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An outbreak of SARS-CoV-2 omicron variant and deaths of three lions in a zoo

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ABSTRACT

There have been reports of the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from humans to various mammalian species. Some infected animals show clinical signs and may even die in rare cases. Outbreaks of SARS-CoV-2 have been reported in zoos where susceptible animals are bred in high population densities. However, there have been few reports of omicron variant outbreaks in zoo animals. From late 2022 to 2023, an outbreak of the SARS-CoV-2 omicron variant occurred in one Japanese zoo. A total of 24 lions were housed in the zoo; 13 of them showed respiratory symptoms, and the three oldest lions died. Molecular and histopathological analyses revealed that the deceased lions were infected with SARS-CoV-2 omicron BF.7.15. Virus-neutralization tests showed that all 21 lions were positive for antibodies against the omicron variant, but not against the delta variant. In addition, three tigers and one bear in the same or neighboring building as the lions possessed antibodies against the omicron variant. This is a very rare report on the outbreak of a SARS-CoV-2 omicron variant infection that resulted in the death of animals. This finding demonstrates the importance of continuous countermeasures to protect non-vaccinated animals from SARS-CoV-2 infection.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the *Betacoronavirus* genus, emerged in 2019 and caused the coronavirus disease 2019 (COVID-19) pandemic, leading to numerous human deaths [1,2]. This virus might have originated from bats by zoonotic transmission and is now circulating among human populations. In addition, reverse zoonotic transmission from humans to susceptible animal species has been also reported [3]. Previously, we documented reverse zoonotic SARS-CoV-2 infection in dogs and cats transmitted from human patients with COVID-19 in Japan, with an approximate infection rate of 15% [4]. In the USA, SARS-CoV-2 has been circulating among deer through reverse zoonotic transmission from humans [5]. Furthermore, the transmission of SARS-CoV-2 from humans to minks on farms has been confirmed in several countries in Europe, followed by spillback to humans [6–8].

SARS-CoV-2 has also infected various mammalian species in zoos, and some of these infected animals showed clinical symptoms and died.

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The virus is thought to have been transmitted from infected zookeepers and then spread among susceptible animals. To date, at least 29 animal species have reportedly been infected with SARS-CoV-2 [3], and some of these animals died due to SARS-CoV-2 infection. The lion (*Panthera leo*) is known to be a susceptible animal that exhibits respiratory signs upon SARS-CoV-2 infection [9–12]. In 2021, two Asiatic lions in a zoological park in India were infected with the SARS-CoV-2 delta variant and exhibited symptoms such as nasal discharge and occasional coughing, followed by their deaths [13]. These reverse zoonotic infections from humans to animals have become a serious problem for zoo animals. However, since the emergence of the omicron variant at the end of 2021, there has been little information on animal infection with SARS-CoV-2.

From the end of 2022 to the beginning of 2023, an outbreak of the SARS-CoV-2 omicron variant occurred among lions at a zoo in Japan. The present study aimed to analyze the outbreak in detail.

Table 1
Characteristics, clinical signs, and clinical course of lions examined in this study.

ID	Sex	Age* (years)	Date of disease onset	Clinical signs	Clinical course
Lion 1	Female	21	8th Jan, 2023	Dyspnea, cough, anorexia	Died on 12th Jan, 2023
Lion 2	Male	19	3rd Jan, 2023	Nasal discharge, anorexia, epistaxis, polyuria, polydipsia	Died on 9th Jan, 2023
Lion 3	Male	19	30th Dec, 2022	Nasal discharge, dyspnea, anorexia	Died on 31st Dec, 2022
Lion 4	Female	19	10th Jan, 2023	Cough	Recovered
Lion 5	Female	17	10th Jan, 2023	Dyspnea, cough	Recovered
Lion 6	Female	16	8th Jan, 2023	Dysnea, cough	Recovered
Lion 7	Female	15	10th Jan, 2023	Cough	Recovered
Lion 8	Female	15	11th Jan, 2023	Cough, nasal discharge	Recovered
Lion 9	Male	13	–	–	–
Lion 10	Female	13	–	–	–
Lion 11	Female	13	–	–	–
Lion 12	Female	13	–	–	–
Lion 13	Male	13	14th Jan, 2023	Cough	Recovered
Lion 14	Male	13	–	–	–
Lion 15	Male	13	–	–	–
Lion 16	Female	13	–	–	–
Lion 17	Female	13	–	–	–
Lion 18	Female	12	15th Jan, 2023	Nasal discharge	Recovered
Lion 19	Female	8	14th Jan, 2023	Cough	Recovered
Lion 20	Female	8	13th Jan 2023	Cough	Recovered
Lion 21	Female	8	17th Jan 2023	Cough	Recovered
Lion 22	Male	5	–	–	–
Lion 23	Male	5	–	–	–
Lion 24	Male	5	–	–	–

*Age on 31st December 2022.

