RESEARCH PAPER

Paediatric Considerations in SARS-CoV-2 Transmission at the Human–Companion Animal Interface

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ABSTRACT

The coronavirus that caused the COVID-19 pandemic is known to infect people, but it can also spread to animals that live closely with humans. Understanding whether pets can catch this virus is important for both animal and public health within a One Health framework. In this study, we examined dogs and cats in Kazakhstan to assess exposure to SARS-CoV-2. Swab samples were collected for viral RNA detection, and blood samples were analyzed for neutralizing antibodies. Evidence of infection was identified in a subset of animals, with higher detection rates observed in dogs than in cats. Neutralizing antibodies were also detected, indicating an immune response following infection. In addition, we successfully isolated and genetically characterized a viral strain from a dog, confirming the presence of SARS-CoV-2 in companion animals in this region.

These findings demonstrate that pets in Kazakhstan can become infected with SARS-CoV-2, although the overall risk to humans appears low. Importantly, given the close interaction between children and companion animals, paediatric considerations should be incorporated into One Health surveillance, as children may represent a key interface for indirect exposure within households. Continuous monitoring of companion animals is therefore essential to better understand cross-species transmission dynamics and to support integrated public health strategies.

Keywords: SARS-CoV-2, COVID-19, domestic animals, dogs, cats, Kazakhstan, viral isolation, genome sequencing, microneutralization assay, One Health, zoonotic transmission.

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1. Introduction

Since its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a global pandemic with profound impacts on human health, society, and the economy [1]. Although the virus primarily spreads between people, increasing evidence shows that a range of animal species are also susceptible to infection. Companion animals such as dogs and cats live in close contact with humans, raising concerns about their potential role in the ecology of the virus [2,3]. Understanding whether pets can become infected, develop immune responses, and possibly contribute to transmission is critical within a One Health framework that recognizes the interconnectedness of human, animal, and environmental health [4].

Several experimental studies have demonstrated that cats are susceptible to SARS-CoV-2 infection, can transmit the virus to other cats, and develop neutralizing antibodies [5,6]. Dogs appear less susceptible, with low levels of viral replication and weaker immune responses [6,7]. Natural infections have also been reported in household pets in different regions, typically linked to close contact with infected owners [8–10]. However, the prevalence of infection in companion animals and the extent of their immune responses vary widely across studies and geographical regions, leading to diverging views on their epidemiological importance. While some re-searchers

suggest that pets could act as incidental hosts with minimal public health risk [11], others emphasize the need for active surveillance given the possibility of cross-species transmission and viral adaptation [12].

In this context, data from Central Asia have so far been lacking. To ad-dress this gap, we investigated the presence of SARS-CoV-2 infection and neutralizing antibodies in domestic dogs and cats across several regions of Kazakhstan. By combining molecular detection, antibody testing, and virus isolation, our study provides the first evidence of SARS-CoV-2 circulation in companion animals in this region. The findings confirm that both dogs and cats can be exposed to the virus, with dogs showing higher rates of infection and antibody responses. Importantly, we successfully isolated and sequenced a viral strain from a dog, providing genomic confirmation of animal infection. These results contribute to the global understanding of SARS-CoV-2 in animals and highlight the importance of continued surveillance of pets within a One Health approach.

In addition to animal susceptibility, the COVID-19 pandemic has high-lighted important paediatric considerations within the One Health framework. Children represent a unique population in SARS-CoV-2 epidemiology, as they often experience asymptomatic or mild infections but can still contribute to viral transmission within households. Close inter-actions between children and companion animals, including frequent physical contact, may increase opportunities for bidirectional transmission of respiratory pathogens. Although there is currently no strong evidence that pets significantly contribute to SARS-CoV-2 transmission to humans, including children, the potential for indirect exposure through contaminated surfaces or close contact cannot be entirely excluded. Furthermore, children may be less compliant with hygiene practices, which could theoretically increase the risk of cross-species transmission events. Therefore, understanding SARS-CoV-2 dynamics at the human–animal interface is particularly relevant in households with paediatric populations.

