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Virology



Clinical, virological, imaging and pathological findings in a SARS CoV-2 antibody positive cat

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 OPEN ACCESS

Received: Dec 15, 2021

Revised: Mar 23, 2022

Accepted: Mar 25, 2022

Published online: May 2, 2022

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ABSTRACT

This paper reports a presumptive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in a cat. A cat with respiratory disease living with three individuals with coronavirus disease 2019 showed bilateral ground-glass opacities in the lung on X-ray and computed tomography. The clinical swabs were negative for SARS-CoV-2 RNA, but the serum was positive for SARS-CoV-2 antibodies. Interstitial pneumonia and prominent type 2 pneumocyte hyperplasia were noted on histopathology. Respiratory tissues were negative for SARS-CoV-2 RNA or antigen, but the cat was positive for feline parvovirus DNA. In conclusion, the respiratory disease and associated pathology in this cat could have been due to exposure to SARS-CoV-2.

Keywords: Cat; SARS-CoV-2; antibody; X-ray; tomography; ground-glass opacity; interstitial pneumonia

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily infects humans, but many studies have shown that animals, including cats, can also be infected naturally or experimentally [1-5]. Because cats live in close contact with humans, there is a great risk of transmission of SARS-CoV-2 from owners suffering from coronavirus disease 2019

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(COVID-19) [5-7]. The present case, a SARS-CoV-2 seropositive cat, likely represents a natural SARS-CoV-2 infection, which are poorly described in cats.

CASE PRESENTATION

A seven-month-old, female domestic crossbred cat showing signs of a respiratory disorder was submitted to a private clinic in Istanbul, Turkey, on March 22, 2021. The owner declared that three people in the same household tested positive for SARS-CoV-2 three weeks earlier. On clinical examination, the cat showed dullness, depression, respiratory signs of coughing, wheezing, and dyspnea accompanied by hyperthermia (39.5°C). As a suspect case of a SARS-CoV-2 infection, the cat was hospitalized in an isolation room after obtaining the owner's consent. Blood analyses revealed high white blood cell and neutrophil counts and an increased total serum protein level (7.4 g/dL).

Latero-Lateral and Vento-Dorsal X-ray images of the thorax showed pulmonary opacification in the right and left lung lobes (opaque regions) (**Fig. 1A**). Computerized tomography (CT) was requested because the cat's owner had COVID-19. The CT revealed two hypodense, heterogeneous lesions measuring approximately 18 × 14 mm and 13 × 10 mm in the transverse plane located in the cranial mediastinum. In both the left and right lung, peripheral and caudal soft tissue densities, consolidation areas, and acinar density increases were observed in all lobes and segments. Therefore, transpneumonia located in the cranial