2. Materials and methods

2.1. Sample collection

Tissue samples from Lions 1, 2, and 3 were collected after their deaths. Zoo veterinarians used the SARS-CoV-2 Rapid Antigen Test (Roche Diagnostics, Basel, Switzerland) to detect the SARS-CoV-2 antigen. Tissue samples were collected by necropsies. For blood collection, lions were restrained in squeeze cages and blood samples were collected from the caudal or saphenous vein. Fecal samples from Lion 5 were collected from the ground of its cage. For further analysis, tissue and serum samples were transported to the Department of Veterinary Science and the Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan.

2.2. Cells

VeroE6/TMPRSS2 cells [14] (JCRB Cell Bank no. JCRB1819) were cultured in Dulbecco's modified Eagle's medium (DMEM)–low glucose (Sigma-Aldrich, MO, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich), 1% penicillin-streptomycin (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), and 1 mg/mL G-418 (Roche Diagnostics) at 37 °C in 5% CO_2. Vero E6-TMPRSS2-T2A-ACE2 cells (BEI resources, catalogue no. NR-54970) were provided by Dr. Barney Graham (National Institute of Health) and Dr. Yoshihiro Kawaoka (The University of Tokyo) and maintained in DMEM–high glucose (DMEM-HG; Sigma-Aldrich) supplemented with 10% FBS, 1% penicillin-streptomycin, 1% non-essential amino acids (Thermo Fisher Scientific, MA, USA), 1 mM sodium pyruvate solution (Thermo Fisher Scientific), and 1 μg/mL puromycin (InvivoGen, CA, USA).

2.3. Viruses

SARS-CoV-2 TY41–702 (omicron BE.1 variant) and TY26–439 (delta variant) were propagated in VeroE6/TMPRSS2 cells and stored at −80 °C until use in virus neutralization (VN) tests. Nucleotide sequence data for these strains are available from GISAID [15] (TY41–702: EPI_ISL_13241867 and TY26–439: EPI_ISL_3374455). SARS-CoV-2 hCoV-19/Lion/Japan/Lion1/2023 (omicron BF.7.15 variant) isolated from the lung tissue of Lion 1 was used for the VN test.

2.4. Real-time reverse transcription-polymerase chain reaction (RT-PCR) for SARS-CoV-2

Tissue samples in DMEM-HG with 2% FBS and antibiotics were homogenized using a BioMasher II (Nippi, Tokyo, Japan) and centrifuged at 1110 ×*g* for 5 min at 4 °C. RNA was extracted from the supernatants, serum samples, and tracheal swabs using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). A real-time RT-PCR assay was conducted with the Quantitect Probe RT-PCR Kit (Qiagen) using the primer set N2 on a LightCycler 480 (Roche Diagnostics) [16]. Samples were considered positive for SARS-CoV-2 RNA when the cycle threshold value was <40.

2.5. RT-PCR for formalin-fixed paraffin-embedded (FFPE) specimens

For RNA extraction from FFPE specimens, either the PureLink FFPE RNA isolation Kit (Invitrogen, Carlsbad, CA) or the Quick-DNA/RNA FFPE Miniprep Kit (Zymo Research, Irvine, CA) was used in accordance with the manufacturer's protocols. Real-time RT-PCR was performed on an Mx3000P or Mx3005P (Agilent Technologies, CA, USA) using the Quantitect Probe RT-PCR Kit (Qiagen) with the specified primer set [17].

