Transmission of SARS-CoV-2 Delta variant (B.1.617.2) from a fully vaccinated human to a canine in Georgia, July 2021

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Abstract
SARS-CoV-2 infection has been described in a wide range of species, including domestic animals such as dogs and cats. Illness in dogs is usually self-limiting, and further diagnostics may not be pursued if clinical signs resolve or they respond to empirical treatment. As new variants emerge, the clinical presentation and role in transmission may vary in animals. This report highlights different clinical presentations and immunological responses in two SARS-CoV-2 Delta-variant-positive dogs with similar exposure to the same fully vaccinated human with a SARS-CoV-2 infection and emphasizes the need for active surveillance and additional One Health research on SARS-CoV-2 variant infections in companion animals and other species.

KEYWORDS
COVID-19, companion animal, Delta, One Health, SARS-CoV-2

1 | INTRODUCTION

SARS-CoV-2 infections in domestic cats and dogs and other animals have been reported globally to the World Organisation for Animal Health (OIE; OIE, 2021). The United States Department of Agriculture maintains an updated list of animal species confirmed positive for SARS-CoV-2 in the United States (USDA, 2021). Seropositivity rates in household settings with exposure to human COVID-19 cases range from 4.5% to 43.8% in cats and 11% to 15.4% in dogs (Fritz et al., 2020; Goryoka et al., 2021; Hamer, Ghai, et al., 2021; Hamer, Pauvolid-Corréa, et al., 2021; Patterson et al., 2020). Experimental infection studies report limited viral shedding in dogs and no evidence of transmission to conspecifics (Bosco-Lauth et al., 2020; Shi et al., 2020). However, these studies did not assess relative differences in susceptibility, transmissibility or pathogenicity of current and emerging variants as reported regarding Alpha (B.1.1.7) and Delta (B.1.617.2) variants in humans (Fisman & Tuite, 2021). Here, we describe the documented transmission of SARS-CoV-2 Delta variant (B.1.617.2) from a fully COVID-19 vaccinated human (CDC, 2021d) to a canine.

2 | MATERIALS AND METHODS

2.1 | Ethics

All procedures involving animals were approved by the Centers for Disease Control and Prevention (CDC; Atlanta, GA) Institutional Animal Care and Use Committee (IACUC; #3104BARMULX).

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2.2 | SARS-CoV-2 rRT-PCR testing

Four swab specimens were collected from each dog at two time points (n = 16), using sterile plastic-handled, polyester-tipped applicators. Specimens were placed in 0.5 ml of viral transport media (VTM) prepared by CDC’s Division of Scientific Resources according to the Standard Operating Procedure #DSR-052–05 (CDC, 2020). Following RNA extraction, all swab specimens were tested using the CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay (Shu et al., 2021), a real-time reverse transcription PCR (rRT-PCR) targeting the matrix 1 (M1) gene and non-structural 2 (NS2) gene for human influenza A and B, respectively, and the nucleocapsid (N) gene for specific detection of SARS-CoV-2.

2.3 | Serology

Blood specimens (n = 6) were collected in 5 ml vacutainer serum separator tubes from the cephalic vein of both dogs at three time points. Serological response against SARS-CoV-2 was determined using a species-independent assay detecting antibody binding to the receptor-binding domain (RBD) of the viral spike (S) protein (Kainulainen et al., 2021).

3 | RESULTS

3.1 | Clinical history and signs of canines exposed to a fully vaccinated SARS-CoV-2-positive owner

A 2-year-old, 31.4 kg (69 lb) neutered male bulldog mix (Dog 1) residing in Georgia, USA, developed a dry, non-productive cough and lethargy on 16 July 2021. On physical exam at onset of clinical signs, Dog 1 was quiet, alert and responsive. An intermittent mild to moderate dry, non-productive cough was noted but tracheal palpation elicited no cough. No other significant findings or history of illness or comorbidities were noted. Dog 1 was prescribed prednisone (1 mg/kg PO, tapered over 10 days) and doxycycline (5 mg/kg PO BID) at onset of clinical signs, continued to exhibit intermittent dry, non-productive cough for 5 days, then recovered uneventfully. A co-housed 11-year-old, 15.6 kg (34.4 lb) neutered male French bulldog (Dog 2) was bright, alert and responsive; he appeared normal with respect to his age and known history of severe, chronic atopic dermatitis, and remained clinically normal.

Three days before clinical onset in Dog 1, the owner reported having mild COVID-19 symptoms (Figure 1), including transient myalgia and mild headache for 3 days, followed by intermittent sneezing, mild upper respiratory congestion and rhinorrhea over the next 3 days. The owner’s upper respiratory symptoms and myalgia resolved within 7 days and no headaches were noted after 10 days of symptom onset. The owner was considered fully vaccinated with two doses of the Moderna (mRNA-1273) COVID-19 vaccine as of 22 February 2021, and tested positive by rRT-PCR for SARS-CoV-2 on a nasopharyngeal swab by a commercial laboratory on 17 July 2021, one day after clinical onset in Dog 1. Both dogs resided in a single-family residence with owner-controlled access to a backyard surrounded by a 6-foot privacy fence. No other humans or animals resided in the household. Routine daily interactions between the owner and both dogs included limited contact while playing, walking on leash, feeding and direct contact through petting. These interactions continued following owner’s symptom onset. Interaction between dogs was frequent and no attempts were made to isolate Dog 1 from Dog 2.

