ZOOLOGICAL RESEARCH

COVID-19-like symptoms observed in Chinese tree shrews infected with SARS-CoV-2

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic continues to pose a global threat to the human population. Identifying animal species susceptible to infection with the SARS-CoV-2/ HCoV-19 pathogen is essential for controlling the outbreak and for testing valid prophylactics or therapeutics based on animal model studies. Here, different aged Chinese tree shrews (adult group, 1 year old; old group, 5–6 years old), which are close relatives to primates, were infected with SARS-CoV-2. X-ray, viral shedding, laboratory, and histological analyses were performed on different days post-inoculation (dpi). Results showed that Chinese tree shrews could be infected by SARS-CoV-2. Lung infiltrates were visible in X-ray radiographs in most infected animals. Viral RNA was consistently detected in lung tissues from infected animals at 3, 5, and 7 dpi, along with alterations in related parameters from routine blood tests and serum biochemistry, including increased levels of aspartate aminotransferase (AST) and blood urea nitrogen (BUN). Histological analysis of lung tissues from animals at 3 dpi (adult group) and 7 dpi (old group) showed thickened alveolar septa and interstitial

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hemorrhage. Several differences were found between the two different aged groups in regard to viral shedding peak. Our results indicate that Chinese tree shrews have the potential to be used as animal models for SARS-CoV-2 infection.

Keywords: SARS-CoV-2/HCoV-19; Tree shrews; Animal model; Susceptibility; COVID-19

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, which is caused by infection with a novel human coronavirus (SARS-CoV-2/HCoV-19/2019-nCoV) (Lu et al., 2020a; Wu et al., 2020; Zhou et al., 2020b; Zhu et al., 2020), continues to exhibit widespread and intense global transmission. As of 08 July 2020, COVID-19 has caused over 539 906 deaths and 11 669 259 cases in 216 countries, areas, or territories (https://www.who.int/emergencies/diseases/novel-coronavirus-2019), with numbers still increasing. Based on recently available evidence, SARS-CoV-2 is considered to have a zoonotic origin (Wong et al., 2020), with bats and pangolins being the most probable natural reservoirs and intermediate hosts, respectively (Lam et al., 2020; Xiao et al., 2020; Zhang et al., 2020b; Zhou et al., 2020a; Zhou et al., 2020b). Hitherto, there are no licensed vaccines or prophylactics or therapeutics available to prevent or treat COVID-19, although results from initial clinical observations are promising for convalescent plasma therapy (Duan et al., 2020) and monoclonal antibody (tocilizumab) treatment, which targets the IL-6 pathway to reduce cytokine storm (Xu et al., 2020). Clinical trials for Remdesivir have not yielded statistically significant clinical benefits in patients with severe COVID-19 according to two recent randomized, double-blind, placebo-controlled, multicenter trials (Goldman et al., 2020; Wang et al., 2020c), although controversies regarding the efficacy of Remdesivir remain (Grein et al., 2020). Similarly, clinical trials of hydroxychloroquine and/or azithromycin treatment in COVID-19 patients have shown conflicting results regarding clinical outcomes and adverse effects (Geleris et al., 2020; Lagier et al., 2020; Million et al., 2020; Tang et al., 2020; Yu et al., 2020a). To better understand the pathogenesis of COVID-19 and to hunt for effective drugs and vaccines, proper animal models are urgently needed.

Several recent studies have attempted to establish proper models for SARS-CoV-2 infection using a variety of animals, including human ACE2 transgenic mice (Bao et al., 2020), ferrets (Kim et al., 2020; Shi et al., 2020), golden Syrian hamsters (Chan et al., 2020), and non-human primates (Gao et al., 2020; Lu et al., 2020b; Munster et al., 2020; Rockx et al., 2020; Shan et al., 2020; Yu et al., 2020b). These studies have broadened our knowledge on the infection and interspecies transmission of SARS-CoV-2 in animals, as well as on drugs (Williamson et al., 2020) and vaccines (Gao et al., 2020) with potential efficacy. Chinese tree shrews (*Tupaia belangeri chinensis*), which are widely distributed in Southeast Asia and South and Southwest China, have a close relationship to non-human primates (Fan et al., 2019). These rat-sized experimental animals have been used extensively in biomedical research (Li et al., 2018; Xiao et al., 2017; Yao, 2017), in particular for viral infections, such as hepatitis B and C (Amako et al., 2010; Wang et al., 2012), avian influenza (Xu et al., 2019), and HSV-1 virus infections (Li et al., 2016). Comparison of angiotensin converting enzyme 2 (ACE2, which serves as the SARS-CoV-2 receptor (Zhou et al., 2020b)) protein sequences shows high sequence identity between humans and tree shrews (up to 81%), suggesting that tree shrews may be susceptible to SARS-CoV-2 infection.

In the current study, we investigated whether Chinese tree shrews can be infected by SARS-CoV-2 and therefore used to create a valid animal model for SARS-CoV-2 infection and COVID-19. Our study found direct evidence that Chinese tree shrews are susceptible to SARS-CoV-2 infection and infected animals display viral shedding, lung lesions, and alterations in blood biochemical indices.