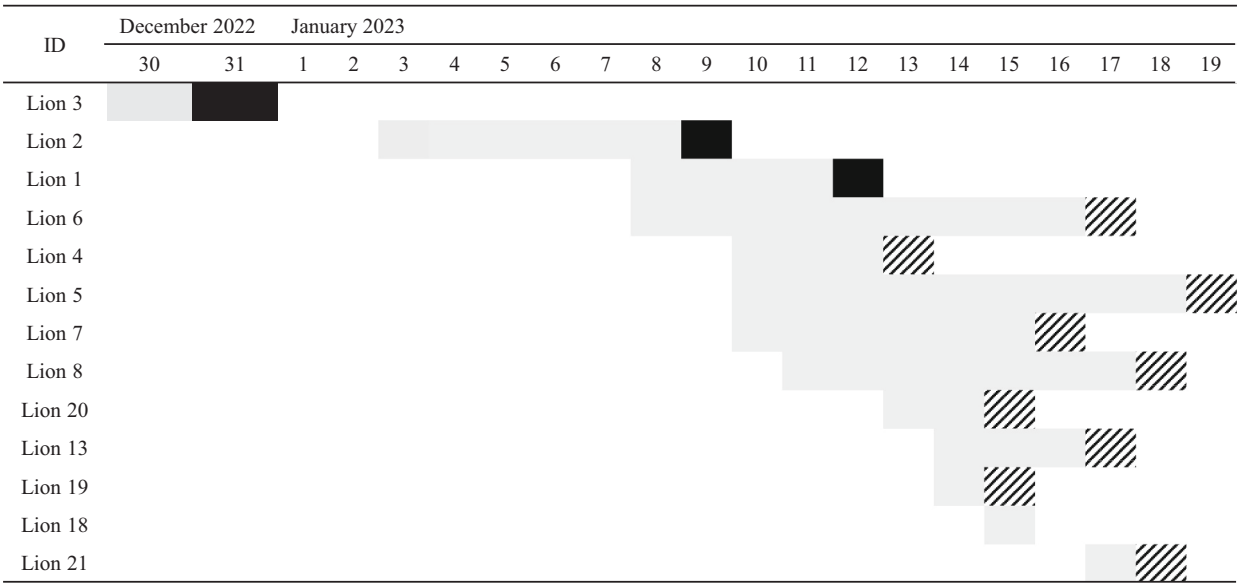

Fig. 1. Progress of the SARS-CoV-2 outbreak in lions.
Gray boxes, black boxes, and boxes with slashes represent the dates of disease onset, death, and recovery, respectively.

2.6. Virus isolation

Serum samples, oral swabs, and the supernatant from homogenized lung tissue samples were filtered through 0.45-μm filters (Corning, Corning, NY, USA). Vero E6-TMPRSS2-T2A-ACE2 cells in 6-well plates (Sumitomo Bakelite, Tokyo, Japan) were inoculated with the filtrates and incubated at 37 °C overnight. The culture medium was then replaced with fresh DMEM-HG containing 2% FBS and antibiotics. The cells were incubated at 37 °C until a cytopathic effect was observed.

2.7. Sequence analysis

Complete genome sequencing for SARS-CoV-2 was carried out as described in our previous study [4]. All whole-genome sequences obtained in this research were deposited in the GISAID database. The Pango lineages of the viral sequences were determined using Nextclade (https://clades.nextstrain.org/).

2.8. Histopathology and immunohistochemistry

Formalin-fixed tissue samples were routinely processed and embedded in paraffin. Tissue sections of 2-μm thickness were cut and stained with hematoxylin and eosin for histopathological examination. For immunohistochemistry, 2-μm tissue sections were deparaffinized and heated at 121 °C for 10 min in retrieval solution with a pH of 6.0 (Nichirei Bioscience, Tokyo, Japan). After washing with phosphate-buffered saline (PBS), endogenous peroxidase was quenched with 3% hydrogen peroxide in PBS. Blocking was then performed with 5% skim milk in PBS for 30 min, after which the tissue sections were incubated with in-house rabbit anti-SARS-CoV-2 N antibody [18] at 4 °C overnight. The slides were then washed with PBS and incubated with Histofine Simple Stain MAX PO (R) (Nichirei Bioscience) at room temperature for 40 min. After washing with PBS, positive signals were visualized using Histofine Simple stain DAB solution (Nichirei Biosicence), and the sections were counterstained with hematoxylin.