Dog 1 was previously healthy, received annual preventive veterinary care, was up to date on core vaccines, tested negative for heartworm antigen on 17 March 2021 and received monthly oral heartworm and flea preventatives. He had attended a dog day-care facility within the 7 days preceding his reported clinical signs. Dog 2 had a history of severe, chronic atopic dermatitis controlled with medications, and received annual preventive veterinary care and dermatology consults as needed. He was up to date on core vaccines, tested negative for heartworm antigen on 25 August 2021 and received monthly oral heartworm and flea preventatives. He had no history of contacts with humans or animals outside the household within the 18 days prior to his first rRT-PCR test. Dog 2’s medications included oclacitinib maleate (8 mg) PO SID, 0.5 ml allergen-specific immunotherapy SQ weekly and intermittent use of oral prednisone (0.75 mg/kg PO SID, tapered over 10 days) for breakthrough dermatitis events. Dog 2 also received 10 mg of prednisone PO every other day for a breakthrough dermatitis event when the owner reported experiencing COVID-19 symptoms (previous breakthrough dermatitis event in May 2021).

3.2 | Viral RNA detection and serology in canines

After confirming SARS-CoV-2 infection in the owner and following consultation with state public and animal health officials, both dogs were tested for SARS-CoV-2 through investigations by the CDC in
In collaboration with state public and animal health officials. At the start of the investigation, the owner was referred to CDC’s Animals and COVID-19 website for federal guidance on managing pets with SARS-CoV-2 (CDC, 2021c). Diagnostic nasal, oropharyngeal and rectal swabs and non-diagnostic fur swabs were collected from both dogs on 21 July and 4 August 2021. On 21 July, nasal, oropharyngeal and fur swabs from Dog 1 (cycle threshold (Ct) values 36.65, 38.30 and 34.08, respectively; cut-off = 40) and Dog 2 (Ct 19.92, 29.36 and 34.60, respectively) were positive by rRT-PCR for SARS-CoV-2. Rectal swabs from both dogs were negative. On 4 August, all swab specimens for both dogs were negative. The owner tested negative by rRT-PCR on a nasopharyngeal swab on 5 August.

Serology was performed on blood specimens collected on 4, 11 and 25 August 2021. Dog 1 was seropositive for SARS-CoV-2 with moderate signal strength (82, 53 and 32.9 times over background, respectively; cut-off = 40) and Dog 2 (Ct 19.92, 29.36 and 34.60, respectively) were positive by rRT-PCR for SARS-CoV-2. Rectal swabs from both dogs were negative. On 4 August, all swab specimens for both dogs were negative. The owner tested negative by rRT-PCR on a nasopharyngeal swab 5 August.

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3.3 | Viral relatedness between canine and human specimens

To investigate the viral relatedness between human and canine SARS-CoV-2 positive specimens, sequences from four specimens (two per dog) were compared to sequence data from the owner’s specimen generated by a commercial laboratory under contract with CDC. Dog 2’s nasal and oropharyngeal specimens were processed for whole genome sequencing by Illumina MiSeq following previously published methods (Paden et al., 2020; Shepard et al., 2016); 98% (nasal) and 100% (oropharyngeal) of the full genome sequences were recovered. The owner’s specimen was sequenced by Illumina NovaSeq using the Illumina COVIDSeq test, recovering 98% of the full genome sequence. All three genome sequences were assigned to lineage AY.25 (a sublineage of B.1.617.2, the Delta variant) using Pangolin (3.1.11) with pangoLEARN version 2021–08–24 (Rambaut et al., 2020). Dog 1’s nasal, oropharyngeal and fur specimens had high rRT-PCR Ct values (>34), so they were processed for S gene sequencing by nested PCR followed by Sanger sequencing. Partial sequences from the nasal and fur specimens for Dog 1 covered positions 21,218–22,080, within the 3,822-nucleotide S gene. Available sequences for the five specimens representing Dogs 1 and 2 and the owner were identical, except for ambiguous bases (R = A or G; Y = T or C). All five sequences shared multiple Delta-variant signature mutations in the S gene, including E156-, F157- and R158G, compared to the SARS-CoV-2 reference genome (strain Wuhan-Hu-1: NC_045512.2). The other two signature mutations (T19R and G142D) in the S gene were identified in four genomes each; status of the fifth genome for each mutation was unclear due to a sequencing gap (Dog 2 nasal swab and human sample; Table 1). From 1 July through 21 July, only 14% of human specimens sequenced in Georgia and uploaded to GISAID (https://www.gisaid.org/; accessed 17 January 2022) were identified as Delta-variant lineage AY.25. Sequence analysis and the date of clinical onset in Dog 1 indicate a human-to-canine transmission event in this household.
Currently, little is known regarding the incidence, spectrum and severity of disease signs caused by new variant strains in companion animals compared to parental SARS-CoV-2 strains circulating earlier in the pandemic. Infection with the Delta variant in companion animals has not yet been described experimentally, and data from natural infections are limited. Several studies investigated serological responses in both experimental and natural infection of domestic animal species; of those reviewed, dogs with natural or experimental exposures that tested positive for SARS-CoV-2 by rRT-PCR and were screened for SARS-CoV-2 antibodies all had congruent antibody titre results (Bosco-Lauth et al., 2020; Calvet et al., 2021; Hamer, Ghai, et al., 2021; Hamer, Pauvolid-Corrêa, et al., 2021; van der Leij et al., 2021). Here, Dog 2 was positive for SARS-CoV-2 by rRT-PCR, but antibodies were not detected. Possible explanations include a long-term history of oral oclacitinib maleate administration, mutations in the RBD altering assay sensitivity for Delta or a true absence of antibodies despite infection. Evidence of immunocompetence in Dog 2 and detection of a moderate serological response in Dog 1 suggests that immunosuppression or assay limitations are less likely and that this case may represent undetectable SARS-CoV-2 antibody titres, as reported in asymptomatic SARS-CoV-2 infections in people (Chan et al., 2020; Marchi et al., 2021).

