOGICAL EXAMINATION

Lung tissues collected from euthanized animals were fixed in 10% neutral-buffered formalin for histological analysis. Paraffin-embedded tissue blocks were sectioned into 5–6 μ m slices using a HEOTION ERM 3100 semi-automatic rotary microtome and MS-2 microtome. Sections were stained with hematoxylin and eosin (H and E) using a Leica S4040 tissue processor. Prepared slides were examined under a binocular microscope (MBI-6) at varying magnifications to assess structural changes in pulmonary tissue (Hu *et al.*, 2013).

ETHICS STATEMENT

All animal studies were conducted per national and international laws and guidelines for handling laboratory animals. The protocol was approved by the Committee on the Ethics of Experiments on Animals of the Research Institute for Biological Safety Problems (RIBSP) (permit number: 0912/25). The animal experiments in this study adheres to the ARRIVE guidelines to ensure transparency, reproducibility and animal welfare.

All SARS-CoV-2 challenge experiments were carried out at RIBSP's Biosafety Level 3 (BSL-3) and Animal Biosafety Level 3 (ABSL-3) facilities.

STATISTICAL ANALYSIS

All experimental data was analysed using Graphpad Prism

RESULTS

CLINICAL MONITORING AND HEMATOLOGICAL EVALUATION

During the observation period, the general clinical condition of dogs in the experimental group was moderately depressed during the initial post-infection days.

Hematological and biochemical parameters were assessed on days 3, 7, 14, and 21 post-infection (Table 1).

Table 1: Clinical, hematological, and biochemical findings in dogs infected with the virus strain (SARS-CoV-2/Indian-Delta/KZ_Almaty/07.2021).

Hematological parameters	Study days				
	Normal	III day	VII day	XIV day	XXI day
Hemoglobin, g/dl	12.0–18.0	11.02 ± 6.66	11.00 ± 5.56	14.5 ± 7.56	15.5 ± 7.56
Hematocrit, %	37–55	35.15 ± 0.02	35.25 ± 0.19	43.25 ± 0.17	45.25 ± 0.19
Red blood cells, ×10 ⁶ /μL	5.0–8.5	4.1 ± 0.5	4.0 ± 0.2	5.4 ± 0.3	5.3 ± 0.4
Eosinophils, %	2–10	4.4 ± 0.1	3.9 ± 0.9	3.5 ± 0.3	3.8 ± 0.3
Monocytes, %	3–10	2.9 ± 1.2	2.3 ± 1.2	3.1 ± 1.2	3.1 ± 1.4
Lymphocytes, %	12–30	10.7 ± 1.0	9.7 ± 0.7	12.7 ± 1.0	11.7 ± 1.0
Leukocytes	4.9–15.4	2.6 ± 0.2	2.5 ± 0.7	2.9 ± 0.6	3.1 ± 0.6
Blood biochemistry parameters	Study days				
	Normal	III day	VII day	XIV day	XXI day
Total protein, g/dL	5.5–7.5	7.6 ± 0.4	7.1 ± 5.7	7.4 ± 1.2	7.3 ± 1.0
Urea, mg/dL	7–27	6.6 ± 0.8	6.2 ± 1.4	6.6 ± 1.1	6.6 ± 1.1
Creatinine, mg/dL	0.5–1.6	1.4 ± 4.1	0.9 ± 5.1	0.5 ± 2.3	1.7 ± 4.1
Glucose, mg/dL	70–140	75.0 ± 1.9	78.5 ± 1.4	78.2 ± 2.2	77.2 ± 2.2
Bilirubin, total, mg/dL	0.1–0.6	0.009 ± 0.001	0.009 ± 0.002	0.010 ± 0.004	0.010 ± 0.004
AST, U/L	5–55	52.25 ± 0.06	51.45 ± 0.32	51.92 ± 0.05	51.92 ± 0.03
ALT, U/L	10–125	10.07 ± 0.01	11.05 ± 0.01	11.31 ± 0.03	11.31 ± 0.00

Note: AST: aspartate aminotransferase; ALT: alanine aminotransferase.

The results revealed a notable decrease in white blood cell count (– 46%), red blood cells (– 5%), and lymphocytes (– 13%), along with a 5% reduction in hematocrit is below the lower limit of the normal range on days 3 and 7 in infected animals. These changes coincided with mild diarrhea and lethargy.