2.9. VN testing

To measure the VN antibody titer, a VN test was conducted using our standard methods [4]. The VN titer was determined as the highest dilution that achieved 50% inhibition of the cytopathic effect.

3. Results

3.1. Clinical features and progressions in lions

Of a total of 24 lions in the zoo, 13 showed respiratory symptoms (Table 1, Fig. 1). On 3rd January 2023 the first case, Lion 2 (19-year-old male), showed nasal discharge and anorexia with leukocytosis (white blood cell count 28.7×10^3 cells/μL) and a high blood urea nitrogen (BUN) of 90.7 mg/dL and was treated with ampicillin, amoxicillin, and rehydration. On 4th January 2023, Lion 2 showed polyuria and epistaxis. On 7th January 2023, biochemical tests showed leukocytosis (25.8×10^3 cells/μL) and a high BUN (87.5 mg/dL). Lion 2 died on 9th January 2023.

The second case, Lion 1 (21-year-old female), had a cough, anorexia, and dyspnea on 8th January 2023. On 9th January 2023, her symptoms slightly improved and antibiotic treatment (ampicillin) was started. However, her respiratory symptoms worsened and she died on 12th January 2023.

Lion 6 (16-year-old female) had a cough on 8th January 2023 and recovered on 17th January 2023. From 10th to 17th January 2023, nine other lions (Lions 4, 5, 7, 8, 13, 18, 19, 20, and 21) showed respiratory symptoms, but all recovered within 1 to 9 days after the onset of their symptoms. Just before the SARS-CoV-2 outbreak, Lion 3 (19-year-old male) died of unknown causes on 31st December 2022. One day before his death, Lion 3 showed nasal discharge, dyspnea, anorexia, leukopenia, elevated liver enzymes, increased creatinine phosphokinase, increased total protein, and increased BUN, and was treated with antibiotics and rehydration.

3.2. Detection of SARS-CoV-2 in diseased lions

Real-time RT-PCR was performed to detect SARS-CoV-2 RNA in both fresh and FFPE samples from three deceased lions (Lions 1, 2, and 3) and one affected lion (Lion 5) (Table 2). The results showed the presence of SARS-CoV-2 RNA in all four lions. High viral RNA copy numbers exceeding 50,000 copies were observed in fresh respiratory samples from Lions 1 and 2. In contrast, Lion 3 was PCR-positive only in FFPE cecum specimens, with a low copy number only slightly above the detection limit. Lion 5 showed low RNA copy numbers in fecal samples collected on the 20th and 22nd of January 2023. To rule out the possibility of other infections, RT-PCR was performed to detect influenza A

Table 2
SARS-CoV-2 gene detection and virus isolation from diseased lions.

ID	Date of sample collection	Sample	Real-time RT-PCR (copies/reaction)	Virus isolation (GISAID no.)
Lion 1	12th Jan, 2023	Lung	>50,000	Isolated (EPI_ISL_19084637)
		Lung (FFPE[a])	3227	N.D.
		Liver	4305	N.D.
		Spleen	4165	N.D.
		Serum	+	Not isolated
Lion 2	9th Jan, 2023	Lung	>50,000	Isolated (EPI_ISL_19084638)
		Tracheal swab	>50,000	Isolated (EPI_ISL_19084639)
		Liver	+	N.D.
		Spleen	559	N.D.
		Serum	+	Not isolated
Lion 3	31st Dec, 2022	Heart (FFPE)	−	N.D.
		Duodenum (FFPE)	−	N.D.
		Pancreas (FFPE)	−	N.D.
		Bronchioles (FFPE)	−	N.D.
		Lung (FFPE)	−	N.D.
		Liver (FFPE)	−	N.D.
		Kidney (FFPE)	−	N.D.
		Cecum (FFPE)	75	N.D.
		Stomach (FFPE)	−	N.D.
		Spleen (FFPE)	−	N.D.
		Adrenal gland (FFPE)	−	N.D.
		Gall bladder (FFPE)	−	N.D.
Lion 5	20th Jan, 2023	Feces	117	N.D.
	22nd Jan, 2023	Feces	+	N.D.
	30th Jan, 2023	Feces	−	N.D.

FFPE: formalin-fixed paraffin-embedded samples; N.D.: not done; +: positive real-time RT-PCR result with a copy number of <50 copies per reaction; −: result below the detection limit.