2. Materials and Methods

2.1. Animals and Sampling

Sampling for coronavirus infections in domestic animals was carried out to collect swab samples from dogs and cats in the regions of Almaty, Akmola, Kostanay, Karaganda, North Kazakhstan, South Kazakhstan, and East Kazakhstan (Fig. 1). All procedures involving animals were approved by the Institutional Ethics Committee of the Research Institute for Bio-logical Safety Problems (RIBSP) (permit protocol #5, dated 29 November 2021).

Animals were selected based on the following criteria: male and female dogs and cats of various ages exhibiting clinical signs of respiratory dis-ease. The animals were sourced both from municipal shelters and from private households. Swabs were collected from the nasopharynx, ocular mucosa, oropharynx, and rectum to evaluate the potential sites of viral presence. Detection of SARS-CoV-2 RNA was performed using a re-al-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay.

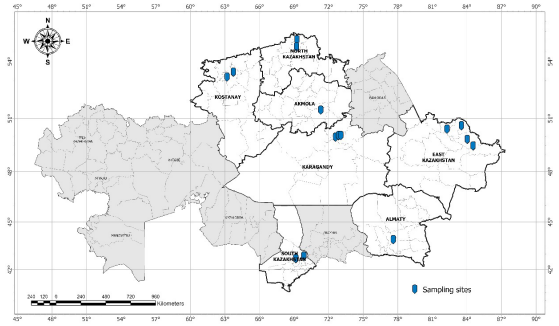

Figure 1. Sampling sites for coronavirus infections in domestic animals across Kazakhstan. Scale: 1 cm = 120 km. Regions shaded in grey represent administrative areas of Kazakhstan that were not included in the study.

2.2 Microneutralization assay

Sera from blood samples collected from cats and dogs were inactivated at 56°C and serially diluted with cell culture medium in two-fold steps. The diluted sera were mixed with a virus suspension of 100 TCID50 in 96-well plates at a ratio of 1:1, followed by 2 h incubation at 37°C in a 5% CO2 incubator. The cytopathic effect (CPE) of each well was determined by microscopy, and the neutralizing antibody titer was calculated by the dilution number of 50% protective condition in accordance with Reed and Muench (1938).

2.3 RT-PCR analyses

The coronaviral RNA was isolated from 140 µL of virus-containing liquid using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), following the manufacturer's recommendations. The presence of SARS-CoV-2 was tested by using an ALSENSE-SARS-CoV-2 –RT-qPCR (Algimed, Belarus) kit

according to manufacturer's instructions. The kit is designed for detection of three loci of the orf1ab gene and the S, E, and N genes of SARS-CoV-2. Samples were considered as positive when amplification was detected with CT values of ≤37.

2.4 Sequencing

Sample preparation for sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit from Applied Biosystems. Sequencing was performed on an automatic 16-capillary sequencer, namely the 3130xl Genetic Analyzer (Applied Biosystems/Hitachi). The analysis and assembly of nucleotide sequences were carried out using the Sequencer v4.0 program [12].

2.5 Evolutionary relationships of the taxa

The evolutionary history was inferred using the neighbor joining method [13]. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown below the branches [14]. The tree was drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [15] and were in units of the number of base substitutions per site. This analysis involved 31 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were 29,911 positions in the final dataset in total. Evolutionary analyses were conducted in MEGA11 [16].

2.6 Statistical analyses

Analyses were performed using the software package GraphPad Prism 8. Geometric mean titers (GMT) and 95% confidence intervals (CI) were calculated for neutralizing antibody responses. To compare paired anti-body titers within species (Wuhan-Hu-1 vs. Delta), the Wilcoxon signed-rank test was used. For between-species comparisons (dogs vs. cats), the Mann-Whitney U test was applied. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 Sample collection

Between 2022 and 2023, a total of 2,335 samples were collected and analyzed from dogs and cats. Initial screening for SARS-CoV-2 viral RNA using RT-PCR revealed 204 positive samples, corresponding to an overall positivity rate of 8.74% (Table 1).