MATERIALS AND METHODS

Animals and ethics statement

Healthy adult tree shrews (adult group, 1 year old, *n*=13, including nine males and four females; old group, 5–6 years old, *n*=7, including three males and four females) were sourced from the Experimental Animal Core Facility of the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS). All experiments with live SARS-CoV-2 were performed in the animal biosafety level 3 (ABSL3) facility in KIZ, CAS. The animal studies were performed in accordance with the regulations of the Guide for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China. The Institutional Animal Care and Use Committee of KIZ, CAS, approved all protocols used in this study (Approval No: SMKX-20200301-02).

Virus strain

The SARS-CoV-2 strain 107 was obtained from the Guangdong Provincial Center for Disease Control and Prevention, Guangdong Province, China. The virus was amplified in Vero-E6 cells. Median tissue culture infective dose ($TCID_{50}$) was used to assess virus infectivity, and titers were calculated by the Reed-Muench method (Reed & Muench, 1938).

Animal experiments and laboratory tests

After anaesthetization with ketamine, we performed clinical examinations for each tree shrew to obtain baseline information. Collection of rectal and throat swabs, X-ray tests, laboratory assessments, routine blood tests (i.e., white blood cell count, including lymphocytes, monocytes, and granulocytes), and serum biochemistry tests (i.e., blood urea nitrogen (BUN), total protein, globulin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT)) were performed. Each animal was then inoculated with a total of 300 μL (1×10^7 $TCID_{50}$) of SARS-CoV-2 by oral (240 μL),

intranasal (20 μL per nostril), and ocular (10 μL per eye) routes. The animals were randomly selected at the indicated days post-inoculation (dpi) for clinical examination after anaesthetization (Figure 1). Briefly, throat and anal swabs were added to 1 mL DMEM medium (Gibco, USA) at various time points (adult group: 3, 5, 7, and 14 dpi; old group: 3, 5, 7 and 11 dpi), after which the animals received chest X-ray tests. Blood samples were collected from each animal for routine blood tests and serum biochemistry before euthanasia. Necropsies were then performed to test viral loads and pathological lesions in different tissues, with seven lung lobes, conjunctiva, kidney, urinary bladder, small intestines (duodenum, jejunum, ileum), testes, and ovaries collected for viral RNA detection. We performed hematoxylin and eosin (H&E) staining of lung tissues, which were fixed in 4% paraformaldehyde and embedded in paraffin for further processing. Double-blind assessment of tissue sections was conducted by a pathologist, who scored the tissues for alveolar edema, interstitial edema, hemorrhage, and inflammatory infiltration. Slides were viewed using a Leica microscope (Leica, Germany) with Leica application suite 4.9 software to capture images. The immunofluorescence process was described in our previous study (Zhang et al., 2019). In brief, the sections were deparaffinized in xylene and rehydrated through a graded ethanol series; for antigen retrieval, sections immersed in saline sodium citrate buffer were microwave heated for 6 min three times. This was followed by cooling to room temperature, washing with 1×phosphate-buffered saline (PBS) containing 0.05% Tween-20 (1×PBST), and blocked with 10% bovine serum albumin (BSA) at 37 °C for 60 min. Anti-SARS-CoV-2 nucleoprotein (NP) (1:200; 40143-R019, Sino Biological, USA) was diluted in BSA and incubated overnight at 4 °C. The sections were then washed, and immunoreactivity was detected using FITC-conjugated secondary antibody (1:500; KPL, 172-1506; incubation for 1 h at room temperature). The sections were directly counterstained with 5 μg/mL 4', 6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature and washed with 1×PBST three times. Slides were visualized under an Olympus FluoView 1000 confocal microscope (Olympus, Japan).

Quantitative real-time RT-PCR (qRT-PCR) to detect SARS-CoV-2 RNA

Total RNA was extracted from swabs and serum samples using a High Pure Viral RNA Kit (Roche, Germany) in accordance with the manufacturer's instructions. TRIzol Reagent (Thermo, USA) was applied for RNA isolation using homogenized tissues. We followed the manufacturer's protocols for one-step RT-PCR using a THUNDERBIRD Probe One-Step qRT-PCR Kit (TOYOBO, Japan) to detect SARS-CoV-2 RNA. Previously reported primers targeting the N protein were used, including (5'-GGGGAACTTCTCCTG