[a] Real-time RT-PCR targeting the SARS-CoV-2 N gene was performed using RNA extracted from FFPE specimens.

virus and canine distemper virus (CDV). A highly pathogenic avian influenza virus had infected birds in the zoo just before the SARS-CoV-2 outbreak. Furthermore, CDV was known to have infected wild animals around the zoo. However, no genes for influenza A virus and CDV were detected in Lions 1 and 2 (data not shown).

3.3. Nucleotide sequences of SARS-CoV-2 detected in lions

The complete nucleotide sequences of SARS-CoV-2 from three samples from Lions 1 and 2 were determined using next-generation sequencing (Table 2). All determined sequences were identical and classified into the BF.7.15 lineage, which was the main SARS-CoV-2 variant detected in Japan from October 2022 to July 2023 [19]. When compared with other sequences within the BF.7.15 lineage, no unique amino acid changes were observed in the lion samples (data not shown). Three viruses were isolated from the lung of Lion 1 and from the lung and trachea of Lion 2 using Vero E6-TMPRSS2-T2A-ACE2 cells, which are highly sensitive to SARS-CoV-2 infection. One isolate from the lung tissue of Lion 1, hCoV-19/Lion/Japan/Lion1/2023, was used for the VN test.

3.4. Histopathological analysis of respiratory tissue from deceased animals

Tissue samples from three deceased lions underwent histopathological analyses (Fig. 2). The lung of Lion 1 showed massive pulmonary edema and infiltration of neutrophils and macrophages in the alveolar septae and alveolar spaces. Some alveolar septae showed necrosis. These findings were compatible with the acute phase of diffuse alveolar damage. In the lung of Lion 2, the alveolar epithelial cells were mainly composed of type II pneumocytes and the alveolar septae were thickened with edema, infiltration of lymphocytes and macrophages, and proliferation of fibroblasts, indicating the chronic phases of interstitial pneumonia, which is the typical pneumonia pattern in viral infection. The lung of Lion 3 was in the organizing phase of chronic interstitial pneumonia, characterized by prominent fibrosis, proliferation of type II pneumocytes, and infiltration of lymphocytes in the interstitium. Immunohistochemistry detected numerous SARS-CoV-2 N-positive cells in the bronchioles of Lions 1 and 2, but not Lion 3 (Fig. 2).

3.5. Detection of VN antibodies against omicron variants of SARS-CoV-2 in lions

To confirm SARS-CoV-2 infection in the lions, a VN test was performed using serum samples from 23 lions, except Lion 3 (Table 3). A total of 21 lions had detectable VN antibodies against the omicron BF.7.15 lineage isolated from Lion 1, while two lions (Lions 1 and 2) who died of acute infection did not. The same trend was observed in VN tests against TY41–702, which belongs to the other omicron strain BE.1 lineage and is closely related to BF.7.15. However, VN antibodies against the delta variant, TY26–439, which was the major variant in Japan just before the emergence of omicron variant, were below the limit of detection in all lions.

3.6. Detection of VN antibodies against SARS-CoV-2 in other animal species

Further serological studies were conducted to investigate the possibility that SARS-CoV-2 could have spread to other animal species in the same or neighboring buildings with the lions. The VN test was performed using serum samples from three tigers, four cheetahs, and three bears that were collected before or after the outbreak. The tigers were housed in the same building as the lions and sometimes shared cages with lions for veterinary services. Cheetahs and bears were housed in neighboring buildings with a distance of 5 m. The VN tests showed that three tigers and one bear were seropositive for the omicron BF.7.15 strain (Table 4). None of the animals possessed VN antibodies against the delta variant.

4. Discussion

Our findings document an outbreak of SARS-CoV-2 in lions and other zoo animals. To date, there is only one report of lions dying due to infection with the SARS-CoV-2 delta variant [13], while the present study is the first to report lions dying due to infection with the omicron variant. After the emergence of the omicron variant, fatal animal cases have rarely been reported. This is likely because the omicron variant is attenuated and has lower pathogenicity in humans and animals compared with previous variants. Early studies have assessed the clinical severity of the omicron variant and indicate that it causes milder symptoms than the delta variant in humans [20]. *Ex vivo* experiments using human lung tissue showed that omicron's replication efficiency in lung parenchyma is lower than that of the D614G and delta variants, coinciding with the lower pathogenicity of the omicron variant in