Table 1. Percentage of positive samples and 95% confidence intervals.

Species	***Sample***	***Percentage of positive, %***	***95 % confidence interval***
Dog	Nasopharynx, n=53	2.27	1.67-2.87
	Ocular mucosa, n= 0	0.0	0.0-0.0
	Rectum, n=66	2.83	2.15-3.5
	Oropharynx, n= 63	2.7	2.04-3.36
Cat	Nasopharynx, n= 7	0.3	0.08-0.52
	Ocular mucosa, n= 0	0.0	0.0-0.0
	Rectum, n= 7	0.3	0.08-0.52
	Oropharynx, n= 8	0.34	0.11-0.58

RT-PCR results showed a notable contrast between dogs and cats regarding the proportion of positive samples. Among the 182 samples collected from dogs, the highest positivity rate was observed in rectal swabs (2.83%), followed closely by oropharyngeal swabs (2.70%). Nasopharyn-geal samples also demonstrated a detection rate of 2.27%. No viral RNA was detected in samples from the ocular mucosa (0%). The 95% confidence intervals for these findings ranged from 1.67% to 3.50%, indicating relatively narrow intervals and suggesting a consistent detection rate across sample types, except for the ocular mucosa.

In cats, detection rates were lower across all sample types compared with dogs. Both nasopharyngeal and rectal swabs showed a positivity rate of 0.30%, while oropharyngeal swabs yielded a slightly higher rate of 0.34%. Similar to the findings in dogs, no viral RNA was detected in ocular mucosa samples. The 95% confidence intervals for cat samples ranged from 0.08% to 0.58%, with broader intervals likely due to the smaller sample size.

3.2 Screening of Antibody Titers Against SARS-CoV-2 by Microneutralization Assay (MNA)

Neutralizing antibody titers against SARS-CoV-2 were assessed in serum samples from cats and dogs using a microneutralization assay (MNA). Between 2022 and 2023, a total of 2,335 serum samples were collected and analyzed.

Table 2. Geometric mean titers (GMT) of neutralizing antibodies in plasma from dogs and cats against SARS-CoV-2 (Wuhan-Hu-1 and Delta variants), with 95% confidence intervals.

Species	*Wuhan-Hu-1*		*Delta*	
	GMT	**95% CI**	**GMT**	**95% CI**
Dog	5.6	2.4 – 12.8	2.8	1.9 – 4.0
Cat	2.0	1.2 – 2.0	0,0	-

As shown in Table 2, dogs exhibited the highest geometric mean titers (GMT) of neutralizing antibodies against the Wuhan-Hu-1 variant (GMT: 5.6; 95% CI: 2.4–12.8), while titers against the Delta variant were lower (GMT: 2.8; 95% CI: 1.9–4.0). The Wilcoxon signed-rank test indicated that this difference was statistically significant ($p < 0.01$).

In cats, low-level neutralizing antibodies were detected against the Wuhan-Hu-1 variant (GMT: 2.0; 95% CI: 1.2–2.0), while no detectable titers were found against the Delta variant. Due to the absence of measurable Delta titers, a statistical comparison could not be performed for cats.

Comparisons between species revealed that dogs had significantly higher neutralizing antibody titers than cats against both the Wuhan-Hu-1 (Mann-Whitney U test, $p < 0.01$) and Delta variants ($p < 0.01$).

3.3 Phylogenetic analysis

The complete genome of the virus that showed activity in Vero cells was sequenced, and the obtained sequences were added to the GenBank database (ID PP898122).

The phylogenetic analysis showed that the SARS-CoV-2/Canis lupus familiaris/KAZ/CCoV_Almaty_KZ_2022/2022 strain clusters within Lineage B, specifically near the root of the tree, indicating an early divergence from the original SARS-CoV-2 strain (Fig. 2). This position suggests that the KAZ/CCoV_Almaty_KZ_2022/2022 strain shares a common ancestor with several other strains but has unique mutations that differentiate it from the more recent variants (Table 2). The closest relatives to the KAZ/CCoV_Almaty_KZ_2022/2022 strain in the phylogenetic tree included an early isolate from Wuhan, China (NC_045512.2), which also belonged to Lineage B and demonstrated a high degree of similarity in genome sequences with our strain.