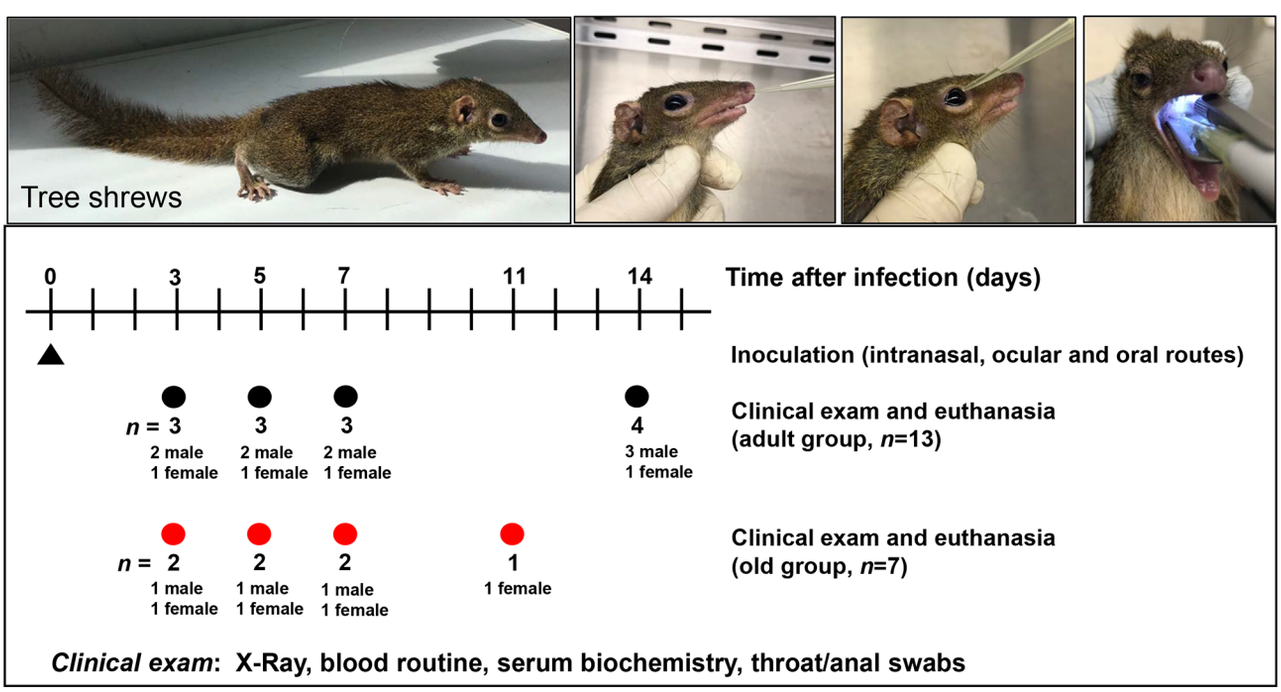

Figure 1 Schematic of experimental design

Tree shrews were divided into two groups, i.e., adult group (*n*=13, black circles) and old group (*n*=7, red circles), and inoculated with SARS-CoV-2 (black triangle) via intranasal, ocular, and oral routes. At 0, 3, 5, 7, 11 (old group), or 14 (adult group) days post-inoculation (dpi), clinical examinations were performed to show tree shrew status. Animals from each group were euthanized and necropsied at indicated dpi for virological and pathological assays.

CTAGAAT-3'/5'-CAGACATTTTGCTCTCAAGCTG-3') and probe FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3' (Wang et al., 2020a). In each run, serial dilutions of the SARS-CoV-2 RNA reference standard (National Institute of Metrology, China) were used in parallel to calculate copy numbers in each sample.

Statistical analysis

Comparisons between different groups were conducted using two-tailed Student's *t* test (GraphPad Prism v7). The data are presented as means±standard deviation (*SD*). A *P*-value of <0.05 was considered statistically significant.

RESULTS

In total, 13 adult tree shrews (~1 year old) and seven old tree shrews (5–6 years old) were used for this study. Each animal was inoculated with SARS-CoV-2 strain 107 (total 300 μL (1×10^7 $TCID_{50}$)) by oral (240 μL), intranasal (20 μL per nostril), and ocular (10 μL per eye) routes (Figure 1). After inoculation with SARS-CoV-2, tree shrews were randomly selected on a specific schedule for X-ray, routine blood work and serum biochemistry tests (Figure 1). Selected tree shrews were euthanized for necropsy and examination of pathological changes at indicated time points. As shown in Figure 2, lung infiltrates became visible on the X-rays from 3 dpi and were sustained to 14 dpi (adult group) or 11 dpi (old group). We used different sampling times for the adult and old groups at the last time point (14 dpi vs. 11 dpi) simply because the old animals refused to eat and drink due to stress during the experiments. Most infected animals (17/20, 85%) presented pulmonary infiltrates according to X-ray tests (Figure 2).