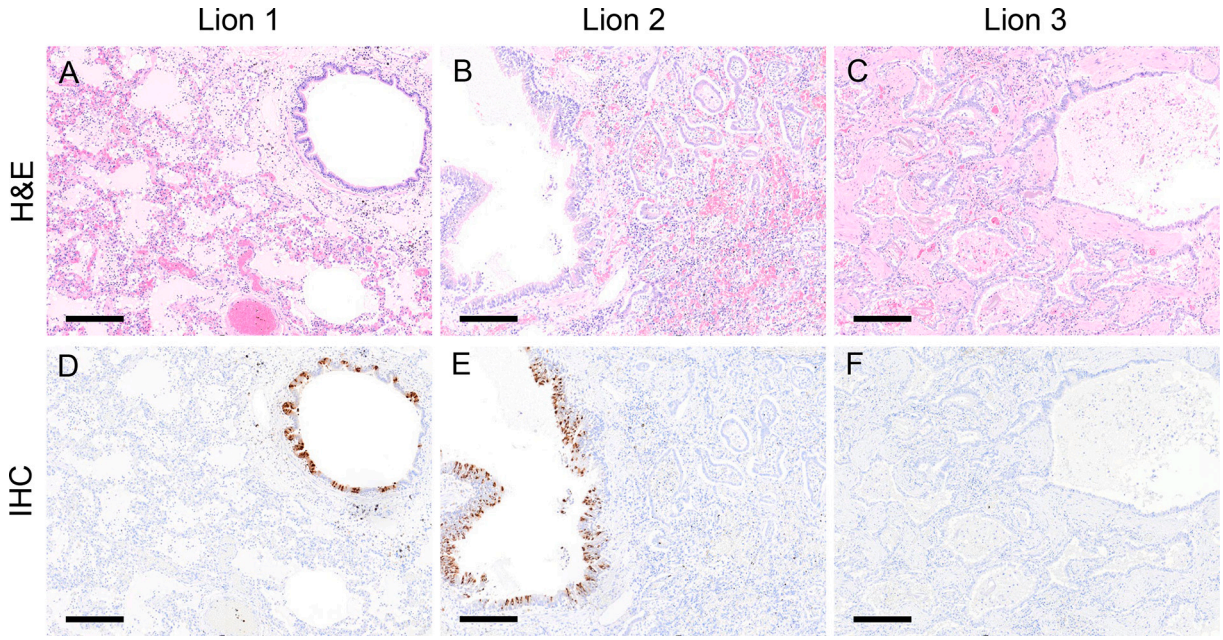

Fig. 2. Histopathological analysis of lung tissues from deceased lions.
(A, B, C) Hematoxylin and eosin (H&E) staining of paraffin-embedded lung samples from Lions 1, 2, and 3, respectively. (D, E, F) Immunohistochemical (IHC) staining of bronchioles reveals SARS-CoV-2-infected cells in Lions 1 and 2, but not in Lion 3. Bars indicate 200 μm.

humans [21]. The attenuation of the omicron variant was also confirmed in *in vivo* animal experiments. Several studies have shown that omicron infection causes milder body weight loss and lower lethality in hamsters, and less severe pneumonia compared with other variants [22,23]. These viral features likely contributed to the low number of animal fatalities due to omicron variant infection. Therefore, the present report of lion deaths provides valuable information on the pathogenicity of the omicron variant in animals under natural conditions.

The deceased lions were the three oldest lions in the zoo, while the next five oldest individuals also exhibited symptoms; this indicates that, like humans, the symptoms in lions tend to worsen with increasing age [24,25]. The two keepers of these lions and tigers were diagnosed with COVID-19 on the 4th and 9th of January 2023, respectively, at the same time as the lions' deaths. From December 2022 to January 2023, the other workers in this zoo also exhibited symptoms of COVID-19. Although the source of the SARS-CoV-2 infection in the lions has not been clarified, it is likely that these lions were infected by the zookeepers. In addition, there were 2 weeks between the death of the first reported lion (Lion 3) and the onset of symptoms in the last affected lion (Lion 21) (Table 1, Fig. 1). This delay in the onset of symptoms among lions suggests the possibility that the virus was transmitted between lions.