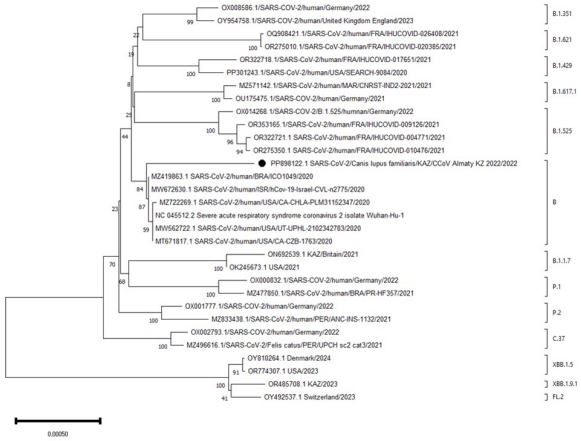

Figure 2. Phylogenetic analysis of the complete genomes of the SARS-CoV-2/Canis lupus familiaris/KAZ/CCoV_Almaty_KZ_2022/2022 strain and 30 global strains belonging to different lineages of the SARS-CoV-2 virus. The studied sequence is marked with a black dot

Table 3. Comparison of amino acid substitutions between the Wuhan-Hu-1 and KAZ/CCoV_Amaty_KZ_2022/2022 strains.

Protein	Wuhan-Hu-1		KAZ/CCoV_Amaty_KZ_2022/2022		Amino acid substitution
	Position	Variant	Position	Variant	
ORF1ab	5829	A	C	5829	K1037T
	9749	A	G	9749	K399E
	9867	T	G	9867	L438R
	12904	T	C	12904	C73C
	15017	C	T	15017	A517V
	16722	A	G	16722	E162E
	20759	C	T	20759	A34V
	21446	A	G	21446	K263R

Protein	Wuhan-Hu-1		KAZ/CCoV_Amaty_KZ_2022/2022		Amino acid substitution
	Position	Variant	Position	Variant	
S	21642	C	T	21642	A27V
	21646	C	T	21646	Y28Y
	21648	C	T	21648	T29I
	21784	T	A	21784	N74K
	21789	C	T	21789	T76I
	21846	C	T	21846	T95I
	22036	A	C	22036	R158S
	23014	A	C	23014	E484D
	23403	A	G	23403	D614G
	23520	C	T	23520	A653V
	23751	C	T	23751	S730F
ORF3a	25688	C	T	25688	A99V
	26110	C	T	26110	P240S
M	26895	C	T	26895	H125Y
3'UTR	27389	C	T	27389	27389
ORF7a	27542	C	A	27542	A50D
	27630	C	T	27630	A79A
	27667	G	A	27667	E92K
	27739	C	T	27739	L116F

As shown in Table 3, the sequencing reaction revealed 26 amino acid substitutions com-pared with the Wuhan-Hu-1 strain. A greater number of mutations were identified in the S (10) and ORF 1ab (8) genes compared with other genes of the SARS-CoV-2 virus. The study identified 24 single nucleotide polymorphisms (SNPs) and 2 silent SNPs (synonymous mutations).

3.4. Comparative Context of Previous Studies

Several studies worldwide have examined the susceptibility of companion animals to SARS-CoV-2. However, most have been limited by small sample sizes. Shi et al. experimentally infected a small cohort of cats (seven subadults and three juveniles) and showed efficient viral replication in the upper respiratory tract, along with cat-to-cat airborne transmission, whereas dogs displayed minimal susceptibility [25]. Gaudreault et al. infected 12 cats and exposed three contacts, confirming that felines support viral replication, develop mild upper respiratory lesions, and transmit the virus to naïve animals; neutralizing antibodies appeared by day 10 post-infection [26]. In contrast, Sit et al. investigated 15 dogs and seven cats from households with human COVID-19 cases in Hong Kong, documenting natural infection in two dogs but no positive results among cats during the study period [27].