Throat and anal swabs were obtained from each animal on

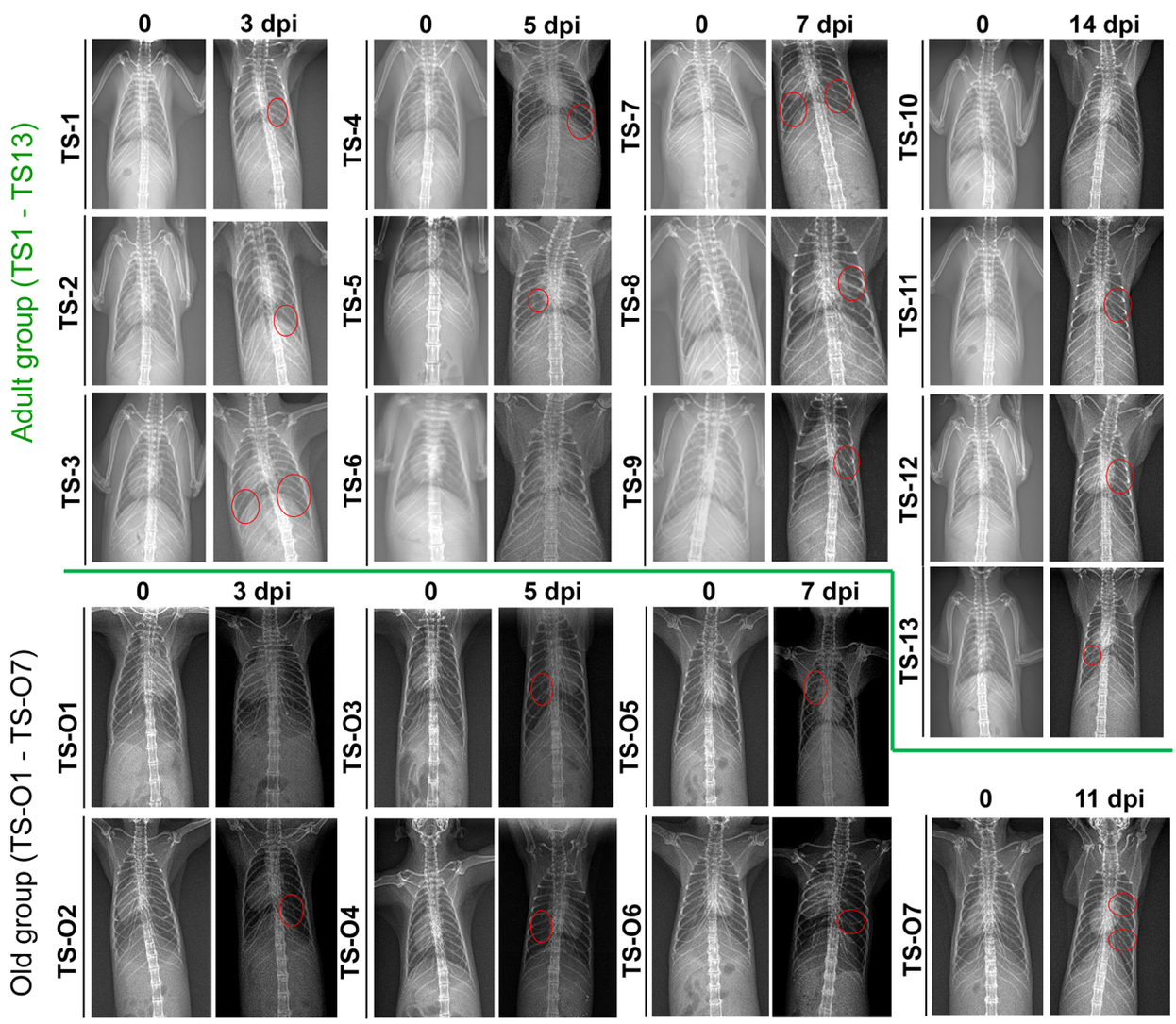

Figure 2 Chest X-rays of tree shrews before and after SARS-CoV-2 inoculation

Circled areas on radiographs are regions showing lung infiltrates.

the days of examination. However, SARS-CoV-2 RNA was undetectable in all swabs from the infected tree shrews. Viral RNA was also undetectable in serum samples of the infected animals (Figure 3A). However, measurement of viral loads in different lobes of the lung and other tissues (conjunctiva, kidney, urinary bladder, small intestines, testes, and ovaries) showed that most lung lobes had higher numbers of viral RNA copies (Figure 3B) in the adult group. For the different examination days, viral RNA in the lung tissue samples peaked at 3 dpi, ranging from 1 360 to 931 777 copies/g, then gradually decreased to undetectable at 14 dpi. In the old group, however, viral RNA was only detected in a few lobes of the lung, with a peak appearing at 7 dpi. Moreover, a low viral copy number was observed in the small intestine (5/20, 25%) and conjunctiva tissues (2/20, 10%) in a small proportion of individuals (Figure 3C). We detected the IgM and neutralizing antibodies in these infected tree shrews but were unable to successfully detect related antibodies (data not shown). Thus, taken together, our results indicate that tree shrews are susceptible to SARS-CoV-2 infection.

We performed histological analysis of lung tissues from animals at 3 dpi in the adult group and 7 dpi in the old group, as these time points showed the highest viral loads in their respective groups. The main gross lesions involved sporadic or massive pulmonary punctate hemorrhage (Figure 4A). Sections of all seven lung lobes showed thickened alveolar septa and interstitial hemorrhage (Figure 4B). Occasionally, alveoli contained small numbers of pulmonary lymphocytes and neutrophils. Immunofluorescence using a murine antibody against SARS-CoV NP demonstrated the presence of viral antigen in a small number of pneumocytes (Figure 4C).

The overall pattern of laboratory assessments in animals before and after SAR-CoV-2 inoculation (Tables 1, 2) exhibited several features. First, the white blood cell (P= 0.001 7), lymphocyte (P=0.023), monocyte (P=0.017 6), and granulocyte counts (P=0.002 8) were obviously elevated after infection in the adult group (Table 1). This pattern is different from that of patients with COVID-19, in which lymphocytopenia is common (Guan et al., 2020). However, we did observe a significant decrease in monocytes (P=0.000 9) in the old group after SARS-CoV infection (Table 2) (Figure 5A). The exact reason for this age-related difference remains to be determined. Second, BUN and albumin were significantly altered in both groups of animals after viral infection. Moreover, there was a marked increase in AST with SARS-CoV-2 infection in the adult group (Figure 5B). These observations reflected impaired liver and renal function, consistent with reported laboratory findings that AST is elevated during COVID-19 progression (Zhang et al., 2020a). Further histological and immunohistochemical analyses and viral detection should be performed on liver and kidney tissues of infected tree shrews to confirm impaired liver and renal function and the presence of viral loads in these organs.