Delta variant is associated with increased transmission rates in humans (CDC, 2021b; Li et al., 2022); the estimated average reproduction number (R0), or the expected number of secondary cases from one primary case in a susceptible population, is 5.1, significantly higher than early pandemic strains (estimated R0 2.79; Liu & Rocklov, 2021). Whether the genomic changes facilitating transmission in humans also result in increased spread within other species is unknown but as SARS-CoV-2 variants emerge in human populations, these variants are expected to be identified in animals (Hamer, Ghai, et al., 2021; Hamer, Pauvolid-Corrêa, et al., 2021; Yaglom et al., 2021).

The greatest risk of SARS-CoV-2 transmission remains among unvaccinated people who are more likely to become infected and transmit the virus (CDC, 2021b). Currently, there is no evidence that animals play a significant role in spreading SARS-CoV-2 to people (CDC, 2021a). However, if emerging variants continue to become more transmissible, SARS-CoV-2-susceptible animal species will remain a risk for animal reservoirs, spillback infections to humans and development of species-specific mutations or variants (Díaz et al., 2021), despite increasing COVID-19 vaccination rates in humans. Active surveillance for SARS-CoV-2 in susceptible animal species (including companion animals that have close and frequent contact with people) and subsequent genomic comparative analysis, along with additional experimental research, are needed to further characterize infections and determine putative evolving roles in transmission considering novel SARS-CoV-2 variants with differing viral kinetics and host dynamics. Applying a One Health approach when conducting epidemiological investigations involving SARS-CoV-2 infections in animals is critically needed to better understand the impact of animals on public health, to protect human and animal health and to determine the role of spillover events in the course of the COVID-19 pandemic.

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CONFLICT OF INTEREST
The authors declare that there were no conflicts of interest.

DISCLAIMER
The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC.

DATA AVAILABILITY STATEMENT
Complete or near-complete genome sequences of SARS-CoV-2 obtained in this investigation are available at GenBank (accession nos. OK174325, OK174326 and MZ741014).

**TABLE 1** Summary of SARS-CoV-2 sequence analysis from owner and two household dogs positive for SARS-CoV-2 Delta variant (B.1.617.2)

| Case     | Lineage | Specimen Type | Ct   | S Gene (4K nt) | B.1.617.2 Signature | S Gene Mutations/Deletions |
|----------|---------|---------------|------|----------------|---------------------|---------------------------|
| Owner    | AY.25   | Nasopharyngeal| NR   | Near complete  | T19R               | E156- F157- R158G         |
| Dog 1    | NA      | Nasal         | 36.65| 800s nt        | T19R G142D E156- F157- R158G |
| Dog 1    | NA      | Fur           | 34.08| 800s nt        | T19R G142D E156- F157- R158G |
| Dog 2    | AY.25   | Oropharyngeal | 29.36| Complete       | T19R G142D E156- F157- R158G |
| Dog 2    | AY.25   | Nasal         | 19.92| Near complete  | --                  | G142D E156- F157- R158G  |

Sequencing unsuccessful or not attempted on the following specimens:

| Dog 1    | NA      | Oropharyngeal | 38.30| NA              | NA                  |
| Dog 2    | NA      | Fur           | 34.60| NA              | NA                  |

Abbreviations: --, indicates a deletion; --, sequence data not obtained in region indicated; Ct, cycle threshold; NA, not applicable; NR, not reported.
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