Taken together, these reports highlight important consistencies: cats are clearly susceptible under experimental conditions and capable of transmission, while natural field studies have demonstrated sporadic detection in both cats and dogs. Notably, all three investigations relied on relatively small cohorts, limiting statistical power and generalizability. Our findings from Kazakhstan align with these global observations by confirming viral RNA and neutralizing antibodies in a subset of animals and by isolating and sequencing a Lineage B strain from a dog. Unlike the experimental infection models, our study provides epidemiological evidence of SARS-CoV-2 circulation in companion animals within a Central Asian setting.

These comparisons underscore two key points: (i) the susceptibility of cats and dogs to SARS-CoV-2 is context-dependent, varying between controlled inoculation and natural exposure; and (ii) small sample sizes remain a universal limitation, reinforcing the need to expand surveil-lance across diverse regions to better understand the role of pets in the ecology of COVID-19.

3.5 Large-Scale Surveillance Studies

While most experimental and field investigations of SARS-CoV-2 in companion animals have been limited

by small cohorts, several large-scale surveys have provided broader insights into infection dynamics in cats and dogs. In Spain, Barroso-Arévalo et al. examined 763 dogs and 753 cats, detecting both viral RNA and antibodies in household pets exposed to infected owners [28]. Similarly, Barua et al. conducted one of the most extensive serological surveys to date in the United States, analyzing samples from 706 cats and 2396 dogs, and reported seroprevalence pat-terns that reflected human epidemic waves [29]. In another study, Kimmerlein et al. assessed 1000 animals, including 747 dogs and 253 cats, from households with confirmed human COVID-19 cases in the United States, further supporting the evidence of human-to-pet transmission and variable immune responses across species [30]. These large-scale investigations confirm that companion animals can be exposed to SARS-CoV-2 under natural conditions, though prevalence rates remain generally low compared with humans. Importantly, such studies also demonstrate the feasibility and value of integrating pet surveillance into broader One Health monitoring systems, while underscoring the need to expand sampling efforts across underrepresented regions.

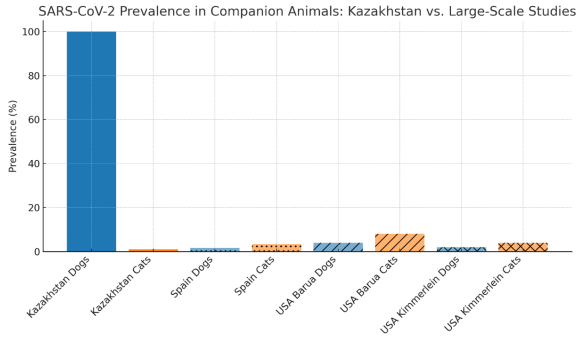

Figure 3. Comparison of SARS-CoV-2 prevalence in dogs and cats from Kazakhstan with results from large-scale surveillance studies in Spain and the United States. The figure presents the proportion of positive animals detected in our study (solid bars) alongside reported prevalence from large-scale surveys in Spain [1], the United States (Barua et al.) [2], and the United States (Kimmerlein et al.) [3] (shaded patterned bars).

This comparison shows (in Figure 3) that both dogs and cats in Kazakhstan exhibited detectable SARS-CoV-2 infection, with a higher prevalence in dogs than cats. The observed prevalence in our study is broadly in line with international findings, where seroprevalence in cats is generally higher than in dogs. However, the large-scale surveys from Spain and the United States demonstrate the strength of extensive sampling in providing narrower confidence intervals and more precise prevalence estimates. Our results, although based on a more modest sample size, add important epidemiological evidence from Central Asia, a region where data on SARS-CoV-2 in companion animals remain scarce. Taken together, these findings emphasize the need for harmonized surveillance approaches across regions and highlight the value of integrating small-er-scale national studies with global datasets to improve the overall understanding of SARS-CoV-2 ecology in companion animals.