DISCUSSION

The COVID-19 pandemic continues to spread worldwide due

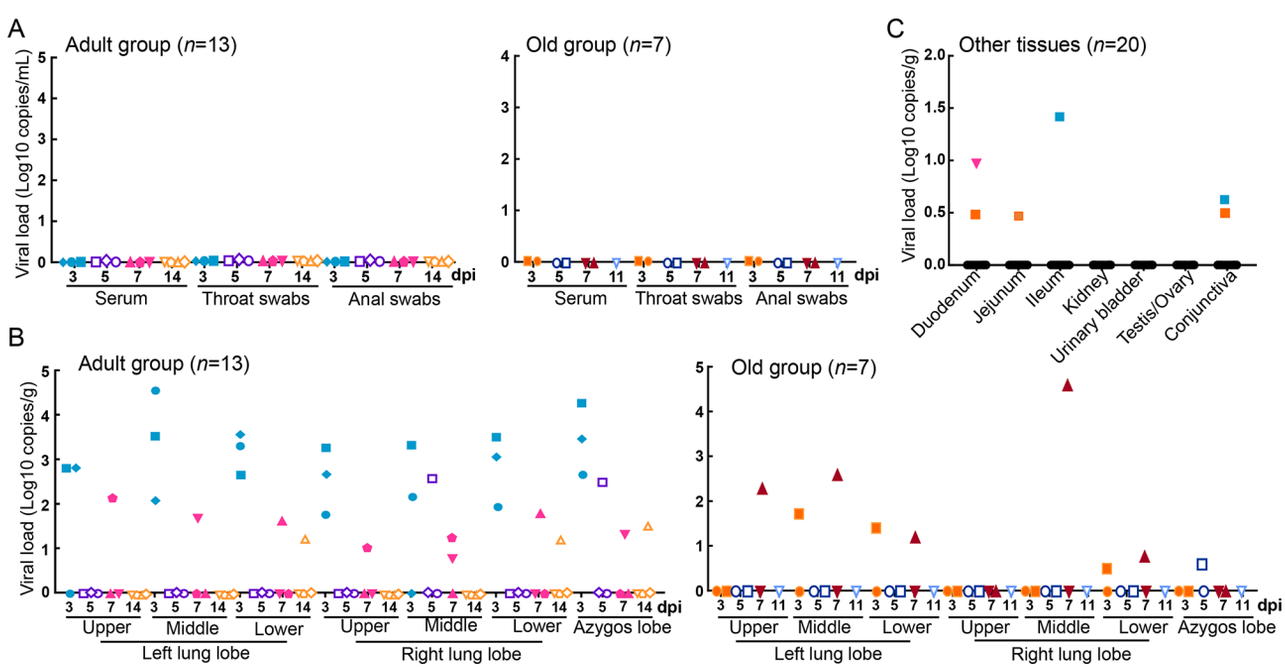

Figure 3 Viral loads of SARS-CoV-2 in Chinese tree shrews

Viral loads in serum and throat/rectal swabs (A), lung lobes (B), and other tissues (C) from infected tree shrews measured using quantitative real-time PCR. Each graphic represents one individual, with color referring to indicated dpi. For instance, three infected animals sacrificed at 3 dpi were defined by square, circle, and diamond, respectively. Marks in blue refer to time point 3 dpi.

to a current lack of approved drugs or vaccines. Recent studies on the infection and transmission of SARS-CoV-2 in different animals have provided new insights into the pathogenesis of SARS-CoV-2 (Bao et al., 2020; Kim et al., 2020; Lu et al., 2020b; Munster et al., 2020; Rockx et al., 2020; Shan et al., 2020; Yu et al., 2020b). Pioneering studies evaluating the clinical benefit of Remdesivir (Williamson et al., 2020) and inactivated vaccine candidate (Gao et al., 2020) in rhesus macaques infected with SARS-CoV-2 have established a good paradigm for researchers to discover and optimize drug and vaccine candidates for the prevention of SARS-CoV-2 infection and for the treatment of COVID-19. In this study, we found that Chinese tree shrews could be infected with SARS-CoV-2, with consistent detection of viral loads in the lung tissues of infected animals. Compared with available non-human primate models (Lu et al., 2020b; Rockx