Biochemical analysis showed no statistically significant differences between the experimental and control groups ($p > 0.05$), with values remaining within normal ranges. Small, non-significant elevations in total protein, bilirubin, and ALT were observed but were not deemed pathologically relevant.

DETECTION OF VIRAL RNA BY RT-PCR

Total RNA was extracted from swabs collected from multiple anatomical sites (nasopharynx, oropharynx, ocular mucosa, rectum) on days 3, 7, 14, and 21 (Table 2). RT-qPCR analysis revealed transient SARS-CoV-2 RNA detection only in rectal swabs on day 3 from two infected animals. No viral RNA was detected in other anatomical sites or at later time points.

Table 2: Detection of SARS-CoV-2 RNA in anatomical swabs via RT-qPCR.

Sample, n=3 technical replicates	Study days			
	III day	VII day	XIV day	XXI day
Nasopharynx	---	---	---	---
Ocular mucosa	---	---	---	---
Rectum	++- (Ct 34.2, 35.1)	+- (Ct 36.8)	---	---
Oropharynx	---	---	---	---

Note: “+” indicates a positive RT-qPCR result; “–” indicates a negative result. n = 3 refers to the number of animals sampled per anatomical site at each time point. Samples were considered positive when amplification was detected with a cycle threshold (Ct) value of ≤ 37 .

As a result of RT-qPCR testing of RNA samples, positive results were obtained on day 3 from two dogs that had received the viral inoculum intranasally. These animals exhibited frequent loose stools with signs of diarrhea beginning on the third day of the study. Clinically, the infected dogs presented with a moderately depressed state and signs of exhaustion. This condition persisted throughout the observation period and was accompanied by minor increases in body temperature, reaching 39.7–40.1°C between days 3 and 8 post-infection. In addition, the experimental group demonstrated progressive weight loss starting from day 4, which began to normalize by day 8 and remained stable for the remainder of the study (Figure 2). In contrast, animals in the control group showed no deviations in somatic or neurological status during the entire observation period.

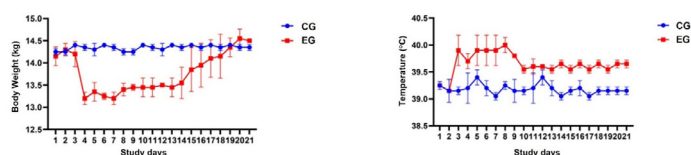


Figure 2: Body weight and temperature changes in dogs post-infection with SARS-CoV-2. Data represent mean $\pm$ SEM (n=5 per group). CG: control group; EG: experimental group.

SEROLOGICAL RESPONSE

Neutralizing antibody titers were assessed using both the microneutralization assay (MNA) and ELISA. MNA detected increasing antibody titers over time, with geometric mean titers (GMTs) rising from 3.03 on day 7 to 84.31 on day 21 (Table 3). In contrast, ELISA only detected antibodies on day 7, suggesting lower sensitivity compared to MNA in this context. All ELISA-positive dogs were negative for viral RNA by RT-PCR at the time of antibody detection, indicating no active viral shedding.

Table 3: Geometric mean titers (GMT) and 95% confidence intervals (CI) from MNA and ELISA in infected dogs.

Test	On day 7 after the first injection		On day 14 after the first injection		On day 21 after the first injection	
	GMT	95% CI	GMT	95% CI	GMT	95% CI
MNA	3.03	3.2-3.2	27.85	32.0-32.0	84.31	89.6-89.6
ELISA	3.03	Not applicable	Not detected	Not applicable	Not detected	Not applicable

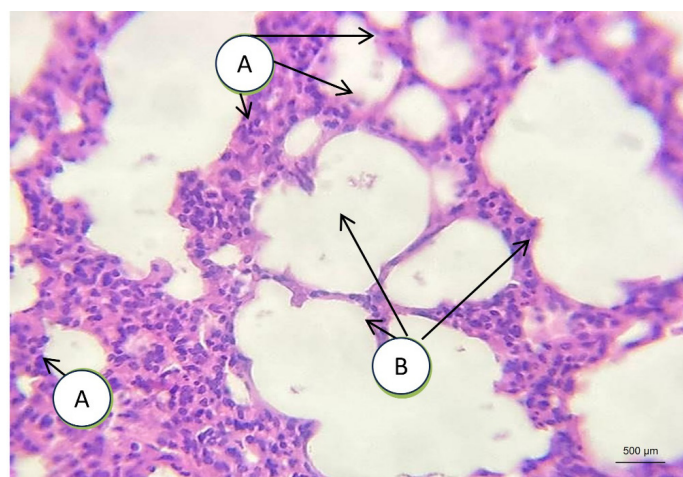


Figure 3: Lung histology on day 7 post-infection. (A) Atelectasis; (B) Alveolar emphysema. Hematoxylin and eosin (H and E) stain. Magnification $\times 40$.