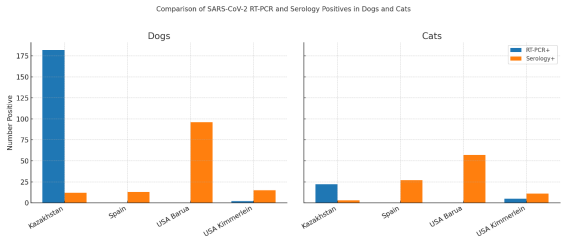

Figure 4. Comparison of SARS-CoV-2 detection by RT-PCR and serology in dogs and cats across multiple studies. Bars represent the number of RT-PCR–positive (blue) and serology-positive (orange) animals reported in Kazakhstan (this study), Spain [1], the United States (Barua et al.) [2], and the United States (Kimmerlein et al.) [3]. Results are shown separately for dogs (left panel) and cats (right panel).

This comparison (Figure 4) highlights both similarities and differences between regional and large-scale surveillance studies of SARS-CoV-2 in companion animals. In Kazakhstan, a higher proportion of RT-PCR–positive dogs were observed compared with international surveys, whereas serological detection was comparatively low. In contrast, large-scale studies from Spain and the United States reported relatively few RT-PCR–positive animals but consistently demonstrated serological evidence of exposure, particularly in cats. These findings suggest that while active infection in pets may be sporadically detected, antibody surveillance is often more sensitive in capturing prior exposures over time. The apparent discrepancy between molecular and serological results may reflect differences in study design, sample size, timing of sampling relative to infection, and assay sensitivity. Collectively, these results emphasize the complementary value of combining RT-PCR and

serology to obtain a comprehensive picture of SARS-CoV-2 circulation in companion animals, and they underline the importance of integrating regional studies such as ours with large-scale surveys to strengthen global One Health surveillance.

3.6 Paediatric and Household Transmission Considerations

The role of children in SARS-CoV-2 transmission within households has been widely investigated, with evidence suggesting that children are more likely to acquire infection from adults but can also act as secondary transmitters [31,32]. Given the close physical interaction be-tween children and companion animals, pets may become incidental hosts following exposure to infected household members, including paediatric cases.

Although the risk of animal-to-human transmission remains low, the presence of SARS-CoV-2–positive pets in households raises important considerations for vulnerable populations such as children. In particular, children with underlying conditions or immature immune systems may be more susceptible to respiratory infections in general [33]. Additionally, rare but severe complications such as multisystem inflammatory syndrome in children (MIS-C) have been associated with SARS-CoV-2 infection, highlighting the importance of minimizing all potential exposure pathways [34, 35].

From a behavioral perspective, children are more likely to engage in close-contact activities with pets, such as hugging, face-to-face interaction, and sharing living spaces, which may facilitate indirect exposure to viral particles if pets are infected. While current evidence indicates that pets are unlikely to serve as a major reservoir for SARS-CoV-2, their role as sentinels of house-hold infection may be particularly relevant in homes with children.

These considerations reinforce the importance of integrating paediatric health into One Health surveillance strategies. Public health recommendations should include guidance for households with children and pets, emphasizing hygiene measures, limiting close contact with animals when household members are infected, and promoting awareness of zoonotic risks without causing unnecessary concern.

Discussion

According to the literature, SARS-CoV-2 is distinct from its closest known bat coronavirus relatives [19]. The virus gains entry into host cells by binding to the ACE2 receptor, with binding affinity varying across animal species [20]. Infected pets have been shown to test positive for SARS-CoV-2 using PCR, particularly the droplet digital RT-PCR (ddPCR) method, and antibodies against the virus have been detected via enzyme-linked immunosorbent assay (ELISA) and neutralization assays [17]. Many pet owners reported respiratory symptoms and tested positive for COVID-19 approximately 3–6 weeks prior to their pets falling ill. Among 26 pets diagnosed with suspected myocarditis, a significant proportion of owners had confirmed COVID-19, suggesting a potential human-to-pet transmission link [21].