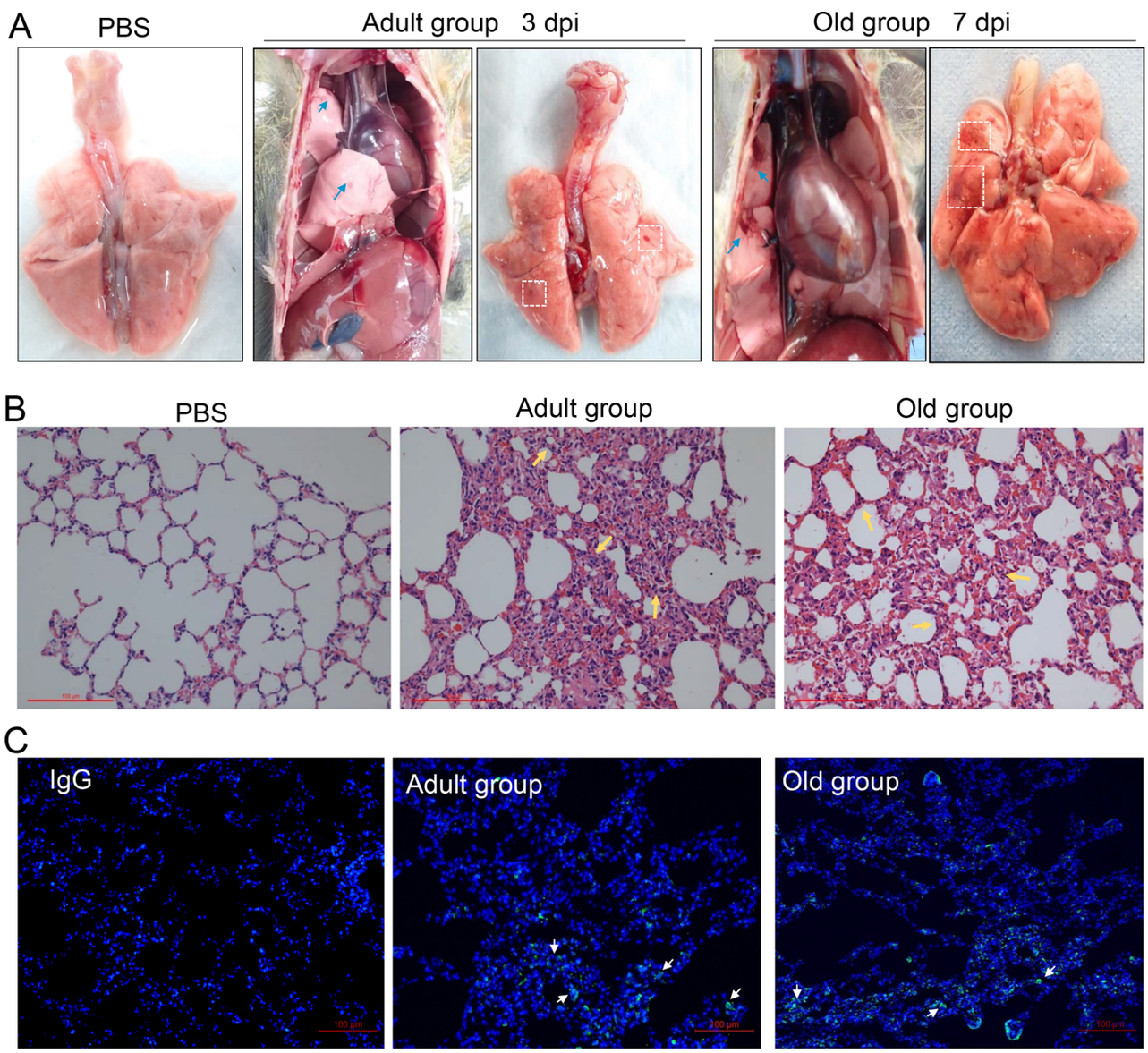

Figure 4 Characterization of lung changes after SARS-CoV-2 infection in Chinese tree shrews

A: Lesions in lungs. Representative view of ventral lungs of infected tree shrew obtained via necropsy at 3 dpi in adult group or at 7dpi in old group compared to healthy control. White box indicates lungs showing focal areas of hyperemia. B, C: Representative hematoxylin and eosin staining (B) and immunofluorescence analysis of viral antigen NP (C) in lung tissues from tree shrews at 3 dpi in adult group or at 7 dpi in old group. Blue arrows in A refer to lesions. Yellow arrows in B indicate pulmonary lymphocytes or neutrophils. White arrows in C indicate SARS-CoV-2 NP staining (green) in pneumocytes, with DAPI staining in blue. PBS and IgG indicate tissue sample from healthy animal in control group treated with PBS but no SARS-CoV-2. Scale bars: 100 μm.

et al., 2020; Shan et al., 2020; Yu et al., 2020b), the tree shrew model for SARS-CoV-2 infection showed similar lung infiltrates as observed in monkeys and humans. Given their genetic closeness to non-human primates (Fan et al., 2019) and fewer ethical concerns compared to studies on monkeys, tree shrews are becoming increasingly important in biomedical research. Based on the results in this study, tree shrews may be a valid model for SARS-CoV-2 infection. Certainly, a direct