SARS-CoV-2 RNA and N antigen were detected in the lungs of Lions 1 and 2, confirming virus replication in respiratory tissues (Table 2, Fig. 2). In addition, histopathological studies showed infiltration of inflammatory cells in the lungs, suggesting that the lions might have died as a result of acute progression. This finding is consistent with the absence of any detectable VN antibodies in Lions 1 and 2 at the time of their deaths. Viral RNA was also detected in liver and spleen samples, but no viral antigens were detected in these organs by immunohistochemical tests (data not shown). It is unknown whether the virus propagated in these organs.

The viral genomes determined from the lion samples showed no significant amino acid changes compared with other strains of human origin in the same clade. This suggests that the lions died from infection with the omicron variant circulating in the human population. Previous studies have shown that SARS-CoV-2 often acquires amino acid changes in spike proteins related to virus entry and antigenicity during replication in animals [26,27]. In the present study, no adaptation-related mutations were identified. Further studies are required to determine whether the omicron strain detected in the present study is pathogenic in lions.

Lion 3 was the first to die, but the viral gene was only detected in the FFPE specimen of the cecum, and the viral copy number was low (Table 2). The immunohistochemical analysis was negative for the SARS-CoV-2 N antigen (Fig. 2). SARS-CoV-2 infection was not initially suspected in Lion 3, and no tissue organ samples were collected for further examination. Therefore, although Lion 3 was infected with SARS-CoV-2, it is unclear whether SARS-CoV-2 infection was the direct cause of death.

The 21 lions had VN antibodies against the omicron variants, but not against the delta variant (Table 3). The omicron variants BF.7.15 and BE.1 are derived from BA.5, and antigenic differences between the BA.5 and delta variants have been reported [28]. These results confirm that an outbreak of SARS-CoV-2 omicron infection occurred among lions. The absence of VN antibody against the omicron variant in Lions 1 and 2 and of VN antibody against the delta variant in all lions also indicated that there was no SARS-CoV-2 infection in this pride of lions before this outbreak. In addition, no detectable VN antibodies in dead lions indicated that lions rapidly died before increase of VN antibody.

Serological tests revealed that all three tigers were infected with SARS-CoV-2 (Table 4). Tigers are known to be susceptible to SARS-CoV-2 and show respiratory symptoms after infection [29]. In this zoo, the tigers were housed in the same building as the lions. According to the zookeepers, insufficient ventilation in this building at the beginning of the outbreak might have increased the risk of SARS-CoV-2 infection in the tigers.

Serum collected from Bear 1 on 6th April 2023 tested positive for VN antibodies against SARS-CoV-2 omicron BF.7.15, indicating that this bear might have been infected with the omicron variant between 11th January and 6th April 2023. Previous studies have reported that angiotensin-converting enzyme 2, a known receptor for SARS-CoV-2, can bind to the spike protein of SARS-CoV-2 in bears at the same level as angiotensin-converting enzyme 2 in dogs [30]. To our knowledge, there have been no reports of SARS-CoV-2 infection in bears. The transmission route to the bear remains unknown; however, some

Table 3
Virus-neutralization test against SARS-CoV-2 using serum from lions.

ID	Date of disease onset	Date of sample collection	Virus-neutralization titer		
			Omicron BF.7.15	Omicron BE.1	Delta
Lion 1	8th Jan, 2023	12th Jan, 2023	<5	<5	<5
Lion 2	3rd Jan, 2023	9th Jan, 2023	<5	<5	<5
Lion 3	30th Dec, 2022	N.D.	N.D.	N.D.	N.D.
Lion 4	10th Jan, 2023	6th Mar, 2023	14	28	<5
Lion 5	10th Jan, 2023	23rd Feb, 2023	80	57	<5
Lion 6	8th Jan, 2023	4th Mar, 2023	40	40	<5
Lion 7	10th Jan, 2023	2nd Mar, 2023	10	14	<5
Lion 8	11th Jan, 2023	2nd Mar, 2023	40	40	<5
Lion 9	–	23rd Feb, 2023	80	57	<5
Lion 10	–	26th Feb, 2023	14	28	<5
Lion 11	–	26th Feb, 2023	5	5	<5
Lion 12	–	26th Feb, 2023	5	<5	<5
Lion 13	14th Jan, 2023	25th Feb, 2023	57	80	<5
Lion 14	–	23rd Feb, 2023	40	28	<5
Lion 15	–	25th Feb, 2023	40	40	<5
Lion 16	–	26th Feb, 2023	20	40	<5
Lion 17	–	26th Feb, 2023	20	14	<5
Lion 18	15th Jan, 2023	2nd Mar, 2023	40	57	<5
Lion 19	14th Jan, 2023	6th Mar, 2023	28	28	<5
Lion 20	13th Jan 2023	6th Mar, 2023	20	28	<5
Lion 21	17th Jan 2023	6th Mar, 2023	7	10	<5
Lion 22	–	5th Mar, 2023	40	40	<5
Lion 23	–	5th Mar, 2023	20	20	<5
Lion 24	–	10th Mar, 2023	40	40	<5