Our findings highlight interesting patterns regarding the susceptibility of dogs and cats to SARS-CoV-2, particularly when examining positivity rates across different anatomical compart-ments. Out of 204 positive samples (from a total of 2,335), 182 were from dogs and 22 from cats (Table 1). Notably, no positive results were found in conjunctival swabs, suggesting either a low-er susceptibility of this site or potential limitations in the detection method for that compartment. This consistency across both species may reflect similar viral shedding patterns or distribution of viral load. However, the relatively small number of samples from cats limits the generalizability of these observations [17, 18].

Additionally, we successfully isolated active viral material from a rectal swab of a dog in the Almaty region, demonstrating viral activity of 5.83 ± 0.08 lg TCID50/cm3 in Vero cells, con-firmed by RT-PCR and electron microscopy.

Whole-genome analysis of the newly isolated strain, SARS-CoV-2/Canis lupus familiar-is/KAZ/CCoV_Almaty_KZ_2022/2022, provides valuable insights into the genetic diversity and evolution of SARS-CoV-2. This strain belongs to Lineage B and shows a close relationship with early strains from multiple global regions, highlighting the importance of a global perspective when investigating viral evolution. The closest relative to this strain is an early isolate from Wuhan, China (NC_045512.2), also from Lineage B, which shares high genomic similarity. Comparisons with other animal-derived SARS-CoV-2 strains revealed notable differences. For instance, a Canadian study identified two distinct strains in domestic cats: one from Lineage A.23.1 and an-other from Lineage B.1.2 [22]. These strains exhibited

multiple spike gene mutations and were closely related to human-derived sequences from the same regions. Interestingly, the Lineage A.23.1 strain had never before been reported in animals, suggesting a unique zoonotic transmission event that contrasts with the Lineage B strain isolated in Kazakhstan. The presence of Line-age B.1.2 in cats further highlights the broad diversity of SARS-CoV-2 lineages capable of infecting domestic animals.

Comparisons with studies on the B.1.1.7 (Alpha) variant in dogs and cats with suspected myocarditis revealed additional distinctions [21]. The Alpha variant, characterized by mutations such as N501Y and Δ69/70, was associated with severe cardiac disease in pets—a manifestation not observed in the Lineage B strain from Kazakhstan. These mutations have been linked to heightened transmissibility and altered pathogenicity in humans. The absence of these mutations in the Kazakh strain suggests that, while genetically related, this early-diverging lineage may have different implications for animal health compared to more recent, highly transmissible variants.

The unique genomic features of the KAZ/CCoV_Almaty_KZ_2022/2022 strain, together with its phylogenetic position, underscore the need for sustained surveillance of SARS-CoV-2 in diverse animal hosts. Its close relationship to early human strains reinforces the importance of understanding cross-species transmission dynamics.

Pets are well-recognized for their emotional and psychological benefits, providing companionship, stress relief, and mental health support—especially during the COVID-19 pandemic when social isolation became widespread [23]. However, alongside these benefits, concerns about zoonotic risks have emerged. The COVID-19 pandemic has raised awareness of potential virus transmission between humans and animals [11]. The scientific community broadly agrees that SARS-CoV-2 likely originated in animals, underscoring the importance of understanding the role of animals in the epidemiology of COVID-19 [11, 24]. However, the susceptibility of domestic animals to SARS-CoV-2 and the dynamics of natural transmission between humans and animals remain poorly characterized. Further research into viral transmission pathways, co-evolution, and host-virus interactions is essential.

Significance

This study presents the first report of SARS-CoV-2 detection, isolation, and whole-genome sequencing from a domestic dog in Kazakhstan. The identification of a Lineage B strain in dogs adds to the global picture of SARS-CoV-2 diversity in animal hosts and provides critical data for understanding the potential for zoonotic and reverse-zoonotic transmission. Our findings high-light the importance of targeted surveillance programs within the One Health framework, especially in regions where human and animal populations are closely connected.