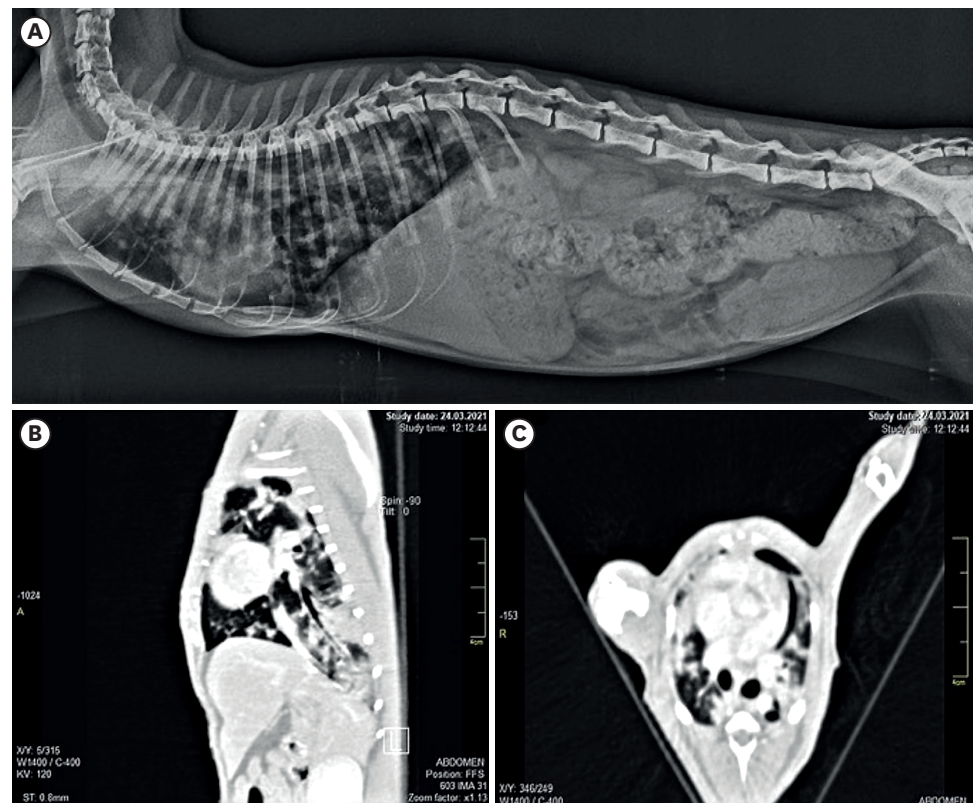


Fig. 1. X-ray and tomographic findings of the cat. (A) Consolidations and opaque regions were observed in the lung on X-ray imaging; (B and C) Ground-glass appearance on tomography.

Conflict of Interest

The J.A.R. laboratory received support from Tonix Pharmaceuticals, Xing Technologies, and Zoetis outside of the reported work.

J.A.R. is an inventor on patents and patent applications on the use of antivirals and vaccines for the treatment and prevention of virus infections, owned by Kansas State University, KS.

Funding

This study was funded by the Scientific and Technological Research Council of Turkey-TUBITAK (Project No: 102S654). Funding for this work was partially provided through grants from the National Bio and Agro-Defense Facility (NBAF) Transition Fund from the State of Kansas (JAR), the Department of Homeland Security Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD), under grant number HSHQDC 16-A-B0006 (JAR), the AMP Core of the Center of Emerging and Zoonotic Infectious Diseases (CEZID) from National Institute of General Medical Sciences (NIGMS) under award number P20GM130448 (JAR), the NIAID Centers of Excellence for Influenza Research and Surveillance under contract number HHSN 272201400006C (JAR), and the NIAID supported Center of Excellence for Influenza Research and Response (CEIRR, contract number 75N93021C00016 to JAR).

mediastinum and chronic organizing pneumonia (consolidation and atelectasis) were considered as differential diagnoses. The lesions (ground-glass opacifications) seen on the CT images (**Fig. 1B and C**) were similar to that seen in COVID-19 pneumonia patients [3,8].

Upon hospitalization, the nasal, pharyngeal, and rectal swabs, and blood with and without EDTA, were taken for virological analyses, and swabs were repeated every three–four days. Medical treatment was given for two weeks, including oseltamivir, prednisolone, omeprazole, amoxicillin, and clavulanic acid, as well as vitamins B, C, D, E, and electrolytes. The cat's overall condition declined, and the cat died after 20 days of hospitalization. A routine postmortem examination was performed, and various internal organs such as lung, trachea, heart, liver, kidney, spleen, pancreas, brain and intestines were collected for histopathological and virological examination.

At postmortem examination, the lungs were firm and characterized by coalescing tan to light red foci and altered buoyancy (**Fig. 2A and B**). The mucosa of the trachea was congested.

The histological examination showed that the pulmonary parenchyma was severely affected by interstitial pneumonia with coalescing areas of necrosis, organizing fibrin exudation (**Fig. 3A, B, and C**), and prominent alveolar type II hyperplasia (**Fig. 3A, B, and D**). Smooth muscle hyperplasia (**Fig. 3B and E**), organizing fibrosis (**Fig. 3E**), and infiltration of mononuclear cells in the alveolar septa. Alveolar spaces lined by prominent type II pneumocytes were often filled with karyorrhectic debris and numerous alveolar macrophages (**Fig. 3D**). The tracheal lumen was partially filled with eosinophilic debris and degenerate neutrophil leukocytes, but there were no alterations in the lining respiratory epithelium or submucosal glands (**Fig. 3F**). A Gram stain was performed, and no bacteria were identified histologically in the affected lung areas. In addition, paraffin-embedded lung and trachea were analyzed at the Louisiana Animal Disease Diagnostic Laboratory in Baton Rouge, LA, USA, for SARS-CoV-2 antigen via immunohistochemistry (IHC) using an anti-nucleocapsid antibody and viral RNA via RNAscope *in situ* hybridization targeting the S gene. No SARS-CoV-2-specific antigen or RNA was detected.