Table 1 Laboratory findings of adult tree shrews infected with SARS-CoV-2

Parameter	Before inoculation (13/13, 100%)	After inoculation					
		Total (13/13, 100%)	*P*-value[a]	3 dpi (3/13, 23%)	5 dpi (3/13, 23%)	7 dpi (3/13, 23%)	14 dpi (4/13, 31%)
White blood cell count (×10^9/L)	1.80±1.12	4.85±3.33	0.0017	1.37±0.70	3.83±1.25	3.77±2.20	8.75±2.62
Lymphocyte count (×10^9/L)	0.85±0.43	1.34±0.51	0.0230	0.70±0.46	2.27±0.90	1.83±0.71	1.50±0.37
Monocyte count (×10^9/L)	0.18±0.09	0.38±0.23	0.0176	0.27±0.21	0.47±0.12	0.50±0.44	0.33±0.05
Granulocyte count (×10^9/L)	0.62±0.46	1.75±1.16	0.0028	0.80±0.70	1.10±0.36	1.43±1.10	3.18±0.14
Lymphocyte percentage (%)	52.64±10.69	51.09±7.86	0.6920	43.90±5.72	59.00±4.15	52.30±10.70	49.65±4.38
Monocyte percentage (%)	13.36±3.92	10.55±4.71	0.1727	14.57±1.17	12.63±3.77	11.67±4.01	5.15±2.63
Granulocyte percentage (%)	34.40±9.02	33.65±7.16	0.9175	41.53±4.56	28.37±2.30	36.03±8.47	29.93±5.07
BUN (mg/dL)	16.46±4.18	23.65±8.43	0.0196	26.00±8.19	23.00±6.25	16.67±11.06	27.50±7.59
TP (g/dL)	6.56±0.28	6.40±0.77	0.4890	5.57±0.38	6.13±0.90	6.87±0.46	6.88±0.57
ALB (g/dL)	3.74±0.44	3.20±0.65	0.0134	2.50±0.26	2.97±0.35	3.60±0.46	3.60±0.71
Globulin (g/dL)	2.82±0.27	3.17±0.26	0.0044	3.07±0.15	3.17±0.55	3.23±0.12	3.20±0.16
ALT (U/L)	151.10±79.21	187.80±123.40	0.2662	141.70±80.59	138.3±77.69	247.70±55.77	214.50±203.40
AST (U/L)	197.00±44.99	467.80±286.20	0.0035	238.30±73.35	583.30±227.00	363.30±75.22	631.50±415.20

[a]: *P*-values indicate differences before and after inoculation (total). $P<0.05$ was considered statistically significant. Data are means±*SD* (Test No./Total No., %). Paired Student's *t* test. BUN: Blood urea nitrogen, TP: Total protein, ALB: Albumin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Table 2 Laboratory findings of old tree shrews infected with SARS-CoV-2

Parameter	Before inoculation (7/7, 100%)	After inoculation					
		Total (7/7, 100%)	*P*-value[a]	3 dpi (2/7, 28%)	5 dpi (2/7, 28%)	7 dpi (2/7, 28%)	14 dpi (1/7, 14%)
White blood cell count (×10^9/L)	1.45±0.53	3.12±2.25	0.0754	0.80±0.28	6.05±0.49	2.75±1.34	2.90
Lymphocyte count (×10^9/L)	0.45±0.26	1.67±1.68	0.1101	0.65±0.49	3.2±2.26	1.85±0.21	0.30
Monocyte count (×10^9/L)	0.21±0.07	0.20±0.16	0.8347	0.05±0.07	0.35±0.21	0.25±0.07	0.10
Granulocyte count (×10^9/L)	0.77±0.31	1.014±0.95	0.5320	0.50±0.28	0.50±0. 28	1.05±0.49	3.00
Lymphocyte percentage (%)	30.77±11.78	46.47±19.90	0.0977	44±8.34	56.80±37.34	51.2±3.11	22.30
Monocyte percentage (%)	16.53±4.19	8.41±2.52	0.0009	8.75±1.62	5.70±2.82	9.10±0.42	11.80
Granulocyte percentage) (%)	50.01±7.85	47.23±22.05	0.7583	54.25±16.62	47.9±49.21	39.70±2.68	46.90
BUN (mg/dL)	19.83±5.44	31.29±11.51	0.0087	36.50±6.36	43.00±4.24	21.5±3.53	17.00
TP (g/dL)	6.42±0.36	5.74±0.79	0.0200	5.45±1.06	5.55±0.92	6.50±0.28	5.20
ALB (g/dL)	3.26±0.35	2.50±0.55	0.0017	2.40±0.85	2.35±0.49	2.95±0.49	2.10
Globulin (g/dL)	3.13±0.21	3.25±0.29	0.3080	3.05±0.21	3.2±0.42	3.55±.021	3.20
ALT (U/L)	202.40±77.66	180.90±122.70	0.6428	187.00±86.27	115.50±33.23	264.50±238.30	132.00
AST (U/L)	229.00±31.97	361.30±316.20	0.1606	192.00±42.43	271.00±183.80	657.50±556.50	288.00

[a]: *P*-values indicate differences before and after inoculation (total). $P<0.05$ was considered statistically significant. Data are means±*SD* (Test No./Total No., %). Paired Student's *t* test. BUN: Blood urea nitrogen, TP: Total protein, ALB: Albumin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

comparison of the current tree shrew model with other similar-sized animal models commonly used in the laboratory, such as ferrets (Kim et al., 2020; Shi et al., 2020) and golden Syrian hamsters (Chan et al., 2020), could help clarify whether tree shrews are superior to other animal models in mimicking the hallmarks of disease observed from human infection with SARS-CoV-2.