HISTOPATHOLOGICAL FINDINGS

To assess pathological changes associated with SARS-CoV-2 infection, histological examination of lung tissues was performed on days 7, 14, and 21 post-infection. All dogs that tested positive for SARS-CoV-2 antibodies via ELISA were subjected to nasal swab collection and subse-

quent RT-PCR testing. None of the swabs tested positive, indicating the absence of active viral shedding at the time of sampling.

On day 7 post-infection, histological analysis of lung tissues from infected animals revealed alternating areas of emphysema and atelectasis, as well as bronchial spasms. Catarrhal inflammation was observed in the bronchi and alveoli, accompanied by mild leukocytic and macrophage infiltration (Figure 3).

Further examination revealed that the lumens of respiratory bronchioles and alveoli were expanded, with interalveolar septa showing signs of thinning and occasional rupture. Endplates were club-shaped and thickened, and vessel walls appeared sclerotic and thickened. Blood vessels were dilated and engorged (Figures 4 and 5).

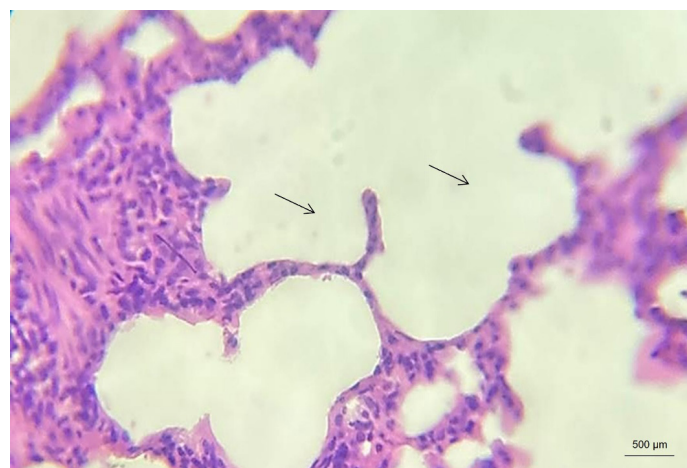


Figure 4: Chronic obstructive centrilobular emphysema. Thinning and lysis of alveolar septal elastic fibers; hyperelastosis of endplates (indicated by arrows). H and E stain. Magnification $\times 40$.

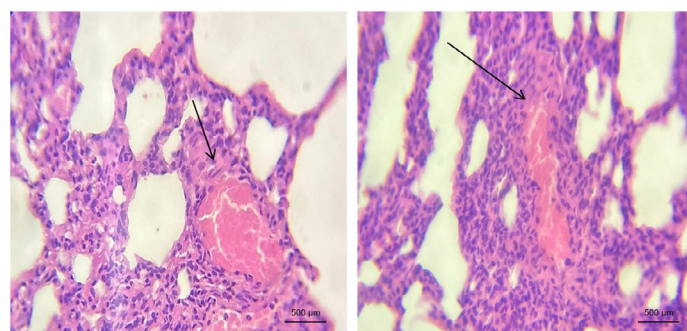


Figure 5: Pulmonary vasculature showing congestion. H and E stain. Magnification $\times 40$.

By day 21 post-infection, notable structural changes in the lungs of the infected group included epithelial simplification in bronchi, thickened alveolar and vascular walls, and signs of epithelial proliferation and edema within the bronchial lumens (Figure 6).

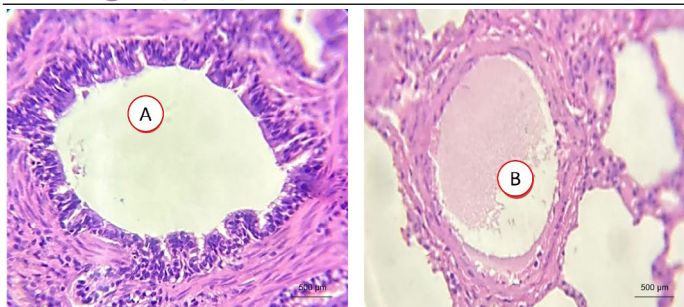


Figure 6: Lung histology on day 21 post-infection. (A) Dilated bronchus with epithelial proliferation; (B) Hemorrhagic inflammation. H and E stain. Magnification $\times 40$.