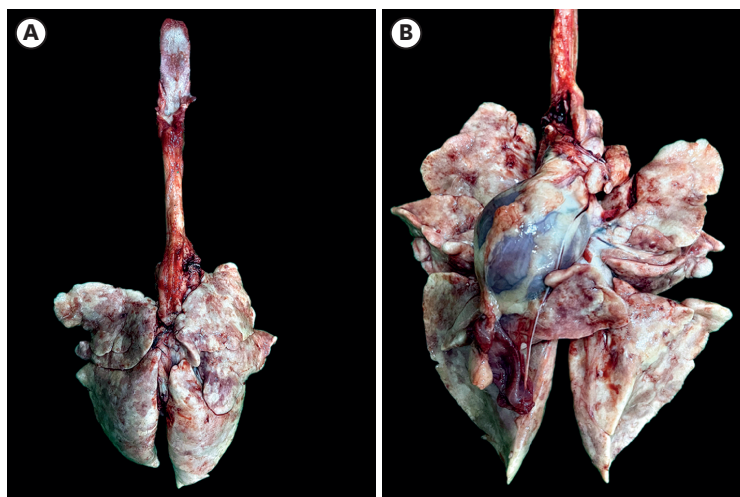


Fig. 2. Gross view of the lungs of the SARS-CoV-2 seropositive cat. (A) Dorsal and (B) ventral surfaces. The pulmonary parenchyma was firm and characterized by coalescing tan to light red, occasionally slightly raised foci throughout, and altered buoyancy.