However, there are several limitations regarding the tree shrew model for SARS-CoV-2 infection in this study. First, we did not include juvenile tree shrews, who may be more susceptible to SARS-CoV-2 infection, as age is a risk factor for COVID-19 (Yang et al., 2020) and we did observe differences regarding viral shedding and laboratory tests between adult and old groups. Interestingly, the old tree shrews seemed to be less susceptible to SARS-CoV-2 infection compared with the adult tree shrews based on viral loads in the lung tissues. This pattern is quite different from previous reports, which suggest that SARS-CoV-2 is more virulent in older humans than in younger humans (Guan et al., 2020; Yang et al., 2020). As the sample size of the old group was relatively small, this observation needs to be further validated. Second, we did not provide body temperature data as we could not achieve reliable measurements to confirm whether infected animals had a fever. Other typical symptoms of COVID-19, such as cough, myalgia, dyspnea, fatigue, and headache (Guan et al., 2020), were also not tested due to a lack of related models. Third, we did not characterize cytokine profiling due to a lack of immunological reagents and did not perform detailed analyses of pathological alterations in all tissues of infected animals. The detection of SARS-CoV-2 RNA in intestinal tissues of infected animals deserves further investigation, including histopathological and immunohistochemical analyses and single-cell RNA sequencing. Similarly, as SARS-CoV-2 is a respiratory pathogen and animals are also infected via this route, analysis of nasal turbinates would also be of benefit to improve knowledge on viral shedding. Fourth, the animals were sacrificed at different time points for lung pathology and viral loads. However, it might be improper to compare different animals sacrificed at different times, particularly as no inbred line of tree shrews is currently available and animals may have a diverse genetic background. Finally, we did not include a group receiving drug treatments, such as Remdesivir or chloroquine, which have been found to inhibit the replication of SARS-CoV-2 in cellular inhibition assays (Wang et al., 2020b), to further evaluate the efficacy of the model for drug tests. Future studies will be carried out to address these needs.

It should be mentioned that during the preparation of our manuscript, we noted a report on SARS-CoV-2 infection in Chinese tree shrews by Zhao and coworkers (Zhao et al., 2020). They used a different procedure compared with ours as they aimed to undertake longitudinal observations of infected animals. Zhao et al. (2020) found no obvious clinical signs in these animals except for an increase in body temperature in some infected tree shrews and mild pulmonary alterations. They could not consistently detect viral shedding at all time points based on nasal, throat, and anal swabs or blood samples. We believe the different procedures used in our study and that of Zhao et al. (2020) may account for the different patterns reported.

In summary, we found that Chinese tree shrews are susceptible to SARS-CoV-2 infection, with lung lesions and alterations in blood biochemical indices observed. Thus, we believe that the Chinese tree shrew infection model could

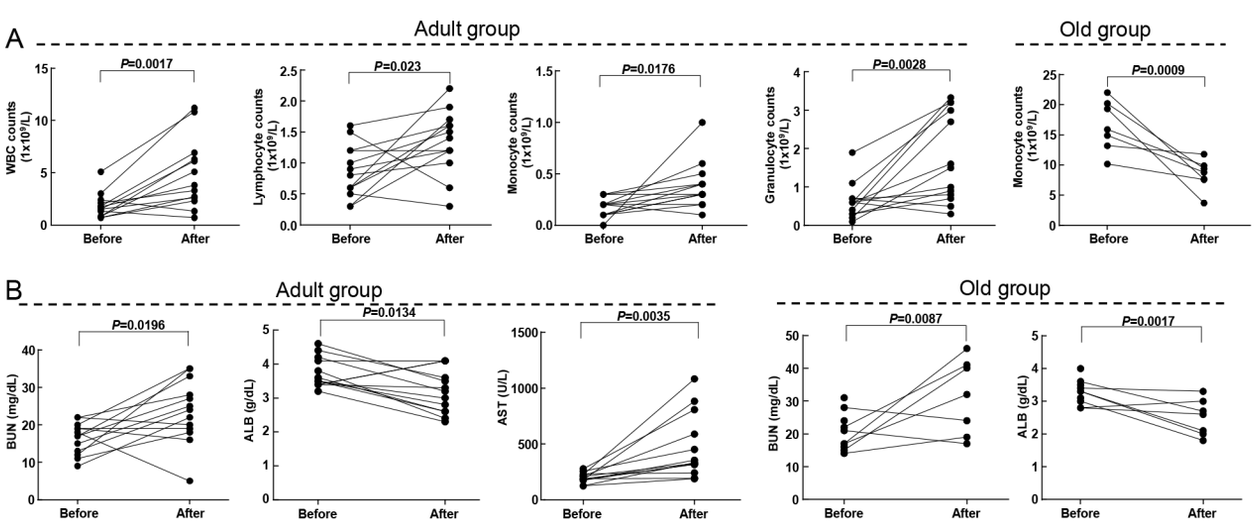

Figure 5 Laboratory findings of altered blood counts (A) and serum biochemistry (B) in tree shrews before and after SARS-CoV-2 infection

Values are means±*SD*. *P*-values were calculated using paired Student's *t* test. Each circle represents one individual, with "Before" and "After" referring to indicated parameter in same animal before SARS-CoV-2 infection and at time of euthanasia, respectively. We grouped all infected animals in each group together, regardless of different infection times of different animals.

potentially be used for drug screening and vaccine evaluation.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Y.G.Y., Y.T.Z, L.X., and D.D.Y. designed the study and prepared the manuscript. L.X., D.D.Y., Y.H.M., Y.L.Y., R.H.L., X.L.F., J.B.H., X.H.W., and M.H.L. performed the experiments. L.X., D.D.Y., and H.R.C analyzed the data and prepared the figures. C.W.K. provided the virus. All authors read and approved the final version of the manuscript.

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