The histopathological profile at this stage was dominated by dystrophic changes in ciliated epithelial cells, including basophilic cytoplasmic inclusions and cellular desquamation. Additional findings included epithelial metaplasia, mononuclear infiltration, hyaline membrane formation, and alveolar spaces filled with exudate, red blood cells, and occasional leukocytes. Capillaries in the interalveolar septa were markedly dilated and engorged with blood. Some alveoli were dilated with septal rupture and vascular wall thickening.

In contrast, histological sections from dogs in the control group revealed normal lung architecture (Figure 7).

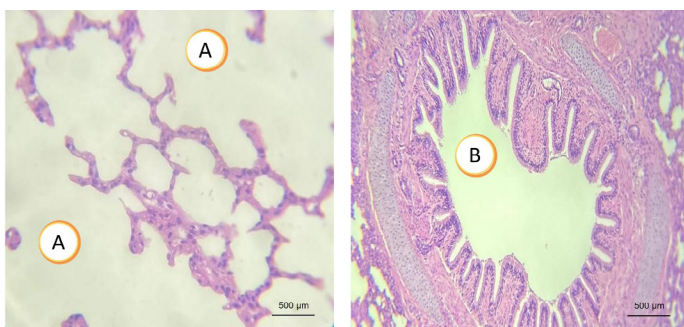


Figure 7: Lung histology in a control dog. (A) Normal expanded alveolar spaces; (B) Cross-section of a bronchus without pathology. H and E stain. Magnification $\times 40$.

Alveoli were airy and expanded, with thin interalveolar septa. Many alveoli appeared to merge, forming large air cavities. The vascular structures were dilated and engorged but displayed no pathological abnormalities. The bronchiolar epithelium retained a typical cylindrical morphology, with preserved structural integrity, muscle fibers, and cartilage plates.

DISCUSSION

The emergence of a novel coronavirus, later named SARS-CoV-2, in December 2019 marked the beginning of the COVID-19 pandemic. On February 11, 2020, the World Health Organization (WHO) officially designated the

disease as COVID-19 (CoronaVirus Disease 2019), caused by SARS-CoV-2 (Almaghaslah *et al.*, 2020). Since then, the virus has demonstrated an extraordinary ability to spread across human populations and, in some instances, to infect animals.

Although there is currently no conclusive evidence that companion animals significantly contribute to the spread of SARS-CoV-2 to humans, various studies have reported natural and experimental infections in animals such as cats, dogs, tigers, and lions (Gautam *et al.*, 2020). Zoonotic and reverse-zoonotic transmission has been confirmed, especially in mink farms in Denmark, the Netherlands, the USA, and Spain, illustrating the virus's capacity to cross species barriers (Gautam *et al.*, 2020; Shi *et al.*, 2020).

Experimental studies have also demonstrated the susceptibility of several species, including Syrian hamsters, ferrets, cats, dogs, non-human primates, and shrews to SARS-CoV-2 infection (Shi *et al.*, 2020; Nurpeisova *et al.*, 2022; Abay *et al.*, 2024). This wide host range suggests that SARS-CoV-2 has considerable zoonotic potential, with the ability to adapt across species (Leroy *et al.*, 2020; Mahdy *et al.*, 2020; Zhao *et al.*, 2020).

While the majority of studies suggest limited transmission of SARS-CoV-2 from infected companion animals to humans, serological surveys have detected antibodies in cats and dogs, suggesting prior exposure rather than active infection. Prevalence rates range from 10% to 12.8% in dogs and 4.5% to 43.8% in cats, with antibody detection rates often exceeding those for viral RNA, suggesting prior rather than active infection (Costagliola *et al.*, 2020; Patterson *et al.*, 2020).

Among canine coronaviruses (CCoVs), alpha-coronaviruses are the most well-known, causing enteric infections in dogs. These viruses typically infect the gastrointestinal tract and are transmitted via the fecal-oral route. Clinical disease is more commonly observed in young or immunocompromised animals, while adult dogs may remain asymptomatic carriers (Zobba *et al.*, 2021). Importantly, these canine-specific coronaviruses are not known to infect humans.