N.D.: not done.

zookeepers worked in both the building that housed the lions and the building that housed the bears.

5. Conclusion

We report the first lion deaths caused by the SARS-CoV-2 omicron variant. Although the omicron variant was thought to be attenuated [20–23], our findings suggest that this variant still retains sufficient virulence to cause death in lions. To protect susceptible and unvaccinated animal species from SARS-CoV-2, it is essential to prevent human-to-animal transmission of the virus. The present study indicates that a continuous countermeasure, the One Health approach, against SARS-CoV-2 is still recommended to protect human and animals from the infection.

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Table 4
Results of virus neutralization test against SARS-CoV-2 using serum from animals housed near the lions.

ID	Species	Sex	Age (years)	Date of sample collection	Virus-neutralization titer	
					Omicron BF.7.15	Delta
Tiger 1	Amur tiger (*Panthera tigris altaica*)	Male	5	18th Apr, 2023	14	<5
Tiger 2	Bengal tiger (*Panthera tigris tigris*)	Female	6	26th Apr, 2023	28	<5
Tiger 3	Amur tiger (*Panthera tigris altaica*)	Female	11	26th Apr, 2023	7	<5
Cheetah 1	Cheetah (*Acinonyx jubatus*)	Female	9	5th Dec, 2022	<5	<5
				5th Jan, 2023	<5	<5
				21st Jan, 2023	<5	<5
Cheetah 2	Cheetah (*Acinonyx jubatus*)	Female	11	6th Dec, 2022	<5	<5
				24th Jan, 2023	<5	<5
Cheetah 3	Cheetah (*Acinonyx jubatus*)	Female	11	12th Dec, 2022	<5	<5
				23rd Jan, 2023	<5	<5
				22nd Feb, 2023	<5	<5
Cheetah 4	Cheetah (*Acinonyx jubatus*)	Female	4	14th Dec, 2022	<5	<5
				1st Feb, 2023	<5	<5
				16th Feb, 2023	<5	<5
Bear 1	Asian black bear (*Ursus thibetanus*)	Female	25	11th Jan, 2023	<5	<5
				6th Apr, 2023	14	<5
Bear 2	Ezo brown bear (*Ursus arctos yesoensis*)	Female	18	11th Jan, 2023	<5	<5
				24th Mar, 2023	<5	<5
Bear 3	Ezo brown bear (*Ursus arctos yesoensis)*	Female	27	16th Jan, 2023	<5	<5
				25th Mar, 2023	<5	<5

CRediT authorship contribution statement

Yudai Kuroda: Writing – original draft, Investigation. **Miki Ozaki:** Writing – original draft, Investigation. **Yusuke Sakai:** Writing – review & editing, Investigation. **Eri Uchida-Fujii:** Writing – review & editing, Investigation. **Ikumi Hanada:** Writing – review & editing, Investigation. **Tsukasa Yamamoto:** Writing – review & editing, Investigation. **Kango Tatemoto:** Writing – review & editing, Investigation. **Yuichiro Hirata:** Writing – review & editing, Investigation. **Yuko Sato:** Writing – review & editing, Investigation. **Harutaka Katano:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Noriyo Nagata:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hirofumi Kato:** Writing – review & editing, Investigation. **Tomoe Shimada:** Writing – review & editing, Supervision, Funding

acquisition, Conceptualization. **Tadaki Suzuki:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Tatsuko Nakao:** Writing – review & editing, Supervision, Conceptualization. **Ken Maeda:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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