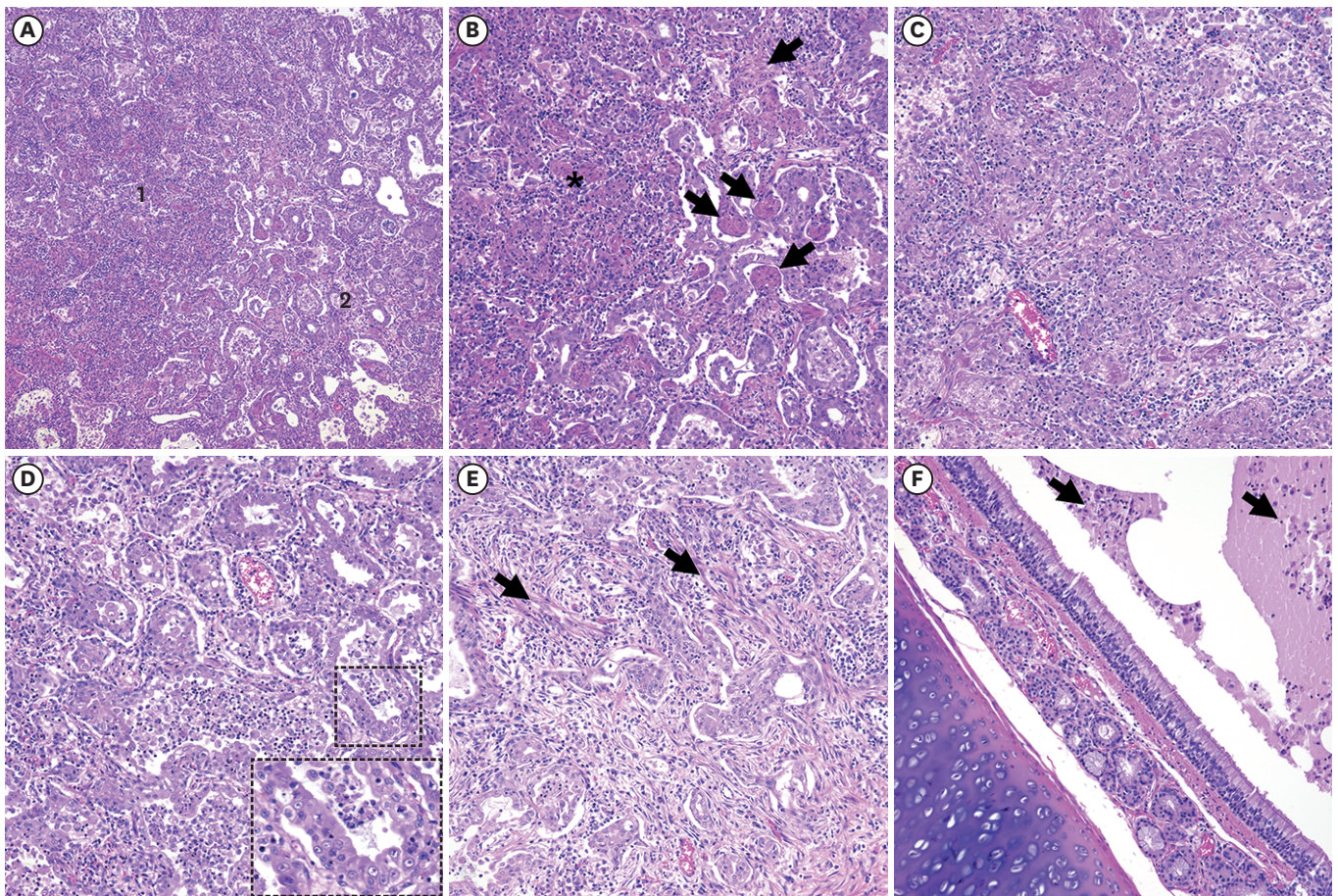


Fig. 3. Histologic changes in the lung and trachea of the SARS-CoV-2 seropositive cat. The pulmonary parenchyma (A-E) was severely affected by coalescing areas of necrosis and organizing fibrin exudation (A [1], B [asterisk], and C), along with areas with prominent alveolar type 2 hyperplasia (A [2], B and D). Repaired alveolar septa were characterized by pronounced smooth muscle hyperplasia (B [arrows] and E (arrows)) and fibrosis (E). Alveolar spaces lined by prominent type 2 pneumocytes (D) were often filled with eosinophilic debris and numerous foamy macrophages (D, inset). The tracheal lumen (F) was partially filled with eosinophilic debris and mixed degenerate leukocytes (arrows). The lining of the respiratory epithelium and subjacent tracheal glands were within normal limits. H&E and Fast Red, 5X (A) and 10X (B-F) original objective.

The cat's serum was analyzed for antibodies to SARS-CoV-2 by an *in-house* enzyme-linked immunosorbent assay using recombinant S1 and RBD proteins, as described previously [7]. Antibodies to both SARS-CoV-2-S1 (OD value: 0.729) and -RBD (OD value: 0.908) proteins were detected in the cat serum. Neutralizing antibodies to SARS-CoV-2 were also detected using a commercial SARS-CoV-2 Surrogate Virus Neutralization Test Kit (GenScript, USA). No antibodies were detected when the serum was analyzed for antibodies to FeCoV (Bionote, Anigen, FeCoV Antibody).

Real-time reverse transcription polymerase chain reaction (RT-PCR) targeting the N gene (2019-nCoV RUO kit, IDT, Cat No: 10006713, USA) was performed on nasal, oropharyngeal, and rectal swabs taken from the cat during hospitalization and tissues such as liver, lung, spleen, kidney, small intestine, and brain; all were negative for the presence of SARS-CoV-2-RNA [2]. The liver and lung tissues were positive for feline parvovirus DNA but negative for FeCoV RNA, feline morbillivirus RNA, and FIV and FeLV proviral DNA by *in-house* RT-PCR assays.