Under the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health, understanding cross-species transmission of SARS-CoV-2 is of critical importance (Zappulli *et al.*, 2020). The close relationship between humans and companion animals necessitates studies like this one, aimed at evaluating the susceptibility of domestic dogs to SARS-CoV-2 under controlled experimental conditions.

In this study, dogs were intranasally inoculated with a human-derived SARS-CoV-2 Delta variant (SARS-CoV-2/

Indian-Delta/KZ_Almaty/07.2021). Hematological analysis revealed transient reductions in leukocyte, erythrocyte, and lymphocyte counts, as well as hematocrit values, particularly on days 3 and 7 post-infection. These findings may reflect mild immune suppression or early-stage inflammation but were not pathognomonic.

Although RT-qPCR confirmed transient viral RNA presence in rectal swabs on day 3 in two dogs from the experimental group, this detection was not sustained at later time points. These dogs also exhibited clinical signs such as diarrhea, moderate lethargy, weight loss, and fever (up to 40.1°C), suggesting a transient systemic response. However, RT-PCR failed to detect viral RNA in nasal or oropharyngeal swabs, and subsequent testing of all ELISA-positive animals was PCR-negative, indicating no ongoing viral replication or shedding.

Serological analysis demonstrated the development of a specific immune response, with rising neutralizing antibody titers detected by microneutralization assay, and a modest early response captured by ELISA. Nonetheless, the absence of sustained viral RNA detection suggests that viral replication in dogs, if it occurs at all, is minimal and self-limited.

Histopathological examination provided further insight. While some inflammatory and structural changes, such as hemorrhagic inflammation, alveolar emphysema, and atelectasis were observed in infected animals, these changes were mild and not consistent with the severe pulmonary pathology typical of COVID-19 pneumonia in humans. Dogs in the control group showed normal lung architecture.

Collectively, these findings indicate that although dogs can mount an immunological response to intranasally administered SARS-CoV-2, they do not support productive viral replication. Histological evidence of lung pathology was limited and nonspecific. Therefore, companion dogs are unlikely to act as intermediate hosts or reservoirs for SARS-CoV-2.

This study contributes to a growing body of evidence suggesting that while SARS-CoV-2 may cross species barriers under experimental or natural conditions, not all exposed species are capable of supporting efficient replication or onward transmission. As coronaviruses continue to evolve, monitoring interspecies dynamics remains a key component of public health preparedness.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that companion dogs intranasal-

ly inoculated with the SARS-CoV-2 Delta variant strain (SARS-CoV-2/Indian-Delta/KZ_Almaty/07.2021) exhibited transient clinical signs, mild hematological alterations, and low-level viral RNA detection in rectal swabs on day 3 post-infection. Despite the detection of specific antibodies and minor histological changes in the lungs, there was no evidence of sustained viral replication or shedding.

The absence of SARS-CoV-2 RNA in nasal and oropharyngeal swabs and the lack of significant pulmonary pathology suggest that dogs are unlikely to serve as amplifying hosts. These findings support the conclusion that while dogs may develop a transient immune response to SARS-CoV-2, they are unlikely to play a significant role in the epidemiology or transmission of the virus to humans or other animals.

Continued surveillance of SARS-CoV-2 in domestic and wild animal populations remains essential in the context of the One Health approach. Further studies involving different viral variants, transmission settings, and species are necessary to fully elucidate the interspecies transmission dynamics of SARS-CoV-2.

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NOVELTY STATEMENTS

This is the first experimental study showing that dogs infected with the SARS-CoV-2 Delta variant do not support sustained viral replication or shedding, suggesting they are unlikely to contribute to the virus's transmission cycle.

AUTHOR'S CONTRIBUTIONS

Conceptualization, Lespek Kutumbetov, Aslan Kerimbayev, Markhabat Kassenov and Kunsulu Zakarya; methodology, Kuanysh Jekebekov, Kamshat Shorayeva, Sandugash Sadikaliyeva and Balzhan Myrzakhmetova; investigation, Syrym Kopeyev, Elina Kalimolda, Yeraly Shayakhmetov, Asankadyr Zhunushov and Alisher Omurtay; data curation, Sandugash Sadikaliyeva; validation, Sergazy Nurabayev and Hansang Yoo; supervision, Berik Khairullin; writing—original draft preparation, Ainur Nurpeisova and Zhandos Abay; writing—review and editing, Ainur Nurpeisova and Zhandos Abay. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The funders

had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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