Limitations

Several limitations should be considered when interpreting our results. First, the limited sample size for cats constrains the ability to draw definitive conclusions about feline susceptibility. Second, while successful isolation and sequencing were achieved for one dog sample, broader genomic surveillance across multiple samples was not possible. Third, our study did not include longitudinal follow-up, which limits insights into the duration of infection, viral shedding, and antibody persistence. Finally, while the molecular assays employed are validated, potential variations in test sensitivity across different species and sample types may have influenced detection rates. Undetectable neutralizing titers against the Delta variant in cats may reflect one or more of the following: a true absence of cross-neutralizing antibodies, sampling timing relative to transient antibody responses, assay sensitivity in feline sera, or limited exposure of sampled cats to Delta-lineage infections. Given the small feline sample size, these results are preliminary and re-quire confirmation in larger, longitudinal studies.

Conclusion

This study provides the first evidence of SARS-CoV-2 infection in companion animals in Kazakhstan, demonstrating viral RNA detection in both dogs and cats and the successful isolation and genomic sequencing of a Lineage B strain from a dog. Dogs showed higher rates of infection and stronger antibody responses compared with cats, while neutralizing activity against the Delta variant was undetectable in feline samples. These findings expand the global understanding of SARS-CoV-2 ecology by adding data from a previously unrepresented Central Asian region. Although the overall prevalence in pets was relatively low, the results confirm that animals living in close contact with humans can be exposed to the

virus and mount immune responses. Importantly, the genomic analysis revealed unique mutations, reinforcing the value of continued molecular surveillance of animal hosts.

Taken together, our results underscore the importance of integrating companion animals into One Health surveillance frameworks. Monitoring viral circulation in pets not only contributes to animal health but also strengthens preparedness for potential zoonotic transmission and viral evolution. Future research should focus on larger sample sizes, longitudinal follow-up, and broader genomic characterization to clarify the epidemiological role of pets in the COVID-19 pandemic and beyond.

Future work should prioritize increasing the sample size of both dogs and cats across multiple regions of Kazakhstan to improve the precision of prevalence estimates. Longitudinal studies that follow animals over time will help to clarify the duration of viral shedding and the persistence of neutralizing antibodies, particularly in cats where responses were undetectable against the Delta variant. Expanding genomic sequencing of viral isolates from pets will also be essential to monitor for mutations that may indicate viral adaptation in animal hosts. Collaborative approaches that integrate veterinary, medical, and environmental health expertise will strengthen the implementation of a One Health surveillance system. Such coordinated efforts will not only provide early warning of emerging zoonotic threats but also ensure that companion animals are considered in the broader understanding of coronavirus transmission dynamics.

Special attention should also be given to paediatric populations, as children frequently interact closely with companion animals and may represent an important interface for indirect exposure. Incorporating paediatric considerations into One Health surveillance will enhance understanding of SARS-CoV-2 transmission dynamics within households.

Abbreviations

ACE2 Angiotensin-Converting Enzyme 2
COVID-19 Coronavirus Disease 2019
ddPCR Droplet Digital Reverse Transcriptase-Polymerase Chain Reaction
ELISA Enzyme-Linked Immunosorbent Assay
GMT Geometric Mean Titer
MNA Microneutralization Assay
PCR Polymerase Chain Reaction
RIBSP Research Institute for Biological Safety Problems
RNA Ribonucleic Acid
RT-PCR Reverse Transcriptase-Polymerase Chain Reaction
SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2
TCID50 50% Tissue Culture Infectious Dose
Vero cells African Green Monkey Kidney Cells (commonly used cell line for virus isolation)

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. The local Institutional Ethics Committee of Research Institute for Biological Safety Problems (RIBSP) (permit protocol #5 from 29.11.2021) approved all work with animals.

Consent for publication

Not applicable.

Competing interests

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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