DISCUSSION

The clinical signs, X-ray and CT images, postmortem examination, and histopathological findings of this case confirmed the diagnosis of severe pneumonia. Three people living in the same household as the cat were positive for SARS-CoV-2, indicating that the cat could have acquired SARS-CoV-2 from the household members. However, SARS-CoV-2 specific RNA was not detected in the clinical swabs nor from the various tissues taken at postmortem examination. In addition, no SARS-CoV-2-specific nucleocapsid protein or viral RNA were detected by IHC and RNAscope *in situ* hybridization in the respiratory tract tissues. The absence of SARS-CoV-2 specific markers at the time of testing was attributed to the cat being presented in a late phase of infection when either only small amounts or no SARS-CoV-2 RNA is expected to be present in the animal [2]. Interestingly, the cat had antibodies to the SARS-CoV-2 S1 and RBD proteins, as well as SARS-CoV-2 neutralizing antibodies. In addition, the lung lesions (pneumonia) were similar to those described previously for SARS-CoV-2 infected cats and humans [1,2,8-13]. The X-ray and CT findings of the lung were also similar to those described for cats and humans infected with SARS-CoV-2 [1,3,11,12]. In particular, the CT findings of the cat's lung with ground-glass opacity were similar to those noted in the lung of COVID-19 human patients [3,8]. Although the lung lesions present in the X-ray images for SARS-CoV-2 infected cats have been reported [11], there are no reports showing the appearance of ground-glass opacities in CT images of SARS-CoV-2 infected cats.

Although the clinical and image findings were similar to SARS-CoV-2 infections, it is unclear if the clinical and pathological changes of this cat were due to a natural, reverse zoonotic SARS-CoV-2 infection because of the absence of SARS-CoV-2 markers (except for SARS-CoV-2-specific antibodies) in clinical samples and the respiratory tract. Importantly, although the animal was negative for many feline pathogens, including FeCoV, the cat was positive for feline parvovirus, which causes feline panleukopenia (FP) in cats. The clinical signs and pneumonia observed in the cat did not resemble FP. Therefore, a potential SARS-CoV-2 infection combined with a feline parvovirus infection [1,14] may have been the cause of the cat's death; the viral infections may have worsened the cat's general health status. The main cause for the death of this animal is unclear because this cat was infected with both, feline parvovirus and SARS-CoV-2.

Diffuse type 2 pneumocyte hyperplasia, necrosis, fibrosis, and smooth muscle hyperplasia are the histologic hallmarks of the present study, indicating a late phase of a severe progressive respiratory disease; similar histological changes have been reported for SARS-CoV-2 infected cats before [1,4,13]. Similarly, severe pulmonary pathology consistent with interstitial pneumonia and diffuse-type 2 pneumocyte hyperplasia are findings of COVID-19 infection in humans [9,10].

Although SARS-CoV-2 antigen or RNA was not detected in the formalin-fixed paraffin-embedded lung and tracheal tissues by the IHC examination and RNAscope ISH, the presence of prominent pneumocyte type-2 hyperplasia, smooth muscle hyperplasia, and mononuclear interstitial inflammation could indicate a late phase of SARS-CoV-2 infection. No bacteria were identified in the lung sections evaluated via a Gram stain. While toxic inhalants could have triggered a similar reparative response in the lungs, exposure to such chemicals was not evident from the case history and household.

In human COVID-19 cases, viral RNA has been detected in many organs, such as the brain, lung, heart, liver, and kidneys [9,10]. In a previous study on a naturally infected cat, SARS-

CoV-2 RNA was detected in the lung, heart, kidney, liver, spleen, and intestine [1]. In contrast, SARS-CoV-2 RNA was detected only in tracheal swabs and the lymph nodes but not in the internal organs of another cat also naturally infected with SARS-CoV-2 [5]. No SARS-CoV-2 RNA was detected in the case analyzed in this study. This could be because the replication and shedding of virus after infection was only for a short duration [15], and the present case was in a later stage of the disease.

In conclusion, the anamnestic, virologic, imaging, and postmortem findings indicate that the cat could have suffered from late-stage SARS-CoV-2 infection based on the following: (i) the animal was SARS-CoV-2 seropositive; (ii) lived in a household with several COVID-19 patients; and (iii) displayed respiratory signs, COVID-19-like lung pathology and tomographic findings showing ground-glass opacity. While there is no unequivocal evidence that the SARS-CoV-2 infection was the underlying cause of death in this cat, this study highlights the need for confirming suspected natural feline cases early after clinical signs develop.

ACKNOWLEDGEMENTS

The authors would also like to acknowledge the Histology and Immunohistochemistry section at the Louisiana Animal Disease Diagnostic Laboratory for their technical assistance.

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